

FIRST BRAZIL-JAPAN CANCER MEETING

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ABSTRACTS

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Cancer strategy in Japan

Shaw Watanabe¹

Cancer is the first cause of death in Japan since 1978, and the number of cancer patients are constantly increasing. In 1992, more than 210,000 people died of cancer making up about 27% of all deaths. In 2000, the total population will be 128 million, and more than 14 million people will be over 79. There will be about 480 thousand cancer patients. Nearly half of them should be aged more than 70 years.

Stomach cancer and uterine cervix cancer have been the most frequent cancer in Japan. Mass-screening programs for both cancers have been introduced in 1965, and since then, both mortality and incidence has been decreasing. Effects of mass-screening shall be introduced. Survival of cancer patients has been improved, but mostly due to increasing proportion of early cancer. Intractable cancer, such as lung, liver, bile duct and pancreas cancer, becomes problem. Primary prevention, in addition to the endeavor to clarify the mechanism of carcinogenesis, calls more attention.

Comprehensive 10-year strategy of Cancer Control which started 10 years ago brings an enormous development of basic research, especially in genetic studies. Next 10-year strategy for Cancer Control and other anticancer strategy in Japan will be discussed.

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Molecular basis of stomach carcinogenesis

E. Tahara²

Genetic alterations in multiple oncogenes and multiple tumor suppressor genes are observed in gastric cancer. Among

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them, those most commonly implicated in both well differentiated or intestinal type and poorly differentiated or diffuse type gastric cancers are inactivation (mutations and allele loss) of the p53 gene and activation (aberrant expression and amplification) of the c-met gene.

Interestingly, the p53 mutation spectrum in primary gastric carcinoma is characterized by frequent mutation at an A:T pair, extremely high transversion incidence, no transition at CpG, and no G:C to G:C to T:A transversion. 6.0 kb transcript of the c-met, which is preferentially detected in tumor, is closely linked with the tumor progression. In addition, mutations and allele loss of the APC gene are frequently associated with well differentiated adenocarcinoma. LOH at the DCC locus and LOH of the bcl-2 gene also are common events of well differentiated adenocarcinoma. Conversely, the development of poorly differentiated adenocarcinoma including scirrhous carcinoma, in addition to changes in the p53 and c-met genes, requires reduction or dysfunction of cadherin. The K-sam gene is amplified preferentially in poorly differentiated adenocarcinoma or scirrhous carcinoma. K-sam amplification in scirrhous carcinoma often occurs independently of c-met amplification which is detected in 38% of scirrhous carcinoma. Overexpression of bcl-2 protein is found only in poorly differentiated adenocarcinoma or signet ring cell carcinoma. Of particular interest is the high frequent loss of allele at D7595 on chromosome 7q which occurs regardless of histological types. Intestinal metaplasia and adenoma of the stomach also contain mutations of the p53, APC and K-ras genes.

Moreover, cripto, a novel gene of EGF gene family, is overexpressed in intestinal metaplasia as well differentiated adenocarcinoma. What is more exciting is that the deletion of the 2.2 kb gene is detected in almost all intestinal metaplasia and well differentiated adenocarcinoma.

These results indicate that different genetic pathways of stomach carcinogenesis may exist for well differentiated and poorly differentiated gastric cancers. Some of the former may develop by a cumulative series of gene alterations similar to those of colorectal cancer.

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1. Laser endoscopic treatment for gastrointestinal cancer

Yanao Oguro³

Endoscopic treatment for gastrointestinal cancer using laser has been performed with good results. The ratio of radical endoscopic treatment with laser to surgery for early gastric cancer was 14.4:100.0, according to our study throughout Japan. This means that the laser treatment is establishing a foothold in the treatment of an early gastric cancer. By vaporization with Nd:YAG laser, some types of early gastric cancer can be treated radically and inoperable, advanced gastric cancer can be treated symptomatically, resulting in hemostasis, recanalization and tumor reduction. The photodynamic therapy, PDT, combined with intravenous administration of hematoporphyrin derivatives and irradiation of an argon dye laser, has the effect of selectively destroying cancerous tissue and is especially useful to the radical treatment of an early gastric cancer and a superficial esophageal cancer with an obscure borderline. We had studied on the endoscopic treatment for gastric tumor throughout Japan in 1987, and covered 1.653 cases of early gastric cancer, in which laser endoscopic treatment was most frequently used. In the follow-up study made more than one year after radical endoscopic treatment, the biopsy negative ratio was 83%-85% by Nd:YAG laser and PDT. With contact method, the effectiveness of laser irradiation is much more exact than with the non-contact method, and the same effect can be obtained at lower laser power. The effectiveness of laserthermia has been confirmed experimentally and clinically in early and advanced gastric cancer without severe accidents, such as a perforation.

2. Recent advances in diagnosis and treatment of early gastric cancer

Yanao Oguro³

(1) Recent advances in diagnosis: recent topics in the diagnosis of an early gastric cancer are electronic endoscopy developed in the U.S.A. and in Japan, the standard endoscopic ultrasonography developed in Japan and then micro-sonoprobe in the U.S.A. and oncogenic diagnosis using biopsy specimens developed in Japan. These will be presented at my lecture.

(2) Recent advances in treatment: successful radical endoscopic treatment of early gastric cancer has recently progressed using chiefly the high frequency electric current method and the laser method. The forms of treatment can be classified into endoscopic mucosal resection, EMR and non-mucosal resection, non-EMR. The high frequency electric current method is a typical method for mucosal resection and makes it possible to confirm by pathology the complete

curability of the excised and collected lesion, but it has limits in terms of size, location and conditions of the lesion. Of the high frequency electric current method, there are two chief methods used which are endoscopic polypectomy and EMR, so called strip biopsy. Radical endoscopic treatment for early gastric cancer has recently become the treatment of choice for lesions, even in patients without surgical risk, because of the decreased quality of life which may follow after surgery.

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Preoperative gastric cancer staging

Ruy G. Bevilacqua⁴

At the Department of Surgery of Hospital A.C. Camargo the policy in gastric cancer is resection, even in patients with advanced tumors. After the preoperative staging patients are considered "operable" when there is a reasonable presumption of resectability or there is a preoperative indication of a palliative by-pass. The staging is critical, since 57% of the patients in the institution are classified as stage IV.

In the 70's and 80's preoperative abdominal staging procedures included liver scintiscans, abdominal ultrasonography and laparoscopy. Of the 471 stage IV patients seen at that time, 333 were considered "operable", but 138 had exclusively exploratory laparotomies. These figures generated a high failure rate of 41%, more frequent in proximal tumors.

These data confirmed an earlier observation at the same Department that laparoscopy alone allowed incorrect evaluations in 41.9% of the gastric cancers. The main reasons for failure were: fixation on the posterior gastric wall, deep hepatic metastasis and non-identified peritoneal metastasis in areas not accessible to direct vision.

In the recent years preoperative abdominal staging included CT scans. This has not improved greatly accuracy, since this method fails in 45% of the cases.

In the recent months we considered the inclusion of endoscopic ultrasonography (EUS). However we were impressed with the observations at Memorial Sloan Kettering Cancer Center that EUS was correct in only 37% of the patients, with a significant tendency to overstage. This possibility was then discarded.

We therefore decided to include videolaparoscopy since May 1993. The method is currently being evaluated. It was possible to conclude that it allows better visual access, better visceral handling, access to peritoneal recesses, and allows lysis of adhesions and eventually the performance of palliative by-pass procedures. We expect to soon have the chance to also utilize videolaparoscopic ultrasound equipment.

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Ultraviolet ray and skin cancer

Shaw Watanabe¹

Incidence of skin cancer in Brazil belongs to the highest group in the world. It is important to know whether or not the incidence of non-melanocytic skin cancer and malignant melanoma will increase as a result of increasing irradiation of ultraviolet ray (UV) which has been caused by the enlargement of ozone holes. Study groups for the Effects of Ultraviolet Ray Increase on Human Health were established by the Environmental Agency in 1990 under the Global Environment Research Program to clarify the adverse effects of UV for Japanese.

We tried to find a good biomarker to detect high risk group of solar keratosis by immunohistochemical staining method with antipyrimidine dimer antibody and antibody to p53 gene product (pAb1901).

The p53 gene product accumulated in the nuclei in nearly 30% cases of solar keratosis. A combination of the polymerase chain reaction and single-strand conformation polymorphism (PCR-SSCP) analysis to investigate aberrations of the p53 gene in SKs and SCCs. By PCR-SSCP, 8/27 (30%) of SKs and 2/5 (40%) SCCs showed structural abnormalities of the p53 gene. Most of the mutations were single-nucleotide missense mutations the rests showed nonsense mutation, double-nucleotide substitution to stop codon, and base deletions to loose p53 production.

As the new project has started this year, a mass-screening for the skin lesions has carried out with physical examination and brief interview with standard questionnaire to know the risk of solar keratosis and skin cancer. These project shall be introduced.

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Aberrant glycosylation in malignant melanoma

Roger Chammas⁵

Over the past 6 decades the prevalence of melanoma increased 15 times all over the world. Melanoma is known for its aggressive biological behavior and high morbidity. Melanoma is a spectral disease and attempts to define molecular correlates of different phases of melanoma tumor progression are current concerns of cancer researchers. A common feature of malignant transformation is the expression of large glycans at cell surface. These large glycans, usually referred as aberrant, decorate lipids and proteins. Although structurally defined for a long time, the function of these glycans are still unknown.

Experimental models of malignant melanoma are known, and much has been done towards understanding the molecular basis of melanoma invasive and metastatic phenotype. Cell surface glycoconjugates are clearly related to the metastatic behavior. Among these glycoconjugates are extracellular matrix receptors which define the ability of neoplastic cells to adhere, migrate or extravasate, and thus to metastasize. We have been focusing on laminin binding integrins, and the role of complex tri- and tetraantennary structures (a pattern of aberrant glycosylation) in their functions. This particular glycosylation pattern can be probed using leucoagglutinin from *Phaseolus vulgaris* (L-PHA). L-PHA reactive oligosaccharides are preferential substrates for polylactosamine extensions, which in turn are substrates for fucosyltransferases, leading to the formation of Lewis antigens.

Murine melanoma cell spreading, but not cell adhesion, on laminin surfaces are dependent on L-PHA reactive oligosaccharides present in integrins. Cell spreading follows cell adhesion and determines firm adhesion and oriented motility. Swainsonine, an alkaloid which inhibits complex glycan formation, inhibits melanoma cell spreading on laminin. Swainsonine also inhibits experimental metastasis formation.

Human melanoma cells also present L-PHA reactive glycans at cell surface. Four major polypeptides bear tri- and tetraantennary in their structure. Again, integrins are among these polypeptides. Lectin-histochemistry was done in patient samples, in order to analyze the prognostic or diagnostic value of this glycosylation pattern in melanomas. Although L-PHA reactivity lacked prognostic significance, metastatic melanocytes were more L-PHA reactive than melanocytes in primary tumors. However, there are some experimental evidences that normal melanocytes are L-PHA negative. Presently, the hypothesis that along malignant transformation the expression of glycosidases involved with complex glycans synthesis in upregulated is under investigation.

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Loss of heterozygosity affecting the APC and MCC genetic loci in patients with primary breast carcinomas

Arnaldo C. Medeiros, Maria Aparecida Nagai, Mario Mourão Neto, Ricardo R. Brentani⁶

Tumour suppressor genes APC and MCC were identified recently, and the cDNA was cloned from chromosome 5q21. Mutations in the APC gene give rise to familial adenomatous polyposis (FAP) and occur in many, perhaps the majority of sporadic colon cancers. Loss of heterozygosity has been

described in other human cancer just like lung and esophageal cancers. Here we show loss heterozygosity in 87 patients with breast cancer for APC and/or MCC loci using the PCR-LOH assay. LOH affecting the APC exon 11 and 15 loci was 9 of 35 (25%) and 4 of 34 (11%) of heterozygous patients respectively. LOH at the MCC exon 10 locus was 7 of 40 (17%) of informative samples. These data suggest that allelic deletion of APC and/or MCC probably is involved in the pathogenesis and/or progression of a subset of breast cancers.

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Flow cytometric DNA analysis and hormonal receptor status in breast carcinoma

Maria A.A. Koike, Rosimeire A. Roela, Edson M. Barbosa and Miriam H. H. Federico⁷

The prognostic value of DNA ploidy in breast cancer relative to other prognostic factors is unsettled. We studied the nuclear DNA content and hormonal receptor levels of 58 frozen operable breast carcinomas using flow cytometry and a radiolabeled hormone binding assay. Tumour ploidy and S-phase fraction (SPF) was calculated in the DNA histogram. We found a non-statistically significant predominance of diploid cancer (65%) in the ER+PR+ subgroup as compared to the ER-PR- subgroup (33%). The high percentage of hypodiploid tumours (12%) compared to the literature (2%), might be reflecting regional differences. Lower SPF were significantly correlated with features determining good prognosis like hormone receptor positivity or nodal status. Ploidy and DNA index presented a poor degree of correlation with these variables. We conclude that analysis of SPF and ploidy could be useful in the prognostic assessment of these cancer patients.

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Estrogen and progesterone receptors in breast cancer among Brazilian women of different racial groups

Maria Mitzi Brentani⁸

Determination of estrogen (ER) and progesterone (PR) receptors in tumors of breast carcinoma patients has been established as a relatively reliable mean to predict the effectiveness of endocrine treatment. The concentration of these receptors in different groups of patients varies and is probably influenced by factors such as age, reproductive status and race. The racial factor, although unexplained, might be associated with variations in the environment, dietary habits and genetic factors.

The purpose of this report is to compare the incidence and level of ER and PR in breast carcinomas obtained at mastectomy from 3.950 consecutive patients from 3 different racial groups resident in São Paulo: white (81.3%), black (16.4%) and ethnic japanese (2.3%).

There is a different distribution of age of onset of the disease in the total population, with higher rates among younger japanese and black patients than among white patients. The proportion of white postmenopausal patients (67%) was higher than the corresponding figures for black (58.5%) and japanese (53.5%). Japanese patients presented a 37.5% frequency of small tumors as compared with 11.4% in white and black patients.

In the japanese population there was a higher percentage of patients discovered at an earlier stage of disease (66.4% in stages I + II, vs 47.2% for white and 30.2% for black patients).

ER and PR were estimated by means of multipoint dextran-coated charcoal assays. No difference in ER status (% positivity and level) were detected in premenopausal breast cancer patients of different races. In postmenopausal women, 76% of japanese were found to have ER+ tumors compared with 56% and 53.6% in black and white patients respectively. Japanese patients showed the highest percentage of PR+ tumors (63%) independently of menopausal status, compared to black (36.7%) and white patients, (48%) as well as the highest proportion of ER+ PR+ tumors (50%).

The stage of the disease do not appear to affect receptor status in all groups. There is a trend for tumors from stage I patients (black and japanese) more often contain ER+.

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Digital cytometry in head and neck cancer

Roberto Ibrahim⁷

The management of the patients with head and neck neoplasias depends largely on the location of the primary tumor, the extent of the disease and the histologic type. The therapeutic alternatives include surgery, radiotherapy, chemotherapy and a combination of these.

In spite of important advances in recent years in the functional improvement and esthetic results, survival of the patients, especially those with squamous cell carcinomas, has been relatively disappointing.

The TNM staging system is used by tradition for the analysis of groups of patients, but is not always useful as a prognostic factor. It is very important to know the factors which behave as indicators of aggressiveness of head and neck tumors, and in this sense new prognostic indices have been investigated in recent years. Among these markers, the tumor

cell DNA content has been extensively studied. Several authors suggest that there is a correlation between the amount of DNA in the tumor cell nuclei and the biologic behavior of the neoplasia. The quantification of the nuclear DNA has been most frequently obtained by flow cytometry. However, in 1989, Wallin et al. introduced the technique of DNA image cytometry. Among the advantages of this new method, we can cite the following: easy performance without complex preparation techniques, the possibility of more elaborate calculations and analyses, the detection of a rate aneuploid event (James & Atkinson, 1989), and direct visualization of the abnormal cells both in fresh and in paraffin-fixed material. However, the interpretation of the histograms still needs improvement. The model that we use for this interpretation is based on the concepts developed by Bocking et al. (1984).

We began our study evaluating the nuclear DNA of differentiated adenocarcinomas of the thyroid, because besides having a larger personal material, these tumors have been extensively studied from the standpoints of epidemiology, histopathology, biochemistry, immunology, diagnosis and treatment, with important contributions in the knowledge of their biology.

With the objective of evaluating the DNA content by image cytometry in the prognosis of differentiated follicular adenocarcinomas of the thyroid, we studied 117 patients submitted to total thyroidectomy (Camargo et al., American Journal of Surgery 164:640, 1992). Seventy (60%) were papillary, 36 (30%) were follicular, and 11 (10%) were Hürthle cell adenocarcinomas. The mean length of follow-up was 57.7 months.

Adverse prognostic factors according to multi-variate analysis were adjacent tissue infiltration ($p = 0.0004$), histologic type ($p = 0.0049$), and patient age ($p = 0.033$).

The nuclear DNA content of tumor cells and of morphologically normal adjacent tissue was assessed by image cytometry, and correlations between nuclear DNA content and prognostic factors were examined.

Fifty-four (75%) adenocarcinomas were classified as aneuploid, 9 (13%) as diploid, and 9 (12%) as borderline. Thirty-four (60%) specimens of morphologically normal adjacent tissue were classified as aneuploid, 18 (32%) as diploid, and 5 (8%) as borderline.

The correlation between tumor ploidy and selected prognostic factors was statistically significant for patient age ($p = 0.0004$) and histologic type ($p = 0.033$).

Despite the fact that ploidy could not be identified as a prognostic factor, we suggest that, because of its correlation with age and histologic type, it might prove prognostic if the number of patients were increased. We also emphasize the importance of evaluating morphologically normal adjacent

tissue because of the high rates of aneuploidy in these areas.

Presently, we are engaged in the study of the DNA content of the squamous cell carcinomas of the head and neck, with still preliminary results. Thirty-two patients (24 males and 8 females, ranging in age between 45 and 74 years) were studied. The T stage is as follows: T₁ (n=2), T₂ (n=13), T₃ (n=10), T₄ (n=7). All patients were submitted to surgery as the initial treatment and 10 of them that postoperative radiotherapy.

Twelve patients had carcinoma of the larynx and 20 of the oral cavity (tongue, floor of mouth, retromolar trigone). The cells taken as controls were lymphocytes of the inflammatory infiltrate. The follow-up was 6 to 32 months. Seven patients died (4 deaths related to the disease and 3 from other causes). There was no correlation between DNA ploidy and the degree of differentiation, age, gender, T stage, tumor location and survival (limited analysis, because of short follow-up to date). The patients were divided into three groups from the standpoint of recurrence: no recurrence, local recurrence and distant metastasis. There was correlation with aneuploidy (p=0.04). There was also correlation between aneuploidy and nodal involvement (N stage).

The literature shows that a significant number of head and neck squamous cell carcinomas are aneuploidy. Ploidy is not a marker of malignity and in some studies keeps a correlation with parameters of biological aggressiveness. There is not a unanimous opinion as to the fact that patients with diploid tumors have a greater survival. We can say that multi-institutional studies involving more patients are necessary to obtain precise conclusions.

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Amplification of genes 11q13-q14 region in head and neck carcinoma

Orlando Parise Jr.⁴

Amplifications of 11q13 region (11q13 amp) are frequently observed in head & neck squamous cell carcinomas (HNSCC). Thirty-one HNSCC patients (pts) were evaluated by southern blot with the probes TYR, D11S833E, FGF3, CYCD1, D11S97, and GSTpi. The mean age was 59.1 years (41-77); male/female: 25/6; tumor sites: oral cavity 4, oropharynx 6, larynx 9, and hypopharynx 12; T1-T2 8, T3-T4 23; N0-N1 17, N2-N3 14. CYCD1 and FGF3 were the most frequently amplified loci (17 pts) of 19 pts with 11q13 amp ranging 2x to 9x. Of 0 N0 pts, 3 (33%) had 11q13 amp versus 13 (76%) of 17 N1-N3 pts. In 25 pts classed T>N, 14 (56%) had 11q13 amp versus 5 (83%) of 6 pts N>=T. Concerning loci associations, 4 (66%) of 6 T1-T2 pts presented 2 amplified loci; 7 (64%) of 11 T3-T4 pts presented 3-5 loci.

Of 21 well differentiated HNSCC, 12 (57%) had 11q13 amp versus 7 (70%) of 10 moderately and poorly differentiated. Survival (Kaplan Meier) was 72.9% to pts without 11q13 amp and 44.9% to pts with 11q13 amp. None of the differences was significant (p<=0.05, Yates and Logrank test) probably due to the small number of pts. Eleven-q13 amp can be prognostic marker and related to loco-regional spread on HNSCC.

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HPV in cancer and papillomas of the head and neck

Luisa Lina Villa⁵

The possible role of human papillomaviruses (HPV) in the etiology of head and neck tumors has been only recently appreciated. Studies have shown that HPV DNA can be detected both in oral leukoplakias and oral cancers with frequencies ranging from 20 to 60% of the specimens. Oral warts may also contain HPVs of the same type as those found in genital lesions, namely HPV 6 and HPV 11. Interestingly, HPV 13 has only been found in focal epithelial hyperplasia, suggesting its viral etiology probably associated with an hereditary background. A variety of factors contribute for the risk of development of oral cancer including smoking, alcohol consumption, dental restoratives, yeast infections, and possibly the interaction with HPV.

The viral etiology of recurrent laryngeal papillomatosis has been confirmed by several methods. We have shown by in situ hybridization and PCR that HPV 6 and 11 DNA was present in all specimens isolated both from children and adults. On the other hand, very low frequencies of HPV positive cases were found in a series of laryngeal carcinomas. Therefore, the role of these viruses in malignant conversion in the upper respiratory tract await additional investigations.

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Collagen type II gene regulation

Sérgio Linne⁹

The transcription of the collagen II gene is regulated by multiple elements. The promoter of this gene contains a TATA box and several GGGCGG sequences repeated between -50 and -300. This gene also contains an enhancer, located in the first intron, which seems to be responsible for the tissue specificity. Silencer elements were also identified upstream from the promoter region.

In order to identify the cis-acting sequences present in the

promoter and enhancer regions, fragments from different sizes were subcloned in plasmids containing CAT gene. The results showed that a fragment spanning from -75 to -110 contained most of the promoter activity, and a 550 base pair fragment is required for full enhancer activity. By using gel shift analysis we could identify a 30 bp sequence which is important for enhancer activity.

We have also studied the effects of three steroid hormones in the transcriptional activity of the collagen II gene. Dexamethasone (10^{-7} M) and retinoic acid (10^{-6} M) inhibited the collagen II promoter-enhancer mediated CAT activity, whereas vitamin D increased activity. The results were also confirmed by northern analysis of cells treated with the three steroid hormones. Since these steroid hormones are known to modulate the transcriptional activity of several other genes, through receptor mediated DNA binding, it is possible that the receptors for these steroid hormones are directly binding to sequences in the collagen II gene.

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Brazilian multi-institutional studies on head and neck cancer

Luiz Paulo Kowalski⁴

Incidence and mortality rates for head and neck cancer in some areas in Brazil are among the highest in the world. The risk of oral cancer in São Paulo is second only to those observed in selected areas of India (Mirra and Franco, 1985). A case-control study of risk factors for upper aereal and digestive system (URDS) cancers was the first collaborative multi-institutional epidemiologic study conducted in Brazil. The participant institutions were: Ludwig Institute for Cancer Research, Hospital Araújo Jorge, Hospital Erasto Gaertner, and Hospital Heliópolis. It started in 1987 and was concluded in 1989 (Franco et al., 1989). Tobacco and alcohol consumption were the strongest risk factors of oral cancers. The adjusted relative risks (RR) for ever vs. never smokers varied according to the type of tobacco consumption (6.3, industrial brand cigarettes; 13.9, pipe; 7, hand-rolled cigarettes). The risk associated with alcohol was mostly evident for "cachaça" and wine. Other important risk factors were: drinking "chimarrão", use of wood stove for cooking, frequent consumption of grilled meat an manioc, and poor oral hygiene characteristics. A significant protective effect was observed for consumption of carotene-rich vegetables and citric fruits.

The risk of presenting with advanced stage vs. early stage disease was evaluated in an analysis of 336 consecutive patients with oral and oropharyngeal carcinomas included in

the first Brazilian multi-institutional head and neck cancer study (Kowalski et al, 1993). Income and educational levels were not associated with stage distribution. The risk of having advanced disease was dependent upon male gender. An other important determinant of advanced stage was tumor location on the less visible surfaces of the oral cavity and oropharynx. There was a clear increase in delay of referral among cases who were seen by more than one health professional. The following factors were independently associated with the risk of advanced disease for lip carcinoma: a painful ulcer, alcoholism, and delay caused by a non-specialist medical doctor. The risk factors associated to advanced oral carcinoma were: type of lesion, odynophagia/dysphagia, delay caused by a dentist and delay caused by a non-specialist medical doctor. Two of the most important immediate consequences of advanced stage were a conspicuous increase in treatment costs and a longer hospital stay.

Since 1990 the Brazilian Head and Neck Cancer Study Group has 10 participant institutions: Ludwig Institute for Cancer Research (São Paulo), Hospital A.C. Camargo (São Paulo), Hospital A. Jorge (Goiânia), Hospital das Clínicas (São Paulo), Hospital E. Gaertner (Curitiba), Hospital Heliópolis (São Paulo), Hospital N. Laureano (João Pessoa), H. Santa Rita (Porto Alegre), H. São Paulo (São Paulo), and H. Universitário (São Paulo). The main object of two prospective trials is the evaluation of different elective treatment approaches to the neck in laryngeal and oral cancer.

The first study was designed to compare standard treatment (modified radical neck dissection with preservation of XI nerve, sternomastoid muscle and internal jugular vein) (MRND) to lateral neck dissection (LND) on the management of clinically negative neck in larynx cancer patients. This study began in June 1990 and will be concluded on December 1993. So far 120 patients have already been included on the trial. The first 91 who had completed planned treatment will be analyzed. All patients had not previously treated T2-T4 N0 supraglottic or transglottic squamous cell carcinoma. There was no significant imbalance between groups with respect to demographic, clinical, pathological, and other therapeutic variables. Forty-seven patients were submitted to MRND and 44 to a LND. The false negative rate was 23%, and most positive nodes were sited at levels II and III. Complications were similar in both groups. To date there were 2 local and 1 neck recurrence, 1 contralateral metastasis, 2 paraostomal recurrences and 1 patient had local, bilateral neck and stomal recurrences. The 36-month actuarial survival rates were 74% in MRND group and 61.6% in LND group ($P = 0.9912$). Although there were no significant statistical differences on the rates of complications and recurrences, and the preliminary

survival results were similar in both groups, a longer follow-up and a larger series in mandatory to validate definitive conclusions.

The second study was designed to compare standard treatment (modified radical classical neck dissection) (MRND) to supraomohyoid neck dissection (SOH) on the management of clinically negative neck in oral cancer patients. This study began in June 1990 and will be concluded in June 1993. So far 95 patients have already been included on the trial. The first 70 who had completed planned treatment will be analyzed. All patients had not previously treated T2-T4 N0 squamous cell carcinoma of the oral tongue (24 cases), floor of the mouth (22 cases), inferior gingiva (8 cases), or retromolar trigone (16 cases). There was no significant imbalance between groups with respect to demographic, clinical or pathological variables. The false negative rate was 20%, and most positive nodes were sited at level II and III. Complications were seen in 35.9% MRND patients and in 22.6% of SOH patients ($P = 0.297$). Mean total duration of hospitalization were 13 days in MRND patients and 11.4 days in SOH. To date 9 and 5 patients presented local or neck recurrences. The 12 month actuarial survival rates were 79.7% in MRND group and 96.7% in SOH group ($P = 0.413$).

Patient accrual will be concluded at the end of 1993. The group is planning to develop new studies in the following areas: chemoprevention, prognostic implications of several biomarkers, organ preservation trials, evaluation of quality of life parameters after combined treatment, and treatment of small primary tumors with huge neck metastasis.

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Leukemic cell differentiation

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Hemopoetic cell proliferation depends on critical interactions with components of bone marrow microenvironment. Fibronectin (FN) and laminin (LN) are well characterized extracellular matrix glycoproteins present in bone marrow. Several receptors mediate their numerous biological activities including members of integrin family

known as very late antigens (VLA). (¹²⁵I) labelled laminin molecules and 125kDa fibronectin fragments were used to demonstrate the presence of a high number of laminin receptors (LNR) in myeloid blast cells as compared to lymphoid blasts, whereas fibronectin binding sites (FNR) were present equally in the two groups. Chemically differentiated H160 promyelocytic leukemia cells increased their content of LNR during differentiation irrespective of the inducer used. ¹²⁵I-laminin binding to lymphocytes and leukemic cells was inhibited by the P1 fragment (from the centre of the laminin cross), whereas granulocytes and in vitro differentiated HL60 leukemia cells presented a dual binding pattern for laminin fragments P1 and E8 (from the long arm of laminin). The pattern of expression of fibronectin receptors VLA4 and VLA5 also changed after in vitro differentiation. VLA5 was down-regulated whereas VLA4 was maintained. We also found low VLA4 and VLA5 levels in bone marrow blasts as compared to peripheral blood leukemic cells. We conclude that the magnitude of LNR levels and variation of P1 and E8 receptors appear to be linked to lineage maturation in hematopoietic cells. Differential expression of VLA4 and VLA5 integrin molecules might be modulating interaction with FN within hemopoietic tissue.

Differentiation is usually coupled to an inhibition of proliferation in the hemopoietic model. The vitamin D3 receptor (VDR), a member of the family of nuclear receptors, has also been demonstrated in many hematopoietic cells. A direct relationship between VDR levels and proliferative activity was described by others in lymphocytes. In order to clarify the role of VDR in myeloid cells, we studied VDR expression in two situations where differentiation is dissociated from proliferation. H160 treated with recombinant gamma interferon (IFN) (differentiated to monocytes without changes in proliferation) presented lower VDR levels. On the other hand HL60 grown until density saturation did not change their VDR content. Our data suggest that VDR levels might be better related to differentiation than to proliferation in the model of HL60 cell differentiation.

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Medical follow-up from Cesium 137 accident of Goiânia

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On September 13 1987 a radioactive 137 Cesium source was removed from a teletherapy unit in an abandoned clinic in Goiânia. This source was broken and opened by ordinary people. They sold the source for a junkyard owner and this man gave small pieces of the Cesium powder for friends and relatives.

On November, 29 the accident was discovered by a physicist monitoring the Sanitary Vigilance in Goiânia. The health state secretary call the National Nuclear Energy Comission to control the accident isolating the contaminated areas and manage the contaminated victims. Approximately 112.000 people were monitored in the city: around 10% of the Goiânia Population. From this monitored people it was found 249 people were contaminated either internally or externally. One hundred twenty people had light contamination and were rapidly decontaminated. One hundred twenty nine had moderate to severe internal or external contamination. From this group 80 required careful medical assistance, 70 person with low dose were managed as outpatients.

Twenty person out of the 50 were hospitalized at Goiânia and 14 of these 20 were transfered to specialized hospital in Rio de Janeiro. Fourteen patients developed some level of bone marrow failure, four patients died in the first month after the accident with infeccion secondary to radiation acute syndrome.

The group of victims was divided in there groups as follows:

Group I: Patients with cytogenetic dosimetry above on equal 0,2 Gy, with radiodermatitis and internal contamination above or equal to 50 Mci (06 persons);

Group II: Patients with cytogenetic dosimetry below 0,2 Gy, without radiodermatitis and internal contamination below to 50 Mci (46 persons);

Group III: Professional who worked in the accident without citogenetic dosimetry and without internal contamination (514 persons).

After five years of medical follow-up we have the following clinical findings:

1988 - Radiodermatitis (in treatment) surgical reconstructions. Hematological control, bone marrow aspiration and biopsy, leucopenias.

1989 - Radiodermatitis recurrences, phalange amputation, hematological normal.

Male and female - hipertension and gastritis (*H. pylori*). Psychological disturbances (suicide).

1990 - Gastritis, sexual diseases, urinary infection, hypertension, shongyloidiasis, renal litiasis, alcoholism.

1991 - Group I: Lentigo in area of radiodermatitis.

Group III: Breast cancer female, nasopharynx cancer, skin cancer.

1992 - Gastritis (*H. pylori*), psychiatric disturbance, hypertension, alcoholism.

1993 - Hypertension, gastritis, alcoholism.

Observing this findings we conclude in his type of medical follow-up: It is important to have a control group in the follow-up;

Radiodermatitis - the surgical intervention is important for the early recovery;

Bone marrow aspiration yearly in the high dose group (100 cGy);

- Sperm count yearly (> 100 cGy);

- Cancer prevention program;

- Routinely psychological and psychiatric evaluation of patients and the team;

Good relationships between physicist and health team.

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