Hospital A.C.Camargo

INCITO: National Institute of Science and Technology in Oncogenomics

Hereditary Cancer and Familial Aggregation: clinical and molecular profiles from Brazilian patients and their relatives with high cancer risk

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PARTIAL REPORT CONCERNING THE PERIOD

16/1/2009 TO 16/1/2010

Summary

A. Basic Research

A.1. Hereditary Cancer Syndromes and Familial Aggregation of Cancer

1.1. Copy number variations (CNVs) in Hereditary Cancer Syndrome and Familial Aggregations: Colorectal Cancer - Lynch Syndrome, Breast and Ovarian Carcinoma, Li-Fraumeni Syndrome

1.2. Wide genome screening for gene mutation by combining exon-enriched genomic fragment and deep sequencing in hereditary nonpolyposis colorectal carcinomas.

A.2. Tumor Biology

2.1. Expression profile-based test for breast cancer prognosis using protein-coding and noncoding genes

2.2. Expression profile-based test for prostate cancer prognosis using protein-coding and noncoding genes

2.3. Integration of vascular genomics and proteomics for diagnosis and therapy of cancer

B: Clinical Trials

B.1. A Phase I Study of ^{18}F-FluoroAcetate Sodium (^{18}F- FAS) as a PET Imaging Agent for Tumor Detection (prostate and breast carcinoma).

B.2. Prospective nonrandomized Phase II study to identify response markers to neoadjuvant chemotherapy and association with radiotherapy and cetuximab in patients with epidermoid carcinoma of the oropharynx.

B.3. Prospective comparative Phase I single-center clinical study to evaluate the effectiveness and safety of the formulation for topical use of F8 protein derived from *Hevea brasiliensis* in the treatment of pressure ulcers.

C: Patient Recruitment and Follow-up, Training, Education Programs, Diffusion and Technology Transfer

C.1. Establishment of research protocols for recruitment and follow-up of individuals at high risk of hereditary carcinomas

C.2. Epidemiological data collection. Database management

C.3. Training and Education Programs

References

Our publications on the subject

Students involved in the project

Development schedule of the project according to the objectives originally proposed

Introduction

In this report we present the accomplishments achieved by the INCITO in 2009 and future directions. This is the first year of the third period and new projects are under development. Therefore, this report addresses only the new data and recent alterations. Most of the technology to update the Research Center is now in the process of acquisition and the results of some projects are preliminary. To make this report more easily understandable to the readers, it follows the same topic organization of the initial proposal presented in 2008**.**

A. Basic Research

A.1. Hereditary Cancer Syndromes and Familial Aggregation of Cancer

1.1. Copy number variations (CNVs) in Hereditary Cancer Syndrome and Familial Aggregations: Colorectal Cancer - Lynch Syndrome, Breast and Ovarian Carcinomas and Li-Fraumeni Syndrome

Summary

It is accepted that 5 to 10% of all cancers are hereditary or familial (Garber & Offit, 2005). The majority of hereditary neoplasias related to breast cancer are associated with germinative mutations in the genes *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 (Lin et al, 1999; Lipton et al, 2001; Walsh et al, 2006; Lynch et al, 2009).

Li-Fraumeni syndrome and its variant Li-Fraumeni-like (LFS/LFL) is a rare autosomal disease related to germinative mutation in the gene *TP53* (Malkin et al., 1992). The cancers most frequently associated with germinative mutations in *TP53* are breast cancer, bone and soft tissue sarcomas, brain tumors, adrenocortical carcinomas (ADC) (Birch et al, 1998) and leukemia. The R337H genetic alteration was previously reported as specific to adrenocortical tumors in Brazilian patients (Ribeiro et al, 2001). Recently, our group evaluated the prevalence of the R337H alteration in 750 healthy women who participated in a screening program for breast cancer in Porto Alegre, southern Brazil. R337H was detected in two women, indicating a frequency of 0.0015%. The carriers reported family history of cancer at multiple sites (Palmero et al, 2008). We suggest that the R337H alteration could have low penetrance predisposition to multiple cancers in southwestern Brazil and a frequency 10-20-fold greater than other mutations in *TP53* commonly associated with LFS (Achatz et al, 2007).

Breast cancer and colorectal cancer (CRC) are among the most common malignant neoplasias in developed and developing countries. Breast cancer is the second most frequent malignant neoplasia worldwide and the first among women. For the year 2010, the National Cancer Institute (*Instituto Nacional do Câncer*, INCA) estimated 49,240 new breast cancer cases. In the Southeastern region, this neoplasia has the highest incidence among women, with an estimated risk of 38,470 new cases per 100,000 women. CRC is the third most frequent tumor in women, with an estimated 14,800 new cases for the year 2010. In the Southeastern region, this is the third most frequent neoplasia in women, with 12,440 new cases per 100,000 women (INCA).

HNPCC can be classified as Lynch Syndrome I (SL I) and Lynch Syndrome II (SL II), according to the presence or absence of extracolonic cancers (Watson & Lynch, 1993). The former is associated with inherited predisposition to the dominant autosomal nonpolyposis CRC at a young age, predilection for the proximal colon and multiple primary CRCs. Besides presenting the same characteristics as the former, the latter is also associated with extracolonic cancer, particularly with carcinomas of the endometrium, ovary, stomach, hepatobiliary tract, pancreas, urethra, renal pelvis, brain and breast (Lynch et al, 1988; Watson & Lynch, 1994; Risinger et al, 1996; Boyd et al, 1999; Lynch & Lynch, 2000; Lynch et al, 2009).

Lynch syndrome represents 3% of CRC cases (Hampel et al, 2008). Moreover, it is a dominant autosomal disease with high penetrance (approximately 85%),

characterized by early development of CRC and by its association with other types of epithelial tumors.

Mutations in two high penetrance genes, specifically *BRCA1* and *BRAC2*, are responsible for approximately 16% of familial risk of breast cancer. In Hereditary Breast and Ovarian Cancer Syndrome (OMIM #114480), 60 to 80% of the mutations occur in the tumor suppressor genes *BRCA1* and *BRAC2* (Miki et al, 1994; Easton et al, 1995; Scott et al, 2003). Even considering that subsequent studies cannot identify another high penetrance gene related to predisposition to breast cancer, several genes that confer low to moderate risk of developing breast cancer have already been identified. Hereditary breast cancer could be part of numerous cancer syndromes (Tan et al, 2008). Besides *BRCA1* and *BRAC2*, other genes could be involved in Hereditary Breast Carcinoma Syndrome.

Some studies report that patients with mutation in the gene *BRCA1* have a 4.11-fold greater risk of developing CRC. Other studies indicate that the risk of patients with breast developing CRC is greater than that verified in the general population without disease (Ford et al, 1994; Burke et al, 1997; Olsen et al, 1999; Lin et al, 1999). Meijers-Heijboer et al (2003) verified that in certain families of patients who presented deletion in the gene *CHEK2* and were diagnosed with breast carcinoma, CRC also developed. Mutation 1100delC in the gene *CHEK2* is associated with increased risk of developing cancer, since it promotes blockage of the kinase function of this gene (Kilpivaara et al, 2003). This mutation is present in high frequencies in families with cases of hereditary breast and colon cancer (HBCC) (Isinger et al, 2006); moreover, this mutation increases susceptibility to breast and colon cancers (Wasielewski et al, 2009). Apparently, this gene acts in synergy with susceptibility genes, within a more complex polygenic model (Meijers-Heijboer et al, 2003).

According to Langerod et al (2007), more than half of all cancer cases present mutations in the gene *TP53*; however, the frequency of these depends on tumor type and subtype. In breast carcinoma, this frequency varies between 20 and 30%. Breast tumor is one of the tumors most associated with mutations in the gene *TP53*; however, CRC also presents an association with mutations in this gene (Petitjean et al, 2007). Mutations in *TP53* in breast cancer are also associated with worse prognosis, resistance to chemotherapeutic drugs and early development of the disease.

There is evidence to suggest that certain cancers could be the result of structural variations that involve abundant DNA fragments in the human genome, related to genetic variability and, therefore, capable of influencing the susceptibility to these complex diseases.

Structural variations in the human genome have been known for many years, but only recently have the dimension and impact of these alterations on the variability of the genome become evident, principally resulting from dissemination of the use of the technique array-based comparative genomic hybridization (array-CGH) and other large-scale investigation technologies of the genome. The copy number variations (CNVs) of segments of DNA of the human genome are defined as large DNA sequences (1 Kb to 2-3 Mb) that differ in the number of copies between different genomes (for review: Sebat, 2007). Although less scientific investigation has been applied to CNVs than to single nucleotide polymorphisms (SNPs), CNVs have an important relation with human diversity and could influence susceptibility to complex diseases, like cancer (Lee et al , 2007; Beckmann et al, 2007; McCarroll & Altshuler, 2007).

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 majority of studies involving hereditary cancer syndromes are based on North American and European populations, as such, limited information exists concerning the South American populations. The Department of Oncogenetics of the AC Carmago Hospital was created in 2000 and since then, more than 3,500 patients and family members have received Genetic Counseling. The Oncotree software was developed by the hospital's Bioinformatics Laboratory as a clinical data management software, used to monitor family history data. In the AC Carmago Cancer Hospital (*Hospital do Câncer AC Camargo*, HCACC), peripheral blood DNA samples of patients with hereditary cancer or family history or familial aggregation of cancer have been recruited over the years. 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 who are negative for mutations in the principal candidate genes are also evaluated by array-CGH. The objective is to publicize rare genomic imbalances that could contain new hereditary predisposition genes, aimed at defining or identifying new markers of risk of susceptibility to cancer.

Methods

Direct Sequencing

Genomic DNA was isolated from peripheral blood and screened for mutations in *BRCA1* (U14680 or NM_007294.3), *BRCA2* (U43746 or NM_000059.1), and *CHEK2* (NM_007194.3) in probands from families with Breast and Ovarian Cancer Syndrome. For Lynch syndrome, *MLH1* (NM_000249.2), *MSH2* (NM_000251.1) and *MSH6* (NM_000179.1) were also screened for mutations. Full sequence determination in both forward and reverse directions was performed for the all genes, except for *CHEK2*, where only deletion 1,100 in the mRNA sequence (1100delC) was evaluated. These evaluations were performed by direct sequencing in DNA of blood samples from 75 patients with hereditary breast cancer (breast/ovarian and breast/colorectal hereditary syndromes) and 113 patients that fulfilled the criteria for Lynch (or HNPCC) Syndrome. The classification of *BRCA1* and *BRCA2* alterations was performed using the Breast Cancer Information Core database (BIC – *http://research .nhgri.nih.gov/bic/*). For classification of *MLH1, MSH2* and *MSH6* the following databases were used Leiden Open Variation Database - LOVD (http://chromium.liacs.nl/LOVD2/colon_cancer/home.php), MMR gene unclassified variants database (http://www.mmrmissense.net/) and dbSNP (http://www.ncbi.nlm.nih.gov/snp/). *TP53* mutations (exons 4–9) were evaluated by denaturing high performance liquid chromatography (DHPLC). Exons 2, 3, 10 and 11 were analyzed by direct sequencing. All mutations were confirmed by a second, independent analysis.

Array-CGH

Although the use of Agilent 244K arrays (a slide containing a single array with ~240.000 oligonucleotides) in this project had been previously indicated, Agilent Technologies (Santa Clara, CA) released higher density arrays last year, specifically Agilent SurePrint G3 Human CGH Microarray 4 x 180K platforms. This slide contains 4 arrays, each comprising ~170,000 coding and noncoding human sequences, annotated against UCSC hg18 (NCBI Build 36, March 2006). The overall average probe spacing is 17 Kb, although the distribution is uneven, with higher density in coding regions than in the backbone. The prices per **slide** (not per array) with 4 x 180K or 244K platforms are similar. The coverage of the new 180K is about 75% of the 244K array, while the costs are reduced in

approximately 25% in comparison with the 244k; therefore, we decided to use the 4x180K in this project.

Test DNAs were extracted by standard protocols from peripheral blood lymphocytes of probands. Gender matching commercial DNA (Promega, Mannheim, Germany) was used as reference. Samples were differentially labeled by random priming either with Cy3- or Cy5-dCTPs (BlueGnome Ltd, Cambridge, UK). Hybridization and washing were performed according to the manufacturer's protocols (Agilent Technologies). Slides were scanned using the Agilent DNA microarray scanner with a 48-slide system. This scanner has been upgraded and permits High-Resolution scanning at 2, 3, 5 or 10 micron resolution, as required by the 180K platform (2-3 micron). The data was extracted with the Feature Extraction version 10.7 and analyzed using Genomic Workbench 5.0 software (Agilent Technologies), with the statistical algorithm ADM-2 and sensitivity threshold of 6.7. As an analysis criterion, the presence of at least three consecutive probes with aberrant values was used. DNA copy number changes were compared both to our reference dataset (currently 40 women not affected by cancer or history of familial cancer) and to the Database of Genomic Variants (DGV; *http://projects.tcag.ca/variation/*).

Patients

Using direct sequencing of specific genes, 75 patients with breast cancer (associated with ovarian or colorectal cancer), 113 patients with Lynch Syndrome and 121 probands of Li-Fraumeni Syndrome and Li-Fraumeni-like were evaluated.

For analysis of CNVs, samples from 52 patients of families with predisposition for breast-ovarian cancer (BO), 64 patients of families with LFS and LFL and 38 patients belonging to families with breast-colon syndrome.

Results

Gene Screening for Mutations

Among the 75 patients with hereditary breast cancer (ovarian or colorectal), seven presented clinically relevant alterations, three in the *BRCA2* and four in the *BRCA1* gene. No alterations in the 1,100 position for *CHEK2* were verified. Among the 113 Lynch syndrome patients, 34 pathogenic mutations were detected, 16 in *MLH1*, 17 in *MSH2* and only one in *MSH6*. In Tables 1 and 2, the alterations of each gene associated with hereditary breast and Lynch syndromes, respectively, are presented.

Table 1: Alterations identified in patients with hereditary breast-ovarian cancer and breast cancer[#] and colorectal syndrome[&].

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-001 [#]	BRCA1	5203delTT	18	yes
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-002 [#]	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-003 [#]	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.5972C>T, p.T1915M	11	unknown
SM-006 [&]	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.8371T>C, p.S2835P	20	no
SM-007 [#&]	BRCA1	undetected		
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-008 [#]	BRCA1	undetected		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		6174delT	11	yes
SM-009 [#&]	BRCA1	undetected		
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-010 [#&]	BRCA1	c.5002T>C, p.M1628T	16	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-011 [#&]	BRCA1	c.2731C>T, p.P871L	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-012 #&	BRCA1	undetected		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-013 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.2024T>C, p.F599S	10	undescribed
SM-014 #&	BRCA1	c.1186A>G, p.Q356R	11	unknown
		c.4654G>T, p.S1512I	15	no
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-015 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-016 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-017 #	BRCA1	c.3376T>G, p.L1086X	11	yes
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-018 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-019 #&	BRCA1	c.3238G>A, p.S1040N	11	unknown
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
		c.9079G>A, p.A2951T	22	no
SM-020 #	BRCA1	c.5002T>C, p.M1628T	16	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2578A>G, p.M784V	11	unknown

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-021 #	BRCA1	c.1186A>G, p.Q356R	11	unknown
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4962G>A, p.A1615T	16	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-022 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-023 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
		c.5075G>A, p.M1652I	16	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-024 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1192A>C, p.K322Q	10	unknown
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	unknown
		c.2457T>C, p.H743H	11	unknown
		c.2578A>G, p.M784V	11	unknown
		c.3199A>G, p.N991D	11	no
SM-025 #	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-026 &	BRCA1	c.3238G>A, p.S1040N	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-027 #&	BRCA1	c.3238G>A, p.S1040N	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-028 #&	BRCA1	undetected		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-029 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.2024T>C, p.F599S	10	undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-030 #&	BRCA1	c.1186A>G, p.Q356R	11	unknown
		c.3830A>G, p.I1237M	11	unknown
		c.4962G>A, p.A1615T	16	unknown
	BRCA2	c.353A>G, p.Y42C	3	unknown
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.10338G>A, p.R3370R	27	unknown
SM-031 #&	BRCA1	c.1186A>G, p.Q356R	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.8377G>T, p.2717S	18	no
SM-032 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-033 &	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-034 #&	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
		c.5075G>A, p.M1652I	16	unknown
	BRCA2	c.2024T>C, p.F599S	10	undescribed
SM-035 #	BRCA1	c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2016T>C, p.D596D	10	unknown
		c.2024T>C, p.F599S	10	undescribed
SM-036 #&	BRCA1	c.3238G>A, p.S1040N	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-037 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
		c.5467T>C, M1783T	22	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.6575A>G, p.D2110A	11	unknown
SM-038 #&	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	Undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-039 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2578A>G, p.M784V	11	unknown
SM-040 #	BRCA1	c.3232A>G, p.E1038G	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.8377G>T, p.2717S	18	no
SM-041 #&	BRCA1	c.2196G>A, p.D693N	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.1711G>A, p.A495P	10	undescribed
		c.2024T>C, p.F599S	10	undescribed
SM-042 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-043 #	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.2024T>C, p.F599S	10	undescribed
		c.9313G>A, p.A3029T	23	unknown
SM-044 &	BRCA1	undetected		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-045 #	BRCA1	c.2731C>T, p.P871L	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.5972T>C, p.M1915T	11	unknown
SM-046 #	BRCA1	c.4158A>G, p.R1347G	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.7697T>C, p.I2490T	15	unknown
		c.9709A>T, p.K3161X	25	yes
SM-047 &	BRCA1	c.3238G>A, p.S1040N	11	unknown
		c.4461A>G, p.S1448G	13	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-048 #&	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c.3199A>G, p.N991D	11	no
SM-049 #	BRCA1	c.2196G>A, p.D693N	11	no
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-050 #&	BRCA1	c.2090A>G, p.Q657Q	11	unknown
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-051 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.772G>C	7	undescribed
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-053 #&	BRCA1	c.5002T>C, p.M1628T	16	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-054 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-055 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-056 &	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-057 &	BRCA1	No detect		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-058 #	BRCA1	c.2731C>T, p.P871L	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-059 &	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-060 #&	BRCA1	No detected		
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-061 #	BRCA1	c.4158A>G, p.R1347G	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.9709A>T, p.K3161X	25	yes
SM-062 #	BRCA1	c.1605C>T, p.R496C	11	unknown
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-063 &	BRCA1	c.5002T>C, p.M1628T	16	unknown
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
SM-064 #&	BRCA1	c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1093A>C, p.N289H	10	no
		c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
		c3199A>G, p.N991D	11	no
		c.9079G>A, p.A2951T	22	no
SM-065 &	BRCA1	c.2731C>T, p.P871L	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.2457T>C, p.H743H	11	unknown
SM-066 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-067 #&	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed

Proband	*Genes*	*Alteration identified*	*Exon*	*Clinically Important*
SM-068 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-069 #	BRCA1	c.5242C>A, p.A1708E	18	yes
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-070 &	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-073 #	BRCA1	c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-074 #&	BRCA1	2080delA	11	yes
		c.2731C>T, p.P871L	11	no
		c.3232A>G, p.E1038G	11	no
		c.3667A>G, p.K1138R	11	no
		c.4956A>G, p.S1613G	16	no
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-080 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.5972C>T, p.T1915M	11	unknown
SM-090 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-094 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-097 #	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
		c.4827A>C, p.K1533N	11	unknown
SM-099	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed
SM-100 #&	BRCA1			
	BRCA2	c.1342C>A, p.H372N	10	no
		c.2024T>C, p.F599S	10	undescribed

Table 2: Alterations identified in patients with Lynch syndrome.

Proband	*Gene*	*Alteration identified*	*Exon*	*Classification of Pathogenicity*	*References*
SL-001	MLH1	c.545+3(A->G)	6	Pathogenic	Pensotti V. Genes Chromosomes Cancer. 1997;19(3):135-42
		c.2152 C>T p.His718Tyr	19	Nonpathogenic	Takahashi M. Cancer Res 2007: 4595-604
	MSH2	not detected			
	MSH6	*not performed*			
SL-002	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-003	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-004	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005, 537-549.
	MSH2	c.1447G>T p.Glu483X	9	Pathogenic	Lagerstedt Robinson. J Natl Cancer Inst. 2007;99(4):291-9
	MSH6	*not performed*			
SL-005	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	c. 2525_2526delAG (p.Glu842ValfsX3)	15	Pathogenic	
	MSH6	*not performed*			
SL-006	MLH1	not detected			
	MSH2	c.1447G>T p.Glu483X	9	Pathogenic	Lagerstedt R. J Natl Cancer Inst. 2007;99(4):291-9
	MSH6	*not performed*			
SL-008	MLH1	not detected			
	MSH2	c.1667delT p. Leu556X	11	Pathogenic	
	MSH6	*not performed*			
SL-011	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-012	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-013	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-014	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-015	MLH1	not detected			
	MSH2	c.2187G>T p.Met729Ile	13	Pathogenic	First described - SIFT and Polyphen
	MSH6	*not performed*			
SL-016	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-017	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-018	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-019	MLH1	c.2224C>T p.Gln742X	19	Pathogenic	First described
	MSH2	not detected			
	MSH6	*not performed*			
SL-020	MLH1	not detected			
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			
SL-021	MLH1	c.779T>G p.Leu260Arg	9	Pathogenic	Montera M. J Med Genet. 2000;37(7):E7
	MSH2	not detected			
	MSH6	*not performed*			
SL-022	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-023	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-024	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-025	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	c.2131C>T p .Arg711X	13	Pathogenic	Kurzawski et al., Clin Genet. 2006;69(1):40-7
	MSH6	*not performed*			
SL-026	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-027	MLH1	*c.*655A>G + 655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	c.965G>A p.Gly322Asp	6	Nonpathogenic	Drotschmann K. Current Biol. 1999: 907-910.
	MSH6	not detected			
SL-028	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2				
	MSH6				
SL-029	MLH1	c.588+2T>A	7	Pathogenic	First described
		*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-030	MLH1	c.655A>G p.Ile219Val	8	NonPathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	c.3974_3976delAGA +c.3974_3976delAGA p.Lys1325MetfsX10	9	Unknown	

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-031	MLH1	c.791-6_793delgtttagATC	10	Pathogenic	First described
		*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-032	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-033	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-034	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-035	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-036	MLH1	c.2027 T>C p.Leu676Pro	18	Pathogenic	First discribed
	MSH2	not detected			
	MSH6	*not performed*			
SL-037	MLH1				
	MSH2				
	MSH6				
SL-038	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
		c.1852_1853delAAIns GC p.Lys618Ala	16	Nonpathogenic	Takahashi M. Cancer Res. 2007: 4595-604
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			
SL-039	MLH1	not detected			
	MSH2	c.1967_1970dup ACTT p.Phe657LeufsX3	12	Pathogenic	
	MSH6	*not performed*			
SL-040	MLH1	c.655A>G + c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol.2005: 537-549.
	MSH2	c.1444A>T p.Arg482X	9	Pathogenic	First described
	MSH6	*not performed*			
SL-041	MLH1	not detected			
	MSH2	not detected			
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-042	MLH1	c.655A>G + c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol.2005: 537-549.
	MSH2				
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-043	MLH1				
	MSH2				
	MSH6				
SL-044	MLH1	not detected			
	MSH2	c.2785C>T p. Arg929X	16	Unknown	
		c.942+3 A>T	5	Pathogenic	Rahner et al. J Pathol. 2008; 214(1):10-6.
	MSH6	*not performed*			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-045	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2				
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-046	MLH1				
	MSH2				
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-047	MLH1	not detected			
	MSH2	not detected			
	MSH6	C.2379_2380delTG p.Ala794HisfsX9	4	Pathogenic	
SL-049	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-050	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-051	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	c.965G>A p.Gly322Asp	6	Nonpathogenic	Drotschmann K. Current Biol. 1999: 907-910.
	MSH6	not detected			
SL-052	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-053	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-054	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
		c.1853delAinsTTCTT p.Lys618IlefsX4	16	Pathogenic	First described
	MSH2	not detected			
	MSH6	*not performed*			
SL-056	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-058	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-059	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-060	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	c.2633T>C p.Val878Ala	4	Unknown	Cyr JL, Heinen CD. J Biol Chem 2008: 31641–31648. Barnetson RA. Hum Mutation 2008: 367-74.
SL-061	MLH1	not detected			
	MSH2	not detected			
	MSH6	c.431G>T p.Ser144Ile		Nonpathogenic	Kariola R. Hum Molec Genet. 2002: 1303-1310.

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-062	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-063	MLH1	not detected			
	MSH2	c.23C>T p.Thr8Met	1	Nonpathogenic	Ramensky V. Nucleic Acids Res 2002: 3894-900.
	MSH6	not detected			
SL-064	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-065	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-066	MLH1	not detected			
	MSH2	not detected			
	MSH6	c.1186C>G Leu396Val	4	Nonpathogenic	Kolodner RD. Cancer Res. 1999: 5058-5074.
SL-067	MLH1	c.1820T>A p.Leu607His	16	Nonpathogenic	Takahashi M. Cancer Res. 2007: 4595-604.
	MSH2	not detected			
	MSH6	not detected			
SL-068	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-069	MLH1	not detected			
	MSH2	not detected			
	MSH6	c.124C>T p.Pro42Leu	1	Unknown	
		c.1338A>T p.Glu446Asp	4	Unknown	
		c.1932G>C p. Arg644Ser	4	Unknown	
SL-070	MLH1	not detected			
	MSH2	not detected			
	MSH6	c.3682G>C p.Ala1228Pro	8	Unknown	
SL-071	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-072	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			
SL-073	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-074	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-075	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-076	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	c.116G>A p.Gly32Glu	1	Nonpathogenic	
SL-077	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-078	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	c.3911G>A p.Arg1304Lys	9	Unknown	
SL-079	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-080	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-081	MLH1	*c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-082	MLH1	c.2152 C>T p.His718Tyr	19	Nonpathogenic	Takahashi M. Cancer Res 2007: 4595-604
	MSH2	not detected			
	MSH6	not detected			
SL-083	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-084	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-085	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-086	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-087	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-088	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	c.2633T>C p.Val878Ala	4	Unknown	Cyr JL, Heinen CD. J Biol Chem 2008: 31641–31648. Barnetson RA. Hum Mutation 2008: 367-74.
SL-089	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-090	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-091	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-092	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol. 2005: 537-549.
		c.1975C>T p.Arg659X	17	Pathogenic	Peltomäki P Familial Cancer. 2001;1(1):9-15
	MSH2	not detected			
	MSH6	*not performed*			
SL-093	MLH1	c.2041G>A p.Ala681Thr	18	Pathogenic	Takahashi M. Cancer Res 2007: 4595-604
	MSH2	not detected			
	MSH6	*not performed*			
SL-094	MLH1	c.1459C>T p. Arg487X	13	Pathogenic	Casey G JAMA. 2005 16;293(7):799-809
	MSH2	not detected			
	MSH6	*not performed*			
SL-095	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-096	MLH1	*c.*655A>G + *c.*655A>G p. Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-097	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol2005: 537-549.
		c.1639_1643dup TTATA p.Leu549TyrfsX44	14	Pathogenic	
	MSH2	not detected			
	MSH6	*not performed*			
SL-2-1	MLH1	c.545+3A>G	6	Pathogenic	Pensotti V. Genes Chromosomes Cancer. 1997;19(3):135-42
	MSH2	not detected			
	MSH6	*not performed*			
SL-2-2	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	c.187delG p.Val63fsX1	1	Pathogenic	Mangold et al. Int J Cancer. 2005;116(5):692-702.
	MSH6	*not performed*			
SL-2-3	MLH1	not detected			
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			
SL-2-4	MLH1	not detected			
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-2-5	MLH1	c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	not detected			
SL-2-6	MLH1	c.1276C>T p.Gln426X	12	Pathogenic	First described
		c.655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-2-7	MLH1	not detected			
	MSH2	not detected			
	MSH6	not detected			
SL-2-8	MLH1	c.677G>A p.Arg226Gln	8	Pathogenic	Pagenstecher C. Hum Genet 2006: 9-22.
	MSH2	not detected			
	MSH6	not detected			
SL-2-9	MLH1	c.1963A>G p.Ile655Val	17	Nonpathogenic	Takahashi M. Cancer Res 2007: 4595-604.
		c.2146G>A p.Val716Met	19	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	c.2152C>T p.Gln718X	13	Pathogenic	Terdiman et al., Gastroenterol. 2002;122(4):940-7
	MSH6	*not performed*			
SL-2-10	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	c.175dupC	1	Pathogenic	
	MSH6	*not performed*			
SL-2-11	MLH1	not detected			
	MSH2	not detected			
	MSH6	*not performed*			
SL-2-12	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-2-13	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-3-1	MLH1	*not performed*			
	MSH2	not detected			
	MSH6	*not performed*			
SL-3-3	MLH1	not detected			
	MSH2	not detected			
	MSH6	*not performed*			
SL-3-4	MLH1	not detected			
	MSH2	not detected			
	MSH6	*not performed*			
SL-3-6	MLH1	c.665delA p.Asn222MetfsX7	8	Pathogenic	First described
	MSH2	*not performed*			
	MSH6	*not performed*			
SL-3-7	MLH1	not detected			
	MSH2	not detected			
	MSH6	*not performed*			

Proband	*Gene*	*Alteration*	*Exon*	*Classification of Pathogenicity*	*Reference*
SL-3-8	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-4-18	MLH1	c.677G>A p.Arg226Gln	8	Pathogenic	Pagenstecher C. Human Genet 2006: 9-22.
	MSH2	not detected			
	MSH6	*not performed*			
SL-4-21	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			
SL-4-23	MLH1	*c.*655A>G p.Ile219Val	8	Nonpathogenic	Raevaara TE. Gastroenterol 2005: 537-549.
	MSH2	not detected			
	MSH6	*not performed*			

Peripheral blood specimens from the index patient and family members were collected, leukocyte DNA was purified and stored for *TP53* mutation detection. Interviews were conducted by trained geneticists, as part of genetic counseling, to collect information on cancers in first-, second-, and third-degree relatives. All subjects signed a term of free informed consent. Medical and histopathological records and death certificates were reviewed, when available, to collect age at diagnosis, tumor site and type. Preliminary results show that 27 out of 121 LFS/LFL probands carried a germline *TP53* mutation. A total of 15 apparently unrelated families carried the same pathogenic germline mutation in the tetramerization domain at codon 337 (R337H), 8 presented different mutations in the DNA binding domain, 2 had splice site mutations and 2 carried deletions. The test was offered to all family members that belonged to a family with a detected mutation. A total of 144 family members were tested and 59 carried germline mutations; among these, 51 carried the R337H mutation. The follow-up of individuals with LFS recommended by the National Cancer Comprehensive Network (NCCN) is offered.

Although they fulfilled all the clinical criteria for LFS/LFL, mutations in *TP53* were not detected in the remaining 97 families.

Dataset of Genomic Variants in Brazilian Individuals

CNV profiles of a cohort of 40 unrelated women from families with molecular diagnosis of Fragile-X Syndrome were evaluated, under the assumption that since the cause of mental retardation has already been determined in these families, the distribution of CNVs should be similar to the general population. Although the DGV (Database of Genomic Variants) is the most commonly used standard database of control individuals for CNV comparisons, the compiled data were obtained from different platforms, though never from a Brazilian sample. Analysis of normal individuals envisages the construction of a CNV panel from a Brazilian population. This data will be used both as a control set for analysis of the cancer predisposition families and to evaluate whether and how this study can be compared with the data documented in the DGV.

In this preliminary report, "rare alterations" were defined, those located in regions covered by ≤3 individual CNVs described in the DGV and containing coding regions; however, the criteria may be changed in the final analysis.

The 40 control samples presented a total of 273 CNV regions, corresponding to an average of 6.8±3.7 CNV regions per individual. Regarding the rare CNVs, a total of 17 were detected among 14 out of 40 (35%) controls, while the remaining 26 controls did not show any rare CNVs.

Evaluation of individuals with familial predisposition to breast-ovarian cancer Syndrome and Li-Fraumeni Syndrome

The probands of breast-ovarian cancer familial aggregates were screened for *BRCA1* and *BRCA2* gene mutations and the Li-Fraumeni Syndrome/LFL patients were screened for *TP53* mutation by sequencing.

A total of 52 individuals from families with breast-ovarian cancer predisposition were investigated by CNVs. The procedure detected 416 CNV segments, corresponding to an average of 8.2±4.4 CNV regions per individual. Regarding the rare CNVs, a total of 33 were detected in 22 (~42%) patients, while the remaining 30 patients did not show any "rare alterations", although one of them exhibited a whole extra chromosome X.

In breast-ovarian cancer syndrome patients, several genomic imbalances were observed comprising entire genes or intragenic rearrangements, many of which may

play a role in cancer. Genomic segments with altered copy numbers detected in patients and that differ regarding frequency and gene content are presented in Figures 1-3. Figure 1 illustrates an intragenic deletion encompassing exons 10 and 11 of *CTNNA3*. The product of this gene may be involved in the formation of stretch-resistant cell-cell adhesion complexes. *CTNNA3* monoallelic expression was reported in bladder carcinoma (Meehan et al, 2007). This is an example of a genomic imbalance affecting a region covered by CNVs containing a gene related to cancer.

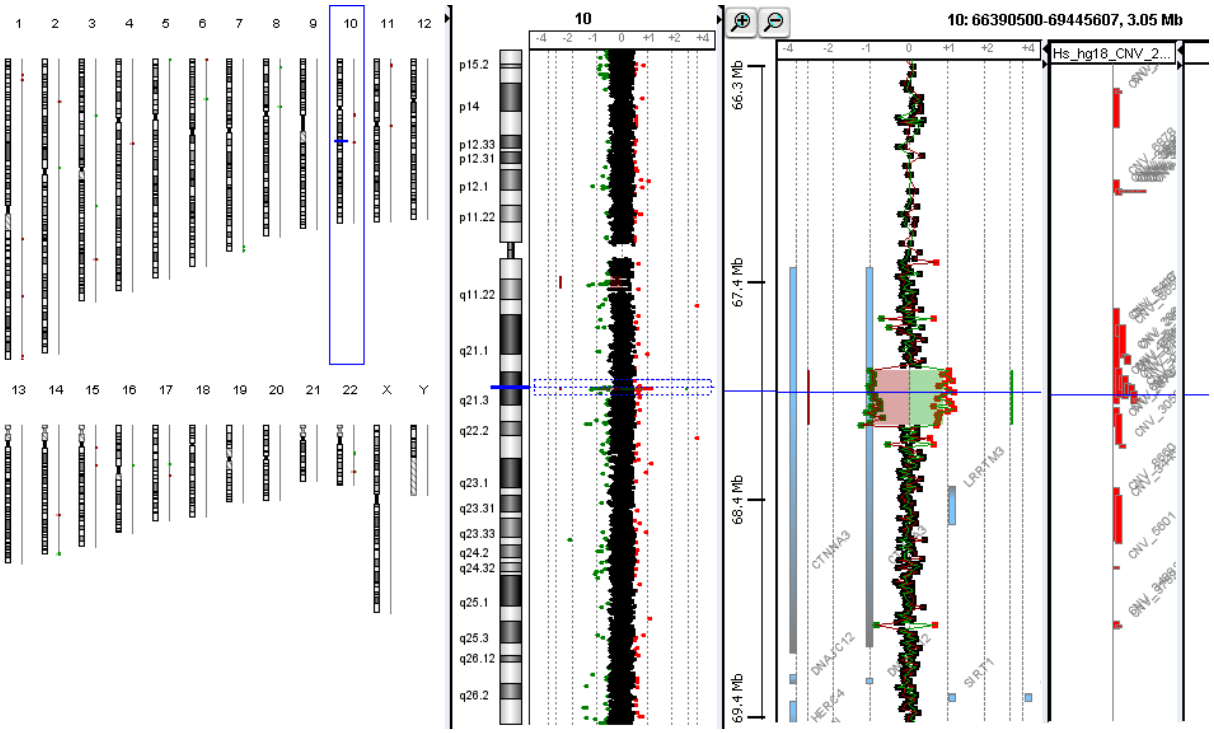


Figure 1. *CTNNA3* intragenic deletion in Patient 3. On the left, a CNV panel of the whole-genome of the patient; in the middle, the entire chromosome 10 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right. The alteration appears as mirrored images due to the superposition of two hybridizations with reversed labeling. Images extracted from the Genomic Workbench software.

Figure 2 depicts the intragenic deletion of *FHIT*. FHIT protein was determined to be altered in numerous tumor types due to deletions in a coding region of chromosome 3p14.2, including the fragile site locus FRA3B. Although a low number of documented CNVs is registered in the DGV, this genomic imbalance is considered a "rare alteration". A similar intragenic deletion in one sample of our dataset control samples was observed.

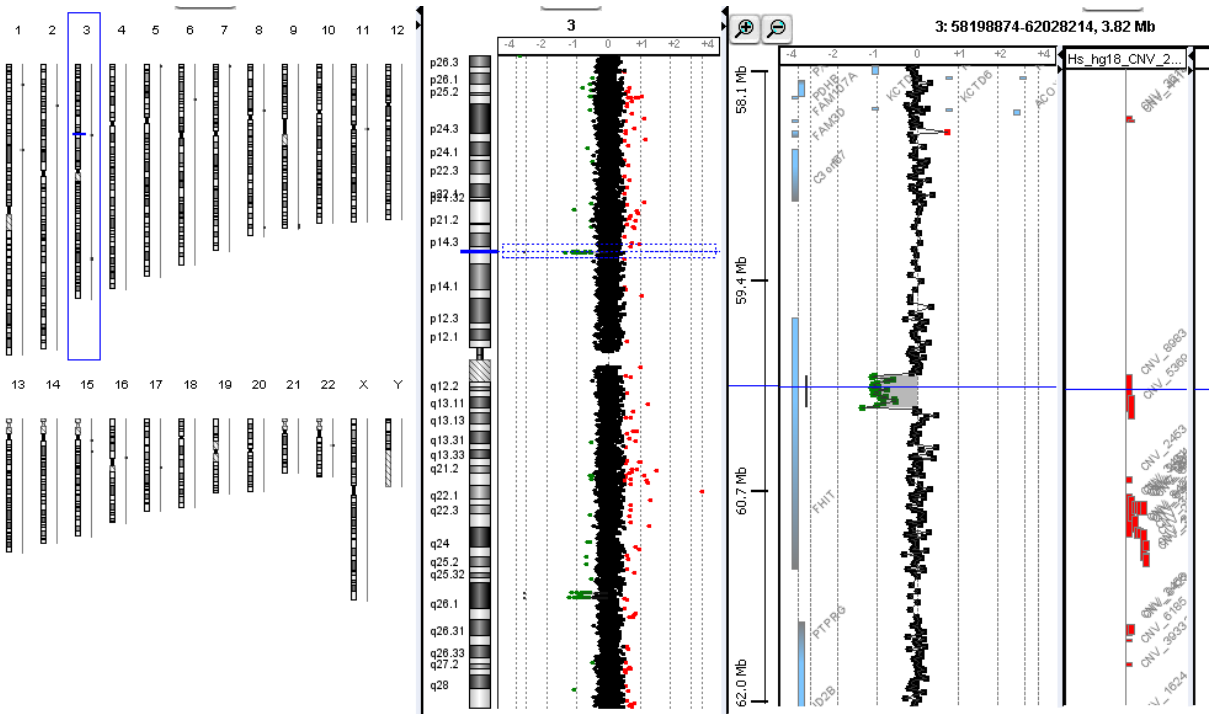


Figure 2. *FHIT* intragenic deletion in Patient 8. On the left, a CNV panel of the whole-genome of the patient; in the middle, the entire chromosome 3 is depicted, and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microdeletion at 3p14.2. Images extracted from the Genomic Workbench software.

In addition, genomic imbalances affecting genes apparently not related to cancer were observed, located in regions with no reports of CNVs in the normal population. Figure 3 shows an example of this situation with a microduplication of the potassium channel gene *KCNH8*, spanning from exon 1 to exon 7, in a genomic segment not covered by common CNVs described in the DGV or in our control dataset.

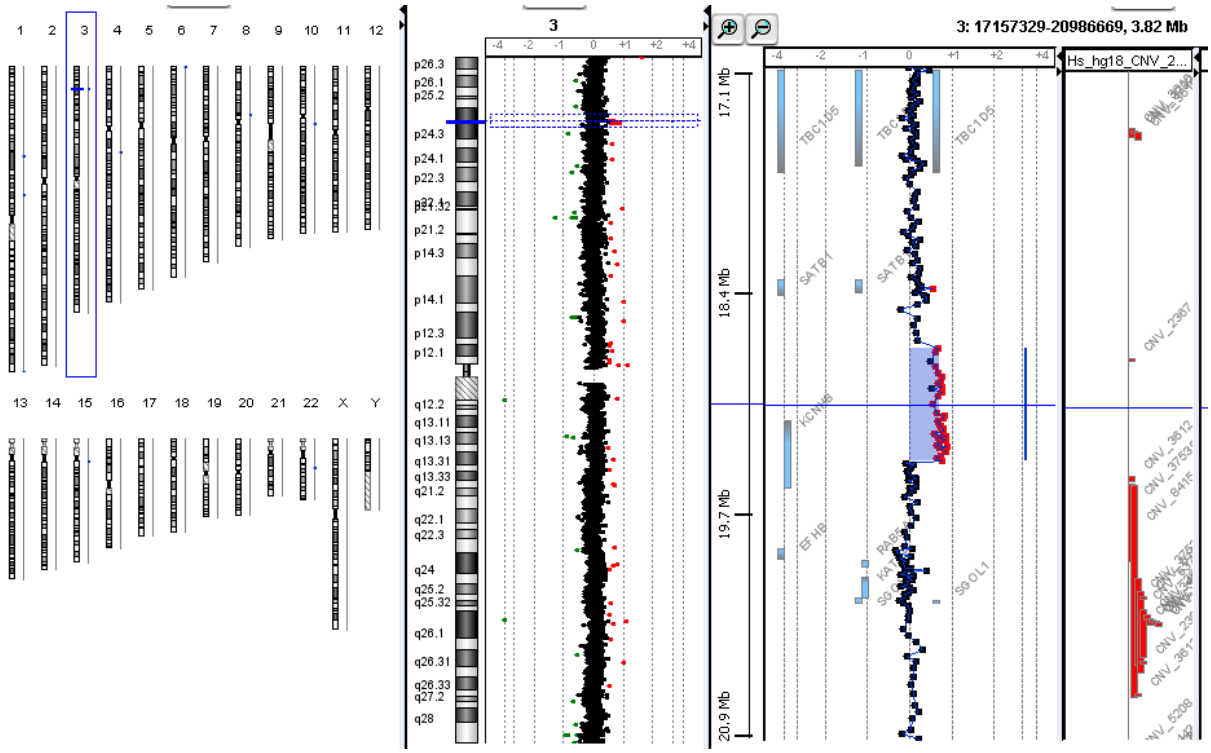


Figure 3. *KCNH8* microduplication in Patient 35. On the left, a CNV panel of the whole-genome of the patient; in the middle, the entire chromosome 3 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microduplication at 3p24.3. Images extracted from the Genomic Workbench software.

Li-Fraumeni and Li-Fraumeni-like Syndromes

Samples of 63 unrelated patients belonging to Li-Fraumeni and Li-Fraumeni-like (LFS/LFL) families were analyzed by oligoarray-CGH, of which 10 were carriers of germinative mutations in gene *TP53* and the remaining proved negative for the sequencing of exons of this gene. A total of 586 variations in the copy numbers of DNA segments were identified in these 63 patients (mean of 9.3±3.9 CNVs per individual). Among the alterations, 80 were rare genomic imbalances detected in 37 (~59%) patients, variants located in regions totally or partially covered by three or less CNVs described in the DGV, and containing one or more genes.

A total of 179 genes were located within the rare genomic imbalances and the putative functions of these genes were classified using the software EASE Version 2.0 (http://david.abcc.ncifcrf.gov/ease/ease.jsp), based on the Gene Ontology Database (http://www.geneontology.org/). Next, based on their putative function, the genes were organized into six different biological classes in a hierarchical manner. Genes that were

classified by the software into more than one biological process were assigned in just one category, according to the following hierarchical criterion: cell growth and/or maintenance; cell communication; transcription; metabolism; and apoptosis. Those which were not classified in any of these five categories were classified as "others". This showed that genes involved in cell growth and/or maintenance (14.5%), cell communication (15.5%), transcription (15.5%) and metabolism (11.7%) represent the majority of genes in our sample (Figure 4). Some examples of genomic segments detected with altered copy numbers in LFS patients are presented in Figures 5-7.

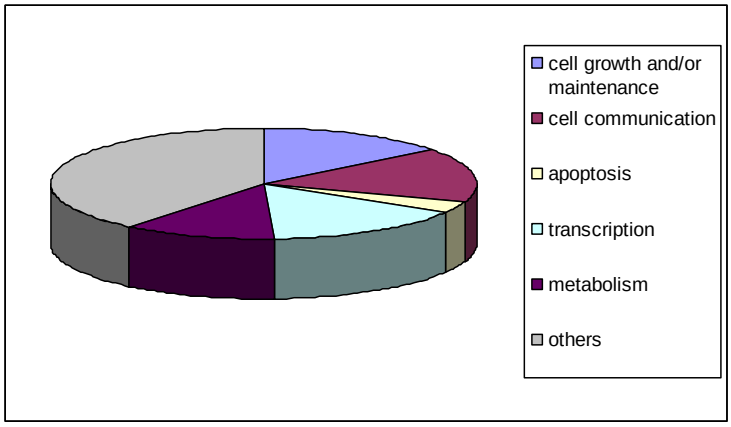


Figure 4. Genes potentially implicated in LFS functionally classified within a biological process category.

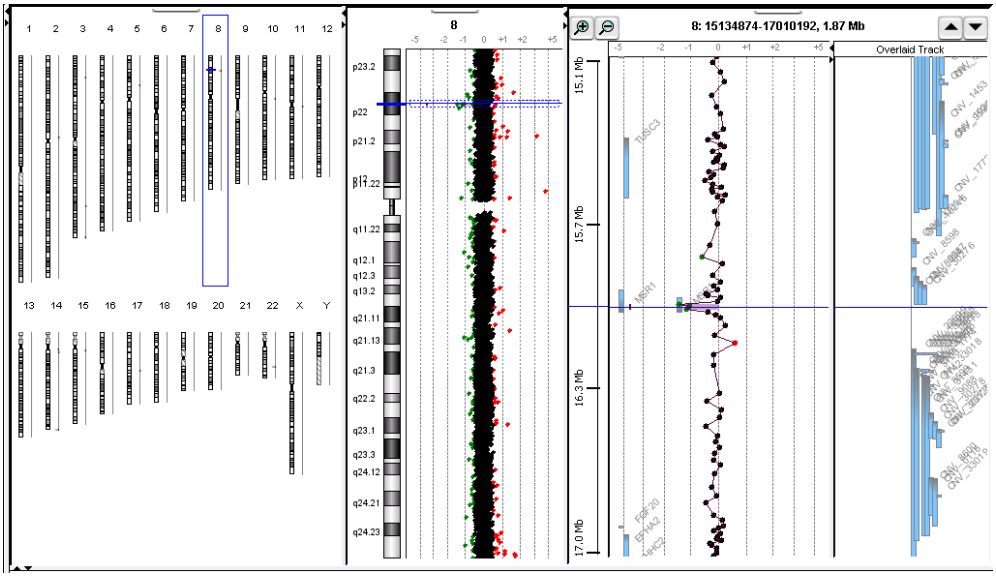


Figure 5 *MSR1* microdeletion in Patient Y14. On the left, a CNV panel of the whole-genome of the patient; in the middle, the entire chromosome 8 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microdeletion at 8p22. Images extracted from the Genomic Workbench software.

Figure 5 illustrates a microdeletion comprising exons 2, 3, 4 and 5 of *MSR1*. This gene encodes class A macrophage scavenger receptors, which include three different types (1, 2, 3) generated by alternative splicing of this gene. These receptors or isoforms are macrophage-specific trimeric integral membrane glycoproteins and have been implicated in many macrophage-associated physiological and pathological processes, including atherosclerosis, Alzheimer's disease and host defense (Plat & Gordon, 2001). Both rare germ-line mutations and common sequence variants of the macrophage scavenger receptor 1 (*MSR1*) gene have recently been implicated as potential prostate cancer susceptibility factors (Sun et al, 2007; Lindmark et al, 2004; Xu et al, 2002; Xu et al, 2000). This deletion is an example of a genomic imbalance affecting a region that contains only a potential cancer marker and no CNVs.

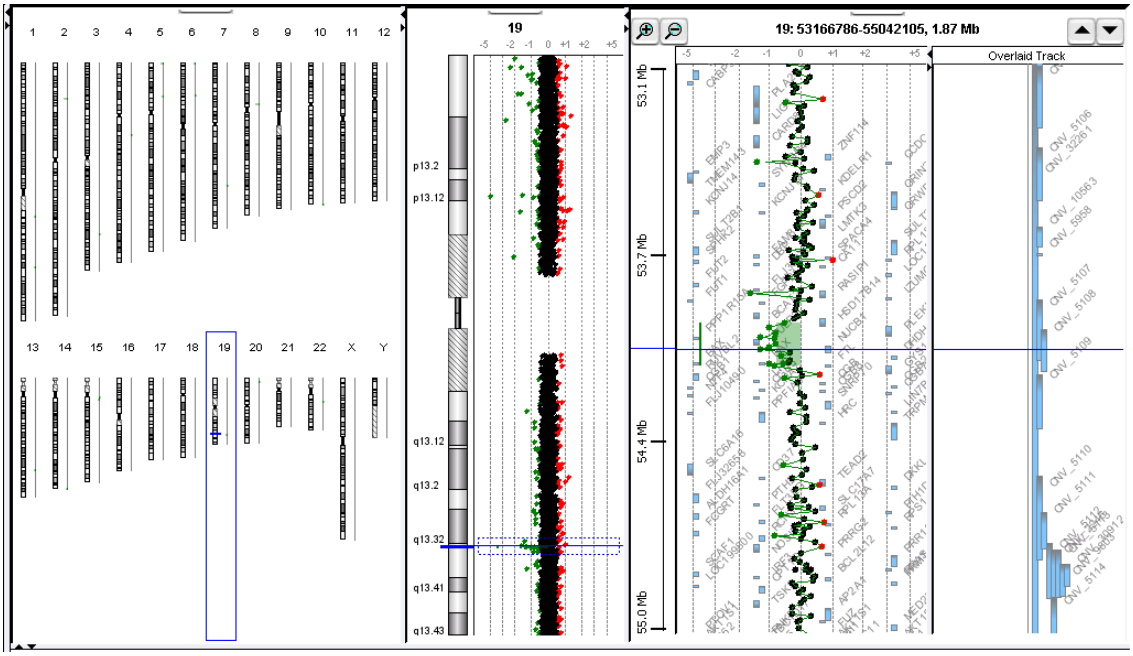


Figure 6. Microdeletion in Patient Y42 comprising 8 genes, including *PPP1R15A* and *BAX* genes. On the left, the CNV profile of the whole-genome of the patient; in the middle, the entire chromosome 19 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microdeletion at 19q13.33. Images extracted from the Genomic Workbench software.

Figure 6 shows a microdeletion in chromosome 19 that involves several genes including *PPP1R15A* and *BAX*. The *PPP1R15A* gene, also called *GADD34,* is a gene whose transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents (Wu et al, 2002). The induction of this gene by ionizing radiation occurs in certain cell lines regardless of *TP53* status and its protein response is correlated with apoptosis following ionizing radiation (Liebermann & Hoffman, 2008). Conversely, the absence of or reduction in the expression of *PPP1R15A* was correlated to malignant transformation of nevus to melanoma (Korabiowska et al, 1997).

BAX is a proapoptotic member of the *BCL-2* family of genes that regulates programmed cell death. *BAX* induces the release of cytochrome c, activation of *CASP3*, and thereby apoptosis. Its expression is elevated in certain tissues after apoptotic stimuli and can be directly regulated by *TP53* (Brady & Gil-Gómez, 1998).

Impaired *BAX* expression has been reported in breast cancer (Bargou et al, 1996), hepatocellular carcinomas (Beerheide et al, 2000) and in a number of other tumor types. Somatic frameshift mutations in the *BAX* gene have been described in colon cancers (Rampino et al, 1997), gastric carcinomas and endometrial atypical hyperplasia (Ouyang et al, 1998), while certain hematopoietic malignancies have been shown to possess loss-of-function mutations of *BAX* (Meijerink et al, 1998). Additionally, the decrease in BAX expression might be involved in the tumorigenesis of HNPCC (Sakao et al, 1998), gastric (Anagnostopoulos et al, 2005) and uterine cancer (Soufla et al, 2005). For several different tumors, low BAX expression level was demonstrated to be a negative prognostic factor for patient survival (Mrozek et al, 2003; Schelwies et al, 2002; Sturm et al, 2001; Prokop et al, 2000; Sturm et al, 1999).

Decreased BAX levels in tumors are not surprising given the fact that the *BAX* gene is a transcriptional target of the tumor suppressor *TP53*, which is mutated in the majority of human cancers (Miyashita et al, 1995). Conversely, the overexpression of proapoptotic proteins, such as BAX, promote apoptosis and sensitize tumor cells to various anticancer therapies (Li et al, 2007; Kim et al, 2001; Kymionis et al, 2001).

Figure 7 illustrates an intragenic deletion encompassing exon 14 and the noncoding exon 15 of *FANCC.* The Fanconi anemia complementation group (FANC) proteins participate in the DNA repair pathway by homologous recombination and it is currently formed by 13 genes (Taniguchi & Dandrea, 2002). Defects in *FANCC* are a

cause of Fanconi anemia (Gibson et al, 1994), a recessive disorder characterized by progressive bone marrow failure (pancytopenia), a diverse assortment of congenital malformations (Krasnoshtein et al, 1996) and predisposition to the development of malignancies (Sinha et al, 2008; Berwick et al, 2007; Rogers et al, 2004).

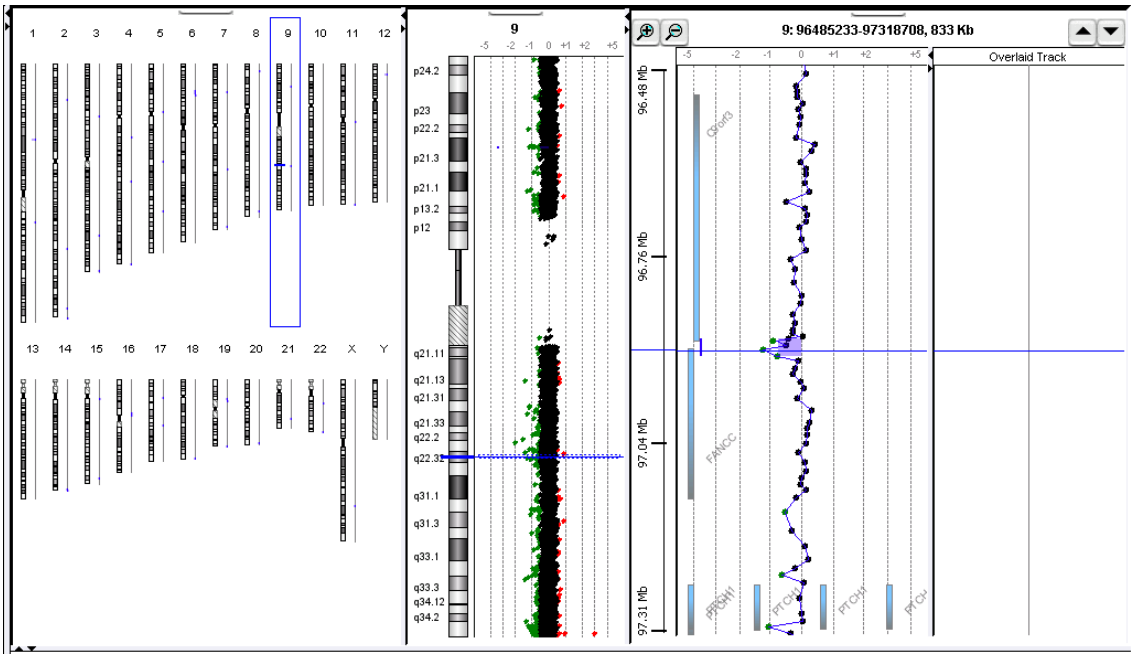


Figure 7. *FANCC* microdeletion in Patient Y129. On the left, a CNV panel of the whole-genome of the patient; in the middle, the entire chromosome 9 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microdeletion at 9q22.32. Images extracted from the Genomic Workbench software.

In the context of Li Fraumeni syndrome, the *FANCC* gene could have an important role, since it presents two *TP53* binding sites, one in the promoter region and the other in its coding region, placing *FANCC* on the list of genes that interact with *TP53* (Liebetrau et al, 1997).

Groups of families with familial predisposition for breast-colon cancer

The cases were divided into two groups: group 1 composed of 20 women diagnosed with breast or colon cancer; and group 2 composed of 18 women diagnosed with breast and colon cancer. Analysis detected 328 alterations (mean ± SD: 8.6±4.8 per individual) compared to the control group composed of Brazilian women, showing 273 alterations (6.8±3.7 per individual). Group 1 presented 146 alterations and group 2, 181

alterations. This result is the equivalent of a mean of 7.3±5.1 and 10.1±4.0 CNV regions per individual in groups 1 and 2, respectively.

Twenty-four alterations considered "rare" were detected in 11/20 patients in group 1 and 28 "rare" alterations in 12/18 patients in group 2 (Table 2). The mean age at diagnosis was 53.5±8.6 years-old. Of these 24 "rare" alterations, eight were observed in both groups.

From data obtained in a second analysis, categorization of the rare alterations was performed according to the presence or absence of previously described CNV regions and genes. These alterations are presented in Table 3.

Some examples of CNVs detected in the present study are presented in Figure 8. In this figure, a large deletion can be observed involving the short arm of chromosome 19. Among the genomic losses exemplified below are: the deletion at 3p12.3 (present in both cases) and involving the gene *ROBO1* (Figure 9); the deletion at 7q36.3 (Figure 10); and the deletion at 2q31.

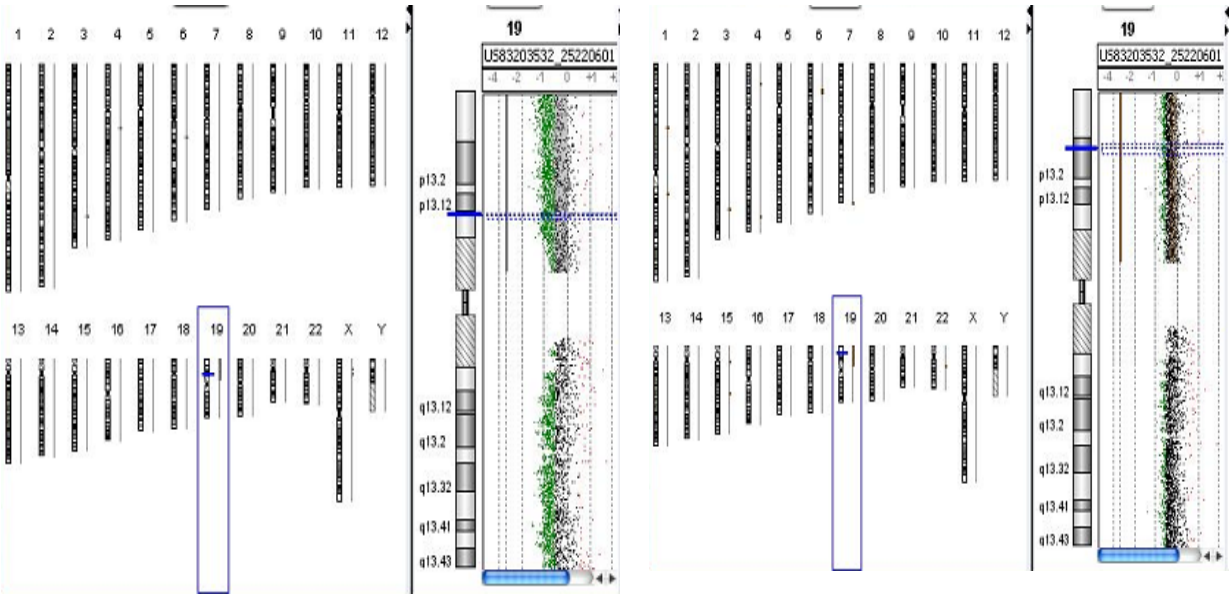


Figure 8. Ideogram hybridization pattern generated by the software DNA Analytics. In the box, the deletion on the short arm of chromosome 19 can be identified (in green) in two case studies. On the right, the entire chromosome 19 is depicted. Images extracted from the Genomic Workbench software.

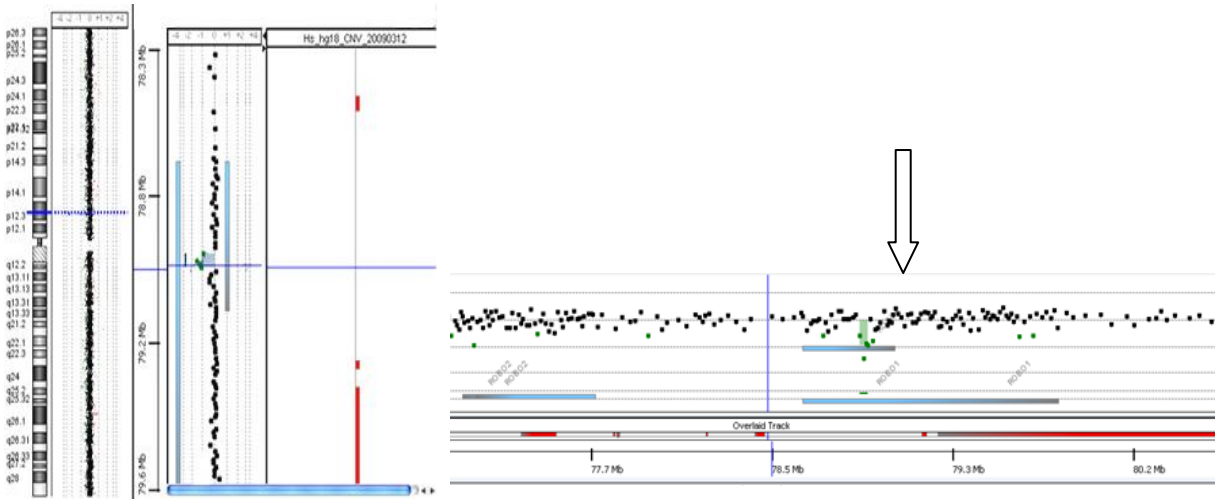


Figure 9. Deletion in region 3p12.3 (arrow), generated by aCGH analysis, detected in two individuals from two families unrelated with breast-colon cancer. On the left, the CNV profile of chromosome 3of the patient; in the right, the chromosome 3p12.3 is depicted and each dot represents one oligoarray probe; showing the microdeletion. Images extracted from the Genomic Workbench software.

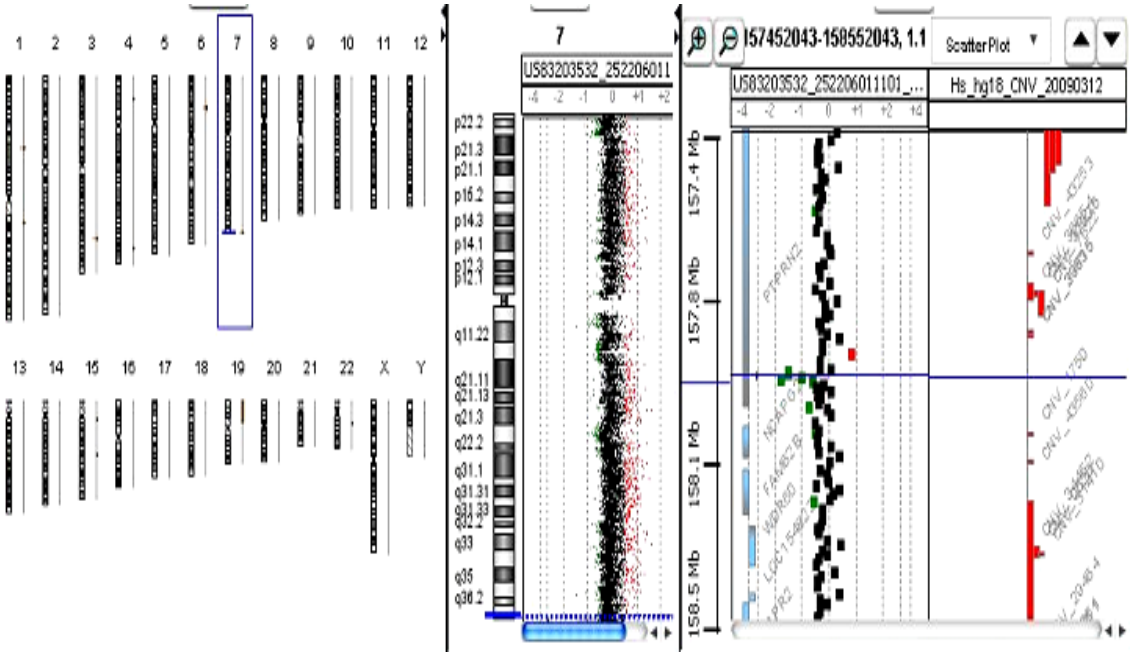


Figure 10. Deletion in region 7q36.3, generated by aCGH analysis. On the left, the CNV profile of the whole-genome of the patient; in the middle, the entire chromosome 7 is depicted and each dot represents one oligoarray probe; the blue box in the middle image is enlarged in the detailed image on the right, showing the microdeletion at 7q36.3. Images extracted from the Genomic Workbench software.

Table 3 presents the genomic alterations present only in regions with genes described in both groups 1 and 2, in which common or rare CNVs present in the databases used for comparisons were not detected. In group 1, 8 alterations were observed, including 5 genomic gains and 4 losses. The region 14q32.11 possesses one case presenting deletion and another presenting a gain. In group 2, 7 alterations were observed, including 4 gains and 3 losses. Among these 15 regions of genomic imbalances, 2 of them are common to both groups: deletion at 3p12.3 and amplification at 11p11.2.

Members of the families in cases involving alterations in the number of genomic copies not present in the databases (DGV or the Brazilian sample) have been invited for further analysis. Currently, three members have been included and are being analyzed by aCGH. In addition, the tumor samples of patients presenting constitutive alterations (described in Table 1) will also be analyzed.

Table 3. Alterations in the copy numbers detected in the samples from groups 1 and 2

Group 1		**Group 2**	
Chromosome Location	**DEL/G**	**Chromosome Location**	**DEL/G**
3p12.3	DEL	2q31.1	DEL
4q12	G	3p12.3	DEL
11p11.2	G	4p13	G
14q32.11	DEL/G	7q36.3	DEL
14q32.2	DEL	11p11.2	G
20p11.21	DEL	13q14.3	G
21q22.3	DEL	19p13.3	G
Xp22.2	G		

DEL: deletion or genomic loss; G: genomic gain

Since two cases showing deletion of the gene *ROBO1* were detected in probands of families with Breast-Colon Cancer Syndrome, parallel analysis of protein expression was performed using a tissue microarray (TMA) containing 248 cases of breast

mammary ductal and lobular carcinomas. In this analysis, 34% of the samples presented absence of staining for the protein, 24% weak staining, 26% moderate and 16% presented strong immunostaining. Moreover, 22% and 44% of these cases presented 1/3 and above 2/3 of stained area, respectively (Figure 11).

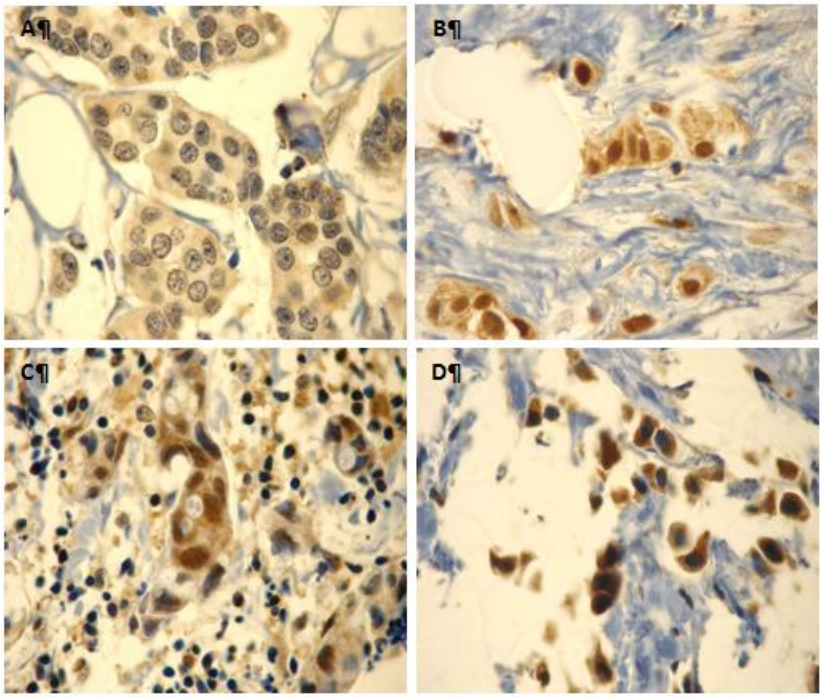


Figure 11. Pattern of ROBO1 protein expression in the nucleus of breast carcinoma cells. The scores used in the evaluation of the intensity of nuclear immunoexpression: A) Negative, 40X; B) 1 (weak staining); C) 2 (moderate staining); D) 3 (strong staining), (1000X IMH).

No significant association was observed between *ROBO1* expression and clinical data, such as age, family cancer history, the presence or absence of hormone receptors (ER, PR and HER-2), nuclear grade, staging, histological type, tumor size, lymph node involvement or the presence or absence of metastasis. A significant association was observed between histological grade and positive staining for ROBO1 protein (*P*= 0.022). In addition, 111/144 cases (77%) negative for staining presented histological grade G2-G3.

The patients were further classified according to family cancer history data, positive (45 cases) and negative (197 cases), to verify any possible association with ROBO1 protein expression; however, no significant association was observed in this

analysis. Although not significant, the majority of cases involving breast cancer and absent or low ROBO1 protein expression presented histological grade 3 and 4 (111 cases), tumor size 3 and 4 (91 cases) and lymph nodes involvement (86 cases).

These data indicate that a significant proportion of familial or sporadic breast cancers present absent or low ROBO1 protein expression and that these cases are associated with characteristics of worse disease prognosis.

Partial Discussion

Li-Fraumeni and Li-Fraumeni-Like Syndrome

In collaboration with the IARC/WHO, we haplotyped unrelated *TP53* R337H carriers using a set of 29 tag SNPs encompassing the whole *TP53* gene, which revealed that all the patients carried the same rare haplotype.

The *TP53 R337H* families are mostly distributed along a road axis between São Paulo and Porto Alegre, over 1200 km apart, historically known as the main settlement route in the XVIII and XIX centuries. *TP53 R337H* carries a lifetime risk of cancer of 70% at age 60, and 15% at age 30, lower than classic LFS TP53 mutations. *TP53 R337H* is predicted to cause up to 2-3000 annual cancers currently not identified as familial in the population of Southern Brazil (Garitano et al, 2009). Given the high population density in these areas, mutations might be present in several hundred thousand individuals, explaining the high frequency of very rare cancers, such as adrenal cortical carcinoma in children, a typical cancer associated with inheritance of the *TP53* mutation, which is about 15 times more frequent in Southern Brazil than in Western Europe. Since the area of distribution of *TP53 R337H* is the most populated area of Brazil, with a population of over 105 million, this allele may be present in about 300,000 individuals.

This situation poses an unprecedented public health problem in this area, since it current estimates suggest that the *TP53 R337H* mutation may be responsible for about 1% of all cancer cases in this population. This study was a collaborative work involving Dr. Pierre Hainaut (from the IARC/WHO) and Dr. Patricia Prolla (from the National Institute in Porto Alegre, INAGEMP). The occurrence of such a mutation should be addressed as a major health issue and families carrying this mutation should be identified and followed using early screening strategies. It is essential to develop a solid understanding of the individual risk patterns in order to offer adequate screening and

surveillance strategies, as well as provide appropriate advice to carrier families. Cancer patterns in families positive for *TP53 R337H* suggest strong genetic modifying effects, making it difficult to predict individual risk. Since protocols for cancer-risk management in Li-Fraumeni or related syndromes are debatable, extreme care should prevail in predictive testing of minors for *TP53 R337H*. A detailed assessment of the risks, benefits and costs is needed to ensure that medical, social and ethical justifications for newborn screening are met (Achatz et al, 2009).

Next, our group aims to verify the prevalence of germline *TP53 R337H* in patients with multiple primary cancers and evaluate the cancer burden related to *TP53 R337H* mutation and rare tumors. The results will provide further insight into the role of genetic variation in both inherited and sporadic tumor development.

Copy Number Variations

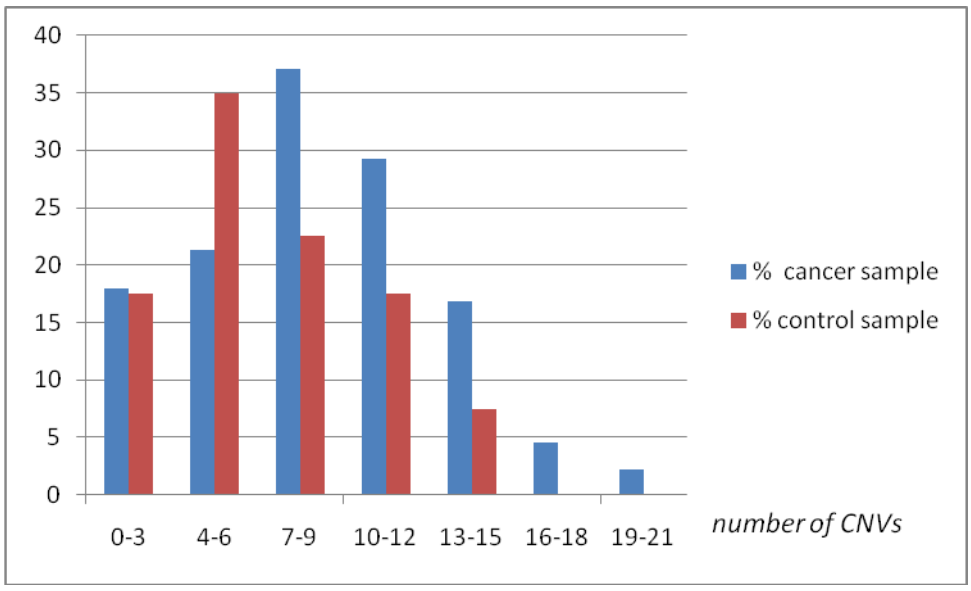
Copy number variations have been shown to have the potential to indirectly influence an individual's susceptibility to cancer; for example, by varying the gene dosage of tumor suppressors or oncogenes. An immediate role of CNVs is observed in tumor cells, which frequently present alterations that are absent in the normal cells of the patient. These alterations should be not considered *variants*, because they are not within the spectrum of normal human variation. Although chromosomal abnormalities are frequently described in tumor cells, gains or losses in small genomic regions can also contribute to cancer cell growth.

Recently, several authors have indicated genetic CNVs associated with potential candidate loci for family cancer gene predisposition. In a pilot candidate-gene association study, Shlien et al (2008) verified a cancer CNV at the gene *MLLT4* (a Ras target that regulates cell–cell adhesion) that appears to be associated with the Li-Fraumeni cancer predisposition disorder (LFS). The frequency of the CNV at *MLLT4* is significantly increased in LFS (P¼0.006, Fisher's exact test): three of the 19 LFS probands (15.8%; observed/expected: 3/0.4¼7.5) harbored the CNV duplication, whereas only 12 out of 710 healthy individuals from the reference population (1.69%; observed/expected: 12/14.6¼0.82) harbored the CNV.

Thean et al (2009) performed screening for mutations in 61 family members (more than 300 individuals for *APC*, *axin* and *B-catenin*). The authors observed no CNVs in their comparison between affected members and health controls. However, they

detected one CNV, involved in genomic loss (111Kb), mapped at 3q26.1 in 8 polyps compared to peripheral blood of patients affected. No genes were mapped in this region. A 2Mb evaluation of the genomic region revealed the gene PPMIL, which is responsible for encoding a serine/threonine phosphatase and acts on signaling routes of *TGF-β* and *BMP*. Analysis of the *PPMIL* transcript showed diminished expression in all 6 polyps and tumors compared to normal mucosa of the affected members. The authors suggested that a CNV region at 3q26 contained an upstream element that regulates the expression of tumor suppressor candidate gene. Thus, the authors presented a new mechanism involving colorectal tumors associated with negative mutation in the gene *APC*.

The initial results using aCGH in 63 patients with LFS/LFL indicate a slight increase in the average CNVs per cancer patient individual (8.2 CNV for breast-ovarian cancer and 9.3 for Li-Fraumeni) compared to the control sample (6.8 CNV), although larger patient samples are required to confirm this trend statistically. The histogram below shows the normalized (in percentage) distributions of CNVs in cancer patients and controls. Comparison between the two distributions shows a shift towards a higher number of CNVs among cancer patients compared to controls.



Although the sample is small, the category of so-called "rare genomic alterations" shows more than twice the CNVs among cancer patients (average of 0.96±1.28 per individual) than among controls (average of 0.42±0.67 per individual).

Among the samples with family history of breast-colon cancer, two cases showed deletion at 3p12.3 involving the *ROBO1* gene. The *ROBO1* gene encodes a receptor belonging to the neural cell adhesion molecule family of receptors. This gene is also expressed in the mammary glands, heart, lungs, pancreas, placenta, prostate and skeletal muscle. Some reports in the literature suggest the *ROBO1* gene is a tumor suppressor (Dallol et al, 2002; Arakawa, 2004). Inactivation of this gene by hypermethylation or low expression (by deletion) could result in cell and tissue disorganization, as reported in preinvasive stage tumors (Sundaresan et al, 1998; Ghosh et al, 2009). Deletion in this chromosomal region has also been reported in lung, kidney and breast cancer and in epithelial dysplasias (Chung et al, 1995; Hung et al, 1995; Sundaresan et al, 1998; Dallol et al, 2002; Arakawa, 2004; Ghosh et al, 2009). Other authors describe abnormal methylation in *ROBO1* in colorectal cancers (Dallol et al, 2002; Dallol et al, 2003; Narayan et al, 2006). In hereditary gastric cancer, it has been observed that the second event associated with inactivation of a tumor suppressor gene is the hypermethylation of the gene (Oliveira et al, 2009). These results indicate that *ROBO1* is associated with breast and colon cancers and that investigating deletion and methylation in *ROBO1* gene in patients with hereditary breast and colon cancer is of enormous interest.

To confirm the absence of or diminished expression of ROBO1 protein in breast carcinomas, immunohistochemistry was used on a series of 248 cases. A high frequency of absent or weak expression was detected in the majority of the samples. More elaborate statistical analysis is ongoing following the analysis of sporadic colorectal carcinomas. An alternative strategy would be to evaluate the methylation pattern of this gene in families that present constitutive deletion.

In one example of breast and colon cancer, deletion was detected involving the *HOXD3* and *HOXD4* genes, mapped at 2q31.1. The *HOX* genes encode transcription factors containing fundamental homeodomains that act in cell regulation, differentiation and migration. The *homeobox* genes perform an important role in organogenesis, embryogenesis and act as regulators of factors that control the expression of a variety of genes involved in cell differentiation (Hamada et al, 2001; Shen et al, 2008). These genes are involved in vascular remodeling processes, which can occur in normal and pathological development, such as angiogenesis induced by tumor cells (Gorski and Walsh, 2000). The *HOX* genes exhibit an altered expression pattern in kidney, lung and colorectal cancers compared to normal tissues (Cillo et al, 1992; Cillo et al, 1993; De Vita

et al, 1993; Tiberio et al, 1994). Moreover, some authors observed altered expression in metastatic cancers of the lung and colon, compared to the primary tumor (Cillo, 1994). Raman et al (2000) verified loss of *HOX* gene expression in breast tumors. A patient carrying the deletion in the 2q31.1 region was diagnosed with breast cancer at 56 years of age and colorectal cancer at 58; tumor staging was T4N1M0 and T2N0M0, respectively. Analysis of the patient's heredogram showed three relatives diagnosed with breast cancer and one with gastric cancer. Although there are no studies reporting any association between loss of expression of the genes *HOXD3* and *HOXD4* with breast and colon tumors, it is evident in the literature that altered *homeobox* gene expression is associated with these types of tumors.

The *PTPRN2* gene, mapped at 7q36.3, encodes a transmembrane protein of the tyrosine phosphatase family (PTP). This family of genes regulates a variety of cellular processes, including cell growth and differentiation and the cell cycle, and has been associated with tumor development (Nordgard et al, 2008). Anglim (2008) identified abnormal methylation on *PTPRN2* in tumor tissues. Rush et al (2004) identified hypermethylation in patients with chronic lymphocytic leukemia. Thus, *PTPRN2* is a candidate for analysis of methylation and deletion in aggregations and families with breast and colon cancer. Deletion of *PTPRN2* was verified in only one patient in group 2. According to the patient's clinical information, she had been diagnosed with colorectal cancer and then with breast cancer. In the family history, one first-degree relative had uterine carcinoma.

Two cases in this study presented a large deletion in the short arm of chromosome 19. According to the patients' clinical information, both had been diagnosed with breast cancer and one of them had also had colon cancer. Based on patient heredograms, it was possible to identify that four women (grandmother, mother, aunt and sister) in the family of one of these women had been diagnosed with breast cancer and a man (uncle) had had colon cancer. Another important piece of information and relevant to our study was that one of the patients had a history of miscarriage. The sister of this patient, who had also suffered a miscarriage, was invited to participate in the study. Members of both these families will be evaluated for CNVs and GTG-banding to evaluate the deletion in 19p. Chromosome 19, in which two large regions of loss in 19p have been observed, is rich in segment duplications and gene cluster families in tandem, which could favor the presence of CNVs in this chromosome (reviewed in Smith

et al., 2008). Recently, analysis of variant sequences in a large population group identified 49 genes associated with cancer within variant regions in more than one individual (reviewed in Shlien & Malkin, 2010). Additionally, a study by our group involving uterine leiomyomas demonstrated that certain regions of chromosomes 11, 14 and 16, rich in CNVs, were significant when integrated analysis of the genomic data and trnscriptomic analysis was performed (data not published). Such findings suggest that CNVs may not only be involved in genomic instability, but also in mechanisms that lead to alterations in the expression of specific genes.

The genomic content of the CNVs of cancer groups needs to be evaluated in relation to their potential role as candidate loci for cancer predisposition disease. To more fully evaluate the impact of each alteration, whenever possible, the genome of other affected family members and/or a DNA sample of the corresponding tumor will be evaluated by array-CGH. Our group is particularly interested in studying families of patients in which rare alterations have been identified. Only CNVs present in all the affected individuals of the same family can be considered predisposing factors (i.e., pathogenic CNVs), even if these are also present at low frequencies in currently unaffected individuals. In addition, a specific CNV *per se* may not represent a predisposing factor, but rather a consequence of a mutation in a cancer gene, known or as yet unidentified. In these cases, any increase in CNVs would be associated with mechanisms, such as a deficiency in apoptosis (e.g., mutation in *TP53*) or DNA repair (e.g., *BRCA1*). In *TP53* mutation carriers, a marked increase in CNVs has been documented (Shlien et al, 2008). However, the authors do not discuss whether only the frequency would be increased, or whether the CNV profiles of these *TP53* mutated individuals would comprise different segments, recurrent or not, affecting genomic segments not normally observed in CNVs common to the general population.

Our group also plans to investigate the status of any rare genomic alterations identified in a patient affected by cancer in the corresponding tumor, aimed at elucidating derived rearrangements, such as deletions in homozygosis or increases in the affected segment.

1.2. Wide genome screening for gene mutation by combining exon-enriched genomic fragment and deep sequencing in hereditary nonpolyposis colorectal carcinomas.

To identify new cancer genes associated with Lynch or HNPCC syndrome and therefore contribute to defining the spectrum of germinative mutations, we proposed to interrogate sections of the genomic sequence that correspond to whole human gene exonic regions using a system based on solid surface for enrichment of desired genomic sections and posterior sequencing in the 454 FLX-Roche platform. Recently, a novel strategy based on solution hybrid selection with biotinylated RNA baits was described (Gnirke et al., 2009) and proved to be more efficient in the enrichment process and in reducing the amount of input fragment required compared to the microarray-based method. Moreover the hybrid-selection selection method permits enrichment of more extensive regions than microarray and therefore, there is no need to select a limited number of genes for this investigation, since the availability procedure theoretically permits all human exons. Thus, we plan to use the SureSelect Human All Exon kit, which also includes sample preparation reagents for next-generation sequencing in either Illumina or SOLiD platforms. This decision was also supported by the fact that our institution has received approval to acquire one of these deep-sequencing platforms (Illumina or SOLiD), which is appropriate for using with this kit. The samples that will be investigated for this strategy were previously screened for classic mutations associated with heritability of the syndrome (*hMLH, hMSH2, hMSH6, PMS2, CHEK2* and *TP53*) through sequencing of the complete gene and do not present any alteration in these genes. At this time, the procedure of importing the reagents and the selection of the 20 non-mutated Lynch cases is ongoing.

A.2. Tumor Biology

2.1. Expression profile-based test for breast cancer prognosis using protein-coding and noncoding genes

2.2. Expression profile-based test for prostate cancer prognosis using protein-coding and noncoding genes

In these projects, the evaluation of prognostic and predictive markers for breast and prostate cancer were proposed, based on a novel set of discriminatory genes, representing protein coding and noncoding transcripts. For this analysis, a unique custom-designed, intron/exon oligoarray platform produced on-demand by Agilent will be used, containing approximately 244k single-strand probes, of which about 200k represents both sense and antisense strands of 100K intronic noncoding messages. Currently, all commercial expression signature-based tests focus on the analysis of protein coding genes only, missing the information buried in ncRNAs. Since the majority of the transcribed components of the human genome generate ncRNAs (ENCODE Project Consortium, 2007), we believe strongly that new prognostic markers in breast and prostate cancer can be identified using this methodology.

Preliminary Results

For assessing the expression profile of both breast and prostate cancer, in order to discover biomarkers, we initially searched the AC Camargo Hospital Biobank for tumor specimens (breast tumors) and/or RNA samples (prostate tumors) of these types of tumor. In Tables 1 and 2, the number of samples available for each tumor type are presented. For breast carcinomas, we decided to performed laser capture microdissection (LCM) in order to determine more homogeneous cell populations and, consequently, appropriate associations between expression profile and tumor cells. At present, 154 prostate carcinomas have been selected and the RNA is available for evaluation.

Table 1. Number of ductal breast tumor specimens from the AC Camargo Hospital Biobank up to 2008.

Molecular Subtypes	**Number of tumor specimens**
HER2 +	17
Luminal A	181
Luminal B	26
TRIPLE NEGATIVE	9
HER2 + (2+) (*)	57
TOTAL	**281**

(*) samples with immunostaining for HER2 that have signal 2+ were not classified into subclasses, since they also have to be evaluated by FISH.

Table 2. Number of prostate tumor specimens and RNA from the AC Camargo Hospital Biobank up to 2009

Tumor	**Number of tumor specimens**	**Number of RNA samples**
Prostate tumor	29	154
Prostatic intraepithelial neoplasia (PIN)	4	5
TOTAL	**31**	**159**

The RNA integrity number (RIN) (by Agilent 2100 Bioanalyzer) was obtained from each prostate tumor and PIN samples presenting a average value and standard deviation (SD) of 7.0 (SD, 0.9) and 6.7 (SD, 0.64), respectively. These values reflect the high quality of RNA, which are suitable for expression profile analyses.

2.3. Integration of vascular genomics and proteomics for diagnosis and therapy of cancer

The goal of this study is identify and validate transcripts upregulated in prostate cancer using large-scale transcriptome analysis (244K platform), aimed at discovering novel upregulated markers. Upregulated genes suggested by this approach will be confirmed in tumor versus nontumor pairs of samples by quantitative RT-PCR. In addition, promoters of the experimentally validated markers will be identified using predictive algorithms and will be confirmed by gene-reporter assays. These promoters will be selected by large-scale transcriptome analysis of tumor cells and tumor vasculature by scanning public databases and by large-scale measurement of genic expression profiles using a custom-designed, intron/exon oligoarray platform, containing 244,000 probes. AAVP chimera vectors, consisting of adeno-associated virus (AAV) and single-stranded bacteriophage (P) M13, exhibit peptides that home to specific targets. The combination of a vector exhibiting peptides that home to tumors with a reporter/suicide transgene (*HSVtk*) under the control of tumor-specific promoter could permit highly specific suppression of tumor growth, as well as its visualization. The duplicity of possibilities reinforces the idea of the co-development of a drug and diagnosis that, if preceded by an individualized evaluation of genic expression (e.g., on the urine of prostate cancer patients), could lead to a personalized diagnosis/treatment based on markers with increased expression in each individual patient. In this study analysis was initiated for the genes *AMACR*, *PCA3* and *PLA2G2A*. The promoters will be experimentally validated by molecular analysis and bioinformatics tools.

This project is being developed in partnership with the MD Anderson Cancer Center, Houston, TX, USA, due to the collaboration established between Dr. W Arap and Dr. Renata Pasqualini. Partial data are presented below involving the results of a study developed under the orientation of Dr. Helena Brentani. The larger project will begin as soon as the results for the analysis of large-scale transcripts are completed.

Regulation in *PCA3/PRUNE2* gene

Introduction

The gene *PCA3* is expressed at high levels in prostate cancer. The transcript is an antisense ncRNA mapped onto the intron of the gene *PRUNE2*. Analysis *in silico* using the Oncomine database detected diminished expression of *PRUNE2* during the progression

of prostate cancer. Based on these findings, the collaboration between the AC Camargo Hospital and the MD Anderson Cancer Center proposed to investigate whether PCA3 was regulating PRUNE2 expression during tumor progression.

The gene *PCA3* is composed of four exons, the last divided into 4a, 4b and 4c. The most frequent variant of *PCA3* is composed by the exons 1, 3, 4a and 4b.

Partial Results

A total of 130 prostate cancer samples were evaluated by qRT-PCR using primers specific for antisense transcript (P2.2, Figure 1) and the *HPRT* gene was used as a normalizer. Diminished coexpression was observed by qRT-PCR (Figure 2). Analysis of the protein was also performed by ELISA.

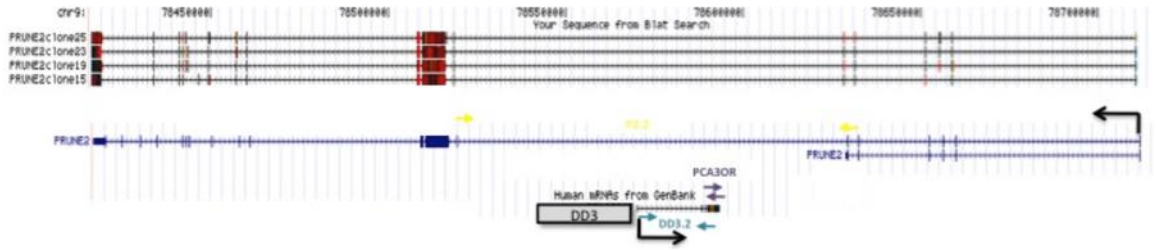


Figure 1. Location of the initiators used to evaluate the regulation of *PCA3*/*PRUNE2*.

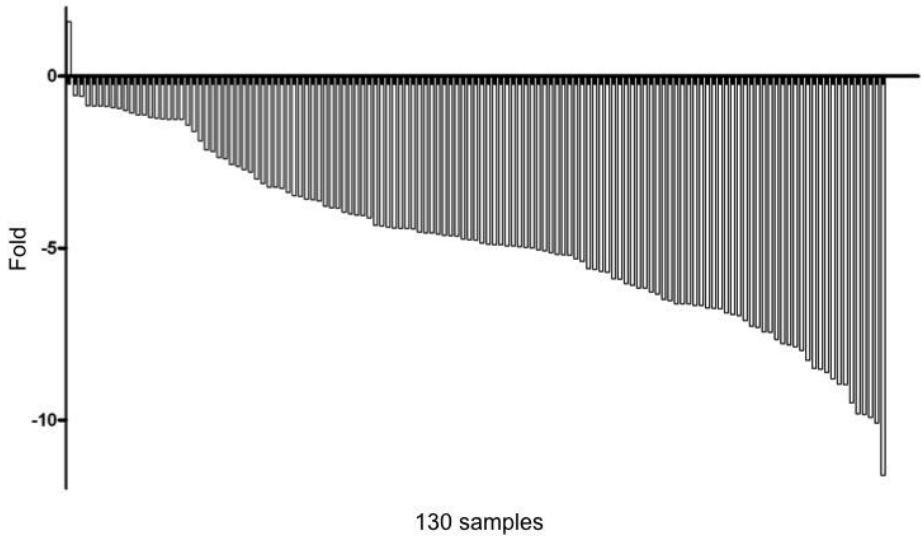


Figure 2. Downexpression pattern of the transcript *PCA3*/*PRUNE2*.

The splicing variants of this gene were also evaluated, revealing a new variant with the retention of the intron mapped between exons 3 and 4. The differential expression levels of this variant were investigated by paired comparison between tumor and normal tissue. Among the 7 paired samples, the variant showing retention of the intron was restricted to normal tissue. Another 5 pairs presented the variant in normal and tumor tissue; however, the levels were greater in normal tissue. One case showed a variant without retention of the intron in tumor and normal tissues and another showed the presence of two transcripts (with and without the intron) in tumor tissue.

Since the most frequent *PCA3* variants are composed of exons 1, 3, 4a and 4b, the diagnostic test based on expression levels of this gene in urinary sediment (GEN-PROBE, based on Groskopf et al., 2006), retention of the intron between exons 3 and 4, could lead to improvement in the diagnostic test. Currently, new transcripts have been detected showing differential expression that could interfere in the results of this diagnostic test and improve its accuracy.

B: Clinical Trials

B.1. A Phase I Study of ^{18}F-FluoroAcetate Sodium (^{18}F- FAS) as a PET Imaging Agent for Tumor Detection (prostate and breast carcinoma)

In this Phase I study, 18 patients with prostate and breast carcinomas will be selected. The safety of the patients will be monitored throughout the study. Dynamic PET imaging of the primary tumor and whole body static PET imaging will be acquired primarily to evaluate the agent's dosimetry, biodistribution, pharmacokinetics and metabolism. The sensitivity of the agent in detecting primary lesions, regional pelvic lymph nodes and distal metastases will be evaluated. The 18F-fluoroacetate PET scan will be compared to standard care scans, including MRI, CT, bone scintigraphy, 18F-FDG PET, X-ray and ultrasound. The product, injectable 18F-fluoroacetate sodium, is being manufactured by the radiopharmacy sector of the Nuclear and Energy Research Institute (*Instituto de Pesquisas Energéticas e Nucleares*, IPEN) in São Paulo, led by Dr. Jair Mengatti, a participant in this project. This project is under development and ^{18}F-FDG is under evaluation by the IPEN.

B.2. Prospective nonrandomized Phase II study to identify response markers to neoadjuvant chemotherapy and association with radiotherapy and cetuximab in patients with epidermoid carcinoma of the oropharynx

Modified to:

B.2. Prospective nonrandomized Phase II study to identify response markers to neoadjuvant chemotherapy and association with radiotherapy and cisplatin in patients with epidermoid carcinoma of the oropharynx

The original Project was designed to include the use of cetuximab on patients with oropharyngeal cancer. At least two clinical trials (Bonner et al; Vermorken et al) have demonstrated the efficacy of this drug; however, alternative therapies, including the association of two or three drugs administered as neoadjuvant therapy, followed by concurrent radio- and chemotherapy (Posner et al; Vermorken et al) has also proved to be effective and probably at a lower cost than cetuximab. Given the high cost of the drug and the difficulty in ascertaining an expected industrial partnership (due to a similar protocol ongoing in Europe, sponsored by Merck), we decided to use samples from patients submitted to the therapy considered as standard, involving two or three drugs, thus altering the project originally proposed. Despite this modification, interest in identifying the response markers within the standard protocol is justifiable and relevant. The new project was analyzed and approved by the Research Ethics Committee of AC Camargo Hospital.

Justification of the New Study

This prospective nonrandomized phase II study aims to evaluate a combination of docetaxel, cisplatin and 5-fluorouracil (5FU) administered as neoadjuvant therapy, followed by radiotherapy concurrent with cisplatin. The project was modified due to difficulties in obtaining a significant number of patients admitted to the Hospital who were able to follow the proposed protocol. Following the establishment of our partnership with Barretos Cancer Hospital, we intend to conclude case selection by the end of the second year of the project. Moreover, integrative genomic and transcriptome analyses in tumor biopsies will be included, aimed at identifying molecular markers associated with the response to therapy.

Introduction

Head and neck carcinomas comprise malignancies arising in the upper respiratory and digestive tracts and are a relatively frequent type of cancer in worldwide. They most frequently affect men over 50 years of age with a history of chronic smoking and alcohol consumption. In the majority of cases, diagnosis is delayed. The most frequent site is in the oral cavity (44%), followed by the larynx (31%) and the pharynx (25%).

Head and neck cancers have a great chance of cure when diagnosed early, but the majority of patients are diagnosed when the disease is already advanced. Despite the recent advances in therapy, patient prognosis involving these carcinomas has only improved marginally over the last three decades. Moreover, the rates of locoregional recurrence, metastasis and second primary cancer remain high (Vaamond et al, 2003). These tumors are associated with various genetic and epigenetic alterations and important studies are being conducted to identify biomarkers for prognosis and resistance to therapy.

The treatment currently considered standard for advanced oropharyngeal tumors involves concomitant chemo- and radiotherapy or surgery followed by adjuvant chemo- and radiotherapy. However, only 30 to 50% of patients with locally advanced disease survive more than three years, even with the advances in surgical techniques and the recognized benefits of therapy combined with radio- and chemotherapy (Mendenhall et al, 2008; Pedruzzi et al, 2009).

In the last few decades, chemotherapy associated with radiotherapy has come to be considered the standard treatment for patients with unresectable disease and for patients who are candidates for organ preservation, both in the US and in numerous European countries. Studies involving combined regimes (chemo- and radiotherapy) show better results compared to sequential treatment (induction chemotherapy followed by isolated radiotherapy) (Merlano et al, 1996; Adelstein et al, 1997; Pignon et al, 2000), with an 8% increase in overall survival and improved locoregional disease control (Adelstein et al, 2000).

Among head and neck cancer patients, 15 to 30% develop metastases. Studies have shown that combined therapy involving cisplatin associated with radiotherapy does not impact the incidence of distance metastasis, so taxanes were incorporated into the induction regime with 5FU and cisplatin (Forastiere et al, 2001; Choong & Vokes, 2008).

Currently, a great deal of interest exists in evaluating the value of induction chemotherapy, involving drugs considered to be more active, followed by combined therapy (chemotherapy associated with radiotherapy). Induction regimes containing three drugs (docetaxel, 5FU and cisplatin) have shown better results compared to cisplatin and 5FU. However, the role of 5FU during the induction phase has been questioned, since its addition is associated with a reduction in the dosage of the other drugs, which are currently considered more active against head and neck cancer. Despite the possibility that the use of combined therapies presents greater toxicity (Posner et al, 2007), recent studies confirm the potential for increased rates of regional disease control without compromising the quality of life of the patients (Curran et al, 2007).

There are a few reports in the literature regarding biological predictors of the response to radio- and/or chemotherapy in oropharyngeal carcinomas and the majority of these are based on heterogenous tumor groups of the upper aerodigestive tract (Weiss, 1993; Miyashita et al, 1994; Gallo et al, 1996; Jordan et al, 1996; Raybaud-Diogène et al, 1997; Gallo et al, 1999; Xie et al, 1999; Dijkema et al, 2000; Raybaud et al, 2000; Grabenbauer et al, 2000; Couture et al, 2002; Yamazaki et al, 2003). The selection of a homogenous group of patients with greater probability of responding to radiation and/or chemotherapy could contribute to modifying the therapeutic behavior of patients with oropharyngeal carcinomas.

Genomic analyses include a variety of tools that can determine specific overall changes in biological parameters. Analyses that evaluate DNA, RNA or protein levels are powerful tools for characterizing gene function and the mechanisms of gene regulation, while facilitating disease classification, biomarker identification, risk stratification and the discovery of drugs. Alterations in DNA copy numbers are common in cancer and can lead to alterations in the expression and function of the genes that reside in the affected genome region. Identifying regions with genomic copy number alterations and, particularly, the genes involved, offers the basis of a clearer understanding of cancer development and, more importantly, can be a useful tool in the identification of markers that permit adequate and personalized clinical management of patients with cancer.

This randomized phase II study aims to evaluate a combination of docetaxel, cisplatin and 5-fluorouracil or docetaxel and cisplatin administered as neoadjuvant therapy followed by radiotherapy and cisplatin. This protocol aims to preserve the pharynx in patients with oropharyngeal carcinoma (stages II-IV, with no distant metastasis), who are potential candidates for surgery.

The study objectives are: (1) investigate biological markers that could act as potential predictors of patient response to chemo- and/or radiotherapy; (2) genotype all the samples for HPV; (3) evaluate the impact of these markers on patient survival; (4) evaluate the impact caused by the treatment, using a quality of life questionnaire developed by the University of Washington, USA, which will be applied during posttreatment follow-up.

Material and Methods

In this study, a total of 40 patients will be selected who are carriers of primary squamous cell carcinoma (SCC) with no prior treatment, aged between 18 to 70 years-old, whose tumors are at a clinically advanced stage (T2, T3 and T4), N0 to N3, with no distant metastasis (M0), diagnosed and treated in the Department of Head and Neck Surgery and Otorhinolaryngology of the AC Camargo Hospital, São Paulo, and the Barretos Cancer Hospital, Barretos, SP, Brazil.

Patient selection seeks potential candidates for radical surgery, with clinical status that would permit both radical surgery (ASA I to III) and neoadjuvant chemo- or radiotherapy associated with carboplatin, performance status of 70 or more according to the Karnofsky scale, estimated life expectancy of at least 6 months, adequate medullar

reserves (leukocytes ≥ 3,500/mm^3; neutrophils ≥ 1,500/mm^3; platelets ≥ 100,000/mm^3; hemoglobin ≥ 9.0 g/dl), adequate hepatic function (total bilirubin ≤ 1.5 times the upper limit of normal; GOT and GPT ≤ 3 times the ULN), adequate renal function (creatinine ≤ 1.5 times the ULN), adherence of the patient to treatment and geographical proximity that permits adequate follow-up for voluntary participation in the study, as specified in the free informed consent form. The project was submitted to and approved by the Research Ethics Committees in both hospitals.

The exclusion criteria in relation to patient participation are the presence of active or uncompensated heart disease, acute myocardial infarction in the six months prior to potential inclusion, the presence of active infection and concomitant systemic diseases (considered serious according to the investigator's criteria), the presence of severe psychiatric disease, another primary neoplasia (except *in situ* carcinoma of the uterine cervix or adequately treated skin basal cell carcinoma). Participation of the patient in another experimental protocol excludes them from this study.

Patient clinical information and physical exams, such as weight, height, performance status (Karnofsky), vital signs, direct measurement of measurable tumors, existing signs and symptoms (MD Anderson Symptom Inventory), hematological exam (hemogram with platelets), biochemical exams (Na, K, Mg, urea, creatinine, Ca, albumin, alkaline phosphatase, GOT, GPT, LDH, bilirubin, urinalysis and pregnancy test for the women), creatinine purification (if indicated), quality of life questionnaire of the University of Washington validated for the Portuguese language, computerized tomography or magnetic resonance imaging, X-ray or thorax tomography, as indicated, will be carefully conducted and included in a standardized form. When available, the results of the imaging exams should be confirmed by PET-CT, locoregional exam, tumor fragment biopsy and blood sample collection for histological and molecular biology analysis.

The forms including the term of free informed consent, Karnofsky scale (performance status), toxicity criteria (I to IV), WHO criteria for response evaluation, quality of life questionnaire (University of Washington) and MD Anderson Symptom Inventory duly validated in Portuguese are attached to this project.

Treatment Protocol

The protocol under study is the realization of chemotherapy treatment in the neoadjuvant phase with cisplatin associated with paclitaxel, both administered on day 1 (D1) of 21-day schedules for a total of three cycles. The doses and administration routes are: 80 mg/m^2 D1 of cisplatin, EV for 2 hours in an infusion pump, diluted in a solution of 250 ml of 0.9% SF and 250 ml of 10% mannitol; associated with paclitaxel, EV for 3 hours in an infusion pump, diluted in 500 ml of 0.9% SF in a polyolefin or glass flask, with a polyethylene-lined nitroglycerin feed (an in-line cellulose acetate filter, with 0.22 micra pores will be used). Pre-cisplatin hydration is achieved with 1000 ml of 0.9% SF infused for 1 hour and after completing cisplatin, 500 ml of 0.9% SF will be infused. The antiemetics used in this protocol are: 16 mg of ondansetron associated with 20 mg of dexamethasone, diluted in 100 ml of 0.9% SF and infused for 15 min prior to chemotherapy. As premedication for paclitaxel, all patients also receive 50 mg of ranitidine, EV in bolus and 50 mg of diphenhydramine EV in 15 min. Patients presenting peripheral neuropathy grade > 2 or renal dysfunction (creatinine clearance <60 ml/min) post-cisplatin receive carboplatin (AUC = 5) as a substitute for cisplatin. This is diluted in 500 ml of 15% SG and infused for 1 hour and does not require the prehydration defined for cisplatin.

The remaining methods are identical to those presented in the project as originally proposed. The response to treatment data will be obtained at the end of the study.

HPV Analysis

To amplify part of the region of the HPV, the Linear Array HPV Genotyping Test (Roche, Branchburg, USA) kit is used for *in vitro* diagnosis by the PCR technique. Amplification of a wide spectrum of HPV genotypes requires the use of consensus initiators oriented toward the conserved region among different genotypes in the HPV genome. The most conserved region is L1 and several consensus initiator complexes have been described in this region (Molijn et al, 2005), including the complexes GP5+/6+ (Jacobs et al, 1997), MY09/11 (Hildesheim et al, 1994) and PGMY (Gravitt et al, 2000). Amplification of a wide spectrum of HPV genotypes, including 13 high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (total: 37 subtypes),

involves using biotinylated initiators in a polymorphic region conserved among the different HPVs.

After total DNA extraction of the tumor biopsy, amplification is achieved in a mixture of reagents with AmpliTaq Gold DNA polymerase in the presence of Mg^{2+} in an excess of deoxynucleoside 5'-triphosphates (dNTPs) and biotinylated primers for amplification of specific HPV regions (450 pb) and the gene β-globin (268 pb). The amplification cycles are optimized during the experiments.

CGH arrays and cDNA oligo arrays

DNA and cDNA samples of oropharyngeal carcinoma biopsies (untreated prior to collection) are obtained for well-established standard protocols in the AC Camargo Cancer Hospital (HACC). The profiles of copy number variations are obtained using Agilent SurePrint G3 Human CGH Microarray 4 x 180K platforms (Agilent Technologies, Santa Clara, CA). Each platform contains 4 arrays and each of these comprises ~170,000 human coding and noncoding sequences annotated against UCSC hg18 (NCBI Build 36, March 2006). The mean spacing between the probes is 17 Kb and although the distribution is random, there is a greater distribution of coding sequences. Normal commercial DNA (Promega, Mannheim, Germany) paired for sex is used as a reference. Normal RNA is obtained from the normal adjacent tissue in all cases. Analyses of large-scale genic expression are performed using the Human 4x40K Agilent oligoarrays platform, in accordance with the manufacturer's recommendations. The samples are differentially labeled by random priming with Cy3- or Cy5-sCTPs (Agilent Genomic DNA ULS labeling kit). The hybridization and washing protocols are conducted in accordance with the manufacturer's recommendations. Emitted fluorescence is captured using a 48-slide system Agilent DNA microarray scanner. This scanner permits a high scanning resolution of 2, 3, 5 or 10 microns, required for the 180K and 44K platforms (2-3 microns). Data is extracted using the Feature Extraction software, version 10.7, and analyzed by the Genomic Workbench Standard software, Edition 5.0.14 (Agilent Technologies, Santa Clara, CA), with an ADM-2 statistical algorithm set at a sensitivity threshold of 6.0. Sequences are considered significant when at least three consecutive probes present relevant values in the analysis. Alterations in DNA copy numbers are compared with a reference database of healthy Brazilians (80 women currently

registered) and the Database of Genomic Variants (DGV;*http://projects.tcag.ca/variation/*).

Integrated analysis between aCGH and expression analysis data

The genomic data will be compared to large-scale genic expression data in order to identify genes with alterations at the transcriptional level, determined by alterations in DNA copy numbers. The Genomic Workbench Standard program, Edition 5.0.14 (Agilent Technologies, Santa Clara, CA) permits the loading of genomic and transcript expression data. This software permits correlating the array-CGH and genic expression data obtained through experiments on the 4x180K and 44K oligonucleotide platforms (Agilent Technologies, Santa Clara, CA), respectively. Correlation vectors that do not present values close to -1 and 1 will be excluded. The loss and gain levels observed will be compared to increased and diminished genic expression to determine correlated genes present in all cases. Only one prior integration analysis will be performed on the data to observe the relation between the two analyses and how the genes behave when observing their expression and copy number alterations. To identify the regions of loss and gain, when compared to the expression data, the parametric Student t test will be used. In parallel, Pearson's correlation coefficient will be determined.

A second analysis will be performed using the data from the platforms chosen to identify the data complexes that intersect between the sets of analysis obtained using the two programs: ParseUniGene developed in Python and Chess (http://biostone.khu.ac.kr/CHESS/). ParseUniGene combines the analyses and guarantees that a new list is created without gene duplication when all the data present in the matrices are observed and compared to the samples studied. For genes showing multiple representations in the expression data complexes, the sequence that presents the greatest correlation between the platforms is used. This analysis starts from the principle that the genes are present in both platforms and present concordant correlation values, both in their expression and in the copy number alterations; i.e., a coefficient >0.3. Using the CHESS program, it is possible to identify the phenotypes or genomic regions of specific alterations. When the intensity of the alteration signal is obtained, the Student t test is applied to investigate the null hypothesis that the ratio values of the study data show the same distribution in their relation to aCGH and gen expression. After the association study and application of the statistical test, the

significant genes obtained are presented with their p values. To compensate for multiple testing in genome-wide association studies, the results are adjusted by the Bonferroni correction. When the association study is completed, the CHESS program marks the genomic regions, including the significant probes, as a green/red bar for the whole chromosome scale. Finally, for biological interpretation, the significance value is calculated from the hypergeometric distribution for data representation in the KEGG pathway and Gene Otology databases, in which the regions with specific phenotypes are involved.

To determine the pathways and networks that are significantly regulated among the gene expression data, analysis is conducted using the Ingenuity Pathway Analysis (IPA) program (http://www.Ingenuity.com). HUGO gene identifiers are mapped in the available networks in the databases accessed by the Ingenuity program and classified by scores. The score indicates the probability that the genes in the network are together due to random events. Values considered significant are those with a 99% confidence interval.

Preliminary Results

Currently, four oropharyngeal carcinoma samples (FAR1T, FAR2T, FAR3T and FAR4T) have been analyzed by array-CGH in 4x180K slides (Agilent). All the genomic regions detected as altered were compared to the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/) and the Brazilian sample database. "Rare" CNVs were defined as those located in regions not described by the DGV and that contained coding regions. However, these criteria may be altered by the time the final data are completed.

The four samples evaluated present copy number alterations. Sample **FAR1T** presented 58 regions showing significant alterations, of which 52 included one or more mapped genes and 39 included one or more previously described CNVs. Among the altered regions, 6 were gains and 52 were losses (False Discovery Rate; FDR=0.05). Large genomic imbalances were detected in case FAR1T, including losses at 3p, 11q, 16q and 21q and gains at 16q, 20p and 20q.

Sample **FAR2T** presented 15 regions showing significant alterations, of which 12 included one or more mapped genes. Regarding the presence of CNVs, 10 of the genomic regions coincided with the presence of at least one CNV characterized in the DGV database (Table 2). One region was a genomic gain and 14 were losses. The sample

FAR2T presented no large genomic imbalances, though all the genic regions that did not present CNVs were involved with losses.

Sample **FAR3T** presented 42 regions showing significant alterations, of which 36 included one or more mapped genes and 26 included one or more previously described CNVs. Seven genomic gains and 35 losses were detected (FDR=0.05).

Sample **FAR4T** presented 37 regions showing significant alterations, of which 28 included one or more mapped genes and 26 included one or more previously described CNVs. Among the altered regions, 22 were genomic gains and 15 were losses (FDR=0.05).

Table 1 presents a summary of the genomic alterations frequently detected in the four cases analyzed, including those present in at least two cases. Figure 1 is a representative ideogram of the gains (lines on the right) and losses (lines on the left) detected in the oropharyngeal carcinoma cases evaluated so far. It was found large regions of genomic imbalances, mostly involving chromosomes 3, 11, 16 and 20 in case FAR1T and discrete alterations in all the cases studied.

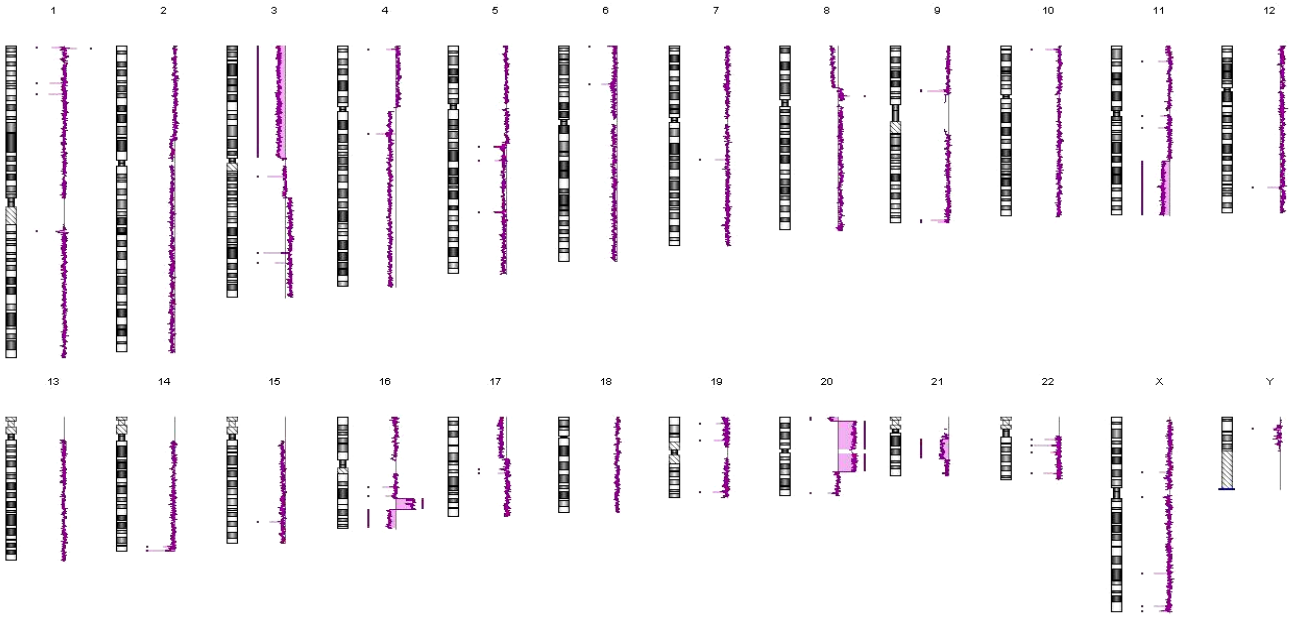
The genes *CREB3* and *GBA2* (9p13.3) were deleted in three out of four cases analyzed. One example of CNV in these tumors is the microamplification involving *PAPD4* (5q14.1) in cases FAR3T and FAR4T. In the remaining altered regions, genes involved in genesis and progression in oropharyngeal carcinomas are mapped (Figure 2).

Table 1. Common genomic alterations (minimum of two cases) among the four cases of oropharyngeal carcinoma evaluated.

CASE	CYTOGENETIC LOCATION	GAIN	LOSS	*GENES*	CNVs described
FAR1T; FAR2T	3q12.3	0	-1,482202	*NFKBIZ*	-
FAR1T; FAR2T	3q26.2	0	-0,828826	*PHC3*	-
FAR1T; FAR3T	4p16.3	0	-0,827189	*C4orf8, TNIP2, SH3BP2*	2
FAR3T; FAR4T	5q14.1	1,560704	0	*PAPD4*	-
FAR1T; FAR3T	7q21.13	0	-1,064555	*STEAP2, C7orf63*	2
FAR1T; FAR2T; FAR3T	9p13.3	0	-1,740918	*CREB3, GBA2*	-
FAR1T; FAR3T	10p15.2	0	-1,116137	*PFKP*	7
FAR1T; FAR3T	11p15.3	0	-0,981891	*MICALCL, PARVA*	-
FAR1T; FAR2T	11q13.1	0	-0,990708	*ATG2A, PPP2R5B, GPHA2*	-
FAR1T; FAR3T	15q25.2 - q25.3	0	-0,884122	*SCAND2, WDR73, NMB*	-
FAR1T; FAR3T	19p13.11	0	-0,837388	*CRLF1, TMEM59L, KLHL26, CRTC1*	-
FAR1T; FAR3T	19p13.3	0	-0,926561	*M6PRBP1*	-
FAR1T; FAR3T	19q13.42	0	-1,240548	*TFPT, PRPF31, CNOT3, LENG1*	-
FAR1T; FAR3T	22q11.21	0	-1,252848	*DGCR14, TSSK2, GSC2*	1
FAR1T; FAR3T	Xp11.3	0	-0,882884	*NDP*	-
FAR1T; FAR3T	Xq25	0	-1,289868	*SH2D1A*	-
FAR1T; FAR2T	Xq28	0	-1,301504	*MAMLD1*	4

– : Absence of CNV described in this region (compared to the Database of Genomic Variants, DGV; *http://projects.tcag.ca/variation/*).

FAR1T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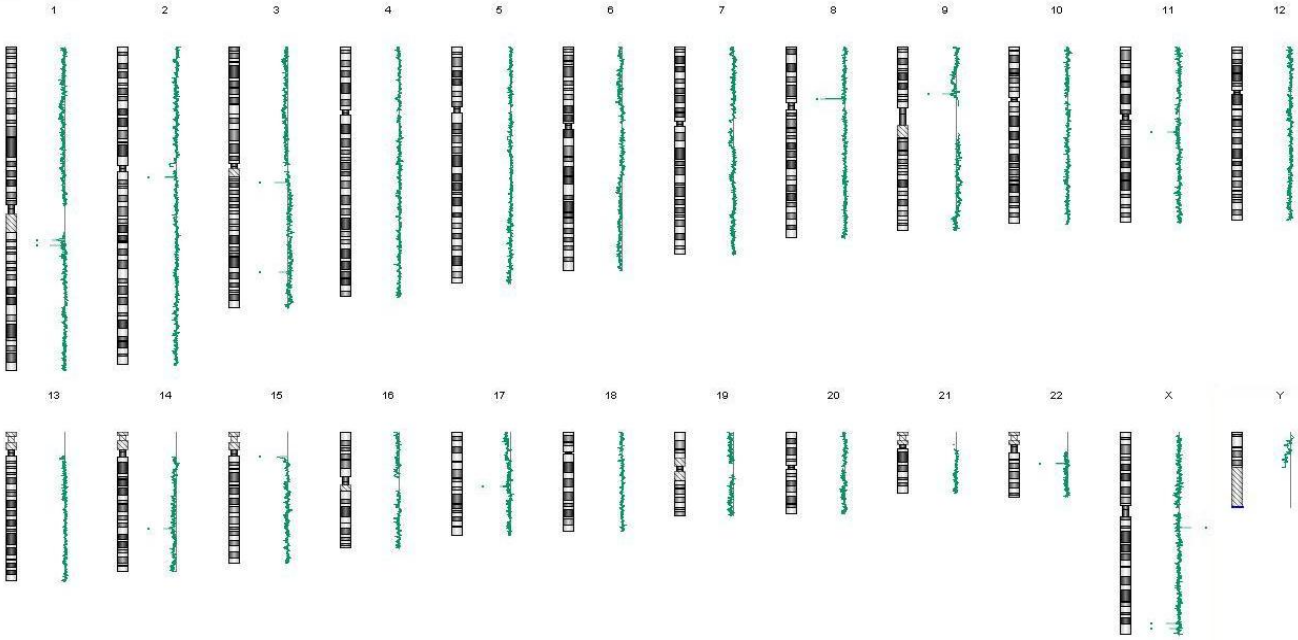
FAR2T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FAR3T

FAR4T

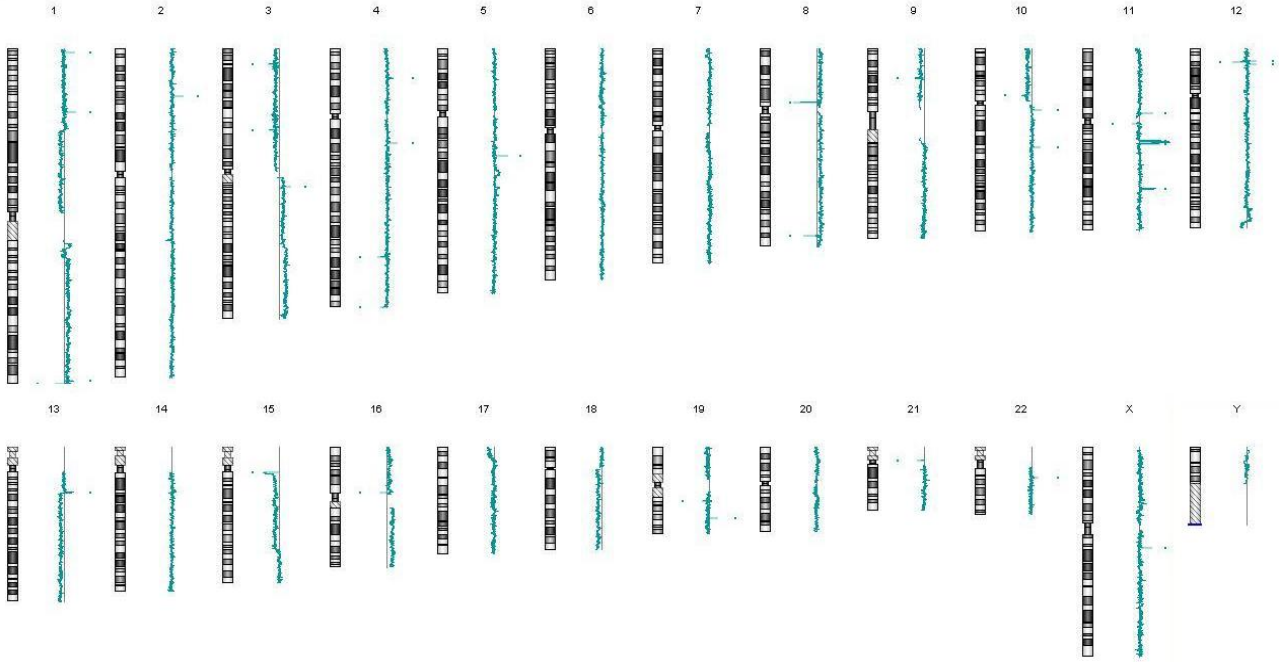

Figure 1. Ideogram representative of the genomic alterations detected in the four cases of oropharyngeal carcinoma evaluated. Regions of gains/amplifications (right) and losses or deletions (left) are presented accurately or as large CNV regions (images generated by the Genomic Workbench program).

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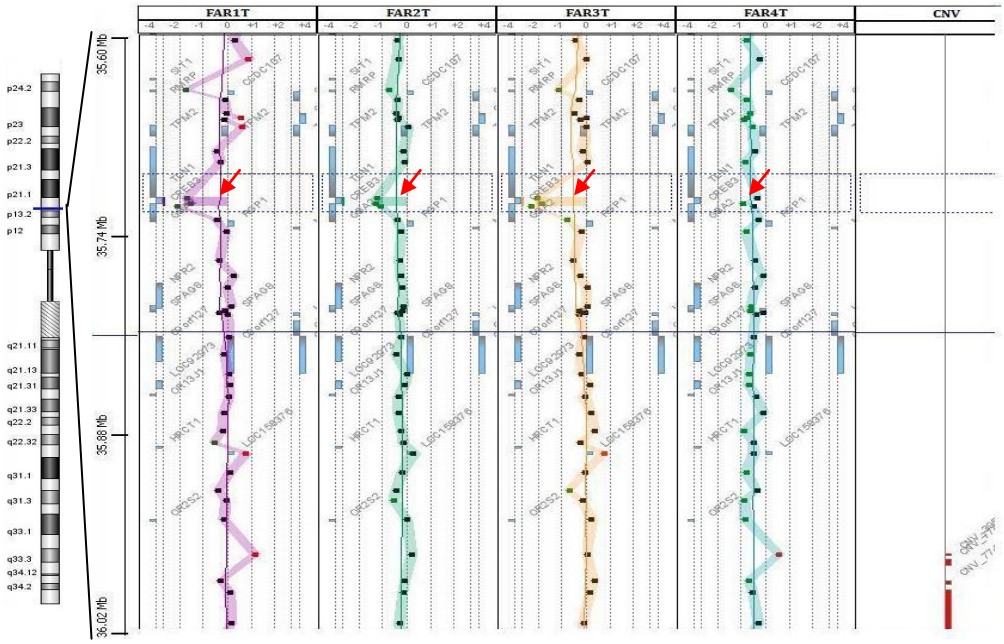

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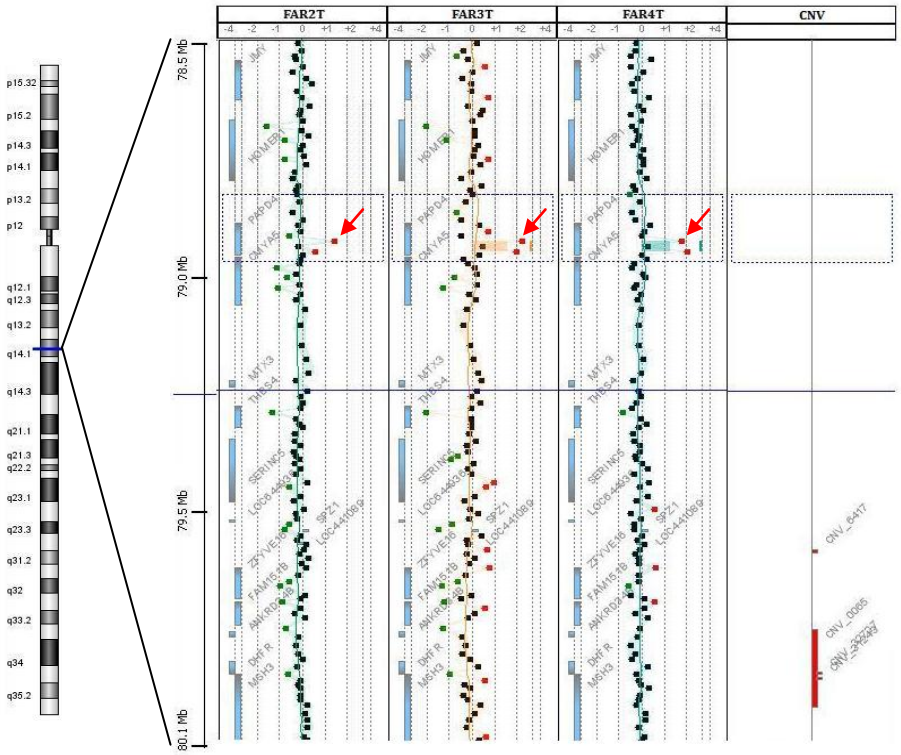

Figure 2. A. Graphic representation of the alterations present in the microdeletion region at 9p13.1 (red arrows), where the genes *CREB3* and *GBA2* are mapped, and the absence of previously described CNVs; **B.** Microamplification of the gene *PAPD4* mapped at 5q14.1 (red arrows). The red dots on the right of the image indicate the regions of gains, black indicates normal regions and green, regions of genomic losses (images generated by the Genomic Workbench program).

Partial Discussion

Among the major risk factors for oropharyngeal carcinoma are excessive smoking and alcohol consumption; however, the mechanisms responsible for carcinogenesis and response to treatment have yet to be elucidated (Gebhart & Liehr, 2000; Argiris & Eng, 2003; Argiris et all, 2008). At present, only four patients have been analyzed who fulfilled the inclusion criteria adopted for the study. The array-CGH methodology allows quantitative detection of genomic copy number alterations at high resolution. Preliminary analysis revealed regions of common and exclusive genomic gains and losses.

There are a few studies in the literature that use array-CGH on head and neck carcinomas, though principally involving carcinomas of the oral cavity. Baldwin et al (2005) used the whole genome tiling-path arrays (containing 32433 BACs) on 20 samples of oral SCCs. Among the alterations, loss at 3p was the most frequent, followed by losses involving chromosomes 4, 5q, 8p, 9p, 10q, 11, 18q and 21q. Gains were frequent at 8q, followed by 3q and smaller regions at 9q, 11q (*CCND1*), 14q and 20q, as well as 7p11.2 (*EGFR*) present in 40% of the cases evaluated. The authors reported previously undescribed alterations, present in 7 to 15 of the 20 cases evaluated, including gains at 3q23, 5p15.2 (*TRIO*), 7p12.3-13, 7q21.2 (*CDK6*), 7q35 and 11q22.2-22.3 (*MMP* cluster) and losses at 2p15, 4q34.3 and 16q23.2. The authors validated some findings by RT-PCR.

In primary SCCs and metastases in cervical lymph nodes, Lui et al (2006) verified genomic gains (*LMYC*, *REL*, *TERC*, *PIK3CA*, *MYB*, *MDR1*, *HRAS*, *GARP*, *CCND2*, *FES*, *HER2, SIS* and *SRY*) and losses (*p44S10*, *TIF1*, *LPL*, *MTAP*, *BMI1*, *EGR2* and *MAP2K5*). Classifier genes were identified between the primary tumor and metastasis (*TGFB2*, *CRBP1*, *PIK3CA*, *HTR1B*, *HRAS*, *ERBB3* and *STK6*) and in comparisons between patients who died and those who survived (*PRKCZ*, *ABL1* and *FGF4*). The authors used protein analysis by immunohistochemistry to validate the findings and reported that the expressive immunoreactivity for FGF4 was associated with worse prognosis, while loss of reactivity for *CRBP1* was characteristic of primary and metastatic SCCs.

In a study also involving oral carcinomas, O'Regan et al (2006) evaluated 20 samples by array-CGH and compared the findings in patients over 40 years of age (Group 1) with those equal to or under 40 (Group 2). Group 1 presented a greater number of gains and losses, in contrast with the minimal number of copy number alterations observed in the nonsmokers in Group 2. Tumors from older patients presented deletions involving 3p and 9p21, as well as gains at regions 3q, 5q, 7p, 8q, 11q and 20q. In Group 2, principally among nonsmokers, no alterations previously reported in oral carcinomas were detected. The

deletion of *CDKN2A* present in 50% of Group 1, was absent in Group 2, demonstrating lower level of genomic instability among younger nonsmoking patients with oral carcinoma compared to other patients with these tumors.

Sparano et al (2006) reported frequent gains (>35%) at 3q, 5p, 8q, 9q and 20q and losses (>40%) at 3p, 8p, 13q and 18q among 21 samples of oral carcinomas evaluated by aCGH. Genes associated with the response pathway to DNA damage were frequently altered, including *BRCA1*, *BRCA2*, *FANCD2* and *FANCG*. Other genes related to hereditary cancer syndromes were reported to be altered, including *VHL*, *MLH1*, *XPC* and *RB1.*

In contrast to previous studies describing oral carcinomas, no common significant regions were observed in the comparison with the present study. This discrepancy could be due to the small number of samples studied, differences in the platforms analyzed or to a lack of distinction between oral cavity and oropharyngeal carcinomas described in the literature.

In the cases presented here, alterations involving chromosome 3 were observed in all cases, although in different regions. Two cases presented loss at 3q26.2, where the *PH3* gene is mapped. This gene is part of the PcG complex (Polycomb group genes) that encodes a diverse complex of regulatory proteins involved in the maintenance of the pattern of expression and that control development. They were initially described in *Drosophila* and later in superior eukaryotes. The PcG complex is responsible for long-term silencing of genes, leading to alterations in the chromatin structure due to deacetylation in the tails of histones and through inhibition of chromatin remodeling via dependency on ATP. This complex can also mediate the silencing of target genes interfering in the remodeling of chromatin SW1/SNF, blocking the initial activity of transcription and recruitment of additional activities of silencing (Dellino et al, 2004; Lavigne et al, 2004). Deshpande et al (2007) suggested that loss of *PH3* in osteosarcomas could favor tumorigenesis by potentially inhibiting the ability of cells to remain in G0. The authors detected diminished *PH3* in 36 out of 56 human osteosarcomas; posterior sequencing analysis revealed a mutation in 9 of the 15 tumors originally evaluated. These data suggest that *PH3* is an important putative tumor suppressor gene.

Loss at 3q12.3 was detected in two grade III oropharyngeal carcinomas, where the *NFKBIZ* gene is mapped. The function of the NFKBIZ protein is associated with response to inflammatory processes and transcription regulation. Mandruzzato et al (2008) reported that this gene was differentially expressed, in a study of genetic signature of good prognosis in 38 melanomas (stage III and IV) evaluated by cDNA microarray. Moreover,

increased expression of the *NFKBIZ* gene was observed in patients with long term survival. In the present study, no data on patient survival are available yet, but one of the patients developed metastasis in the liver. Thus, the loss of this gene may be associated with parameters of worse prognosis.

It was detected losses involving the *TNIP*/*ABIN2* gene, mapped at 4p16.3. Its product binds to the C-terminal zinc-finger domain of A20 (TNFAIP3) and is involved in the activation of the ERK-MAP kinase pathway in several cell types (Van Huffel et al, 2001; Papoutsopoulou et al, 2006). Tadros et al (2003) reported that ABIN-2 inhibits endothelial apoptosis and frees the cells from cell death after growth factor deprivation. Recently, it was reported that ABINs contain an ubiquitin-binding domain that is essential for the inhibition of the inhibitory activity of NF-kappa B and antiapoptotic activities. In this context, it has been proposed that ABINs function as adaptors between ubiquitinated proteins and other regulatory proteins. Alternatively, ABINs could disturb signaling complexes, competing with other ubiquitin-binding proteins to bind to specific ubiquitin targets. Together these findings imply an important role for ABINs in the regulation of immunity and tissue homeostasis (Verstrepen et al, 2009). The *SH3BP2* gene also mapped at 4p16.3, was co-deleted with the *TNIP* gene in two cases. Although its physiological function remains unknown, it has been suggested that it plays the role of an immunoreceptor involved in cell signaling due to its interaction with a series of signaling molecules, including the families Src and Syk of tyrosine kinase proteins, among others. Recently, the locus 3BP2/SH3BP2 presented as muted in a rare disease, cherubism, involved in craniofacial development, suggesting a role for 3BP2 in osteoclasts and in regulating the function of hematopoietic cells (Deckert, 2006).

Gains in *PAPD4*, mapped at 5q14.1, were detected in two cases. This gene is highly conserved and has been associated with the function of binding to ATP and nucleotides, the process of RNA polyadenylation and the processing of mRNA.

Two cases presented losses involving *STEAP2*, mapped at 7q21.13. This gene is a member of the STEAP family and encodes a protein of the cell membrane. The structure of these proteins contains six transmembrane domains and intracellular amino and carboxyl terminals, suggesting that they are involved in the function of ionic channels, protein transportation or gap-junctions. In prostate carcinomas, they have been related to amplifications (data unpublished, personal communication).

The region 9p13.3 was involved in gains in three oropharyngeal carcinomas, where *CREB3* and *GBA2* are mapped. The *CREB3*/*LZIP* gene is related to chemotaxis processes and

transcription regulation. This protein is involved in leukocyte migration, tumor and endoplasmic reticulum suppression and the degradation of proteins associated with stress. The *GBA2* gene is related to metabolic and catabolic processes, and to beta-glucosidase, glucosylceramidase and hydrolase activity, though up to now, no tumor processes have been described.

The *PFKP* gene, mapped at 10p15.2, involved in losses in two cases, is responsible for the synthesis of a key regulatory enzyme in glycolysis. Genomic losses at 11p15.3 (*MICALCL* and *PARVA*) and 11q13.3 (*ATG2A*, *PPP2R5B* and *GPHA2*) were each detected in two cases. The MICALCL protein interacts noncovalently and selectively with a protein kinase activated by mitogens. The *PARVA* gene is responsible for the synthesis of an extracellular matrix adhesion protein that is involved in the regulation of cell morphology and cell survival. The parvin family (alpha and beta) is associated with intracellular pathways that regulate the dynamic actin cytoskeleton. The two parvins appear to perform opposing roles: alpha inhibits cell spreading activities, while beta inhibits the activity of ILK and reverses certain oncogenic effects in tumor cells (Sepulveda & Wu, 2006).

Losses at 15q25.2-25.3 comprising the genes *SCAND* (transcription factor), *WDR73* (conserved in several different species, but of unknown function) and *NMB* were detected in two cases. The NMB protein and its receptor have been described as mitogens that coexpress in proliferative cells of the colon. Although chromosome 19 is rich in common CNVs, genomic losses were detected involving 19p13 and 19q13, which do not coincide with the known CNVs and could be relevant in the tumor process.

Losses in the X chromosome involved *NPD* (growth factor) and *SH2D1A*, a binding protein, with a principal role in bidirectional stimulus of T and B cells; multiple transcript variants and isoforms of this gene have been reported.

The exact function performed by these genes and their correlations in the development and response to clinical treatment will be evaluated in much greater detail following the inclusion of a larger number of cases in the array-CGH and transcript expression analyses.

B.3. Prospective comparative Phase I single-center clinical study to evaluate the effectiveness and safety of the formulation of topical use of F8 protein derived from *Hevea brasiliensis* in the treatment of pressure ulcers

In this project, Pele Nova Biotecnologia S.A. and the team of AC Camargo Hospital had intended to study F8 protein extracted from the serum of natural latex of the Pará rubber tree (Hevea brasiliensis). A total of 40 patients presenting pressure ulcers were to be included in the study. The patients would have been randomized into two groups: the control group of 20 patients attended using the conventional treatment; and the test group of 20 patients treated with the experimental substance. Both groups would have been submitted to daily curatives and rigorous follow-up by doctors and nurses of the research team for the 8 weeks of the study period. Unfortunately, Pele Nova Biotecnologia S.A. demonstrated no further interest in developing the project, despite the previously assumed commitment. In attached we present the formal letter of the company signatory.

C. Patient Recruitment and Follow-up, Training, Education Programs, Diffusion and Technology Transfer

C.1. Establishment of research protocols for the recruitment and follow-up of individuals at high risk of hereditary carcinomas

A protocol for counseling, recording data and monitoring of patients with syndromes of inherited predisposition to cancer was established. These criteria were described in detail above.

1. Counseling, Recording Data and Monitoring System

Patients who meet the Bethesda and Amsterdam criteria are attended at the Hereditary Colorectal Cancer Registry and patients who meet the criteria for the Hereditary Breast-Ovarian, Breast-Colon, and Li-Fraumeni Syndromes are attended in the cancer genetics department.

Probands referred for a cancer genetics evaluation are submitted to Genetic counseling, in a non directive strategy. Cancer risk reduction options are debated, including prophylactic surgery (when indicated) and tests for early detection of cancer. Sharing information with family members is encouraged. For patients undergoing the genetic test, the results are disclosed in the post-test counseling session. Family pedigree is recorded using the Progeny software. The file is then imported into the OncoTree, which records the personal and family history of cancer, including the results of genetic testing and follow-up examinations. OncoTree data are available only to the team responsible for patient care.

2. Criteria for Syndromes´ Clinical Diagnosis

The criteria for diagnosis of the syndromes are described below:

Modified Bethesda Criteria (Umar et al. 2004)

- Colorectal cancer (CRC) diagnosed younger than 50 years of age;
- Individuals with cancer associated with synchronous or metachronous Lynch Syndrome (LS) tumor, regardless of age (CRC, endometrium, stomach, ovary, pancreas, urinary tract, biliary tract, small intestine, sebaceous adenomas, keratoacanthomas);

- CRC with MSI-H histology in patients less than 60 years of age (tumor with lymphocytic infiltrate, lymphocytic reaction Crohn-like, mucinous differentiation in signet ring, medullary growth pattern);
- CRC diagnosed in one or more first-degree relatives with a cancer related to LS, one of the tumors diagnosed before 50 years of age;
- CRC diagnosed in two or more first- or second-degree relatives with the LS-related tumors, regardless of age at diagnosis.

Amsterdam criteria I (Vasen et al. 1991)

- At least three members of a family with CCR;
- One member must be a first-degree relative of the other two;
- At least two generations affected;
- At least one member with CCR and 50 years of age;
- Exclusion of familial adenomatous polyposis.

Amsterdam criteria II (Vasen et al. 1999)

- At least three members of a family with CRC or endometrial cancer, or transitional cell excretory urinary carcinoma (renal pelvis or urethra), or adenocarcinoma of the small intestine;
- One member must be a first-degree relative of the other two;
- At least two generations affected;
- At least one member with CCR and 50 years of age;
- Exclusion of familial adenomatous polyposis.

Breast-Colon Syndrome

For diagnosis of the breast colon syndrome Meijers-Heijboer et alor Naseem et al. criteria are used:

Criteria Meijers-Heijboer (Meijers-Heijboer et al. 2003)

- Two individuals with breast cancer (first- and second-degree relative) with at least one case diagnosed before 60 years of age and one of the following:
- At least one individual with breast cancer and colorectal cancer diagnosed at any age;

- At least one individual with colorectal cancer diagnosed before 50 years of age, a first- or second-degree relative of a patient with breast cancer;
- At least 2 individuals with colorectal cancer diagnosed at any age, first- or second-degree relatives of a patient with breast cancer.

Criteria Naseem (Naseem et al 2006)

At least one of the following criteria:

- An individual with breast cancer and colorectal cancer at any age, and one additional case of breast cancer or colorectal cancer in a first- or second-degree relative;
- An individual with colorectal cancer diagnosed under 50 years of age with a family member with breast cancer diagnosed before 50 years of age or two relatives with breast cancer diagnosed at any age;
- Two individuals with colorectal cancer diagnosed at any age and at least one family member diagnosed with breast cancer before 50 years of age or two cases of breast cancer diagnosed at any age.

Hereditary Breast-Ovarian Cancer Syndrome (NCCN, 2009)

Personal history of breast cancer and most of the items below:

- Diagnosis up to 45 years of age without family history;
- Diagnosis up to 45 years of age with family history
- Diagnosis over 50 years of age with a family member with breast cancer diagnosed before 50 years of age with family history or with epithelial ovarian cancer;
- Two of the primary breast tumors diagnosed before 50 years of age;
- Diagnosis at any age, with two or more relatives diagnosed with breast cancer or ovarian cancer at any age;
- Family member with male breast cancer;
- Personal history of epithelial ovarian tumor / primary peritoneal tumor;
- Members of the families of ethnic groups associated with high frequency of mutation (Ashkenazi Jews, Icelanders, Swedes, Hungarians);
- Other categories except the above

Personal history of epithelial ovarian cancer;

- Diagnosis of ovarian epithelial tumor / primary peritoneal tumor

Personal history of male breast cancer;

- Diagnosis of male breast tumor.

Li-Fraumeni Syndrome

A clinical diagnosis is confirmed when completing one of the criteria below:

Classic Li-Fraumeni Syndrome (Bougeard et al. 2008, Li et al. 1998)

- Sarcoma in childhood or at a young age (before 45 years of age) and;
- First-degree relatives with any cancer at an early age (before 45 years of age) and;
- First- or second-degree relative who has a diagnosis of cancer at a young age (before 45 years of age) or sarcoma at any age.

Birch Criteria (Birch et al. 2001)

- Childhood cancer or sarcoma, CNS or ADR before 45 years of age and;
- First- or second-degree relative with typical Li-Fraumeni tumor (sarcoma, breast cancer, CNS, ADR or leukemia) at any age and;
- First- or second-degree relatives with any cancer before 60 years of age.

Eeles Criteria (Eeles et al. 1998)

- LFL-E1 presence of two first- or second-degree relatives with typical tumors at any age (sarcoma, breast cancer, CNS tumors, leukemia, ADR, melanoma, prostate cancer, pancreatic cancer);
- LFL-E2 sarcoma at any age in the index patient with the following two tumors (which may be present in the same individual) breast cancer at <50 years of age and / or CNS tumors, leukemia, ADR, melanoma, prostate cancer, pancreatic cancer at <60 years of age or sarcoma at any age.

Chompret Criteria (Chompret et al. 2008)

- Sarcoma, CNS, breast cancer or ADR before 36 years and a) a first- or second-degree relative with cancer before 46 years of age OR b) Family member with multiple primary tumors at any age OR;
- Multiple primary tumors, including two tumors (sarcoma, brain, breast cancer or ADR), with the first tumor diagnosed before 36 years of age, regardless of family history OR;
- ADR at any age and regardless of family history;

- Index patient with typical Li-Fraumeni tumor (soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, SNC, ADR, leukemia, and bronchoalveolar carcinoma of the lung) before 46 years of age and;
- First- or second-degree with typical Li-Fraumeni tumor before 56 years of age (except breast cancer if the proband has breast cancer) or multiple tumors OR;
- Index-patient with multiple tumors, with at least two from the spectrum and LFS before 46 years of age OR;
- ADR at any age or breast cancer before 36 years of age without mutations in the BRCA1 or 2.

3. Follow-up protocol according to clinical criteria for inherited predisposition cancer syndromes

Appointments and tests of individuals at high risk of cancer will be conducted according to family history.

Colorectal Cancer

Bethesda Criteria

Procedure	**Frequency**	**Age**
Physical exam	Annual	10 years before diagnosis of youngest individual in the family
Colonoscopy	Annual or Biannual	40 years of age or 10 years before diagnosis of youngest individual in the family

Amsterdam Criteria

Procedure	**Frequency**	**Age**
Physical exam	Annual	20-25 years of age
Endoscopy	Annual or Biannual	35 years of age
Urinary cytology	Annual	30-35 years of age
Abdominal ultrasound	Annual	30-35 years of age
Pelvic ultrasound	Annual	30-35 years of age
Transvaginal ultrasound	Annual	30-35 years of age
Mammography	Annual	40 years of age

Breast-colon Syndrome

Procedure	**Frequency**	**Age**
Physical Exam	Annual	25 years of age
Colonoscopy	Biannual	40 years of age or 10 years before diagnosis of youngest individual in the family
Mammogram	Annual	25 years of age or 10 years before diagnosis of youngest individual in the family
MNR	Annual	25 years of age or 10 years before diagnosis of youngest individual in the family
Transvaginal ultrasound	Annual	25 years of age or 10 years before diagnosis of youngest individual in the family

Li-Fraumeni Syndrome

Procedure	**Frequency**	**Age (yrs old)**
Physical Exam	Annual	20-25
Colonoscopy	Biannual	20-25
Mammogram	Annual	20-25
Abdominal ultrasound	Annual	20-25
Urinary ultrasound	Annual	20-25
Thyroid ultrasound	Annual	20-25
Breast ultrasound	Annual	25
Endoscopy	Annual	25

Hereditary Breast-Ovarian Syndrome

Procedure	**Frequency**	**Age (years old)**
Physical Exam	Annual	20-25
Mammogram	Annual	20-25
MNR	Annual	20-25
Transvaginal ultrasound	Annual	20-25
CA-125	Twice a year	20-25

Diffusion

INCITO Website

MENU 1 – What is INCITO?

The National Institute of Science and Technology in Oncogenomics (*Instituto Nacional de Ciência e Tecnologia em Oncogenômica*, INCITO) of the AC Camargo Hospital is one of the National Institutes of Science and Technology (INCTs), a program coordinated by the National Council of Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*, CNPq) of the Ministry of Health (*Ministério da Saúde*, MCT). In the State of São Paulo, the program is administered in conjunction with the São Paulo Research Foundation (F*undação de Amparo à Pesquisa de São Paulo*, FAPESP).

The mission of INCITO is to prevent and treat cancer by means of the application of innovative concepts and technologies. The objective of the project is to be the best integrated center of research, treatment and teaching in oncology in Latin America; with scientific (epidemiology, tumor biology and clinical trials) teaching and information diffusion projects.

The project, entitled "Hereditary cancer and familial aggregation: clinical and molecular profiles of Brazilian patients and their relatives at high risk of cancer" proposes the clinical and genetic investigation to broaden current understanding of the most frequent cancers in the population associated with hereditary predisposition (prostate, breast and colorectal) and suggest new alternatives for the diagnosis and treatment of these types of cancers.

About INCT

The National Institute of Science and Technology (*Instituto Nacional de Ciência e Tecnologia*, INCT) is a program that promotes scientific advances in Brazil, through financing multicentric research projects. It seeks to articulate the actions of research groups in areas strategic to the country and stimulate the international development of scientific research and technology.

Each project involves at least three institutions from two Brazilian states and besides integrating research groups, the INCT aims to contribute to improving education and the

diffusion of scientific knowledge among the lay population. Thus, the formation of young researchers and support for the installation and functioning of laboratories within Brazilian teaching and research institutions is part of the scope of this program.

MENU 2 – Who we are

INCITO structure

Project Coordinator: Prof. Dr. Luiz Paulo Kowalski (HACC)

Vice-coordinator: Prof. Dr. Sergio Verjovski-Almeida (IQ-USP)

Management Committee

Prof. Dr. André Lopes Carvalho (FPioXII-Barretos)

Prof. Dra. Silvia Regina Rogatto (UNESP-Botucatu)

Prof. Dr. Fernando Augusto Soares (HACC)

Dr. Dirce Maria Carraro (HACC)

Partner Institutions (Brazilian)

São Paulo

A.C. Camargo Hospital, São Paulo, SP

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Fernando Augusto Soares

Dirce Carraro

Helena P Brentani

Maria Izabel Waddington Achatz

Fabio de Oliveira Ferreira

Ademar Lopes

Eduardo Abrantes

Eduardo Nobrega Pereira Lima

Antonio Hugo Marques Campos

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Barretos Cancer Hospital, Barretos, SP

André Lopes Carvalho

Edmundo Mauad

Luciano Souza Viana

Marcos Duarte de Mattos

Amaral Carvalho Hospital, Jaú, SP

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Faculty of Medicine, UNESP-Botucatu, SP

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Rogério Hossne Saad

Maria Aparecida Custódio Domingues

Alexandre Bakonyi

Ceará Cancer Institute, Fortaleza, CE

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Sérgio Joaçaba

Rosane Santana

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Ilce Mara de Syllos Cólus

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Magnus von Doeberitz

Memorial Sloan-Kettering Cancer Center, NY, USA

Jose G Guilem

Virginia Bioinformatics Institute, Virginia, USA

João C. Setubal

Carlos Sarroca

Hospital Italiano, Buenos Aires, Argentina

Carlos Alberto Vaccaro

Instituto Nacional de Enfermedades Neoplasicas, Peru

Abelardo Arias Velásques

MENU 3 – Research Projects

The research projects are divided into scientific, educational and information diffusion (link).

The scientific projects are divided into three areas: epidemiology, tumor biology and clinical trials.

Epidemiology:

- Initiating with organized recruitment and registration, create a databank of patients with a history of hereditary cancer or familial aggregation and obtain samples from these patients in order to perform molecular assays;

- Investigate germinative alterations in DNA (copy numbers of DNA sequences) in family cancer using a model of lower complexity and heterogeneity than the tumors;
- Research genome alterations to identify new genes involved in the etiology of hereditary cancers and, whenever possible, compare these epidemiological and molecular data with Brazilian and Latin American centers;
- Extend these findings using a large-scale sequencing methodology for family and sporadic tumors;

Tumor biology:

- Determine the differential genic expression profile of breast and prostate tumors and correlate these with clinical parameters, such as metastatic potential, among other analyses;

Clinical trials:

- Analyze the potential of the most efficient substance for PET-CT imaging analysis in patients with breast and prostate tumors;
- Identify markers for neoadjuvant chemotherapy response in head and neck cancer;
- Evaluate the potential of F8 protein as a cicatrizant agent.

MENU 4 – Diffusion/Education

Education

The proposal is to establish an interface between basic research and clinical applications, this project involves actions of education and professional training. The objective is to involve participating institutions in the development and application of extension courses.

The first action outlined to achieve such objectives was the creation of the clinical Oncogenetics discipline, within the *stricto sensu* postgraduation at AC Camargo Hospital, and the Oncogenomics discipline, within the Biochemistry postgraduation of the Institute of Chemistry, USP. Both courses received the maximum evaluation - a note of 7 - by CAPES.

The Oncogenetics discipline was implemented in the *senso latu* course of specialization in Oncology Nursing. The course in Nursing in Oncogenetics began in November 2009, ministered in the Distance Learning environment of AC Camargo Hospital.

To promote the transference of knowledge between participating institutions, the AC Camargo Hospital team and national and international partners should minister extension courses for health professionals.

Diffusion

Diffusion actions are aimed at establishing mechanisms to publicize and distribute the research conducted by the group and the benefits to society, by means of the establishment of information programs concerning hereditary cancer.

MENU 5 - Contacts

Head office

INCITO - National Institute of Science and Technology in Oncogenomics (*Instituto Nacional de Ciência e Tecnologia em Oncogenômica*)

Rua Prof. Antonio Prudente, 211 – 2º Subsolo – Centro Administrativo

CEP – 01509-900 – Liberdade – São Paulo – SP

Email: difusão.incito@hcancer.org.br

C.2. Epidemiological data collection. Database management.

The Biotechnology Laboratory (LBHC) at A.C. Camargo Hospital maintains, develops and divulges the use of the science management software LBHC Science Suite, which is composed of six systems: Login (access control), GC (common tables and catalogs), Pinga/Biobank (biobank management), Pinga/Projects (scientific project submission and analysis by the Ethics Committee), Oncotree (family tree registration for hereditary diseases studies) and XUSD (customizable clinical data management).

Besides library updates, technical reviews and improvements in security in all systems, the sample search and request management and molecule repository (DNA/RNA) modules have undergone upgrades to improve their usability, therefore all sample request and samples are now registered and managed through this system; the freezer management module was also improved, enabling users to easily create containers with customized coordinates; Oncotree gained the Progeny 6 and 7 family trees import and export module, which permits the exchange and backup of data; it has been also prepared to draw family trees with using Madeline 2.0; on XUSD, a clinical profile search module was developed, and the possibility of evaluating formulae when filling out the medical record. All these systems were translated into English.

A paper was written describing these systems and their design process is currently under review by a Medical Informatics Journal; stable versions are available under GPL license at http://www.lbhc.hcancer.org.br/wiki/LBHC Science Suite pt. These systems were installed at the Ceará Cancer Institute (*Instituto do Câncer do Ceará*), where a tumor bank is being established. An agreement was confirmed with the Children's Institute (*Instituto da Criança*), which is also installing a blood bank for scientific studies and for which they provide a technician (with a TT4A allowance) to install, adapt and develop the Pinga/Biobank to their requirements. The adoption of these systems by the Santa Casa de Misericórdia de São Paulo is under negotiation.

C.3. Training and Education Programs

One proposal developed within the various projects was that professionals in the area of health, including oncologists, nurses, biotechnicians and psychologists would receive

training in laboratorial and outpatient practice of the procedures and conducts adopted in the management of probands and their families with a history of hereditary cancer or familial aggregations of cancer. During this period, courses focusing on the training and use of the programs specifically tailored for the elaboration of genealogies, family risk calculations, application of the clinical criteria adopted by international consensus and identification of individuals who are eligible for genetic tests were organized. These courses are described below in detail, the first was ministered in the Oncogenetics Courses for postgraduate students and health professionals and the second, more specifically for nurses.

Oncogenetics course

The Oncogenetics course is coordinated by three researchers of the INCITO project (Erika M M Santos, Maria I W Achatz and Silvia R Rogatto). It was approved by the Antonio Prudente Foundation Post-Graduation Council, and one a year will be offered (in August 2010), lasting 72 hours, equivalent to six credits in the Program. The description, aims and content is presented below.

Description

Genes in hereditary and sporadic cancer. Hereditary cancer predisposition syndromes. Genetic counseling in hereditary cancer predisposition syndromes. Psychological and ethical aspects of genetic counseling in oncology.

Aims

The course aims to provide students with basic knowledge concerning cancer genetics, such that by the end of the course, they are able to:

- Identify the mechanisms and genetic components that are related to hereditary cancer predisposition syndromes (HCPS) and differentiate them;
- Recognize criteria and diagnostic tests available for different HCPS;
- Analyze and interpret practices of Genetic Counseling;
- Recognize and discuss the ethical and psychological aspects associated with hereditary cancers.

Schedule and speakers

Module I – Introduction to Cancer Genetics

Date	Time	Hours	Activity	Speaker
08/02	14:00-18:00	3	Lecture: Cancer Genetics	Silvia R Rogatto
		1	Discussion Panel	
08/03	14:00-18:00	3	Lecture: Mendelian Inheritance and Non Traditional	Silvia R Rogatto
		1	Discussion Panel	
08/04	14:00-18:00	2	Lecture: Genetic Counseling	Maria I W Achatz
		2	Lecture: Multidisciplinary Team and Genetic Counseling	Erika M M Santos
08/05	14:00-18:00	3	Lecture: Risk Models in Genetic Counseling	Maria I W Achatz
08/06	17:00-18:00	1	Discussion Panel	Erika M M Santos

Module II – Molecular Investigation

Date	Time	Hours	Activity	Speaker
08/09	14:00-18:00	3	Lecture: Molecular investigation techniques I	Silvia R Rogatto
		1	Discussion Panel	
08/10	14:00-18:00	3	Lecture: Molecular investigation techniques II	Silvia R Rogatto
		3	Discussion Panel	
08/11	14:00-18:00	3	Lecture: Bioinformatics and Oncogenetic Investigation	Eduardo Abrantes
		1	Discussion Panel	

Module III – Hereditary Cancer Predisposition Syndromes (HCPS)

Date	Time	Hours	Activity	Speaker
08/12	14:00-18:00	3	HCPS and breast cancer I Lecture: Hereditary Breast and Ovarian Syndrome	Maria I W Achatz
		1	Discussion Panel	
08/13	14:00-18:00	3	HCPS and breast cancer II Lecture: Li-Fraumeni Syndrome; Cowden Disease; Ataxia- telangiectasia; Breast-colon syndrome	Maria I W Achatz
		1	Discussion Panel	
08/16	14:00-18:00	3	HCPS and colon cancer I Lecture: Lynch Syndrome	Benedito M Rossi
		1	Discussion Panel	Erika M M Santos
08/17	14:00-18:00	3	HCPS and colon cancer II Lecture: Familial Adenomatous Polyposis and others syndromes	Fábio O Ferreira
		1	Discussion Panel	Erika M M Santos

Date	Time	Hours	Activity	Speaker
08/17	14:00-18:00	2	HCPS and gastrointestinal tumors Lecture: hereditary diffuse gastric cancer; hereditary pancreatic cancer1	Maria I W Achatz
		2	HCPS and endocrine tumors Lecture: *MEN1* e *2*	Maria I W Achatz
08/19	14:00-18:00	2	HCPS and Phacomatosis (Neurofibromatosis, VHL, tuberus sclerosis)	Patricia Prolla
		1	HCPS and skin tumors I Lecture: Xeroderma pigmentosum, Gorlin Syndrome	Patricia Prolla
08/20	14:00-18:00	2	HCPS and skin tumors II Lecture: Familial Melanoma	Gilles Landman
		2	Discussion Panel	Maria I W Achatz
08/23	14:00-18:00	2	HCPS and head and neck tumors Lecture: Molecular and epidemiological evidence of head and neck HCPS	Silvia R Rogatto
		2	Discussion panel	Silvia R Rogatto

Module IV – Multidisciplinary Aspects

Date	Time	Hours	Activity	Speaker
08/24	14:00-18:00	2	Lecture: Impact of Hereditary Cancer	Maria T C Lourenço
		2	Discussion panel	Erika M M Santos
08/25	14:00-18:00	1	Lecture: Intervention in High Risk Individuals	Erika M M Santos
		1	Lecture: Education in Health Public Policies in Oncogenetics	E Erika M M Santos
		2	Nutrigenomics and cancer	Thomas Ong
08/26	14:00-18:00	2	Lecture: Ethical Aspects	José Goldim
		1	Lecture: Research Perspectives	Silvia R Rogatto
		1	Course Evaluation	Silvia R Rogatto, Maria I W Achatz, Erika M M Santos

Oncogenetics course for nurses in the Oncology Nursing Specialization Program at HACC and developing an Oncogenetic course for a Distance Learning Format

The course of Oncogenetics is coordinated by two nurses from the Specialization Program in Oncology Nursing at the Antonio Prudente Foundation. The course lasts 14 hours and in 2009 the course was offered on two occasions (from July 1st to August 5th and from October 31st to November 14th 2009), with 70 students attending. The teaching material on the

subject is available to students on the distance learning website run by AC Camargo Hospital. The course was integrated into the specialization program in Module I - Introduction to oncology and therapeutic basis in oncology nursing, and there are plans for three new classes in 2010. The description, aims and program content are presented below.

Description

Application of Genetics in Oncology. Role of the nurse in cancer genetics. Genes and hereditary and sporadic cancer. Cancer risk assessment. Hereditary predisposition syndromes for colorectal and breast cancer.

Aims

The course aims to provide students with basic knowledge concerning cancer genetics, such that by the end of the course, they are able to:

- Identify the role of genetics in cancer prevention, diagnosis, treatment and prognosis;
- Describe the role of the nurse in cancer genetics;
- Identify the genetic mechanisms related to cancer development;
- Describe the purposes of risk assessment and genetic counseling;
- Identify the essential elements in the collection of cancer family history;
- Describe the main features of the hereditary cancer predisposition syndrome related to colon and breast cancer;
- Reflect on the ethical and psychosocial aspects of genetic testing.

Program

Genetic and genomic impact in oncology nursing practice - 2h

Nursing role - 1h

Molecular biology basis - 2h

Basic principles of Genetics - 2h

Cancer biology - 2h

Cancer risk assessment - 3h

Colon and breast hereditary cancer predisposition syndrome - 1h

Genetic testing: ethical and legal aspects - 1h

The establishment of a course within the distance learning environment also forms part of the aim of this project. On November 16th 2009, the first course, “Fundamentals in Cancer Genetics for Nurses" was initiated. Figure 1 shows the initial screen of the course. This course will be given twice in 2010.

Figure 1 - Initial screen of “Fundamentals in Cancer Genetics for Nurses"

The first edition of the course in 2009 was ministered to 32 students. Of the total, 27 are from the State of Sao Paulo; two are from Minas Gerais; one from Para; one from Goiais, and one student lives in Bahia.

Description

Genetics application in oncology. Nursing role. Genes and sporadic and hereditary cancer. Risk assessment. Hereditary cancer predisposition syndromes. Genetic Testing. Oncogenetics services.

Aims

The program aims to provide participants with basic knowledge in cancer genetics, such that by the end of the course the students are able to discuss, describe and understand:

- The impact of oncogenomics in the practice of oncology nursing;

- The components and structure of DNA, RNA and proteins;
- The processes of transcription, translation and replication;
- The concepts related to gene expression;
- The processes related to cell division and cell-cell communication;
- The basic concepts and principles of genetics;
- The characteristics of a malignant cell;
- The role of genetics in tumor development;
- The methods for cancer risk assessment;
- A pedigree with at least three generations;
- The characteristics of hereditary cancer;
- The main syndromes related to predisposition to breast and colorectal cancer;
- The limitations of genetic testing in identifying individuals at risk;
- The functions of oncogenetics services and indications for referral;

and identify:

- Individuals at increased risk of cancer on the basis of family history;
- Strategies to reduce the risk of cancer;
- The use of genetic testing.

Program

Module I: **Impact of Oncogenomics in Nursing Practice.** Oncogenomic application in Prevention, Diagnosis, Staging, Treatment and Prognosis.

Module II. Principles of Molecular Biology. Basic genetic mechanisms: DNA, RNA and protein synthesis and chromosome karyotype. DNA replication. Regulation of gene expression. Cell signaling.

Module III. Genetics. Chromosomal and point mutations. Phenotype and Genotype. Somatic and Germline mutations. Patterns of inheritance. Penetrance and expressivity.

Module IV. Cancer Biology. Definition of neoplasia. Features of a malignant cell (morphological, biochemical). Genes related to cancer: oncogenes, tumor suppressor genes, repair genes.

Module V. Risk Assessment. Proposal of risk assessment. Components of risk assessment. Collection of family history and construction of pedigrees with standard symbols. Features of hereditary cancer. Terminology used in risk assessment. Methods for primary and secondary prevention of cancer in the general population and in high-risk individuals.

Module VI. Hereditary Cancer Predisposition Syndromes. Features of hereditary cancer. Hereditary breast-ovarian cancer syndrome. Lynch Syndrome. Familial Adenomatous Polyposis. Other cancer predisposition syndromes.

Module VII. Genetic testing. Definition of genetic testing. Use of genetic testing. Indications for genetic testing. Interpretations of genetic testing. Ethical, legal, social and psychological testing.

Module VIII. Oncogenetic Services. Structure of cancer genetics service. Purpose of service and fluxogram. How to refer to specialists.

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Manuscripts published related to INCITO

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Marina B. Sapienza – Scientific initiation - FAPESP Grant (concluded)

Interactions with other INCTs

INAGEMP, Porto Alegre, Coordinator: Roberto Giuliani.

Rede Familial de Cancer Familial. Manual Operacional, Ministério da Saúde, 2009, ISBN 978-85-7318-151-71.

Development stages of the project according to the objectives originally proposed

1. Patients and their relatives have been and continue to be recruited to obtain epidemiological data and to collect samples. The Oncotree software was made available to two collaborative centers (Barretos Cancer Hospital and the Botucatu Faculty of Medicine). The biobank samples of the HACC were used in molecular assays, the results of which are presented in this report.
2. The results of the investigation of mutations by direct sequencing of the genes *BRCA1*, *BRCA2*, *MSH1*, *MSH2*, *MSH6*, *CHEK2* and *TP53* in 309 individuals were presented. The sequencing of the *BRCA1* and *BRCA2* genes by tiling-PCR followed by high performance sequencing will be performed in the next phase.
3. Genomic alterations in probands of the Breast and Ovarian, Li-Fraumeni and Breast-Colon Syndromes are under investigation. The results of aCGH from 153 probands were presented. A subgroup of these cases with alterations was selected and members of these families are being contacted and invited to participate in the project. These individuals will also be evaluated by aCGH. Other strategies were also included as analysis proposals in the next phase, such as the inclusion of the evaluation of tumors from some of these individuals, analysis of proteins in altered genes and the investigation of possible second events associated with gene silencing.
4. The Lynch Syndrome project and DNA capture will be initiated in the next phase, as soon as the high performance sequencer is installed in the HACC.
5. Analyses of differential genic expression of coding and noncoding elements in prostate and breast carcinomas should be initiated in the next phase. Currently, the samples have been separated. The breast carcinomas will be submitted to laser microdissection.
6. The product ^{18}F-FAS in its injectable form is being manufactured by the radiopharmacy sector of the IPEN in São Paulo, with the participation of Dr. Jair Mengatti. This project is ongoing and ^{18}F-FAS is under evaluation by the IPEN.
7. The project associated with evaluating the angiogenic potential of protein F8 was cancelled. The company showed no further interest in developing the product (documentation attached).

8. The project “Prospective nonrandomized Phase II study to identify response markers to neoadjuvant chemotherapy and association with radiotherapy and cetuximab in patients with epidermoid carcinoma of the oropharynx” was modified to “Prospective nonrandomized Phase II study to identify response markers to neoadjuvant chemotherapy and association with radiotherapy and cisplatin in patients with epidermoid carcinoma of the oropharynx” . A detailed justification, together with the inclusion of the molecular investigation in search of markers in response to therapy was included in this new version. Partial results (4 of 40 proposed patients) are also included in the report.
9. The Clinical Oncogenetics discipline was implemented within the Postgraduate Program in Sciences (Oncology) and should be ministered in the second semester of 2010.
10. The extension courses in Oncogenetics are in the organization phase led by the HACC team in conjunction with national and international partners.
11. A Clinical Oncogenetics discipline for nurses was created and ministered within the Specialization in Nursing course. The same course is being ministered within the Distance Learning Program of the HACC.
12. A website is currently being organized outlining the INCITO project and information programs concerning hereditary cancer. Furthermore, researchers at Brazilian Partner Institutions should receive training on this theme as a way of promoting the project in their units of origin.