

## Testis Cancer Literature

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**Abstract:** Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the testis cancer.

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### 1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

### Literatures

Adair, S. J., T. M. Carr, et al. (2008). "The TAG family of cancer/testis antigens is widely expressed in a variety of malignancies and gives rise to HLA-A2-restricted epitopes." *J Immunother* **31**(1): 7-17.

The TAG-1, TAG-2a, TAG-2b, and TAG-2c cancer/testis genes, known to be expressed in an unusually high percentage of melanoma cell lines, are shown here to be expressed in a variety of tumor lines of diverse histologic type, including cancers of the brain, breast, colon, lung, ovary, pharynx, and tongue. The genes are also expressed in fresh, uncultured melanoma, and ovarian cancer cells. Epitope prediction algorithms were used to identify potential HLA-A1, HLA-A2, HLA-A3, HLA-B7, and HLA-B8 epitopes, and these potential epitopes were tested for their ability to stimulate a peptide-specific cytotoxic T lymphocyte response using lymphocytes from healthy donors. Two HLA-A2-restricted epitopes (SLGWLFLLL and LLLRLECNV) were identified using this approach. Cytotoxic T lymphocytes specific for each of these peptides were capable of recognizing tumor cells expressing both the corresponding class I major histocompatibility complex encoded molecule and the TAG genes. These results indicate that TAG-derived peptides may be good components of a

therapeutic vaccine designed to target melanoma and a variety of epithelial cell-derived malignancies.

Adair, S. J. and K. T. Hogan (2009). "Treatment of ovarian cancer cell lines with 5-aza-2'-deoxycytidine upregulates the expression of cancer-testis antigens and class I major histocompatibility complex-encoded molecules." *Cancer Immunol Immunother* **58**(4): 589-601.

**PURPOSE:** To test the hypothesis that decrease in DNA methylation will increase the expression of cancer-testis antigens (CTA) and class I major histocompatibility complex (MHC)-encoded molecules by ovarian cancer cells, and thus increase the ability of these cells to be recognized by antigen-reactive CD8(+) T cells. **METHODS:** Human ovarian cancer cell lines were cultured in the presence or absence of varying concentrations of the DNA demethylating agent 5-aza-2'-deoxycytidine (DAC) for 3-7 days. The expression levels of 12 CTA genes were measured using the polymerase chain reaction. The protein expression levels of class I MHC molecules and MAGE-A1 were measured by flow cytometry. T cell reactivity was determined using interferon-gamma ELISpot analysis. **RESULTS:** DAC treatment of ovarian cancer cell lines increased the expression of 11 of 12 CTA genes tested including MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, NY-ESO-1, TAG-1, TAG-2a, TAG-2b, and TAG-2c. In contrast, DAC treatment decreased the already low expression of the MAGE-A2 gene by ovarian cancer cells, a finding not previously observed in cancers of any histological type. DAC treatment increases the expression of class I MHC molecules by the cells. These effects were time-dependent over a 7-day interval, and were dose-dependent up to 1-3 microM for CTA and up to 10 microM for class I MHC molecules. Each cell line tested had a unique pattern of gene upregulation after exposure to DAC. The enhanced expression levels

increased the recognition of 2 of 3 antigens recognized by antigen-reactive CD8(+) T cells. CONCLUSIONS: These results demonstrate the potential utility of combining DAC therapy with vaccine therapy in an attempt to induce the expression of antigens targeted by the vaccine, but they also demonstrate that care must be taken to target inducible antigens.

Ait-Tahar, K., A. P. Liggins, et al. (2009). "Cytolytic T-cell response to the PASD1 cancer testis antigen in patients with diffuse large B-cell lymphoma." *Br J Haematol* **146**(4): 396-407.

The identification of immunogenic cancer testis antigens (CTAs) as immunotherapeutic targets represents one approach to improve treatment options for diffuse large B-cell lymphoma (DLBCL). We previously identified PASD1 [PAS (Per ARNT Sim) domain containing 1 (PASD1)], a DLBCL-associated CTA that was expressed in a range of hematopoietic malignancies. The aim of the present study was to investigate the presence of a cytotoxic T-cell (CTL) response to PASD1 in DLBCL patients. A significant gamma-interferon (IFN) release was detected in 21/29 HLA-A\*0201-positive DLBCL patients (18 de novo DLBCL, two transformed DLBCL and one T-cell rich B-cell lymphoma) following short-term culture of their peripheral blood mononuclear cells stimulated with five HLA-A\*0201-restricted PASD1 peptides. No significant responses were detected in 21 HLA-A\*0201-negative DLBCL patients (12 de novo DLBCL, seven transformed DLBCL, two T-cell rich B-cell lymphoma) or six normal subjects. CTL cell lines were able to lyse PASD1-positive tumour cells in a major histocompatibility complex-Class I dependent manner. The presence of a gamma-IFN response correlated with PASD1 protein expression in the tumour cells in 12/15 cases studied. This is the first report of a CTL response to a CTA in DLBCL. Our results provide additional valuable evidence supporting PASD1 as a potential immunotherapeutic target for the treatment of DLBCL and other malignancies.

Ajiro, M., T. Katagiri, et al. (2009). "Involvement of RQCD1 overexpression, a novel cancer-testis antigen, in the Akt pathway in breast cancer cells." *Int J Oncol* **35**(4): 673-81.

We here report identification and characterization of required for cell differentiation 1 homolog (RQCD1) as a potential therapeutic target for breast cancer. Gene-expression profiling analysis of breast cancer cells, semi-quantitative RT-PCR, Northern blotting and Western blotting confirmed RQCD1 to be frequently up-regulated in breast cancer specimens and breast cancer cell lines. On the other

hand, its expression was very weak or hardly detectable in normal human tissues except testis, indicating this molecule to be a novel cancer-testis antigen. Treatment of breast cancer cell lines with siRNA targeting RQCD1 drastically suppressed cell proliferation. Concordantly, introduction of exogenous RQCD1 into HEK293 cells significantly enhanced cell growth, implying RQCD1 to have an oncogenic activity. Co-immunoprecipitation experiments and immunocytochemical staining revealed an interaction of RQCD1 protein with Grb10 interacting GYF protein 1 (GIGYF1) and 2 (GIGYF2) proteins, involved in regulation of Akt activation, in breast cancer cells. Interestingly, knockdown of either of RQCD1, GIGYF1 or GIGYF2 resulted in significant reduction of the phosphorylation of Akt at Ser 473 in breast cancer cell lines. Our findings suggest that RQCD1 is a potential molecular target for treatment of breast cancer.

Akre, O., A. Pettersson, et al. (2009). "Risk of contralateral testicular cancer among men with unilaterally undescended testis: a meta analysis." *Int J Cancer* **124**(3): 687-9.

The association between undescended testis (cryptorchidism) and testicular cancer is established, but it is not known whether the risk of testicular cancer among men with unilateral maldescent is increased in both testes, or only on the undescended side. This is a meta-analysis of 11 case-control studies and 1 cohort study that all assessed the risk of testicular cancer separately for the undescended and descended testis. We used fixed-effects meta-analysis to calculate pooled estimates and 95% confidence intervals (CIs) for the relative risk. Of 199 tumors in men with unilateral cryptorchidism, 158 (79%) were on the ipsilateral side and 41 (21%) on the contralateral side. The pooled relative risks for testicular cancer in the ipsilateral and contralateral testis were 6.33 (95% CI, 4.30 to 9.31) and 1.74 (95% CI, 1.01 to 2.98), respectively. We conclude that in 1-sided undescended testis, the risk of testicular cancer may be increased in both testes, although to a much greater extent on the ipsilateral side.

Albers, P. (2002). "Surgery in testis cancer: laparoscopic and open techniques." *Curr Opin Urol* **12**(5): 435-40.

PURPOSE OF REVIEW: The review focuses on the current developments of the management of patients with testis cancer regarding surgery. For clinical stage I and stage II disease, the pros and cons of surgery as a diagnostic and therapeutic tool are updated. Additionally, the emerging role of laparoscopic techniques in the staging of the disease is critically discussed. The

review presents the currently changing indications for surgery in addition to chemotherapy in metastatic disease. **RECENT FINDINGS:** The complication rates of primary retroperitoneal lymph node dissection have recently been assessed by the German Testicular Cancer Study Group. These data confirm the excellent results of the Indiana series published some years ago. Laparoscopic surgery has been performed in a larger cohort of patients in specialized centers, and, concomitantly, operative times and complication rates have dropped. Indications for surgery in the post-chemotherapy setting have been more clearly defined recently. Seminoma patients usually do not need surgical removal of the residual tumor after chemotherapy, whereas patients with non-seminoma disease probably need surgery even in cases of complete radiological remission after chemotherapy. In view of the recent data on late relapse, complete surgical removal of residual disease for non-seminoma seems of the utmost importance. **SUMMARY:** Larger series of surgical procedures, laparoscopic as well as open, have helped to define the role of this approach in the management of testis cancer. Long-term data on patients with complete response to initial treatment and late relapse have shown the danger of limiting the treatment of metastatic disease to chemotherapy alone. These data have also shown the importance of proper surgical techniques for all stages of testis cancer.

Albers, P. (2007). "Management of stage I testis cancer." *Eur Urol* **51**(1): 34-43; discussion 43-4.

**OBJECTIVE:** Over the last 5 years the management of stage I testis cancer has changed tremendously. This review focuses on the latest changes in diagnostics and treatment of clinical stage I non-seminomatous and seminomatous germ cell tumors. **METHODS:** A non-structured literature search (MEDLINE) was performed, including recently published papers (up to March 2006) on the subject. **RESULTS:** Organ-sparing surgery has become an accepted approach to treat malignant and nonmalignant tumours in a solitary testis. With certain precautions and adjuvant radiotherapy, this approach has proven to be as effective as orchidectomy. Prognostic factors strongly influence the decision for or against adjuvant treatment in seminoma and non-seminoma. With the help of a risk-adapted approach, about 50% of patients with clinical stage I testis cancer will favour close surveillance instead of immediate adjuvant treatment. Several well-conducted trials have helped to substantiate the management. Surgical staging by retroperitoneal lymph node dissection became an exception. Patients with non-seminoma with high risk for occult metastatic disease will favour adjuvant chemotherapy and in patients

with seminoma radiotherapy with reduced dosage will be challenged by carboplatin monotherapy. **CONCLUSION:** With adequate diagnostics and treatment, 100% of patients with stage I testis cancer will survive. Future research will focus on quality control, adherence to guideline recommendations, and further reduction of treatment to diminish the risk of late sequelae for patients with adjuvant radiotherapy or chemotherapy.

Almeida, L. G., N. J. Sakabe, et al. (2009). "CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens." *Nucleic Acids Res* **37**(Database issue): D816-9.

The potency of the immune response has still to be harnessed effectively to combat human cancers. However, the discovery of T-cell targets in melanomas and other tumors has raised the possibility that cancer vaccines can be used to induce a therapeutically effective immune response against cancer. The targets, cancer-testis (CT) antigens, are immunogenic proteins preferentially expressed in normal gametogenic tissues and different histological types of tumors. Therapeutic cancer vaccines directed against CT antigens are currently in late-stage clinical trials testing whether they can delay or prevent recurrence of lung cancer and melanoma following surgical removal of primary tumors. CT antigens constitute a large, but ill-defined, family of proteins that exhibit a remarkably restricted expression. Currently, there is a considerable amount of information about these proteins, but the data are scattered through the literature and in several bioinformatic databases. The database presented here, CTdatabase (<http://www.cta.lncc.br>), unifies this knowledge to facilitate both the mining of the existing deluge of data, and the identification of proteins alleged to be CT antigens, but that do not have their characteristic restricted expression pattern. CTdatabase is more than a repository of CT antigen data, since all the available information was carefully curated and annotated with most data being specifically processed for CT antigens and stored locally. Starting from a compilation of known CT antigens, CTdatabase provides basic information including gene names and aliases, RefSeq accession numbers, genomic location, known splicing variants, gene duplications and additional family members. Gene expression at the mRNA level in normal and tumor tissues has been collated from publicly available data obtained by several different technologies. Manually curated data related to mRNA and protein expression, and antigen-specific immune responses in cancer patients are also available, together with links to PubMed for relevant CT antigen articles.

Alzheimer, M., T. Drewes, et al. (2005). "The cancer/testis antigen CAGE-1 is a component of the acrosome of spermatids and spermatozoa." *Eur J Cell Biol* **84**(2-3): 445-52.

Cancer/testis antigens (CTAs) are characterized by their restricted expression pattern. In normal individuals their expression is largely restricted to the testis. In the case of cancer patients, CTA expression has also been frequently observed in the tumoral cells. CTAs are considered to be promising targets for immunotherapy. However, almost nothing is known about the properties defined by the vast majority of CTAs. Here, we have investigated the expression pattern and localization of the CTA CAGE-1 during mouse spermatogenesis. We show that protein CAGE-1 is 849 amino acids long. Analysis of the first spermatogenic wave of pubertal mice by RT-PCR and immunoblotting showed that CAGE-1 is predominantly expressed during postmeiotic stages. CAGE-1 localizes to the acrosomal matrix and acrosomal granule, as demonstrated by immunocytochemistry at the light and electron microscopic level. Taken together, our results allowed to define protein CAGE-1 as a novel component of the acrosome of mammalian spermatids and spermatozoa.

Alves, P. M., N. Levy, et al. (2007). "Molecular and immunological evaluation of the expression of cancer/testis gene products in human colorectal cancer." *Cancer Immunol Immunother* **56**(6): 839-47.

Tumor-specific gene products, such as cancer/testis (CT) antigens, constitute promising targets for the development of T cell vaccines. Whereas CT antigens are frequently expressed in melanoma, their expression in colorectal cancers (CRC) remains poorly characterized. Here, we have studied the expression of the CT antigens MAGE-A3, MAGE-A4, MAGE-A10, NY-ESO-1 and SSX2 in CRC because of the presence of well-described HLA-A2-restricted epitopes in their sequences. Our analyses of 41 primary CRC and 14 metastatic liver lesions confirmed the low frequency of expression of these CT antigens. No increased expression frequencies were observed in metastatic tumors compared to primary tumors. Histological analyses of CRC samples revealed heterogeneous expression of individual CT antigens. Finally, evidence of a naturally acquired CT antigen-specific CD8(+) T cell response could be demonstrated. These results show that the expression of CT antigens in a subset of CRC patients induces readily detectable T cell responses.

Andrade, V. C., A. L. Vettore, et al. (2008). "Prognostic impact of cancer/testis antigen expression

in advanced stage multiple myeloma patients." *Cancer Immunol* **8**: 2.

This study aims to analyze the expression of 14 cancer/testis (CT) antigens in multiple myeloma (MM) to identify possible prognostic markers and therapeutic targets. The expression of MAGEA1, MAGEA2, MAGEA3/6, MAGEA4, MAGEA10, MAGEA12, BAGE1, MAGEC1/CT7, the GAGE family, LAGE-1, PRAME, NY-ESO-1, SPA17 and SSX1 was studied by RT-PCR in 15 normal tissues, a pool of 10 normal bone marrow samples, 3 normal tonsils and bone marrow aspirates from 6 normal donors, 3 monoclonal gammopathies of undetermined significance (MGUS), 5 solitary plasmacytomas, 39 MM samples (95% advanced stage) and the MM cell line U266. MAGEC1/CT7 was expressed in bone marrow aspirates from one MGUS and one plasmacytoma. The frequencies at which CT antigen were found to be expressed in MM patients were MAGEC1/CT7 77%, LAGE-1 49%, MAGEA3/6 41%, MAGEA2 36%, GAGE family 33%, NY-ESO-1 33%, BAGE-1 28%, MAGEA1 26%, PRAME 23%, SSX-1 26%, MAGEA12 20.5%, MAGEA4 0%, and MAGEA10 0%. Cox's regression model showed that GAGE family expression and having >6 CT antigens expressed were independent prognostic factors when all patients were analyzed. However, MAGEC1/CT7 expression was the only independent prognostic factor when non-transplanted patients were analyzed. Based on our findings, MAGEC1/CT7, MAGEA3/6 and LAGE-1 are good candidates for immunotherapy, since together they cover 85% of our MM cases. Furthermore, expression of the GAGE family, >6 CT antigens and MAGEC1/CT7 seem to have impact on MM prognosis.

Andrade, V. C., A. L. Vettore, et al. (2009). "Frequency and prognostic relevance of cancer testis antigen 45 expression in multiple myeloma." *Exp Hematol* **37**(4): 446-9.

OBJECTIVE: This study aims to analyze the expression of cancer testis antigen 45 (CT45) in normal tissues and in plasma cell disorders and to identify possible associations with clinical data and prognosis in multiple myeloma (MM) patients. MATERIALS AND METHODS: Expression of CT45 was studied in 20 normal tissues (testis, placenta, skeletal muscle, bladder, lung, spleen, heart, brain and fetal brain, thymus, uterus, stomach, mammary gland, pancreas, prostate, small intestine, kidney, adrenal gland, spinal cord, colon, and one pool of 10 normal bone marrow samples) and bone marrow aspirates from 3 monoclonal gammopathies of undetermined significance, 5 solitary plasmacytomas, 61 newly diagnosed MM patients and MM cell line U266 by reverse transcriptase polymerase chain reaction.

RESULTS: CT45 was positive in 3 of 20 (15%) normal tissues tested: lung, brain (both fetal and adult), and spinal cord. Among monoclonal gammopathies, CT45 was positive in 2 of 5 (40%) solitary plasmacytomas bone marrow aspirates, 10 of 61 (16%) MM bone marrow aspirates, and in the U266 MM cell line. CONCLUSIONS: We did not find associations between bone marrow histology and CT45 expression. However, we demonstrated for the first time that positive expression of CT45 was associated with poor prognostic (International Staging System) and poor outcomes in MM patients, meaning that CT45-positive cases presented seven times more chance of worse evolution than the negative ones.

Artamonova, II and M. S. Gelfand (2004). "Evolution of the exon-intron structure and alternative splicing of the MAGE-A family of cancer/testis antigens." *J Mol Evol* **59**(5): 620-31.

Cancer/testis antigens (CT-antigens) are proteins that are predominantly expressed in cancer and testis and thus are possible targets for immunotherapy. Most of them form large multigene families. The evolution of the MAGE-A family of CT-antigens is characterized by four processes: (1) gene duplications; (2) duplications of the initial exon; (3) point mutations and short insertions/deletions inactivating splicing sites or creating new sites; and (4) deletions removing sites and creating chimeric exons. All this concerns the genomic regions upstream of the coding region, creating a wide diversity of isoforms with different 5'-untranslated regions. Many of these isoforms are gene-specific and have emerged due to point mutations in alternative and constitutive splicing sites. There are also examples of chimeric mRNAs, likely produced by splicing of read-through transcripts. Since there is consistent use of homologous sites for different genes and no random, indiscriminant use of preexisting cryptic sites, it is likely that most observed isoforms are functional, and do not result from relaxed control in transformed cells.

Atanackovic, D., J. Arfsten, et al. (2007). "Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation." *Blood* **109**(3): 1103-12.

Immunotherapies using cancer-testis (CT) antigens as targets represent a potentially useful treatment in patients with multiple myeloma (MM) who commonly show recurrent disease following chemotherapy. We analyzed the expression of 11 CT antigens in bone marrow samples from patients with MM (n=55) and healthy donors (n=32) using reverse transcriptase-polymerase chain reaction (RT-PCR).

CT antigens were frequently expressed in MM with 56% (MAGEC2), 55% (MAGEA3), 35% (SSX1), 20% (SSX4, SSX5), 16% (SSX2), 15% (BAGE), 7% (NY-ESO-1), and 6% (ADAM2, LIPI) expressing the given antigen. Importantly, CT antigens were not expressed in healthy bone marrow. Analyzing patients with MM (n=66) for antibody responses against MAGEA3, SSX2, and NY-ESO-1, we found strong antibody responses against CT antigens preferentially in patients who had received allogeneic stem cell transplantation (alloSCT). Antibody responses against NY-ESO-1 correlated with NY-ESO-1-specific CD4+ and CD8+ T-cell responses against peptide NY-ESO-1(51-62) and CD4+ responses against NY-ESO-1(121-140) in 1 of these patients. These allogeneic immune responses were not detectable in pretransplantation samples and in the patients' stem cell donors, indicating that CT antigens might indeed represent natural targets for graft-versus-myeloma effects. Immune responses induced by alloSCT could be boosted by active CT antigen-specific immunotherapy, which might help to achieve long-lasting remissions in patients with MM.

Atanackovic, D., I. Blum, et al. (2006). "Expression of cancer-testis antigens as possible targets for antigen-specific immunotherapy in head and neck squamous cell carcinoma." *Cancer Biol Ther* **5**(9): 1218-25.

Cancer-Testis (CT) antigens are by definition expressed in tumor but not in healthy tissue except testis and might represent ideal targets for antigen-specific immunotherapy. Here, we present the first comprehensive analysis of CT antigen expression in patients with head and neck squamous cell carcinoma (HNSCC). Tumor samples (N = 51), and adjacent healthy tissue from patients with HNSCC were analyzed for the expression of 23 genes designated CT antigens using RT-PCR. Patient sera (N = 39) were screened for IgG antibody responses against NY-ESO-1, MAGEA3, and SSX2. According to their expression pattern antigens were divided into four groups. ADAM2, LIPI, SLLP1, AKAP3, CTAGE, ZNF165, CAGE, and FTHL17 were expressed in tumor and healthy tissue at comparable frequencies. NY-TLU-57, GAGE1, SAGE1 were expressed more frequently in tumor samples than in healthy tissues. TPTE, LDHC, SPO11 were expressed neither in tumor samples nor in healthy tissue. 9 CT antigens were expressed only in the tumor tissue and may represent ideal candidates for active immunotherapy in HNSCC: MAGEA3 was expressed in 72%, SSX1 in 45%, MAGEC2 in 33%, MAGEC1 in 28%, BAGE in 17%, SSX2 in 16%, SCP1 in 12%, NY-ESO-1 in 6%, and HOM-TES-85 in 4% of cases. 86% of tumor samples expressed at least one, 69% expressed at least two, and 43% expressed at least three of these

antigens. Three patients showed an antibody response against NY-ESO-1. In conclusion, we demonstrate here that HNSCC frequently express CT antigens. Furthermore, a relatively high percentage of tumors express more than one CT antigen opening the perspective for polyvalent antigen-specific immunotherapy.

Atanackovic, D., T. Luetkens, et al. (2009). "Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma." *Clin Cancer Res* **15**(4): 1343-52.

**PURPOSE:** Reliable data on the persistence of tumor expression of cancer-testis (CT) antigens over time and consequent analyses of the effect of CT antigen expression on the clinical course of malignancies are crucial for their evaluation as diagnostic markers and immunotherapeutic targets. **EXPERIMENTAL DESIGN:** Applying conventional reverse transcription-PCR, real-time PCR, and Western blot, we did the first longitudinal study of CT antigen expression in multiple myeloma analyzing 330 bone marrow samples from 129 patients for the expression of four CT antigens (MAGE-C1/CT7, MAGE-C2/CT10, MAGE-A3, and SSX-2). **RESULTS:** CT antigens were frequently and surprisingly persistently expressed, indicating that down-regulation of these immunogenic targets does not represent a common tumor escape mechanism in myeloma. We observed strong correlations of CT antigen expression levels with the clinical course of myeloma patients as indicated by the number of bone marrow-residing plasma cells and peripheral paraprotein levels, suggesting a role for CT antigens as independent tumor markers. Investigating the prognostic value of CT antigen expression in myeloma patients after allogeneic stem cell transplantation, we found that expression of genes, such as MAGE-C1, represents an important indicator of early relapse and dramatically reduced survival. **CONCLUSIONS:** Our findings suggest that CT antigens might promote the progression of multiple myeloma and especially MAGE-C1/CT7, which seems to play the role of a "gatekeeper" gene for other CT antigens, might characterize a more malignant phenotype. Importantly, our study also strongly supports the usefulness of CT antigens as diagnostic and prognostic markers as well as therapeutic targets in myeloma.

Ayyoub, M., R. N. Taub, et al. (2004). "The frequent expression of cancer/testis antigens provides opportunities for immunotherapeutic targeting of sarcoma." *Cancer Immun* **4**: 7.

Sarcomas are rare but aggressive malignant tumors associated with high mortality, for which the

efficacy of standard therapies remains limited. In order to develop immunotherapeutic approaches for the treatment of sarcoma, we studied the relevance of cancer/testis antigens (CTAs), a group of antigens whose expression is developmentally regulated and that is specifically found in some tumor types, as sarcoma vaccine targets. CTA expression was assessed by PCR and/or immunohistochemistry (IHC) in sarcoma tumor samples that included different histological subtypes and sarcoma cell lines. Expression of HLA class I was assessed by IHC in tumor samples and by FACS analysis in cell lines. More than 70% of the tumor samples expressed at least one CTA. The majority of tumors and cell lines expressed normal levels of HLA class I. HLA class I expression in cell lines was enhanced upon treatment with IFN-gamma. CTA expression was enhanced or induced by treatment with the demethylating agent 5-aza-2'-deoxycytidine, resulting in recognition by specific CTLs. Interestingly, a spontaneous humoral and CD8+ T cellular response to the CTA NY-ESO-1 was detected in a synovial sarcoma patient. Together, these findings strongly support the implementation of CTA-based immunotherapy of sarcoma as a means to improve the efficacy of the standard therapy.

Bar-Shira Maymon, B., L. Yogev, et al. (2004). "Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy." *Fertil Steril* **81**(5): 1391-4.

**OBJECTIVE:** To assess Sertoli cell involvement in postchemotherapy azoospermia. **DESIGN:** Case report. **SETTING:** Teaching hospital. **PATIENT(S):** A 31-year-old azoospermic man who underwent cancer cytotoxic chemotherapy for non-Hodgkin's lymphoma at 13 years of age. **INTERVENTION(S):** Testicular biopsy specimens were obtained for sperm recovery in preparation for intracytoplasmic sperm injection. The biopsy specimens were evaluated by quantitative immunohistochemistry for the immature Sertoli cell markers cytokeratin 18 (CK-18) and D2-40. **MAIN OUTCOME MEASURE(S):** Extent of immature Sertoli cells. **RESULT(S):** A fraction of Sertoli cells (13%) in the atrophic tubules of this patient reexpressed the intermediate filament protein CK-18, which is normally absent after puberty, but not the D2-40 antigen, an Mr 40,000 a-linked membrane glycoprotein, whose loss of expression at puberty marks an irreversible step in Sertoli cell maturation. Tubules with normal spermatogenic progression lined by Sertoli cells negative for CK-18 were also observed. **CONCLUSION(S):** A fraction of Sertoli cells of this patient initially progressed to full maturation at puberty and reverted to a dedifferentiated state marked by reexpression of CK-

18 as a consequence of chemotherapy. This inactivation of Sertoli cells caused by the cytotoxicity of the chemotherapeutic drugs may have contributed to the spermatogenic impairment and resulting infertility.

Batchu, R. B., A. M. Moreno, et al. (2003). "High-level expression of cancer/testis antigen NY-ESO-1 and human granulocyte-macrophage colony-stimulating factor in dendritic cells with a bicistronic retroviral vector." *Hum Gene Ther* **14**(14): 1333-45.

Tumor-specific genes delivered to dendritic cells (DCs) have been used for the generation of cytotoxic T cells (CTLs), but their application has been limited on the one hand by low viral titers resulting in low transduction efficiency and poor protein production, and on the other hand by immunogenicity of the selectable marker and poor viability of the DCs. We addressed these limitations by creating a multipurpose master vector (pMV) and cloning the tumor gene NY-ESO-1, which is highly expressed in more than 50% of advanced myeloma patients. pMV was constructed from a Moloney murine leukemia virus (Mo-MuLV)-based retroviral backbone with the following features: (1) an extended packaging signal to achieve high viral titers, (2) a splice acceptor region to facilitate protein production, (3) a nonimmunogenic selectable marker, dihydrofolate reductase-L22Y (DHFR(L22Y)), to exclude the generation of CTLs against the selectable marker, (4) an internal ribosomal entry site between the tumor-specific gene (NY-ESO-1) and the selectable marker DHFR(L22Y) for coexpression of two heterologous gene products from a single bicistronic mRNA, minimizing the possibility of differential expression of these two genes, and (5) human granulocyte-macrophage colony-stimulating factor (hGM-CSF) cDNA driven by the human T-lymphotropic virus promoter to enhance DC function and viability. Recombinant virus of pMV-NY-ESO-1 was generated with vesicular stomatitis virus G envelope protein (VSV-G) in the GP2-293 cell line for efficient transduction. We present evidence that the DC phenotype is unaltered after transduction and that more than 85% of DCs express NY-ESO-1, which secrete approximately 40 ng of GM-CSF per 10(6) DCs.

Baumgaertner, P., N. Rufer, et al. (2006). "Ex vivo detectable human CD8 T-cell responses to cancer-testis antigens." *Cancer Res* **66**(4): 1912-6.

Clinical trials have shown that strong tumor antigen-specific CD8 T-cell responses are difficult to induce but can be achieved for T-cells specific for melanoma differentiation antigens, upon repetitive vaccination with stable emulsions prepared with

synthetic peptides and incomplete Freund's adjuvant. Here, we show in four melanoma patients that ex vivo detectable T-cells and thus strong T-cell responses can also be induced against the more universal cancer-testis antigens NY-ESO-1 and Mage-A10. Interestingly, all patients had ex vivo detectable T-cell responses against multiple antigens after serial vaccinations with three peptides emulsified in incomplete Freund's adjuvant. Antigen-specific T-cells displayed an activated phenotype and secreted IFN $\gamma$ . The robust immune responses provide a solid basis for further development of human T-cell vaccination.

Beck, S. D. and R. S. Foster (2006). "Long-term outcome of retroperitoneal lymph node dissection in the management of testis cancer." *World J Urol* **24**(3): 267-72.

In low volume testicular cancer, (clinical stage A/B1) retroperitoneal lymph node dissection has maintained its therapeutic benefit while minimizing morbidity with the reduction of the surgical template from a full bilateral dissection to a unilateral nerve-sparing surgery. The optimal treatment for low stage disease is largely patient driven with surgery and surveillance considered the primary treatment modalities. In the post chemotherapy population, patients with complete radiographic resolution of retroperitoneal disease are observed at Indiana University as the relapse rate in this population is approximately 5%. Residual masses after chemotherapy should be resected. A modified post chemotherapy dissection is adequate in low volume disease restricted to the primary landing zone of the affected testicle. In chemo-refractory disease, aggressive surgery provides a 5 year survival of 31% for patients with active cancer. Excluding chemo-naïve patients, late relapse disease is managed surgically with 50% being cured of disease.

Beck, S. D., R. S. Foster, et al. (2002). "Teratoma in the orchiectomy specimen and volume of metastasis are predictors of retroperitoneal teratoma in post-chemotherapy nonseminomatous testis cancer." *J Urol* **168**(4 Pt 1): 1402-4.

**PURPOSE:** Patients who require post-chemotherapy retroperitoneal lymph node dissection after induction chemotherapy for metastatic testis cancer derive therapeutic benefit from resection of teratoma but resection of necrosis is not beneficial. We determine if the absence of teratoma in the orchiectomy specimen is a reliable predictor of the absence of teratoma in the retroperitoneum at post-chemotherapy retroperitoneal lymph node dissection. **MATERIALS AND METHODS:** A retrospective review of the Indiana University testis cancer data

base was performed. A total of 644 patients who underwent retroperitoneal lymph node dissection after induction chemotherapy only were selected for study. The presence or absence of teratoma in the orchiectomy specimen and volume of retroperitoneal tumor were analyzed as predictors of retroperitoneal teratoma at post-chemotherapy retroperitoneal lymph node dissection. RESULTS: Of the patients with teratoma in the orchiectomy specimen 85.6% had an element of teratoma in the retroperitoneum, and of those without teratomatous elements in the orchiectomy specimen 48% had teratoma in the retroperitoneum ( $p < 0.00001$ ). Increasing volumes of retroperitoneal tumor were associated with a higher probability of discovering teratoma at post-chemotherapy retroperitoneal lymph node dissection. CONCLUSIONS: The absence of teratoma in the orchiectomy specimen does not reliably predict the absence of teratoma in the surgical specimen at post-chemotherapy retroperitoneal lymph node dissection. Post-chemotherapy surgery is indicated if retroperitoneal tumor remains after chemotherapy irrespective of the presence or absence of teratoma in the orchiectomy specimen.

Bellati, F., C. Napoletano, et al. (2007). "Cancer testis antigen expression in primary and recurrent vulvar cancer: association with prognostic factors." *Eur J Cancer* **43**(17): 2621-7.

Cancer testis tumour associated antigens (C/T-TAAs) were investigated in several gynaecologic and non-gynaecologic neoplasms as possible prognostic markers and targets for immunotherapy. The objective of the present study was to evaluate C/T-TAA expression patterns and prognostic significance in patients affected by vulvar cancer. Melanoma antigen E (MAGE)-A1, MAGE-A4 and NY-ESO-1 expression was determined by immunohistochemistry in paraffin-embedded tissue specimens from 45 primary and 14 recurrent vulvar carcinomas treated with surgery. MAGE-A1, MAGE-A4 and NY-ESO-1 were expressed in 25 (42%), 38 (64%) and 40 (68%) of the 59 samples, respectively. MAGE-A4 was significantly more frequently expressed in tumours with lymph node metastases ( $p < 0.002$ ) and in recurrent tumours ( $p < 0.02$ ). NY-ESO-1 was more highly expressed by moderately or poorly differentiated tumours ( $p < 0.01$ ). This study demonstrates that vulvar cancer frequently expresses C/T-TAAs. Antigen expression correlates with the presence of lymph node metastases and poor tumour differentiation.

Bera, T. K., R. Maitra, et al. (2002). "PATE, a gene expressed in prostate cancer, normal prostate, and

testis, identified by a functional genomic approach." *Proc Natl Acad Sci U S A* **99**(5): 3058-63.

To identify target antigens for prostate cancer therapy, we have combined computer-based screening of the human expressed sequence tag database and experimental expression analysis to identify genes that are expressed in normal prostate and prostate cancer but not in essential human tissues. Using this approach, we identified a gene that is expressed specifically in prostate cancer, normal prostate, and testis. The gene has a 1.5-kb transcript that encodes a protein of 14 kDa. We named this gene PATE (expressed in prostate and testis). In situ hybridization shows that PATE mRNA is expressed in the epithelial cells of prostate cancers and in normal prostate. Transfection of the PATE cDNA with a Myc epitope tag into NIH 3T3 cells and subsequent cell fractionation analysis shows that the PATE protein is localized in the membrane fraction of the cell. Analysis of the amino acid sequence of PATE shows that it has structural similarities to a group of proteins known as three-finger toxins, which includes the extracellular domain of the type beta transforming growth factor receptor. Restricted expression of PATE makes it a potential candidate for the immunotherapy of prostate cancer.

Bera, T. K., D. B. Zimonjic, et al. (2002). "POTE, a highly homologous gene family located on numerous chromosomes and expressed in prostate, ovary, testis, placenta, and prostate cancer." *Proc Natl Acad Sci U S A* **99**(26): 16975-80.

We have identified a gene located on chromosomes 21 that is expressed in normal and neoplastic prostate, and in normal testis, ovary, and placenta. We name this gene POTE (expressed in prostate, ovary, testis, and placenta). The POTE gene has 11 exons and 10 introns and spans approximately equal 32 kb of chromosome 21q11.2 region. The 1.83-kb mRNA of POTE encodes a protein of 66 kDa. Ten paralogs of the gene have been found dispersed among eight different chromosomes (2, 8, 13, 14, 15, 18, 21, and 22) with preservation of ORFs and splice junctions. The synonymous:nonsynonymous ratio indicates that the genes were duplicated rather recently but are diverging at a rate faster than the average for other paralogous genes. In prostate and in testis, at least five different paralogs are expressed. In situ hybridization shows that POTE is expressed in basal and terminal cells of normal prostate epithelium. It is also expressed in some prostate cancers and in the LnCAP prostate cancer cell line. The POTE protein contains seven ankyrin repeats between amino acids 140 and 380. Expression of POTE in prostate cancer and its undetectable expression in normal essential tissues make POTE a candidate for the



immunotherapy of prostate cancer. The existence of a large number of closely related but rapidly diverging members, their location on multiple chromosomes and their limited expression pattern suggest an important role for the POTE gene family in reproductive processes.

Bettoni, F., F. C. Filho, et al. (2009). "Identification of FAM46D as a novel cancer/testis antigen using EST data and serological analysis." *Genomics* **94**(3): 153-60.

Cancer/testis Antigens (CTAs) are immunogenic proteins with a restricted expression pattern in normal tissues and aberrant expression in different types of tumors being considered promising candidates for immunotherapy. We used the alignment between EST sequences and the human genome sequence to identify novel CT genes. By examining the EST tissue composition of known CT clusters we defined parameters for the selection of 1184 EST clusters corresponding to putative CT genes. The expression pattern of 70 CT gene candidates was evaluated by RT-PCR in 21 normal tissues, 17 tumor cell lines and 160 primary tumors. We were able to identify 4 CT genes expressed in different types of tumors. The presence of antibodies against the protein encoded by 1 of these 4 CT genes (FAM46D) was exclusively detected in plasma samples from cancer patients. Due to its restricted expression pattern and immunogenicity FAM46D represents a novel target for cancer immunotherapy.

Biggs, M. L. and S. M. Schwartz (2004). "Differences in testis cancer survival by race and ethnicity: a population-based study, 1973-1999 (United States)." *Cancer Causes Control* **15**(5): 437-44.

**OBJECTIVE:** We examined the relationship between race/ethnicity and testis cancer survival in a population-based setting. **METHODS:** We analyzed 16,086 cases of primary testis cancer diagnosed during 1973-1999 and reported to 12 cancer registries participating in the National Cancer Institute's Surveillance, Epidemiology, and End Results Program. We compared testis cancer-specific survival between patients from different racial/ethnic groups by use of the hazard ratio (HR) and 95% confidence intervals (CI) calculated from Cox proportional hazards models, adjusting for stage, histology, and period of diagnosis. **RESULTS:** Relative to non-Hispanic whites, a greater proportion of African American, Native American, Hawaiian, and Hispanic patients were diagnosed at late stages. There were 886 deaths among 16,086 testis cancer patients and overall 5-year survival was 95%. After adjustment for stage, histology, and period of diagnosis, the risk of dying from testis cancer was increased among African

Americans (HR = 2.3; CI: 1.6-3.2), Native Americans (HR = 2.1; CI: 1.1-3.9), Filipinos (HR = 3.6; CI: 1.3-9.5), Hawaiians (HR = 2.4; CI: 1.4-4.1), and Hispanics (HR = 1.4; CI: 1.1-1.8), compared to non-Hispanic whites. **CONCLUSION:** These findings are consistent with previous reports of race/ethnic disparities in stage at diagnosis and survival in testis cancer patients as well as other cancer patients. Further research is needed to understand the reasons underlying these disparities.

Bodey, B. (2002). "Cancer-testis antigens: promising targets for antigen directed antineoplastic immunotherapy." *Expert Opin Biol Ther* **2**(6): 577-84.

During the last decade, the aberrant expression of normal testicular proteins in neoplastically transformed cells became common knowledge. Cancer-testis antigens (CTAs) represent a novel family of immunogenic proteins. The genes MAGE, BAGE, GAGE, LAGE and NY-ESO-1 code for antigens that are recognised on various neoplastically transformed cells by autologous, cytolytic CD8 (+) T lymphocytes. The MAGE genes were initially analysed from melanomas and turned out to have an almost exclusively neoplasm specific expression pattern. In normal adult tissues, most 23 human MAGE genes are expressed only in the testis, with expression patterns suggesting that this gene family is involved in germ cell development. The SSX (synovial sarcoma on X chromosome) gene family, located on the X chromosome, encode a family of highly homologous nuclear proteins. A number of observations confirmed that all five SSX genes were expressed in normal testis. The newly detected CTA, NY-ESO-1, is regarded as one of the most immunogenic antigens ever isolated, inducing spontaneous host immune responses in 50% of patients with NY-ESO-1-expressing neoplasms. The identification of neoplasm-associated markers recognised by cellular or humoral effectors of the immune system has opened new perspectives for antigen directed, individualised antineoplastic immunotherapy. In preparation for this new era of targeted immunotherapy, a number of neoplasm-associated antigen families have been identified as targets for CD8+, cytolytic T lymphocytes in vitro and in vivo : (1) CTAs expressed in various neoplasms and in normal testis, restricted to male germ cells; (2) melanocyte differentiation antigens; (3) point mutations of normal genes; (4) antigens overexpressed in neoplastic tissues; and (5) viral antigens. Immunotherapeutic protocols directed against the CTAs have already been initiated to analyse the induction of antigen-specific cellular and humoral immune responses in vivo.

Bodey, B., V. Bodey, et al. (2008). "Expression in childhood primary brain tumors of NY-ESO-1, a cancer/testis antigen: an immunohistochemical study." *In Vivo* **22**(1): 83-7.

**BACKGROUND:** NY-ESO-1 is a human gene that codes for antigens that are expressed in malignancies of various histological types, but not in normal tissues, except the testes. The expression of NY-ESO-1 in intracranial brain tumors including astrocytomas (ASTRs) and medulloblastomas (MEDs)/primitive neuroectodermal tumors (PNETs) was examined since the expression of NY-ESO-1 has only previously been explored in depth in neuroblastomas. **MATERIALS AND METHODS:** During our immunohistochemical study, a sensitive, four-step, alkaline phosphatase-conjugated antigen detection technique was employed. The expression of NY-ESO-1 was thereby examined in 6 cases of MED/PNET and 14 cases of ASTR. **RESULTS:** All 6 MED/PNET cases demonstrated high levels of immunoreactivity (overexpression) with the highest immunostaining intensity grades A and B. In the astrocytic tumors of various subtypes examined, the level of NY-ESO-1 expression was not as strong as that in MEDs/PNETs. However, there was a significant increase in expression level when comparing low-grade pilocytic ASTRs to high-grade anaplastic ASTRs and glioblastomas. **CONCLUSION:** As evidenced by our results, NY-ESO-1 overexpression increases as the malignancy grade of the astrocytic tumors increases. These data suggest that antigen-directed immunotherapy of primary brain tumors could target cancer/testis antigens (CTAs), especially those expressed at higher frequency such as NY-ESO-1.

Bodey, B., S. E. Siegel, et al. (2002). "MAGE-1, a cancer/testis-antigen, expression in childhood astrocytomas as an indicator of tumor progression." *In Vivo* **16**(6): 583-8.

During the last decade, the aberrant expression of normal testicular proteins in neoplastically transformed cells became common knowledge. Cancer/testis-antigens (CTAs) represent a novel family of immunogenic proteins. The MAGE genes were initially analyzed from melanomas and turned out to have an almost exclusively neoplasm-specific expression pattern. This expression pattern might contribute to the genetic instability of neoplastically transformed cells. In normal adult tissues, most 23 human MAGE genes are expressed only in the testis, but only in the mitotic spermatogonia (germ cells) and in the primary spermatocytes. The immunocytochemistry was carried out on routine, formalin-fixed, paraffin-wax-embedded 3 to 4 mm thick astrocytoma (ASTR) tissue

sections. A four step, indirect, biotin-streptavidin based method was employed with alkaline phosphatase enzyme conjugation. Immunocytochemical presence and cellular localization of the MAGE-1 CT-antigen, employing anti-MAGE-1 MoAB was observed only in anaplastic, high-grade ASTRs (100%), certainly including glioblastomas, in this study. The immunoreactivity was always heterogeneous, showing a cytoplasmic pattern and loosely grouped cells with similar staining characteristics being detected within the cellular and hormonal microenvironment of the ASTRs. We never identified MAGE-1, CT-antigen expression in the lowest grade, pilocytic ASTRs. The MAGE-1 CTA expression levels may also be used to evaluate the malignant and dedifferentiation tendencies of low-grade ASTRs and predict the likelihood of mutations of the genome and further dedifferentiation towards even more malignant anaplastic ASTR and glioblastoma multiforme IPs.

Bolli, M., E. Schultz-Thater, et al. (2005). "NY-ESO-1/LAGE-1 coexpression with MAGE-A cancer/testis antigens: a tissue microarray study." *Int J Cancer* **115**(6): 960-6.

The characterization of the expression pattern of different families of cancer/testis (C/T) antigens in different tumors, at the protein level, might be of relevance in the development of multiantigen vaccine preparations for active specific immunotherapy. We have used tissue microarray (TMA) technology to explore in large numbers of tumor specimens the expression of NY-ESO-1/LAGE-1 C/T antigens and its correlation with MAGE-A expression by using D8.38 and 57B monoclonal antibodies (MAb). The epitopes recognized by these reagents in C/T antigens were identified by molecular mapping by using a bacterial expression system. Out of 2,052 samples, 119 (5.8%) scored positive upon staining with D8.38 NY-ESO-1/LAGE-1-specific MAb. Expression in >10% of cases was detectable in melanoma and basaloma (31.6 and 18.2%, respectively), large cell carcinomas and adenocarcinomas of the lung (17.8 and 10.5%, respectively), stomach adenocarcinomas of the intestinal type (13.2%), pT2-4 bladder TCC (18.2%), nonseminomatous carcinomas of the testis (10.4%) and liposarcomas (15.4%). Simultaneous expression of NY-ESO-1/LAGE-1 and MAGE-A C/T antigens was then addressed in a TMA where 101/845 and 73/845 samples (12 and 8.6%, respectively) showed evidence of MAGE-A or NY-ESO-1/LAGE-1 specific staining, respectively. In 35/845 specimens (4.1%) concomitant expression of MAGE-A and NY-ESO-1/LAGE-1 was observed (p = 0.0002). Discrepancies in the expression of NY-ESO-1/LAGE-1 and MAGE-A were conspicuously detectable in

squamous cell carcinomas of the skin (MAGE-A positive but NY-ESO-1/LAGE-1 negative) and in liposarcomas (NY-ESO-1/LAGE-1 positive, but MAGE-A negative). Taken together, these data suggest novel areas of application of C/T antigens targeted active specific immunotherapy possibly based on multiantigen vaccine preparations.

Bor, P., J. Hindkjaer, et al. (2006). "Screening for Y microdeletions in men with testicular cancer and undescended testis." *J Assist Reprod Genet* **23**(1): 41-5.

**PURPOSE:** To investigate a possible association between testicular cancer or undescended testis and Y microdeletions. **METHODS:** It was designed as a retrospective clinical study. A total of 225 men with testicular cancer or undescended testis were included to study. Fertile men (n = 200) were investigated as a control. Genomic DNA, which was extracted from blood samples were investigated with a fluorescent multiplex PCR protocol for screening for Y microdeletions. **RESULTS:** A single STS missing was found in eight men; one from the control group (sY153), seven from the patients group. The positive cases showed a single STS missing of marker sY153 and sY139 in testicular cancer (6/185) and undescended testis (1/40) patients, respectively. **CONCLUSIONS:** Since no contiguous, real Y microdeletions were found in the study population, it seems that Y microdeletions are not a likely common etiological cause of poor spermatogenesis in testicular cancer and undescended testis. However, it remains to be determined whether men having a single STS missing have a risk of developing testis cancer or having undescended testis.

Bredenbeck, A., V. M. Hollstein, et al. (2008). "Coordinated expression of clustered cancer/testis genes encoded in a large inverted repeat DNA structure." *Gene* **415**(1-2): 68-73.

Cancer/testis antigens (CTA) are expressed in cancers and testis or placenta only and, therefore are considered promising targets for cancer immunotherapy and diagnosis. One family of CTA is the MAGEA family which comprises 13 members and was shown to be expressed synchronously with members from the CSAG (TRAG-3) family of CTA. The MAGEA genes are arranged in 4 subclusters located on the X chromosome. Subcluster III exposes a remarkable gene organization with an inverted repeat (IR) DNA structure of a triplicated couplet of a MAGEA gene and a CSAG gene. Analyzing the mRNA expression pattern of all genes of the MAGEA and CSAG family of cancer/testis genes, we show that the MAGEA and CSAG genes encoded in the large IR are expressed coordinately and independent from the

MAGEAs encoded outside the IR. These results reinforce our hypothesis that the large MAGEA/CSAG-IR DNA structure has an impact on the regulation of gene expression.

Caballero, O. L. and Y. T. Chen (2009). "Cancer/testis (CT) antigens: potential targets for immunotherapy." *Cancer Sci* **100**(11): 2014-21.

Cancer/testis (CT) antigens are protein antigens with normal expression restricted to adult testicular germ cells, and yet are aberrantly activated and expressed in a proportion of various types of human cancer. At least a subset of this group of antigens has been found to elicit spontaneous humoral and cell-mediated immune responses in cancer patients, raising the possibility that these antigens could be cancer vaccine targets. More than 100 CT antigen genes have been reported in the literature, with approximately 30 being members of multigene families on the X chromosome, so-called CT-X genes. Most CT-X genes are expressed at the spermatogonia stage of spermatogenesis, and their functions are mostly unknown. In cancer, the frequency of CT antigen expression is highly variable among different tumor types, but is more often expressed in high-grade late-stage cases in general. Cancer vaccine trials based on CT antigens MAGE-A3 and NY-ESO-1 are currently ongoing, and these antigens may also play a role in antigen-specific adoptive T-cell transfer and in the immunomodulation approach of cancer therapy.

Calabro, L., E. Fonsatti, et al. (2005). "Methylation-regulated expression of cancer testis antigens in primary effusion lymphoma: immunotherapeutic implications." *J Cell Physiol* **202**(2): 474-7.

Primary effusion lymphoma (PEL) is a large B-cell neoplasm with an unfavorable prognosis and limited therapeutic options. In this study, cancer testis antigens (CTA) were investigated as potential immunotherapeutic targets in patients with PEL. Baseline expression of a panel of 11 CTA was highly heterogeneous among five PEL cell lines. In particular, the investigated CTA were not expressed in BC-2 and BC-3 cells, while BC-1, HBL-6, and BCBL-1 cells tested positive for 6, 8, and 9 CTA, respectively. The DNA hypomethylating agent 5-aza-2'-deoxycytidine (5-AZA-CdR) invariably induced or up-regulated the expression of all investigated CTA in all cell lines analyzed. The de novo expression of CTA was still detectable at mRNA and protein level at least 2 months after the end of 5-AZA-CdR treatment. These findings, and the concomitant up-regulation of HLA-class I antigens and ICAM-1 by 5-AZA-CdR, support its clinical use to set-up innovative chemo-immunotherapeutic approaches in PEL.

Casey, R. G., M. Aktar, et al. (2008). "A prospective 10 year audit of a single Irish centre's experience of retroperitoneal lymph node dissection for metastatic testis cancer." *Surgeon* 6(5): 294-6.

**BACKGROUND:** Retro-peritoneal lymph node dissection (RPLND) following chemotherapy is critical in advanced germ cell tumours with residual retro-peritoneal masses. Post-chemotherapy RPLND is more extensive, may require adjacent organ resection and has higher morbidity. The study aim was to analyse patient demographics, clinical stage, surgical procedures and cure rates following RPLND. **METHODS:** An RPLND database (1994-2005) was analysed prospectively for demographics, pre/post-RPLND staging, chemotherapy regimen, cure, follow-up and early/late morbidity and mortality. **RESULTS:** 73 patients were identified (range 17-49 median 25.7). The mean hospital stay was 14.3 days (range 6-50). Clinical stage at presentation was; IV (16), III (19), II (27), I (11) and prior to RPLND was IV (12), III (6), II (55), I (0). Eleven patients with stage I disease progressed prior to RPLND. Seventy-one patients received cisplatin-based chemotherapy with partial response (49), minimal response (14), no response (7), disease progression (3) and 13 patients required salvage chemotherapy. RPLND was bilateral (26), unilateral (36) and suprahilar (11) with nerve sparing in 10. Other major procedures included nephrectomy (22), aortic graft (1), ureterectomy (1) and caval dissection (1). RPLND histology was mature teratoma (MT) (37), fibrosis/necrosis (26), NSGCT (6), seminoma (1), mixed NSGCT/teratoma (1), sarcoma (1) and mixed seminoma/teratoma (1). Early (n = 26) and late (n = 13) morbidity was significant but expected. There was no mortality. Ninety-five per cent had complete remission following RPLND (mean follow-up 30 months). One patient is deceased following relapse. **CONCLUSIONS:** The decision to perform post-chemotherapy RPLND depends on the possibility of viable tumour or teratoma and surgical morbidity. Appropriate case selection and timely intervention in an experienced centre permits optimum outcome.

Chen, Y. T., B. Alpen, et al. (2003). "Identification and characterization of mouse SSX genes: a multigene family on the X chromosome with restricted cancer/testis expression." *Genomics* 82(6): 628-36.

Human SSX was first identified as the gene involved in the t(X;18) translocation in synovial sarcoma. SSX is a multigene family, with 9 complete genes on chromosome Xp11. Normally expressed almost exclusively in testis, SSX mRNA is expressed in various human tumors, defining SSX as a cancer/testis antigen. We have now cloned the mouse ortholog of SSX. Mouse SSX genes can be divided

into Ssxa and Ssxb subfamilies based on sequence homology. Ssxa has only one member, whereas 12 Ssxb genes, Ssxb1 to Ssxb12, were identified by cDNA cloning from mouse testis and mouse tumors. Both Ssxa and Ssxb are located on chromosome X and show tissue-restricted mRNA expression to testis among normal tissues. All putative human and mouse SSX proteins share conserved KRAB and SSX-RD domains. Mouse tumors were found to express some, but not all, Ssxb genes, similar to the SSX activation in human tumors.

Chen, Y. T., M. Hsu, et al. (2009). "Cancer/testis antigen CT45: analysis of mRNA and protein expression in human cancer." *Int J Cancer* 124(12): 2893-8.

CT45 is a cancer/testis gene that we previously identified by massively parallel signature sequencing. Encoded by a multigene family on chromosome X, CT45 showed restricted mRNA expression to normal testis and various cancers. In this study, monoclonal antibodies were generated against recombinant CT45 protein, and CT45 protein expression in normal and tumor tissues was evaluated by immunohistochemical analysis. In adult normal tissue, CT45 expression was restricted to testicular germ cells, detected as a nuclear protein mainly at the stage of primary spermatocytes. In tumors, CT45 protein expression correlated with the mRNA levels detected by quantitative RT-PCR, and most lung cancer and ovarian cancers with CT45 mRNA at levels >1% of testicular expression were CT45 protein-positive. In positive cases, CT45 showed expression patterns that ranged from diffuse strong staining to heterogeneous and patchy expression. In lung cancer, CT45 expression was least frequent in adenocarcinoma, more frequent in squamous cell carcinoma and neuroendocrine tumors. Using tissue microarrays, 376 lung cancer, 219 ovarian cancer and 155 breast cancer were evaluated for CT45 protein expression. The expression frequency was highest in ovarian cancer (37%), followed by lung cancer (13%) and lowest in breast cancer (<5%). Given the focal nature of CT45 expression in many cases, these numbers represented the minimal frequency of expression in these tumor types. In summary, the expression frequency and characteristics of CT45 expression are similar to other CT cancer vaccine targets currently in clinical trials, e.g., NY-ESO-1 and MAGE-A, suggesting CT45 as a potentially useful cancer target.

Chen, Y. T., C. Iseli, et al. (2006). "Identification of a new cancer/testis gene family, CT47, among expressed multicopy genes on the human X

chromosome." Genes Chromosomes Cancer **45**(4): 392-400.

Cancer/testis (CT) genes are normally expressed in germ cells only, yet are reactivated and expressed in some tumors. Of the approximately 40 CT genes or gene families identified to date, 20 are on the X chromosome and are present as multigene families, many with highly conserved members. This indicates that novel CT gene families may be identified by detecting duplicated expressed genes on chromosome X. By searching for transcript clusters that map to multiple locations on the chromosome, followed by in silico analysis of their gene expression profiles, we identified five novel gene families with testis-specific expression and >98% sequence identity among family members. The expression of these genes in normal tissues and various tumor cell lines and specimens was evaluated by qualitative and quantitative RT-PCR, and a novel CT gene family with at least 13 copies was identified on Xq24, designated as CT47. mRNA expression of CT47 was found mainly in the testes, with weak expression in the placenta. Brain tissue was the only positive somatic tissue tested, with an estimated CT47 transcript level 0.09% of that found in testis. Among the tumor specimens tested, CT47 expression was found in approximately 15% of lung cancer and esophageal cancer specimens, but not in colorectal cancer or breast cancer. The putative CT47 protein consists of 288 amino acid residues, with a C-terminus rich in alanine and glutamic acid. The only species other than human in which a gene homologous to CT47 has been detected is the chimpanzee, with the predicted protein showing approximately 80% identity in its carboxy terminal region.

Chen, Y. T., M. J. Scanlan, et al. (2005). "Identification of cancer/testis-antigen genes by massively parallel signature sequencing." Proc Natl Acad Sci U S A **102**(22): 7940-5.

Massively parallel signature sequencing (MPSS) generates millions of short sequence tags corresponding to transcripts from a single RNA preparation. Most MPSS tags can be unambiguously assigned to genes, thereby generating a comprehensive expression profile of the tissue of origin. From the comparison of MPSS data from 32 normal human tissues, we identified 1,056 genes that are predominantly expressed in the testis. Further evaluation by using MPSS tags from cancer cell lines and EST data from a wide variety of tumors identified 202 of these genes as candidates for encoding cancer/testis (CT) antigens. Of these genes, the expression in normal tissues was assessed by RT-PCR in a subset of 166 intron-containing genes, and those with confirmed testis-predominant expression were

further evaluated for their expression in 21 cancer cell lines. Thus, 20 CT or CT-like genes were identified, with several exhibiting expression in five or more of the cancer cell lines examined. One of these genes is a member of a CT gene family that we designated as CT45. The CT45 family comprises six highly similar (>98% cDNA identity) genes that are clustered in tandem within a 125-kb region on Xq26.3. CT45 was found to be frequently expressed in both cancer cell lines and lung cancer specimens. Thus, MPSS analysis has resulted in a significant extension of our knowledge of CT antigens, leading to the discovery of a distinctive X-linked CT-antigen gene family.

Chen, Y. T., C. A. Venditti, et al. (2005). "Identification of CT46/HORMAD1, an immunogenic cancer/testis antigen encoding a putative meiosis-related protein." Cancer Immun **5**: 9.

Transcripts with ESTs derived exclusively or predominantly from testis, and not from other normal tissues, are likely to be products of genes with testis-restricted expression, and are thus potential cancer/testis (CT) antigen genes. A list of 371 genes with such characteristics was compiled by analyzing publicly available EST databases. RT-PCR analysis of normal and tumor tissues was performed to validate an initial selection of 20 of these genes. Several new CT and CT-like genes were identified. One of these, CT46/HORMAD1, is expressed strongly in testis and weakly in placenta; the highest level of expression in other tissues is <1% of testicular expression. The CT46/HORMAD1 gene was expressed in 31% (34/109) of the carcinomas examined, with 11% (12/109) showing expression levels >10% of the testicular level of expression. CT46/HORMAD1 is a single-copy gene on chromosome 1q21.3, encoding a putative protein of 394 aa. Conserved protein domain analysis identified a HORMA domain involved in chromatin binding. The CT46/HORMAD1 protein was found to be homologous to the prototype HORMA domain-containing protein, Hop1, a yeast meiosis-specific protein, as well as to asy1, a meiotic synaptic mutant protein in *Arabidopsis thaliana*.

Chitale, D. A., A. A. Jungbluth, et al. (2005). "Expression of cancer-testis antigens in endometrial carcinomas using a tissue microarray." Mod Pathol **18**(1): 119-26.

Cancer-testis (CT) antigens are expressed in a variety of malignant tumors, but in normal adult tissue, they are only expressed in testicular germ cells. Owing to this tumor-associated expression pattern, these antigens are of major interest as potential targets for immunotherapy and possibly for diagnostic purposes. This study was performed to analyze the expression of four CT antigens, NY-ESO-1, MAGE-

A3, MAGE-A4, and CT7/MAGE-C1, in endometrial carcinoma using immunohistochemistry, and to correlate expression with histologic subtypes, grade, and expression of WT1 and p53. Formalin-fixed paraffin-embedded tissues of 130 endometrial carcinomas of the following types and grades were analyzed using a tissue microarray: 85 endometrioid carcinomas (FIGO grade 1, 39; grade 2, 11; and grade 3, 35), 18 papillary serous carcinomas, 12 clear cell carcinomas, 13 malignant mixed müllerian tumors, one mucinous adenocarcinoma, and one undifferentiated carcinoma. The following anti-CT monoclonal antibodies/antigens were studied by immunohistochemistry: monoclonal antibody ES121/NY-ESO-1, monoclonal antibody M3H67/MAGE-A3, monoclonal antibody 57B/MAGE-A4, and monoclonal antibody CT7-33/CT7. The CT expression data were compared to WT1 and p53 protein expression as analyzed in a previous study. Positive staining with anti-CT monoclonal antibodies was graded as follows: focal, <5% positive cells; 1+, 5-25% cells; 2+, 26-50% cells; 3+, 51-75%; and 4+, >75% cells. The 3+ and 4+ staining patterns were considered homogeneous patterns of potential clinical significance and were scored positive for statistical analysis. In low-grade tumors, the most immunoreactivity was seen with mAb M3H67 but little labeling was observed with the other monoclonal antibodies. In high-grade tumors, monoclonal antibodies M3H67 (25%), 57B (23%), and CT7-33 (20%) showed the highest reactivity, while ES121 showed the lowest immunoreactivity (6%). The staining pattern was mostly heterogeneous. Statistical significance was found solely for the correlation of monoclonal antibody 57B staining and p53 expression. No correlation was found for any anti-CT monoclonal antibody staining and clinical stage or for anti-CT staining and WT1 expression. CT antigens CT7, MAGE-A3 and MAGE-A4, but not NY-ESO-1, are expressed in high-grade endometrial carcinomas, and expression of MAGE-A4 is correlated with the presence of overexpressed p53.

Cho, B., H. Lee, et al. (2003). "Promoter hypomethylation of a novel cancer/testis antigen gene CAGE is correlated with its aberrant expression and is seen in premalignant stage of gastric carcinoma." *Biochem Biophys Res Commun* **307**(1): 52-63.

Previously, we reported the identification and characterization of a novel cancer/testis antigen gene, CAGE(4), that was expressed in various histological types of tumors, but not in normal tissues, with the exception of the testis. To date, molecular mechanisms for the expression of CAGE have never been studied. In our expression analysis, we found that some cancer cell lines did not express CAGE. The

expression of CAGE could be restored in these cell lines by treatment with 5'(-)-aza-2'(-)-deoxycytidine, suggesting that the expression of CAGE is mainly suppressed by hypermethylation. Bisulfite sequencing analysis of the 16 CpG sites of the CAGE promoter in various cancer cell lines and tissues revealed a close relationship between the methylation status of the CAGE promoter and the expression of CAGE. The transient transfection experiments displayed that the methylation of CpG sites inhibited the CAGE promoter activity in luciferase reporter assays. The methylation of the CpG sites inhibited the binding of transcription factors, shown by a mobility shift assay. A methylation-specific PCR analysis revealed that hypomethylation of the CAGE promoter was present at frequencies of more than 60% in breast, gastric, and lung cancers, and hepatocellular carcinomas, and at frequencies of less than 40% in prostate, uterine cervical, and laryngeal cancers. Promoter hypomethylation was found in chronic gastritis (19/55, 34.5%) and liver cirrhosis (13/22, 59%), but not in normal prostate, normal colon, or chronic hepatitis. These results suggest that the methylation status of the CpG sites of CAGE determines its expression, that the hypomethylation of CAGE precedes the development of gastric cancer and hepatocellular carcinoma, and that the high frequencies of hypomethylation of CAGE, in various cancers would be valuable as a cancer diagnostic marker.

Cho, B., Y. Lim, et al. (2002). "Identification and characterization of a novel cancer/testis antigen gene CAGE." *Biochem Biophys Res Commun* **292**(3): 715-26.

We applied serological analysis of cDNA expression library technique to identify cancer-associated genes. We screened cDNA expression libraries of human testis and gastric cancer cell lines with sera of patients with gastric cancers. We identified a gene whose expression is testis-specific among normal tissues. We cloned and characterized this novel gene. It contains D-E-A-D box domain and encodes a putative protein of 630 amino acids with possible helicase activity. It showed wide expression in various cancer tissues and cancer cell lines. The corresponding gene was named cancer-associated gene (CAGE). PCR of human x hamster Radiation Hybrids showed localization of CAGE on the human chromosome Xp22. Transient transfection of CAGE showed predominantly nuclear localization. Both Western blot and plaque assay indicated seroreactivity of CAGE protein. We found that demethylation played a role in the activation of CAGE in some cancer cell lines that do not express it. Cell synchronization experiments showed that the

expression of CAGE was related with cell cycle. This suggests that CAGE might play a role in cellular proliferation. Because CAGE is expressed in a variety of cancers but not in normal tissues except testis, this gene can be a target of antitumor immunotherapy.

Cho, H. J., O. L. Caballero, et al. (2006). "Physical interaction of two cancer-testis antigens, MAGE-C1 (CT7) and NY-ESO-1 (CT6)." *Cancer Immunol* **6**: 12.

Cancer/testis (CT) antigens are the protein products of germ line-associated genes that are activated in a wide variety of tumors and can elicit autologous cellular and humoral immune responses. CT antigens can be divided between those that are encoded on the X chromosome (CT-X antigens) and those that are not (non-X CT antigens). Among the CT-X antigens, the melanoma antigen gene (MAGE) family, defined by a shared MAGE homology domain (MHD), is the largest. CT-X genes are frequently expressed in a coordinate manner in cancer cells, and their expression appears to be modulated by epigenetic mechanisms. The expression of CT-X genes is associated with advanced disease and poor outcome in different tumor types. We used the yeast two-hybrid system to identify putative MHD-interacting proteins. The MHD of MAGE-C1 (CT7) was used as bait to screen a human testis cDNA library. This study identified NY-ESO-1 (CT6) as a MAGE-C1 binding partner. Immunoprecipitation and immunofluorescence staining confirmed MAGE-C1 interaction with NY-ESO-1, and cytoplasmic colocalization of both proteins in melanoma cells. Co-expression of these two genes was found to occur in cancer cell lines from different origins, as well as in primary tumors (multiple myeloma and non-small cell lung cancer samples). This is the first report of direct interaction between two CT antigens and may be pertinent in the light of the frequently coordinated expression of these proteins.

Condomines, M., D. Hose, et al. (2007). "Cancer/testis genes in multiple myeloma: expression patterns and prognosis value determined by microarray analysis." *J Immunol* **178**(5): 3307-15.

Cancer-testis (CT) Ags are expressed in testis and malignant tumors but rarely in nongametogenic tissues. Due to this pattern, they represent attractive targets for cancer vaccination approaches. The aims of the present study are: 1) to assess the expression of CT genes on a pangenomic base in multiple myeloma (MM); 2) to assess the prognosis value of CT gene expression; and 3) to provide selection strategies for CT Ags in clinical vaccination trials. We report the expression pattern of CT genes in purified MM cells (MMC) of 64 patients with newly diagnosed MM and 12 patients with monoclonal gammopathy of

unknown significance, in normal plasma cell and B cell samples, and in 20 MMC lines. Of the 46 CT genes interrogated by the Affymetrix HG-U133 set arrays, 35 are expressed in the MMC of at least one patient. Of these, 25 are located on chromosome X. The expression of six CT genes is associated with a shorter event-free survival. The MMC of 98% of the patients express at least one CT gene, 86% at least two, and 70% at least three CT genes. By using a set of 10 CT genes including KM-HN-1, MAGE-C1, MAGE-A3/6/12, MAGE-A5, MORC, DDX43, SPACA3, SSX-4, GAGE-1-8, and MAGE-C2, a combination of at least three CT genes-desirable for circumventing tumor escape mechanisms-is obtained in the MMC of 67% of the patients. Provided that the immunogenicity of the products of these 10 CT genes is confirmed, gene expression profiling could be useful in identifying which CT Ags could be used to vaccinate a given patient.

Condomines, M., D. Hose, et al. (2009). "Gene expression profiling and real-time PCR analyses identify novel potential cancer-testis antigens in multiple myeloma." *J Immunol* **183**(2): 832-40.

Cancer-testis (CT) Ags are attractive targets for immunotherapeutic strategies since they are aberrantly expressed in malignant cells and not, or in limited number, in somatic tissues, except germ cells. To identify novel CT genes in multiple myeloma, we used Affymetrix HG-U133 gene expression profiles of 5 testis, 64 primary multiple myeloma cells (MMC), and 24 normal tissue samples. A 5-filter method was developed to keep known CT genes while deleting non-CT genes. Starting from 44,928 probe sets, including probe sets for 18 previously described CT genes, we have obtained 82 genes expressed in MMC and testis and not detected in more than 6 normal tissue samples. This list includes 14 of the 18 known CT genes and 68 novel putative CT genes. Real-time RT-PCR was performed for 34 genes in 12 normal tissue samples, 5 MMC samples, and one sample of five pooled testes. It has validated the CT status of 23 of 34 genes (67%). We found one novel "testis-restricted" gene (TEX14, expression in testis and tumor only), eight "tissue-restricted" (mRNA detected in one or two nongametogenic tissues), and seven "differentially expressed" (mRNA detected in three to six nongametogenic tissues) CT genes. Further studies are warranted to determine the immunogenicity of these novel CT Ag candidates.

Cooper, D. E., O. L'Esperance J, et al. (2008). "Testis cancer: a 20-year epidemiological review of the experience at a regional military medical facility." *J Urol* **180**(2): 577-81; discussion 581-2.

**PURPOSE:** Testis cancer is the most common solid malignancy in the young adult population and the incidence in this population is increasing. We present a 20-year epidemiological review of testis cancers treated at our institution. **MATERIALS AND METHODS:** The records of testis cancer cases diagnosed between January 1988 and June 2007 were reviewed. Patient demographics, cancer histology and stage, adjuvant therapy, temporal trends and survival data are presented. Our experience was compared to trends published in the SEER (Surveillance, Epidemiology and End Results) database and the National Cancer Database. **RESULTS:** A total of 338 testis cancers (330 germ cell tumors) were diagnosed during the study period. Median patient age at diagnosis was 26.6 years vs 34 in the SEER database. We observed a temporal increase in stage I tumors (57% to 75%) and a decrease in the proportion of seminomas (52% to 43%) during the study period. In terms of adjuvant therapy for stage I seminoma the use of radiotherapy decreased (91% to 75%), while the use of chemotherapy increased (1.5% to 7.5%). For stage I nonseminomatous germ cell tumors the use of adjuvant chemotherapy increased (12% to 20%), while the use of staging retroperitoneal lymph node dissection decreased (88% to 63%). Five-year cancer specific survival was 97.7%. **CONCLUSIONS:** We are seeing an increase in localized disease at diagnosis, an increase in surveillance for stage I disease and 5-year survival in excess of 95%, similar to data in SEER and the National Cancer Database. However, unlike in SEER and the National Cancer Database, our patients are younger, we are seeing less seminoma and we are performing significantly more staging retroperitoneal lymph node dissection.

Coral, S., L. Sigalotti, et al. (2002). "5-aza-2'-deoxycytidine-induced expression of functional cancer testis antigens in human renal cell carcinoma: immunotherapeutic implications." *Clin Cancer Res* 8(8): 2690-5.

**PURPOSE:** Limited therapeutic options are presently available for advanced renal cell carcinoma (RCC). This study was designed to define the clinical potential of the DNA hypomethylating agent 5-aza-2'-deoxycytidine (5-AZA-CdR) in human RCC, through its control of the expression of "therapeutic targets" of the cancer testis antigen (CTA) family, and of the tumor-associated antigen RAGE-1, in RCC cells. **EXPERIMENTAL DESIGN:** Reverse transcription (RT)-PCR assays of a panel of RCC cells treated with 5-AZA-CdR, investigated the induction of the expression of several CTAs and of RAGE-1. Immunoprecipitation and Western blotting assessed whether the expression of CTA-specific mRNA

induced by 5-AZA-CdR resulted in a translated protein of appropriate molecular weight. The functional activity of de novo expressed CTA was evaluated using (51)Cr release cytotoxicity assays of 5-AZA-CdR-treated HLA-A2-positive RCC cells using HLA-A2-restricted NY-ESO-1-specific CTLs. **RESULTS:** Exposure to 5-AZA-CdR invariably induced the expression of the CTA MAGE-1, -2, -3, and -4, GAGE 1-6, and NY-ESO-1 in all of the RCC cells investigated. De novo expression of NY-ESO-1 was persistent, being still detectable 60 days after the end of treatment, and generated a functional protein efficiently recognized by HLA-A2-restricted NY-ESO-1-specific CTLs. 5-AZA-CdR also induced RAGE-1 expression in RAGE-1-negative RCC and sarcoma cells but not in neoplastic cells of different histology. **CONCLUSIONS:** This study provides the scientific rationale to establish new strategies of chemoimmunotherapy in RCC patients. The well-defined immunogenicity of the investigated CTAs and of RAGE-1 suggest that systemic administration of 5-AZA-CdR represents a promising strategy to enhance the constitutively poor immunogenic potential of RCC cells, and to propose that virtually all RCC patients receive active and/or adoptive CTA- or RAGE-1-based immunotherapy.

Correa, J. J., C. Politis, et al. (2007). "Laparoscopic retroperitoneal lymph node dissection in the management of testis cancer." *Cancer Control* 14(3): 258-64.

**BACKGROUND:** The surgical approach to management of testis cancer has been traditionally through an open incision, but in the last decade, several centers have reported their experience with laparoscopic retroperitoneal lymph node dissection (LRPLND). **METHODS:** We reviewed the English literature, summarized the outcomes, and included our initial experience with the LRPLND procedure. **RESULTS:** Improvements in operative time, complications, and morbidity have occurred as surgical experience has increased. The procedure is more challenging in postchemotherapy patients. Outcomes at our institute are comparable to reported series from other institutions, and LRPLND is our current procedure of choice for RPLND. **CONCLUSIONS:** LRPLND has been shown to be a safe, effective, minimally invasive procedure in the management of testicular cancer patients who require surgery to address the retroperitoneal lymph nodes.

Corvin, S., W. Sturm, et al. (2005). "Laparoscopic retroperitoneal lymph-node dissection with the waterjet is technically feasible and safe in testis-cancer patient." *J Endourol* 19(7): 823-6.



**BACKGROUND AND PURPOSE:** The acceptance of open retroperitoneal lymph node dissection (RPLND) for stage I and II nonseminomatous testicular cancer has decreased because of the intraoperative and postoperative morbidity of the procedure. Laparoscopic RPLND is a minimally invasive and safe alternative for low-stage germ-cell tumors. It is, however, technically demanding and should therefore be performed only in experienced centers. The purpose of the present study was to evaluate the waterjet technique for laparoscopic RPLND. **PATIENTS AND METHODS:** A series of 18 patients with clinical stage I testis cancer (group A) and 7 patients who had received chemotherapy for stage II disease (group B) underwent laparoscopic RPLND at our institution. The procedure was performed identically to the open approach using the modified template according to Weissbach and associates. The waterjet was used for removal of lymphatic tissue from the aorta and the vena cava, as well as from the sympathetic trunk. **RESULTS:** The operation was completed in all patients without conversion to open surgery. The mean operating time was 232 +/- 48 minutes. The waterjet was able to remove lymphatic tissue easily and atraumatically. At pressures of 20 bar, the lymph-node capsule remained completely intact, thus avoiding tumor-cell spread. Antegrade ejaculation could be preserved in all patients, who, to date, show no evidence of disease. **CONCLUSIONS:** The waterjet allows the safe and complete removal of lymphatic tissue, leaving vulnerable anatomic structures intact. It can decrease the learning curve of laparoscopic RPLND and contribute to better acceptance of this procedure.

Costa, F. F., K. Le Blanc, et al. (2007). "Concise review: cancer/testis antigens, stem cells, and cancer." *Stem Cells* **25**(3): 707-11.

In the multistep process of cancer development, the concept that cancer stem cells are derived from normal stem cells that have gradually accumulated various genetic and epigenetic defects is gaining strong evidence. A number of investigations have identified molecular markers that, under normal conditions, are responsible for stem cell homeostasis but are also expressed in tumor "stem cell-like" subpopulations. In this regard, it was recently reported that a group of tumor-specific antigens known as cancer/testis antigens (CTAs) are expressed in human MSCs. It has long been stated that in normal tissue these antigens are exclusively expressed in germ cell precursors; however, based on these results, we suggest that CTAs are expressed at earlier stages during embryogenesis. The tumor-restricted expression of CTAs has led to several

immunotherapeutic trials targeting some of these proteins. The clinical implications that these trials may have on the normal stem cell pools, as well as the immunologic properties of these cells, is to date poorly studied and should be considered.

Cronwright, G., K. Le Blanc, et al. (2005). "Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression." *Cancer Res* **65**(6): 2207-15.

Several families of genes by and large located on the X chromosome encode proteins of unspecified function. Commonly known as cancer/testis (CT) antigens, they are considered, under normal conditions, only to be expressed in cells of the germ line and placenta. CT genes are also often expressed in cancer cells, hence their classification. Here we report that their expression in normal cells is wider spread and can be observed in cells with the potential for self-renewal and pluripotency, namely, stem cells. Several CT genes and their products, CT antigens, including SSX, NY-ESO-1, and N-RAGE, were expressed in undifferentiated mesenchymal stem cells (MSCs) and down-regulated after osteocyte and adipocyte differentiation. To elucidate the possible overlapping function played by these genes in cancer and stem cells, a comparative analysis of the localization of their proteins was made. In addition, localization relative to other MSC markers was examined. This revealed that SSX localizes in the cytoplasm and overlap occurs in regions where matrix metalloproteinase 2 (MMP2) and vimentin accumulate. Nevertheless, it was found that no protein interactions between these molecules occur. Further investigation revealed that the migration of a melanoma cell line (DFW), which expresses SSX, MMP2, and vimentin, decreases when SSX is down-regulated. This decrease in cell migration was paralleled by a reduction in MMP2 levels. Analogous to this, SSX expression is down-regulated in MSCs after differentiation; concomitantly a reduction in MMP2 levels occurs. In addition, E-cadherin expression increases, mimicking a mesenchymal epithelial transition. These results afford SSX a functional role in normal stem cell migration and suggest a potentially similar function in cancer cell metastases.

Dakshinamurthy, A. G., R. Ramesar, et al. (2008). "Infrequent and low expression of cancer-testis antigens located on the X chromosome in colorectal cancer: implications for immunotherapy in South African populations." *Biotechnol J* **3**(11): 1417-23.

Cancer-testis (CT) antigens are a group of tumor antigens that are expressed in the testis and

aberrantly in cancerous tissue but not in somatic tissues. The testis is an immune-privileged site because of the presence of a blood-testis barrier; as a result, CT antigens are considered to be essentially tumor specific and are attractive targets for immunotherapy. CT antigens are classified as the CT-X and the non-X CT antigens depending on the chromosomal location to which the genes are mapped. CT-X antigens are typically highly immunogenic and hence the first step towards tailored immunotherapy is to elucidate the expression profile of CT-X antigens in the respective tumors. In this study we investigated the expression profile of 16 CT-X antigen genes in 34 colorectal cancer (CRC) patients using reverse transcription-polymerase chain reaction. We observed that 12 of the 16 CT-X antigen genes studied did not show expression in any of the CRC samples analyzed. The other 4 CT-X antigen genes showed low frequency of expression and exhibited a highly variable expression profile when compared to other populations. Thus, our study forms the first report on the expression profile of CT-X antigen genes among CRC patients in the genetically diverse South African population. The results of our study suggest that genetic and ethnic variations in population might have a role in the expression of the CT-X antigen genes. Thus our results have significant implications for anti-CT antigen-based immunotherapy trials in this population.

de Wit, N. J., I. M. Cornelissen, et al. (2005). "The MMA1 gene family of cancer-testis antigens has multiple alternative splice variants: characterization of their expression profile, the genomic organization, and transcript properties." *Genes Chromosomes Cancer* **42**(1): 10-21.

Previously, we reported the identification of MMA1A by screening for differential gene expression in two human melanoma cell lines displaying diverse metastatic behavior after subcutaneous inoculation into nude mice. Splice variant MMA1B, which also was identified through database homology searches, showed a high degree of similarity with the MMA1A for exons 1, 2, and 4, but was missing exon 3. Through extensive expression profiling among normal and tumor samples, both MMA1A and -1B were found to belong to the family of cancer-testis antigens. In this study, we identified four additional alternatively spliced MMA1 variants, named MMA1C, MMA1D, MMA1E, and MMA1F. Generally, these novel MMA1 transcripts differ from MMA1A in that exon 2 or exon 3 is enlarged because of the use of alternative splice sites in intron 2 of the MMA1 gene. Moreover, MMA1E also lacks exon 3, as was previously seen in MMA1B. In screening for expression of the novel MMA1 transcripts in normal

and tumor tissues, we demonstrated that MMA1C, -1D, and -1E also are members of the cancer-testis antigen family. MMA1F was found in only one melanoma metastasis sample and therefore is believed to have been expressed incidentally. Furthermore, we comprehensively elucidated the genomic structure of the MMA1 gene and the characteristic features of the alternatively spliced MMA1 transcripts.

de Wit, N. J., P. Verschuure, et al. (2004). "Testis-specific human small heat shock protein HSPB9 is a cancer/testis antigen, and potentially interacts with the dynein subunit TCTEL1." *Eur J Cell Biol* **83**(7): 337-45.

Searching EST databases for new members of the human small heat shock protein family, we recently identified HSPB9, which is expressed exclusively in testis as determined by Northern blotting (Kappe et al., *Biochim. Biophys. Acta* **1520**, 1-6, 2001). Here we confirm this testis-specific expression pattern by RT-PCR in a larger series of normal tissues. Interestingly, while screening HSPB9 ESTs, we also noted expression in tumours, which could be verified by RT-PCR. Protein expression of HSPB9 was also detected in normal human testis and various tumour samples using immunohistochemical staining. We thus conclude that HSPB9 belongs to the steadily growing number of cancer/testis antigens. To get a better understanding of the function of HSPB9, we performed a yeast two-hybrid screen to search for HSPB9-interacting proteins. TCTEL1, a light chain component of cytoplasmic and flagellar dynein, interacted in both the yeast two-hybrid system and in immunoprecipitation experiments with HSPB9. Additionally, immunohistochemical staining showed co-expression of HSPB9 and TCTEL1 in similar stages of spermatogenesis and in tumour cells. The possible functional significance of this interaction is discussed.

de Wit, N. J., U. H. Weidle, et al. (2002). "Expression profiling of MMA-1a and splice variant MMA-1b: new cancer/testis antigens identified in human melanoma." *Int J Cancer* **98**(4): 547-53.

Using high-density oligonucleotide array analysis, we have recently compared the gene expression profiles of 2 human melanoma cell lines with marked difference in metastatic behavior after subcutaneous inoculation into nude mice (de Wit et al., *Melanoma Res*, in press). We identified an expressed sequence tag (EST), which we called malignant melanoma-associated 1 (MMA-1a), showing evident differential expression between the 2 cell lines. The MMA-1a gene is localized on chromosome 21q22.2 and its mRNA exists of 4 exons. Homology search displayed a splice variant of MMA-

1a that lacks exon 3 and that was called MMA-1b. Expression profiles of MMA-1a and MMA-1b are determined by reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Among 30 different normal tissue samples, expression of MMA-1a and MMA-1b was exclusively found in the testis after a first PCR of 30 cycles. Even more sensitive screening achieved by performing multiple semi-nested RT-PCR showed no or very low expression in the other normal tissues tested. During melanocytic tumor progression, MMA-1a and/or MMA-1b exhibited an emergence of expression in primary melanoma (20%) and melanoma metastasis samples (30%) after only 1 round of PCR. Expression of MMA-1a and/or MMA-1b was also identified in other tumor cell lines and fresh tumor samples of variable origin, e.g., lung, liver, bladder and soft tissues (sarcomas). We conclude that MMA-1a and MMA-1b are new members of the family of cancer/testis antigens.

de Wit, R. and K. Fizazi (2006). "Controversies in the management of clinical stage I testis cancer." *J Clin Oncol* **24**(35): 5482-92.

During the last two decades, definitive primary treatments and surveillance with definitive treatment deferred until relapse have demonstrated 98% to 99% cure rates in patients with stage I testis cancer, and these options have obtained firm positions in standard management. The development of optimal management strategies in various countries were at least partly guided by available surgical expertise in retroperitoneal lymph node dissection in the United States, and easy access to reference hospitals in densely populated countries in Western Europe that facilitated close surveillance programs; hence, treatment preferences differ on the two sides of the Atlantic. The success of both approaches is highly dependent on the skills of the practitioner, particularly of surgery and of scrutinized surveillance. As a result, local expertise and familiarity with a chosen modality has strengthened over the years, and investigators have been reluctant to embark on randomized trials designed to compare one modality with another. Such expertise with one particular technique, with the other approach being less familiar territory, has created controversy, because both physicians and patients seek evidence-based data coming from randomized clinical trials on which to make management decisions. Moreover, the reduced risk of relapse resulting from the use of radiotherapy or carboplatin in stage I seminoma and of cisplatin-based chemotherapy in stage I nonseminoma must be balanced against the potential long-term adverse effects in this population of patients with a normal life expectancy. The purpose of this review is to present the currently available data and discuss the merits and

the disadvantages of the various approaches, yielding to the possible conclusion that all options appear to be equal in terms of efficacy, but that modality-associated adverse effects differ.

Dieckmann, K. P., H. Lauke, et al. (2002). "Testicular germ cell cancer despite previous local radiotherapy to the testis." *Eur Urol* **41**(6): 643-9; discussion 649-50.

**BACKGROUND:** Testicular intraepithelial neoplasia (TIN, also carcinoma in situ of the testis) is the uniform precursor of testicular germ cell cancer. Local radiotherapy to the testis with dosages of 18-20 Gy has been found to safely eradicate TIN and germ cells, too. Thus, the general assumption is that the development of invasive germ cell tumours can be prevented by this radiotherapy. **PATIENTS AND METHODS:** Herein, we report two patients with one-sided testicular tumour and biopsy-proven contralateral TIN. Both of them developed germ cell neoplasms in the remaining testis although local radiotherapy with 20 Gy had been applied to the testis. **RESULTS:** One patient developed pure seminoma 7 years after completion of radiotherapy, the other developed a combined tumour consisting of embryonal carcinoma and seminoma after 5 years. Treatment consisted of orchiectomy in each of the cases. Histologically, both had TIN in the testicular tissue surrounding the new growths. **CONCLUSIONS:** Pathogenetically, a small fraction of radioresistant TIN cells overcoming irradiation and progressing to full-blown germ cell cancer in the later course may be the histogenetic clue to explain these unexpected events. Other explanations, though less probable, could be technical radiotherapeutic failure due to targeting problems and a pre-existing radioresistant germ cell tumour in the irradiated testicle.

Dieckmann, K. P., J. Linke, et al. (2007). "Spermatogenesis in the contralateral testis of patients with testicular germ cell cancer: histological evaluation of testicular biopsies and a comparison with healthy males." *BJU Int* **99**(5): 1079-85.

**OBJECTIVE:** To assess histologically signs of testicular dysgenesis (TD) in the contralateral testes of patients with testicular germ cell tumours (GCTs) and to compare these findings with the spermatogenic quality in healthy men, as the contralateral testis is considered to be involved with dysgenetic features such as poor sperm production, and accordingly, GCTs are hypothesized to be part of the 'TD syndrome' (TDS). One testicular biopsy is thought to represent spermatogenesis in the entire testis. We evaluated this view by using testicular two-site biopsies. **PATIENTS AND METHODS:** 2318

patients with testicular GCT had a contralateral testicular two-site biopsy. Testicular biopsies taken on forensic autopsy from 1388 presumably healthy men served as controls. Spermatogenesis was rated histologically according to a modified Johnsen score. Clinical factors were recorded to explore associations with reduced spermatogenesis. Differences in spermatogenesis scoring results among two-site biopsies were noted. Statistical analysis involved Wilcoxon-Mann-Whitney and Jonckheere-Terpstra tests for comparing patients and controls, and for studying associations with clinical factors. Classification and regression-tree analysis was used to explore multivariate associations. **RESULTS:** Histologically, patients had significantly poorer spermatogenesis than healthy men. Clinically, hypospermatogenesis was significantly associated with testicular atrophy, undescended testes, male infertility, and advanced clinical stage; 5.4% of cases (95% confidence interval 4.43-6.27) had discordant findings of >2 points on double biopsy and 9.8% had differences of 1 point. Discordance was significantly associated with poor spermatogenesis and testicular atrophy. **CONCLUSIONS:** We confirmed histologically that there is markedly reduced spermatogenesis in the contralateral testes of patients with GCT. This result lends credence to the view that GCT is part of the so-called TDS. But as hypospermatogenesis is associated with advanced clinical stage, impairment of sperm production might at least partly be acquired secondary to the endocrine activity of GCT. There were clinically relevant discordant results on double biopsy in 5.4%, predominantly in infertile patients and in atrophic testes. Thus the histological evaluation of male infertility is best done by multiple biopsies.

Domae, S., Y. Nakamura, et al. (2009). "Identification of CCDC62-2 as a novel cancer/testis antigen and its immunogenicity." *Int J Cancer* **124**(10): 2347-52.

Cancer/testis (CT) antigens are expressed in normal germ line tissues and various cancers. They are considered promising target molecules for immunotherapy for patients with various cancers. To identify CT antigens, we performed serological identification of antigens by recombinant expression cloning. The humoral immune response of cancer patients against a newly defined antigen was analyzed. A testicular cDNA library was immunoscreened with serum obtained from a gastric adenocarcinoma patient whose primary cancer had regressed once and most liver metastases had disappeared transiently. We isolated 55 positive cDNA clones comprising 23 different genes. They included 4 genes with testis-specific expression profiles in the Unigene database, including coiled-coil domain containing 62

(CCDC62). RT-PCR analysis showed that the expression of 2 splice variants of CCDC62 was restricted to the testis in normal adult tissues. In malignant tissues, CCDC62 variant 2 (CCDC62-2) was aberrantly expressed in a variety of cancers, including stomach cancer. A serological survey of 191 cancer patients with a range of different cancers by ELISA revealed antibodies to CCDC62-2 in 13 patients, including stomach cancer. None of the 41 healthy donor serum samples were reactive in the same test. The serum reaction against CCDC62-2 was confirmed by western blot. CCDC62-2 is a CT antigen that is immunogenic in cancer patients.

Duale, N., B. Lindeman, et al. (2007). "Molecular portrait of cisplatin induced response in human testis cancer cell lines based on gene expression profiles." *Mol Cancer* **6**: 53.

**BACKGROUND:** Testicular germ cell tumors (TGCTs) respond well to cisplatin-based chemotherapy and show a low incidence of acquired resistance compared to most somatic tumors. The reasons for these specific characteristics are not known in detail but seem to be multifactorial. We have studied gene expression profiles of testicular and colon cancer derived cell lines treated with cisplatin. The main goal of this study was to identify novel gene expression profiles with their functional categories and the biochemical pathways that are associated with TGCT cells' response to cisplatin. **RESULTS:** Genes that were differentially expressed between the TGCT cell lines vs the (somatic) HCT116 cell line, after cisplatin treatment, were identified using the significance analysis of microarrays (SAM) method. The response of TGCT cells was strikingly different from that of HCT116, and we identified 1794 genes that were differentially expressed. Functional classification of these genes showed that they participate in a variety of different and widely distributed functional categories and biochemical pathways. Database mining showed significant association of genes (n = 41) induced by cisplatin in our study, and genes previously reported to be expressed in differentiated TGCT cells. We identified 37 p53-responsive genes that were altered after cisplatin exposure. We also identified 40 target genes for two microRNAs, hsa-mir-372 and 373 that may interfere with p53 signaling in TGCTs. The tumor suppressor genes NEO1 and LATS2, and the estrogen receptor gene ESR1, all have binding sites for p53 and hsa-mir-372/373. NEO1 and LATS2 were down-regulated in TGCT cells following cisplatin exposure, while ESR1 was up-regulated in TGCT cells. Cisplatin-induced genes associated with terminal growth arrest through senescence were identified, indicating associations which were not previously

described for TGCT cells. **CONCLUSION:** By linking our gene expression data to publicly available databases and literature, we provide a global pattern of cisplatin induced cellular response that is specific for testicular cancer cell lines. We have identified cisplatin-responsive functional classes and pathways, such as the angiogenesis, Wnt, integrin, and cadherin signaling pathways. The identification of differentially expressed genes in this study may contribute to a better understanding of the unusual sensitivity of TGCT to some DNA-damaging agents.

Dubovsky, J. A., M. R. Albertini, et al. (2007). "MAD-CT-2 identified as a novel melanoma cancer-testis antigen using phage immunoblot analysis." *J Immunother* **30**(7): 675-83.

One focus in the field of tumor immunology is the identification of cancer-specific antigens that might be exploited as therapeutic targets or as immunologic diagnostic markers. Cancer-testis antigens (CTAs) are of particular interest as potential target antigens given that their expression is typically restricted to germ cells among normal tissues, but aberrantly expressed in multiple tumor types. In the current report, we sought to evaluate serum antibody immune responses to a defined panel of CTA from multiple antigen families to identify potential tumor-specific antigens that could potentially serve as candidate target antigens for immunotherapy or diagnostic purposes. This was conducted by screening sera from male patients with metastatic melanoma (n=44) and volunteer blood donors (n=50) against a panel of lambda phage-encoded CTA. We found that IgG antibody responses occurred in 39% of patients with melanoma to at least one of these antigens compared with 4% of controls (P<0.001). We found antibody responses to one antigen, MAD-CT-2, occurred in 27% of patients compared with 0/50 controls (P<0.0001). These findings, along with the demonstration that MAD-CT-2 is expressed in melanoma cell lines, identified MAD-CT-2 as a novel melanoma CTA.

Dubovsky, J. A. and D. G. McNeel (2007). "Inducible expression of a prostate cancer-testis antigen, SSX-2, following treatment with a DNA methylation inhibitor." *Prostate* **67**(16): 1781-90.

**BACKGROUND:** Active immunotherapies are one approach being developed as novel treatments for prostate cancer. Critical to the success of these therapies is the identification of appropriate target antigens. We have been seeking to identify immunologically recognized proteins, cancer-testis antigens (CTA) in particular, in patients with prostate cancer that would be rational target antigens. **METHODS:** Using a previously reported panel of 29

different CTA, we used sera from 98 patients with prostate cancer and 50 healthy male blood donor controls to detect CTA-specific IgG. We then further evaluated the expression of one antigen, SSX-2, in prostate cancer cell lines and tissues. **RESULTS:** We identified IgG specific for NY-ESO-1, LAGE-1, NFX-2, and SSX-2 in at least 1/98 individuals with prostate cancer. We demonstrated that SSX-2 is a prostate CTA, and its expression is associated with metastatic prostate cancer. In addition, we report that the treatment of at least two human prostate cancer cell lines with the DNA methylation inhibitor 5-aza-2'-deoxycytidine induced the expression of SSX-2. In contrast, treatment of a normal prostate epithelial cell line (RWPE-1) with 5-aza-2'-deoxycytidine did not induce SSX-2 expression. **CONCLUSIONS:** Our findings suggest that SSX-2 could be further pursued as an immunotherapeutic target in prostate cancer, and that treatment with 5-aza-2'-deoxycytidine could be exploited to modulate antigen expression in combination with immunotherapeutic approaches.

Dubovsky, J. A., D. G. McNeel, et al. (2009). "Treatment of chronic lymphocytic leukemia with a hypomethylating agent induces expression of NXF2, an immunogenic cancer testis antigen." *Clin Cancer Res* **15**(10): 3406-15.

**PURPOSE:** Critical to the success of active immunotherapy against cancer is the identification of immunologically recognized cancer-specific proteins with low tolerogenic potential. Cancer testis antigens (CTA), in particular, fulfill this requirement as a result of their aberrant expression restricted to cancer cells and lack of expression in normal tissues bypassing tolerogenic mechanisms against self. Although CTAs have been extensively studied in solid malignancies, little is known regarding their expression in chronic lymphocytic leukemia (CLL). **EXPERIMENTAL DESIGN:** Using a two-pronged approach we evaluated the immunogenicity of 29 CTAs in 22 patients with CLL and correlated these results to reverse transcriptase PCR data from CLL cell lines and patient cells. **RESULTS:** We identified IgG-specific antibodies for one antigen, NXF2, and confirmed this response by ELISA and Western blot. We found that treatment of CLL with 5-aza-2'-deoxycytidine can induce expression of NXF2 that lasted for several weeks after treatment. Treatment also increased levels of MHC and costimulatory molecules (CD80, CD86, and CD40) necessary for antigen presentation. In addition, we identified other promising antigens that may have potential immunotherapeutic application. **CONCLUSIONS:** Our findings suggest that NXF2 could be further pursued as an immunotherapeutic target in CLL, and that treatment with demethylating agents could be

exploited to specifically modulate CTA expression and effective antigen presentation in malignant B cells.

Eggerer, S. E., Y. Lotan, et al. (2005). "Magnetic resonance angiography for the nonpalpable testis: a cost and cancer risk analysis." *J Urol* **173**(5): 1745-9; discussion 1749-50.

**PURPOSE:** For the unilateral nonpalpable testis standard management is open surgical or laparoscopic exploration. An ideal imaging technique would reliably identify testicular nubbins and safely allow children to forgo surgical exploration without compromising future health or fertility. Our goal was to perform a cost and risk analysis of magnetic resonance angiography (MRA) for unilateral nonpalpable cryptorchid testes. **MATERIALS AND METHODS:** A search of the English medical literature revealed 3 studies addressing the usefulness of MRA for the nonpalpable testicle. We performed a meta-analysis and applied the results to a hypothetical set of patients using historical testicular localization data. Analysis was then performed using 3 different management protocols-MRA with removal of testicular nubbin tissue, MRA with observation of testicular nubbin tissue and diagnostic laparoscopy. A cancer risk and cost analysis was then performed. **RESULTS:** MRA with observation of testicular nubbin tissue results in 29% of patients avoiding surgery without any increased cost of care. Among the 29% of boys with testicular nubbins left in situ and observed the highest estimated risk was 1 in 300 of cancer developing, and 1 in 5,300 of dying of cancer. **CONCLUSIONS:** A protocol using MRA with observation of inguinal nubbins results in nearly a third of boys avoiding surgical intervention at a similar cost to standard care without any significant increased risk of development of testis cancer.

Egland, K. A., V. Kumar, et al. (2002). "Characterization of overlapping XAGE-1 transcripts encoding a cancer testis antigen expressed in lung, breast, and other types of cancers." *Mol Cancer Ther* **1**(7): 441-50.

Cancer testis (CT) antigens have an expression pattern that is predominantly restricted to testis in normal tissues, yet they are expressed in many different histological types of cancers. One previously described member of the CT antigen family, XAGE-1, was shown to be expressed in Ewing's sarcomas and rhabdomyosarcomas. Here we show that XAGE-1 is also expressed in breast cancer, prostate cancer, and different types of lung cancers, including lung squamous cell carcinoma, adenocarcinoma, small cell lung carcinoma, and non-small cell lung carcinoma. In addition, XAGE-1

mRNA was present in ovarian cancer, melanoma, glioblastoma, T-cell lymphoma, chronic myelogenous leukemia, and histiocytic lymphoma cell lines. We also characterized the XAGE-1 transcript by primer extension analysis and found that transcription of the XAGE-1 gene is initiated from two distinct start sites, resulting in two overlapping transcripts, XAGE-1a and XAGE-1b. XAGE-1a contains two in-frame ATG translational start codons; whereas XAGE-1b initiates downstream of the first ATG start codon. Our results suggest that XAGE-1b is the dominant transcript, and that translation begins with the second ATG start codon, producing a 9 kDa protein. Because XAGE-1 is expressed in such a diverse range of cancers, it has potential to be used as a target for many cancer immunotherapies.

Ehrlich, Y., O. Yossepowitch, et al. (2006). "Distribution of nodal metastases after chemotherapy in nonseminomatous testis cancer: a possible indication for limited dissection." *BJU Int* **97**(6): 1221-4.

**OBJECTIVE:** To assess the clinical and pathological findings of patients treated by bilateral retroperitoneal lymph node dissection (RPLND) after chemotherapy, to identify a subset for whom modified template nodal resection might be contemplated, as bilateral RPLND is the treatment of choice in patients with residual retroperitoneal disease after chemotherapy for nonseminomatous germ-cell tumour (GCT). **PATIENTS AND METHODS:** The medical records were reviewed of 50 consecutive patients who had RPLND after chemotherapy between 1996 and 2005. Bilateral template RPLND was performed uniformly. Extracted lymph nodes were surgically stratified into three distinct anatomical zones by two sagittal planes running in front of the aorta and the inferior vena cava. The pathological findings were correlated with the side of the primary lesion and the extent of metastatic disease before chemotherapy. **RESULTS:** Pathological assessment of the resected lymph nodes revealed teratoma in 28 patients (56%), viable carcinoma in three (6%), and necrosis or fibrosis in 19 (38%). All clinical stage Is, IIA and IIB left-sided primary tumours followed a predictable pattern of spread constricted to a modified left-sided template. Patients with clinical stage IIC and III, or right-sided primary tumour, had a less predictable metastatic pattern, having crossover metastases to the contralateral template. **CONCLUSION:** Bilateral RPLND should be considered as the reference standard in patients with metastatic GCT and residual retroperitoneal mass after completing chemotherapy. However, the present data suggest that a modified template dissection might be considered even after chemotherapy in patients with left-sided primary

tumours and limited nodal involvement at presentation.

Enokizono, J., H. Kusuhara, et al. (2008). "Quantitative investigation of the role of breast cancer resistance protein (Bcrp/Abcg2) in limiting brain and testis penetration of xenobiotic compounds." *Drug Metab Dispos* **36**(6): 995-1002.

The role of breast cancer resistance protein (BCRP/ABCG2) in limiting the brain and testis penetration of xenobiotic compounds in the blood-brain and -testis barriers was investigated using Bcrp(-/-) mice. Tissue/plasma concentration ratios in the brain (K(p,brain)) and testis (K(p,testis)) obtained under steady-state conditions were significantly increased in Bcrp(-/-) mice for PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), N-hydroxyl PhIP, MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline), dantrolene, and prazosin. In addition, the K(p,brain) of triamterene and the K(p,testis) of 4'-hydroxyl PhIP were also significantly increased in Bcrp(-/-) mice. The effect of functional impairment of Bcrp on the brain uptake of PhIP, dantrolene, and daidzein in Bcrp(-/-) mice determined using in situ brain perfusion was weaker than that observed on the K(p) values. In vitro transcellular transport experiments using cell lines expressing mouse Bcrp or P-glycoprotein (Mdr1a/Abcb1a) showed that, among the tested Bcrp substrates, PhIP, MeIQx, prazosin, and triamterene are common substrates of Bcrp and P-glycoprotein. The K(p) values of common substrates exhibited a smaller increase both in the brain and testis of Bcrp(-/-) mice than expected from the in vitro Bcrp activities. The Bcrp-specific substrates were weak acids, whereas basic or neutral BCRP substrates were also P-glycoprotein substrates. These results suggest that BCRP limits the tissue penetration of xenobiotic compounds in the blood-brain and -testis barriers, but its in vivo importance is also modulated by P-glycoprotein activity.

Figueiredo, D. L., R. C. Mamede, et al. (2006). "Expression of cancer testis antigens in head and neck squamous cell carcinomas." *Head Neck* **28**(7): 614-9.

**BACKGROUND:** There is considerable interest in the expression of cancer testis (CT) antigens in human cancers, because they may serve as the basis for diagnostic tests or an immunologic approach to therapy, or as prognostic markers. **METHODS:** On this basis, we evaluated by semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) the expression of genes that code for tumor antigens (melanoma antigen-1 [MAGE-1], MAGE-4, MAGE-10, MAGE-12, B melanoma antigen, CTL-recognized antigen melanoma antigen (CT antigen 2) [LAGE], New York

esophageal squamous cell carcinoma antigen (CT antigen 1) [NYESO-1], and preferentially expressed antigen of melanoma [PRAME]) in surgical samples of the tumors, margins, and lymph nodes (when present) from patients with a diagnosis of head and neck carcinoma. The study was conducted on 33 patients (31 men and two women), aged 31 to 94 years (mean, 56 years), with squamous cell carcinomas located in the mouth (15 cases), larynx (14 cases), and pharynx (four cases). **RESULTS:** The findings were compared with the clinical course and laboratory data. Expression of at least one antigen was observed in 66.6% of cases, with different rates of expression according to tumor staging (100% of T4, 57% of T3, 50% of T1 and T2) and smoking habit. There was a significantly higher expression of multiple genes (two or more) in tumors in advanced stages. **CONCLUSIONS:** We conclude that the tumor-specific antigen genes are expressed in variable frequencies and intensities in the primary lesions of head and neck squamous cell carcinomas and in their metastases, with expression of the PRAME gene being always present in the metastatic lymph nodes. In primary lesions, gene expression correlated with smoking habit and with advanced tumors with a higher malignant potential, with the frequent expression of two or more of these genes.

Foell, J. L., M. Hesse, et al. (2008). "Membrane-associated phospholipase A1 beta (LIPI) Is an Ewing tumour-associated cancer/testis antigen." *Pediatr Blood Cancer* **51**(2): 228-34.

**BACKGROUND:** Cancer/testis antigens (CTA) represent a heterogeneous group of antigens expressed nearly exclusively in tumour cells and testis. Recently, we identified phospholipase A1 beta (a CTA also known as lipase member I, LIPI) as a gene with high expression in Ewing family tumours (EFT). In the present paper we analyzed expression of LIPI in a panel of normal tissues and tumour samples. **PROCEDURE:** The expression of CTA in EFT and normal tissues was analyzed by using DNA microarray datasets. Expression of LIPI in EFT, a panel of other tumour samples, and normal tissues was analyzed by using RT-PCR and quantitative RT-PCR. **RESULTS:** LIPI was expressed in EFT samples but not in other investigated tumour samples. Expression of LIPI in normal tissues was restricted to testis and thyroid. However, expression in these tissues was low compared with EFT. Interestingly testis as well as thyroid expressed all analyzed EFT-associated transcripts, suggesting that these tissues harbour a small cell population with molecular features of EFT. The sensitivity of the LIPI RT-PCR was similar to the sensitivity of the conventional EWSR1-FLI1 RT-PCR, suggesting that LIPI might be

useful as additional diagnostic target structure. CONCLUSIONS: The human cancer/testis antigen LIPI is highly expressed in Ewing family tumours and can be easily detected by RT-PCR or quantitative RT-PCR. LIPI might be an interesting target for the development of future diagnostic tools or treatment strategies.

Fossa, A., L. Alsoe, et al. (2004). "Serological cloning of cancer/testis antigens expressed in prostate cancer using cDNA phage surface display." *Cancer Immunol Immunother* **53**(5): 431-8.

Serological cloning of tumor-associated antigens (TAAs) using patient autoantibodies and tumor cDNA expression libraries (SEREX) has identified a wide array of tumor proteins eliciting B-cell responses in patients. However, alternative cloning strategies with the possibility of high throughput analysis of patient sera and tumor libraries may be of interest. We explored the pJuFo phage surface display system, allowing display of recombinant tumor proteins on the surface of M13 filamentous phage, for cloning of TAAs in prostate cancer (PC). Control experiments established that after a few rounds of selection on immobilized specific IgG, a high degree of enrichment of seroreactive clones was achieved. With an increasing number of selection rounds, a higher yield of positive clones was offset by an apparent loss of diversity in the repertoire of selected clones. Using autologous patient serum IgG in a combined biopanning and immunoscreening approach, we identified 13 different TAAs. Three of these (NY-ESO-1, Lage-1, and Xage-1) were known members of the cancer/testis family of TAAs, and one other protein had previously been isolated by SEREX in cancer types other than PC. Specific IgG responses against NY-ESO-1 were found in sera from 4/20 patients with hormone refractory PC, against Lage-1 in 3/20, and Xage-1 in 1/20. No reactivity against the remaining proteins was detected in other PC patients, and none of the TAAs reacted with serum from healthy subjects. The results demonstrate that phage surface display combined with postselection immunoscreening is suitable for cloning a diverse repertoire of TAAs from tumor tissue cDNA libraries. Furthermore, candidate TAAs for vaccine development of PC were identified.

Fradet, Y., V. Picard, et al. (2005). "Cancer-testis antigen expression in bladder cancer." *Prog Urol* **15**(6 Suppl 1): 1303-13.

PURPOSE: To evaluate the potential of cCancer-t/Testis antigens (CTAs) as targets for immunotherapy of bladder cancer, we evaluated the expression of 9 CTA genes or families of genes in normal urothelia, bladder tumours and bladder cancer

human bladder tissues cell lines. As expression of most CTAs is controlled by epigenetic mechanisms, we also evaluated the effect of the DNA methylase inhibitor 5-aza-2'-deoxycytidine (5-AZA-DC), and/or the histone deacetylase inhibitors Trichostatin A (TSA) on their expression in bladder cancer cell lines. MATERIAL AND METHODS: Expression of NY-ESO-1/LAGE-1, MAGE-A, MAGE-C1, BAGE, HOM-TES-85, SCP-1, SSX-1, SSX-2 and SSX-4 was analyzed by semi-quantitative RT-PCR and Western blotting on 10 normal urothelia, 23 24 superficial and 223 invasive tumours and on 10 cell lines treated with 5-aza-2'-deoxycytidine (5-AZA-DC) and/or Trichostatin A (TSA). RESULTS: Expression of all CTA genes could be observed in at least 1 tumour except for HOM-TES-85 for which mRNA was never detected. MAGE-A, BAGE and NY-ESO-1/LAGE-1 mRNAs were the most frequently detected, respectively in 5677%, 212% and 89% of superficial and in 6461%, 4139% and 276% of invasive tumours. With the exception of MAGE-A, CTA transcripts were rarely detected in the cell lines. However, expression of all CTA genes, except SCP-1, could be induced at various levels by the drugs and 5-AZA-DC was a much more potent inducer than TSA. CONCLUSION: These data suggest that immunotherapy of bladder cancer could target CTAs, especially those expressed at higher frequency such as MAGE-A, BAGE and NY-ESO-1/LAGE-1. Moreover, their induction by chemotherapeutic agents such as 5-AZA-DC, provides a potential pretreatment aimed at inducing the immunogenicity of the tumours.

Frank, C., M. Hundemer, et al. (2008). "Cellular immune responses against the cancer-testis antigen SPAN-XB in healthy donors and patients with multiple myeloma." *Leuk Lymphoma* **49**(4): 779-85.

The cancer-testis antigen SPAN-XB has been recently identified in multiple myeloma (MM). In the present study, we identified and characterized for the first time a cytotoxic cellular immune response against SPAN-XB in healthy donors and patients with MM. Using two independent computer algorithms, two SPAN-XB-derived peptides (peptides 624 and 626) with predicted binding to HLA-A2 were identified. To further improve the immunogenicity of peptide 626 we designed a heteroclitic peptide (peptide 627) by modifying one amino acid on the HLA binding position 2 of peptide 626. Using an IFN-gamma Elispot assay we could demonstrate the presence and functional activity of CD8 peptide specific T cells with all tested peptides. By analysis of peripheral blood of 13 healthy donors and five patients with MM peptide specific T-cell precursors specifically recognizing at least one of the tested peptides could be detected and expanded in 9 of 13 of



tested donors and 3 of 5 tested patients. Importantly, in two donors specific peptides could be generated against the heteroclitic peptide 627 but not against the native peptide 626. We conclude that SPAN-XB-derived peptides can elicit a consistent CD8 T cell response in healthy donors and patients with MM.

Garg, M., D. Chaurasiya, et al. (2007). "Sperm-associated antigen 9, a novel cancer testis antigen, is a potential target for immunotherapy in epithelial ovarian cancer." *Clin Cancer Res* **13**(5): 1421-8.

**PURPOSE:** Cancer testis antigens are a group of tumor antigens with gene expression restricted to male germ cells in the testis and in various cancerous tissues. Recently, we reported a novel testis-specific sperm-associated antigen 9 (SPAG9) gene, a new member of the c-Jun NH(2)-terminal kinase-interacting protein family, having functional role in sperm-egg fusion and mitogen-activated protein kinase signaling pathway. National Center for Biotechnology Information Blast searches revealed SPAG9 nucleotide sequence similarities with expressed sequence tags of various cancerous tissues. In an effort to examine the clinical utility of SPAG9, we investigated the SPAG9 mRNA and protein expression in epithelial ovarian cancer (EOC). Humoral immune response to SPAG9 was also evaluated in EOC patients. **EXPERIMENTAL DESIGN:** We determined the expression profile of SPAG9 transcript by reverse transcription-PCR and RNA in situ hybridization and SPAG9 protein expression by immunohistochemistry in EOC specimens and human ovarian cancer cell lines. Using ELISA and Western blotting, we analyzed specific antibodies for SPAG9 in sera from patients with EOC. **RESULTS:** SPAG9 mRNA and protein expression was detected in 90% of EOC tissues and in all three human ovarian cancer cell lines. Specific SPAG9 antibodies were detected in 67% of EOC patients and not in sera from healthy individuals. **CONCLUSIONS:** Our findings indicate that SPAG9 is highly expressed in EOC and immunogenic in patients. Humoral immune response against SPAG9 in early stages of EOC suggests its important role in early diagnostics. These results collectively suggest that SPAG9, a novel member of cancer testis antigen family, could be a potential target for the development of diagnostic and therapeutic methods in EOC.

Germa-Lluch, J. R., X. Garcia del Muro, et al. (2002). "Clinical pattern and therapeutic results achieved in 1490 patients with germ-cell tumours of the testis: the experience of the Spanish Germ-Cell Cancer Group (GG)." *Eur Urol* **42**(6): 553-62; discussion 562-3.

**OBJECTIVE:** To describe the clinical characteristics and treatment results obtained with the

application of a homogeneous treatment protocol in 1490 patients with germ-cell tumours (GCT) registered in the 55 hospitals belonging to the Spanish Germ-Cell Cancer Group (GG) during the period between January 1994 and April 2001. **METHODS:** In general, surveillance was the common policy for stage I patients without local poor prognosis factors, whereas they received adjuvant chemotherapy in case those factor were present. Chemotherapy schedules used in advanced cases were cisplatin and etoposide (EP) for seminoma and BEP or BOMP-EPI in non-seminoma, according to whether the patient was in the good or poor prognosis IGCCCG (International Germ-Cell Cancer Collaborative Group) group. Excision of residual masses was mandatory in non-seminomatous germ-cell tumour (NSGCT). **RESULTS:** Initial local symptomatology was increased testis size in 90% of cases. Sonography was an excellent diagnostic tool to suggest tumour. Non-seminoma (64.2%) was more frequent than seminoma (35.8%). Approximately 10% had the antecedent of cryptorchidism. Non-seminoma patients were 7 years younger than seminoma. Right testis was involved predominantly. Pre-orchidectomy tumour markers were elevated in 21% of seminoma (betaHGC) and 79% in non-seminoma (alphaFP and/or betaHGC). Scrotum violation occurred in only 1.8%. There were significant differences among stage I and the IGCCCG prognosis groups related to a longer interval between the first symptom and orchietomy. Eighteen percent of non-seminomatous germ-cell tumour belonged to the poor prognosis IGCCCG group. With a median follow-up to 33 months, this series has achieved a 3 year overall survival of 98% for seminoma and 94% for non-seminoma. Only 10% of excised residual masses present after chemotherapy contained malignant cells. **CONCLUSION:** Spanish GCT have a similar clinical pattern to that described in the other occidental countries except for a slight increased proportion of non-seminoma upon seminoma. Co-operative groups as GG are unique structures to obtain quick and wide experience on the treatment of testis tumours, contributing to achieve a high cure rate.

Ghafouri-Fard, S. and M. H. Modarressi (2009). "Cancer-testis antigens: potential targets for cancer immunotherapy." *Arch Iran Med* **12**(4): 395-404.

Cancer-testis antigens are tumor antigens that their expression is almost limited to male germ cells in the testis. Some of cancer-testis antigens are also expressed in the ovary and in trophoblasts. Recently their expression has been seen in different types of tumors. Many pathophysiologic studies suggest that a blood-testis barrier exists in the testis. Because spermatogenesis begins at puberty, new cell-surface antigens are expressed when the immune system has

refined the ability to distinguish self from nonself. So, sperms in the testis do not stimulate immune responses. In addition, although antigen-presenting cells are commonly seen in the interstitial spaces of the testis, these cells are scarcely seen within the seminiferous tubules. So, testis is considered as an immune-privileged site, and testis-specific genes, if expressed in cancers can be immunogenic. For this reason cancer-testis antigens are promising candidates for cancer immunotherapy and have become a major focus for the development of vaccine-based clinical trials in recent years. In addition, these antigens can also be used as biomarkers for early detection of cancers.

Ghochikyan, A., M. Mkrtychyan, et al. (2007). "Elicitation of T cell responses to histologically unrelated tumors by immunization with the novel cancer-testis antigen, brother of the regulator of imprinted sites." *J Immunol* **178**(1): 566-73.

Brother of the regulator of imprinted sites (BORIS) was previously described as a transcription factor for epigenetic reprogramming the expression of which is strictly confined to germ cells of adult testes but is aberrantly activated in the vast majority of neoplastic cells. Considering the critical role of BORIS in cancerogenesis and the fact that its expression pattern may preclude thymic tolerance, we generated DNA- and protein-based mouse BORIS antitumor vaccines using a non-DNA-binding version of the BORIS molecule. Clinical use of BORIS as a vaccine Ag would require that certain safety concerns be met. Specifically, administration of the functional BORIS protein would hypothetically pose a risk of BORIS accelerating the progression of cancer. To alleviate such safety concerns, we have developed vaccines based on the BORIS molecule lacking the DNA-binding zinc fingers domain. To enhance anti-BORIS cellular immune responses, we used a standard molecular adjuvant approach. It consisted of plasmids encoding murine IL-12 and IL-18 for a DNA-based vaccine and conventional Th1 type adjuvant, Quil A, for a protein-based vaccine. Both DNA- and protein-based vaccines induced Ag-specific CD4(+) T cell proliferation with Th1 and Th2 cytokine profiles, respectively. Protein-based, but not DNA-based, BORIS vaccine induced a significant level of Ab production in immunized animals. Importantly, potent anticancer CD8(+)-cytotoxic lymphocytes were generated after immunization with the DNA-based, but not protein-based, BORIS vaccine. These cytolytic responses were observed across a wide range of different mouse cancers including mammary adenocarcinoma, glioma, leukemia, and mastocytoma.

Gilbert, S. M., S. Daignault, et al. (2008). "The use of tumor markers in testis cancer in the United States: a potential quality issue." *Urol Oncol* **26**(2): 153-7.

**BACKGROUND:** Performance measurement is currently being implemented at various levels to address issues related to quality of care. Administrative data and cancer registries provide a measure of health care delivery (processes) at the population level. In testis cancer, the use of tumor markers is an important component of care and may represent a measurable quality indicator. We analyzed the use of testis cancer tumor markers across the United States. **METHODS:** Incident germ cell testis cancer cases between 1998 and 2002 were abstracted from the Surveillance, Epidemiology, and End Results (SEER) program. Tumor marker codes were categorized as performed and not performed. Bivariate and multivariable analyses were performed. Logistic regression was used to determine the independent association of demographic and clinical factors with the failure to measure or document tumor markers. **RESULTS:** In 4,742 testis cancer cases, performance of both alpha fetoprotein and human chorionic gonadotropin was recorded in 44.7% of cases, while performance of alpha fetoprotein, human chorionic gonadotropin, and lactate dehydrogenase together occurred in only 16%. Tumor marker use did not increase considerably between 1998 and 2002, and significant variation existed between SEER sites. In multivariable models, disease stage and the interaction between SEER site and year were significantly associated with tumor marker use. **CONCLUSIONS:** We have identified low and variable rates of tumor marker use in testis cancer cases recorded in SEER. As pay-for-performance evolves, standardized metrics for recommended processes of care (e.g., measurement of tumor markers in patients with testis cancer) may be tied to reimbursement. In this context, failure to document the use of tumor markers will be interpreted as less than optimal care.

Gjerstorff, M. F. and H. J. Ditzel (2008). "An overview of the GAGE cancer/testis antigen family with the inclusion of newly identified members." *Tissue Antigens* **71**(3): 187-92.

GAGE cancer/testis antigens are frequently expressed in many different types of cancer, whereas their expression in normal tissues is limited to the germ cells of the immune-privileged organs, testis and ovary. Thus, GAGE proteins may be attractive candidates for immunotherapy of cancer. This review describes the structure and phylogeny of the GAGE family members and presents a revised nomenclature, which will enable a more clear distinction of genes and gene products. The GAGE gene locus at chromosome X p11.23 consists of at least 16 genes,

each of which is located in one of an equal number of highly conserved tandem repeats, and more genes remain to be identified. These genes are likely the creation of unequal replication under positive selection after the divergence of primates from other mammals. The encoded products are predicted to be highly similar small acidic proteins involved in germ cell biology. When expressed in tumor cells, GAGE proteins can elicit both cellular and humoral immune responses, indicating that they are appropriate targets for cancer immunotherapy. The potential use of GAGE proteins in cancer immunotherapy, including possible limitations, is also discussed.

Gjerstorff, M. F., K. Kock, et al. (2007). "MAGE-A1, GAGE and NY-ESO-1 cancer/testis antigen expression during human gonadal development." *Hum Reprod* **22**(4): 953-60.

**BACKGROUND:** Cancer/testis antigens (CTAs) are expressed in several cancers and during normal adult male germ cell differentiation. Little is known about their role in fetal development of human germ cells. **METHODS:** We examined expression of the CTAs MAGE-A1, GAGE and NY-ESO-1 in fetal gonads by single and double immunohistochemical staining. **RESULTS:** We found that GAGE was expressed in the primordial germ cells of the gonadal primordium, whereas MAGE-A1 and NY-ESO-1 were first detected in germ cells of both testis and ovary after sexual differentiation was initiated. The number of positive germ cells and the staining intensity of all three CTAs peaked during the second trimester and gradually decreased towards birth in both male and female germ cells. In oocytes, MAGE-A1 expression terminated around birth, whereas NY-ESO-1 expression persisted through the neonatal stage and GAGE expression was maintained until adulthood. The population of GAGE-expressing male and female germ cells partially overlapped the population of OCT4-positive cells, whereas MAGE-A1 and NY-ESO-1 were clearly expressed only by OCT4-negative cells. **CONCLUSIONS:** Our results suggest that MAGE-A1 and NY-ESO-1 are associated with highly proliferating germ cells, whereas GAGE proteins have a more general function in germ cells unrelated to any specific developmental stage. The recognition of differential cellular expression of GAGE, MAGE-A1, NY-ESO-1 and OCT4 may help define biologically distinct germ cell subpopulations.

Glazer, C. A., I. M. Smith, et al. (2009). "Integrative discovery of epigenetically derepressed cancer testis antigens in NSCLC." *PLoS One* **4**(12): e8189.

**BACKGROUND:** Cancer/testis antigens (CTAs) were first discovered as immunogenic targets normally expressed in germline cells, but

differentially expressed in a variety of human cancers. In this study, we used an integrative epigenetic screening approach to identify coordinately expressed genes in human non-small cell lung cancer (NSCLC) whose transcription is driven by promoter demethylation. **METHODOLOGY/PRINCIPAL FINDINGS:** Our screening approach found 290 significant genes from the over 47,000 transcripts incorporated in the Affymetrix Human Genome U133 Plus 2.0 expression array. Of the top 55 candidates, 10 showed both differential overexpression and promoter region hypomethylation in NSCLC. Surprisingly, 6 of the 10 genes discovered by this approach were CTAs. Using a separate cohort of primary tumor and normal tissue, we validated NSCLC promoter hypomethylation and increased expression by quantitative RT-PCR for all 10 genes. We noted significant, coordinated coexpression of multiple target genes, as well as coordinated promoter demethylation, in a large set of individual tumors that was associated with the SCC subtype of NSCLC. In addition, we identified 2 novel target genes that exhibited growth-promoting effects in multiple cell lines.

**CONCLUSIONS/SIGNIFICANCE:** Coordinated promoter demethylation in NSCLC is associated with aberrant expression of CTAs and potential, novel candidate protooncogenes that can be identified using integrative discovery techniques. These findings have significant implications for discovery of novel CTAs and CT antigen directed immunotherapy.

Godmann, M., R. Lambrot, et al. (2009). "The dynamic epigenetic program in male germ cells: Its role in spermatogenesis, testis cancer, and its response to the environment." *Microsc Res Tech* **72**(8): 603-19.

Spermatogenesis is a truly remarkable process that requires exquisite control and synchronization of germ cell development. It is prone to frequent error, as paternal infertility contributes to 30-50% of all infertility cases; yet, in many cases, the mechanisms underlying its causes are unknown. Strikingly, aberrant epigenetic profiles, in the form of anomalous DNA and histone modifications, are characteristic of cancerous testis cells. Germ cell development is a critical period during which epigenetic patterns are established and maintained. The progression from diploid spermatogonia to haploid spermatozoa involves stage- and testis-specific gene expression, mitotic and meiotic division, and the histone-protamine transition. All are postulated to engender unique epigenetic controls. In support of this idea are the findings that mouse models with gene deletions for epigenetic modifiers have severely compromised fertility. Underscoring the importance of understanding how epigenetic marks

are set and interpreted is evidence that abnormal epigenetic programming of gametes and embryos contributes to heritable instabilities in subsequent generations. Numerous studies have documented the existence of transgenerational consequences of maternal nutrition, or other environmental exposures, but it is only now recognized that there are sex-specific male-line transgenerational responses in humans and other species. Epigenetic events in the testis have just begun to be studied. New work on the function of specific histone modifications, chromatin modifiers, DNA methylation, and the impact of the environment on developing sperm suggests that the correct setting of the epigenome is required for male reproductive health and the prevention of paternal disease transmission.

Goyer, R. A., J. Liu, et al. (2004). "Cadmium and cancer of prostate and testis." *Biometals* **17**(5): 555-8.

Cancer of the prostate is an important and potentially fatal disease in humans but the etiology is yet undefined. Cadmium and cadmium compounds are known to be human carcinogens based on findings of increased risk to lung cancer among exposed workers, but a relationship between cancer of the prostate and/or testis in humans is unclear in spite of suggestive results in rats. Parenteral administration or oral exposure to cadmium can result in proliferate lesions and tumors of the prostate in rats. The ability of cadmium to produce neoplasms in the prostate of rats is atypically dose-related and only occurs in rats at doses below the threshold for significant testicular toxicity. Testicular androgen production is essential for the maintenance of the prostate and prostate tumors. The rat testis may also develop tumors if cadmium is given parenterally at high doses. Subsequent to testicular hemorrhagic necrosis, there will be loss of testosterone production and hyperplasia and neoplasia of testicular interstitial cells, thought to be a response to trophic hormone release from the pituitary. The pathogenesis of prostatic cadmium carcinogenesis might include aberrant gene expression resulting in stimulation of cell proliferation or blockage of apoptosis. Activation of transcription factors such as the metallothionein gene and activation of some protooncogenes may enhance cell proliferation with damaged DNA. Suppression of DNA repair would add to the population of cells with damaged DNA. Chemically induced apoptosis can be blocked by cadmium, facilitating aberrant cell accumulation.

Grah, J., M. Samija, et al. (2008). "Immunohistochemical expression of cancer/testis antigens (MAGE-A3/4, NY-ESO-1) in non-small cell

lung cancer: the relationship with clinical-pathological features." *Coll Antropol* **32**(3): 731-6.

The aim of this study was to explore the expression of cancer/testis tumor associated antigens (C/T TAAs) MAGE-A 3/4 and NY-ESO-1 in lung squamous cell carcinoma and adenocarcinoma, and to evaluate their association with the standard clinical-pathological features of surgically treated lung cancer patients. The study included 80 patients with non-small cell lung cancer (40 adenocarcinomas, 40 squamous cell carcinomas) who had undergone surgery in the period between 2002 and 2005. The MAGE-A3/4 and NY-ESO-1 antigen expression was analyzed immunohistochemically (IHC). The results showed MAGE-A3/4 and NY-ESO-1 positive staining in 65.1% and 23.3% of squamous cell carcinomas and 18.9% and 10.8% of adenocarcinomas, respectively. A statistically higher MAGE-A3/4 expression was observed in planocellular bronchial carcinoma ( $p < 0.001$ ), while no difference was found in the expression of NY-ESO-1 in adenocarcinoma and planocellular carcinoma ( $p = 0.144$ ). A significant association was found between the MAGE-A3/4 expression and presence of tumor necrosis in squamous cell cancer specimens ( $p = 0.001$ ), but not in adenocarcinoma ( $p = 0.033$ ). A statistically significant association was noted between the NY-ESO-1 expression and positive hilar and mediastinal lymph nodes in adenocarcinoma ( $p = 0.025$ ) whereas it was not the case in squamous cell carcinoma. Non-small cell lung cancer frequently expresses cancer/testis tumor associated antigens. Our results demonstrate that the MAGE-A3/4 and NY-ESO-1 expression was significant associated with prognostic factors of poor outcome of disease (presence of tumor necrosis and lymph node metastasis). As C/T antigens are important for inducing a specific immune reaction in lung cancer patients, there is an intention to form a subgroup of patients in the future, whose treatment would be enhanced by specific immunotherapy based on the observed scientific results.

Groeper, C., F. Gambazzi, et al. (2007). "Cancer/testis antigen expression and specific cytotoxic T lymphocyte responses in non small cell lung cancer." *Int J Cancer* **120**(2): 337-43.

Non small cell lung cancers (NSCLC) express cancer/testis antigens (CTA) genes and MAGE-A expression correlates with poor prognosis in squamous cell carcinomas. We addressed cytotoxic T lymphocytes (CTL) responses to HLA class I restricted CTA epitopes in TIL from NSCLC in an unselected group of 33 patients consecutively undergoing surgery. Expression of MAGE-A1, -A2, -A3, -A4, -A10, -A12 and NY-ESO-1 CTA genes was tested by quantitative RT-PCR. Monoclonal

antibodies (MAb) recognizing MAGE-A and NY-ESO-1 CTA were used to detect CTA by immunohistochemistry. CD8(+) TIL obtained from tumors upon culture with anti CD3 and anti CD28 mAb and IL-2 were stimulated with autologous mature DC (mDC) and HLA-A\*0101 restricted MAGE-A1(161-169) or MAGE-A3(168-176) peptides or HLA-A\*0201 restricted MAGE-A4(230-239), MAGE-A10(254-262), NY-ESO-1(157-165) or multi-MAGE-A (YLEYRQVPV) peptides or a recombinant vaccinia virus (rVV) encoding MAGE-A and NY-ESO-1 HLA-A\*0201 restricted epitopes and CD80 co-stimulatory molecule. Specificity was assessed by <sup>51</sup>Cr release and multimer staining. At least one CTA gene was expressed in tumors from 15/33 patients. In 10 specimens, at least 4 CTA genes were concomitantly expressed. These data were largely confirmed by immunohistochemistry. TIL were expanded from 26/33 specimens and CTA-specific CTL activity was detectable in 7/26 TIL. In 6, however, specific cytotoxicity was weak, (<40% lysis at a 50:1 E:T ratio) and multimer staining was undetectable. In one case, high (>60% lysis at 50:1 E:T ratio) MAGE-A10(254-262) specific, HLA-A\*0201 restricted response was observed. Supportive evidence was provided by corresponding multimer staining. Although CTA genes are frequently expressed in NSCLC, detection of CTL reactivity against CTA epitopes in TIL from nonimmunized NSCLC patients represents a rare event.

Gure, A. O., R. Chua, et al. (2005). "Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer." *Clin Cancer Res* **11**(22): 8055-62.

**PURPOSE:** Cancer-testis genes mapping to the X chromosome have common expression patterns and show similar responses to modulators of epigenetic mechanisms. We asked whether cancer-testis gene expression occurred coordinately, and whether it correlated with variables of disease and clinical outcome of non-small cell lung cancer (NSCLC). **EXPERIMENTAL DESIGN:** Tumors from 523 NSCLC patients undergoing surgery were evaluated for the expression of nine cancer-testis genes (NY-ESO-1, MAGE-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT7/MAGE-C1, SSX2, and SSX4) by semiquantitative PCR. Clinical data available for 447 patients were used to correlate cancer-testis expression to variables of disease and clinical outcome. **RESULTS:** At least one cancer-testis gene was expressed by 90% of squamous carcinoma, 62% of bronchioloalveolar cancer, and 67% of adenocarcinoma samples. Statistically significant coexpression was observed for 34 of the 36 possible cancer-testis combinations. Cancer-testis

gene expression, either cumulatively or individually, showed significant associations with male sex, smoking history, advanced tumor, nodal and pathologic stages, pleural invasion, and the absence of ground glass opacity. Cox regression analysis revealed the expression of NY-ESO-1 and MAGE-A3 as markers of poor prognosis, independent of confounding variables for adenocarcinoma of the lung. **CONCLUSIONS:** Cancer-testis genes are coordinately expressed in NSCLC, and their expression is associated with advanced disease and poor outcome.

Haffner, A. C., A. Tassis, et al. (2002). "Expression of cancer/testis antigens in cutaneous T cell lymphomas." *Int J Cancer* **97**(5): 668-70.

Cancer/testis-antigens (CTA), a novel and expanding family of immunogenic proteins detected by serological screening of recombinant cDNA expression libraries, encompass promising candidate targets for T-cell based immunotherapy. We screened kryo-preserved tissue of cutaneous T cell lymphoma (CTCL, n=36) such as mycosis fungoides (MF, n=17), pleomorphic cutaneous T-cell lymphoma (n=8) and Sezary's syndrome (SS, n= 11) as well as a non-malignant entity (small plaques parapsoriasis, SPP, n=5), for the expression of CTA by RT-PCR and Northern blot hybridization. From a panel of eleven CTA (MAGE-1, MAGE-C1, MAGE-3, BAGE, GAGE, SSX-1, SSX-2, SSX4, SCP-1, NY-ESO-1 and TS85) (HOM-Tes-85), mRNA expression could be detected for SCP-1 in 8/17 MF and 6/8 pleomorphic CTCL patients but was completely absent in small plaques parapsoriasis. SS patients had a more heterogeneous antigen expression pattern: Gage (1/11), MAGE-1 (3/11), MAGE-3 (6/11), MAGE-C1 (5/11), NY-ESO-1 (7/11) and TS85 (5/11), with expression of MAGE-3 confirmed by immunohistochemistry. CTA could provide defined targets for antigen-based vaccination in a high percentage of cases with CTCL. SCP-1 might serve as an additional diagnostic indicator in early and clinically indistinct lesions suspicious for cutaneous T-cell lymphoma.

Haier, J., M. Owzcareck, et al. (2006). "Expression of MAGE-A cancer/testis antigens in esophageal squamous cell carcinomas." *Anticancer Res* **26**(3B): 2281-7.

**BACKGROUND:** Although the diagnosis and therapy of esophageal cancer have improved over the past decade, the prognosis remains dismal. Since MAGE-A cancer/testis antigens (CTA) are potential targets for immunotherapy, this study was aimed at evaluating their expression in these patients and its prognostic value. **MATERIALS AND METHODS:**

Using 57B monoclonal antibody, MAGE-A CTA expression was analyzed in paraffin-embedded tumor specimens of 98 patients with esophageal squamous cell carcinoma or adenocarcinomas who had undergone surgical resection. For all patients, a postoperative follow-up of at least 4 years was available. The expression was quantified using a scoring system considering intensity and homogeneity of the immunostaining. The prognostic relevance of MAGE-A expression was analyzed in univariate analyses as well as Cox proportional hazard regression analysis. RESULTS: 57B positivity could be detected in 38 tumors (38.8%). Positive staining was observed in five out of 32 adenocarcinomas (15.2%) and in 33 out of 66 (50%) squamous cell carcinomas. MAGE-A expression did not correlate with the TNM classification, grading or age of the patients. Both univariate ( $p=0.88$ ) and multivariate analyses ( $p = 0.82$ ) revealed that MAGE-A expression lacked prognostic significance in esophageal carcinomas. CONCLUSION: MAGE-A was expressed in half of the squamous cell carcinomas of the esophagus, but rarely in adenocarcinomas. Although its immunodetection was insufficient for prognostic evaluation, the high expression rate suggests MAGE-A as a potential target for immunotherapy in the first group with the ability for pretherapeutic testing.

Harao, M., S. Hirata, et al. (2008). "HLA-A2-restricted CTL epitopes of a novel lung cancer-associated cancer testis antigen, cell division cycle associated 1, can induce tumor-reactive CTL." *Int J Cancer* **123**(11): 2616-25.

Toward the development of a novel cancer immunotherapy, we have previously identified several tumor-associated antigens (TAAs) and the epitopes recognized by human histocompatibility leukocyte (HLA)-A2/A24-restricted cytotoxic T lymphocyte (CTL). In this study, we tried to identify a TAA of lung cancer (LC) and its HLA-A2 restricted CTL epitopes to provide a target antigen useful for cancer immunotherapy of LC. We identified a novel cancer testis antigen, cell division cycle associated gene 1 (CDCA1), overexpressed in nonsmall cell LC using a cDNA microarray analysis. The expression levels of CDCA1 were also increased in the majority of small cell LC, cholangiocellular cancer, urinary bladder cancer and renal cell cancers. We used HLA-A2.1 transgenic mice to identify the HLA-A2 (A\*0201)-restricted CDCA1 epitopes recognized by mouse CTL, and we investigated whether these peptides could induce CDCA1-reactive CTLs from the peripheral blood mononuclear cells (PBMCs) of HLA-A2-positive donors and a NSCLC patient. Consequently, we found that the CDCA1(65-73)

(YMMPVNSEV) peptide and CDCA1(351-359) (KLATAQFKI) peptide could induce peptide-reactive CTLs in HLA-A2.1 transgenic mice. In HLA-A2(+) donors, in vitro stimulation of PBMC with these peptides could induce peptide-reactive CTLs which killed tumor cell lines endogenously expressing both HLA-A2 and CDCA1. As a result, CDCA1 is a novel cancer-testis antigen overexpressed in LC, cholangiocellular cancer, urinary bladder cancer and renal cell cancers, and CDCA1 may therefore be an ideal TAA useful for the diagnosis and immunotherapy of these cancers.

Hayashi, E., Y. Matsuzaki, et al. (2007). "Identification of a novel cancer-testis antigen CRT2 frequently expressed in various cancers using representational differential analysis." *Clin Cancer Res* **13**(21): 6267-74.

PURPOSE: Cancer-testis antigens are promising targets for cancer immunotherapy. Identification of additional cancer-testis antigens with frequent expression in various cancers was attempted using representational differential analysis (RDA) and immunogenicity evaluation. EXPERIMENTAL DESIGN: cDNAs preferentially expressed in testis were enriched using RDA by subtraction between testis and normal tissues. Thirty clones showing cancer-testis-like expression based on EST database analysis were evaluated by reverse transcription-PCR. A potential antigen, CRT2, was identified and its expression was analyzed with a newly generated anti-CRT2 antibody. The immunogenicity of CRT2 was examined based on reactivity with serum immunoglobulin G (IgG) from cancer patients, using Western blot and ELISA analysis, and on in vitro induction of tumor-reactive CTLs from HLA-A24 transgenic mice and human peripheral blood lymphocytes. RESULTS: CRT2 was expressed in elongated spermatids of testis among normal tissues and in various cancer cell lines and tissues. The recombinant CRT2 protein was recognized by serum IgG from patients with various cancers in Western blot and ELISA analyses. A CRT2-derived peptide was identified as an HLA-A24-restricted T-cell epitope that induced tumor-reactive CTLs. CONCLUSION: CRT2 was identified as a new cancer-testis antigen expressed in elongated spermatids of testis and in cancer tissues (particularly melanoma) that is recognized by serum IgG from cancer patients. An HLA-A24-restricted T-cell epitope capable of inducing tumor-reactive CTLs was identified, suggesting that CRT2 may be useful for cancer diagnosis and immunotherapy.

He, C., Z. Zuo, et al. (2007). "Genome-wide detection of testis- and testicular cancer-specific alternative splicing." *Carcinogenesis* **28**(12): 2484-90.

Alternative pre-messenger RNA (mRNA) splicing is a key molecular event that allows for protein diversity and plays important roles in development and disease. Alternative pre-mRNA splicing regulations during spermatogenesis and alternative pre-mRNA splicing etiology in testicular tumorigenesis are yet to be characterized. By genome-wide analysis, here we describe alternative splicing features that distinguish distinctive patterns of alternative pre-mRNA splicing among human testis, testicular cancer and mouse testis. Through computationally subtractive analysis, we detected 80 testis-specific transcript candidates in human testis, 175 in human testicular cancer and 262 in mouse testis, which were integrated into a database. Reverse transcription-polymerase chain reaction confirmed that most of these transcript candidates from mouse testis were testis specific. Around 40% of the transcripts were from unknown/hypothetical genes, which were useful for further functional analysis. These transcripts were not overlapped, indicating lack of evolutionary conservation. Further chromosome mapping showed distinct chromosomal preference of alternative pre-mRNA splicing events. Comparison analysis indicated that alternative pre-mRNA splicing in human testicular tumor shared some characters/trends with those in mouse testis. Moreover, human testicular tumor tended to use rare splice sites and there were also distinct sequences adjacent dominant splice sites between normal testis and testicular tumor. These special features of alternative pre-mRNA splicing in human testicular tumor suggested that testicular tumorigenesis was involved in multiple steps/levels of alternative splicing events. Using alternative splicing as a potential source for new clinical diagnostic, prognostic and therapeutic strategies for treatment of testicular tumors seems to have a bright prospect.

Heidenreich, A. (2009). "Contralateral testicular biopsy in testis cancer: current concepts and controversies." *BJU Int* **104**(9 Pt B): 1346-50.

Of all patients with unilateral testis cancer, approximately 5% harbour testicular intraepithelial neoplasia (TIN) in their contralateral testicle that will progress into an invasive germ cell tumour over time. The accurate diagnosis of TIN by a random two-site surgical testis biopsy and effective therapy by local radiation has led to the concept of a contralateral screening biopsy in all patients with testis cancer. However, screening and preventive treatment are only indicated if the therapeutic outcome of the screened population is improved, and the physiological

function of the affected organ is not impaired. Based on a critical review of previous reports, some drawbacks of this policy have to be considered and question the routine indication for contralateral testis biopsy: (i) all TIN-negative patients still have to undergo meticulous follow-up for metachronous testis cancer due to a false negative biopsy rate of 0.5-1.0%; (ii) local radiation of TIN results in irreversible infertility due to eradication of spermatogenesis; (iii) local radiation of TIN results in an impairment of endocrine Leydig cell function in 25% of the patients; (iv) therapeutic outcome and prognosis will not be improved in irradiated patients as compared to patients on surveillance; (v) local tumour resection for the management of metachronous testicular cancer represents an effective and viable option. Current reports do not support the strategy of contralateral testis biopsy in all patients with unilateral testicular germ cell tumours. According to the recommendations of the European Germ Cell Cancer Consensus Group, a testis biopsy might be offered to high-risk patients for contralateral TIN (testicular volume <12 mL, history of cryptorchidism, age <30 years).

Heidenreich, A., P. Albers, et al. (2003). "Complications of primary nerve sparing retroperitoneal lymph node dissection for clinical stage I nonseminomatous germ cell tumors of the testis: experience of the German Testicular Cancer Study Group." *J Urol* **169**(5): 1710-4.

**PURPOSE:** Nerve sparing retroperitoneal lymph node dissection has been the standard diagnostic and therapeutic approach to clinical stage I nonseminoma. However, the application of prognostic risk factors and introduction of laparoscopy have recently called into question the clinical usefulness of nerve sparing retroperitoneal lymph node dissection. We assessed the therapeutic efficacy and associated complications of this procedure in patients with clinical stage I nonseminomatous germ cell tumor treated at 7 tertiary referral centers to evaluate its role in the modern management of low stage testis cancer. **MATERIALS AND METHODS:** Between January 1995 and September 2000, 239 patients with clinical stage I nonseminomatous germ cell tumor underwent nerve sparing retroperitoneal lymph node dissection in standardized fields of dissection. For retrospective analysis patient charts were reviewed. A minor complication did not prolong hospital stay and a major complication prolonged hospitalization for at least 2 days. Early complications developed within the first 30 days after retroperitoneal lymph node dissection and late complications occurred from postoperative day 31 and thereafter. **RESULTS:** Nerve sparing retroperitoneal lymph node dissection was performed unilaterally in 209 patients (88.2%) and bilaterally in

30 (11.8%). Median operative time was 214 minutes (range 90 to 395), mean hospital stay was 8 days (range 4 to 39) and mean blood loss was less than 150 ml. A mean of 18.5 lymph nodes (range 9 to 57) were dissected with metastases detected in 67 patients (28%). An average of 2.9 lymph nodes (range 1 to 14) with a mean diameter of 2.6 cm. (range 0.3 to 6.0) showed metastasis. Disease was pathological stage I in 172 patients (71.7%), 52 (17.6%) had 3 or fewer metastatic lymph nodes, and 15 (6.3%) had 4 to 5 and 10 (4.2%) had greater than 5 positive lymph nodes. Minor complications occurred in 14.2% of the cases and major complications were observed in 5.4%. Antegrade ejaculation was preserved in 93.3% of the patients, recurrence developed in 14 (5.8%) and retroperitoneal recurrence was observed in 3 (1.2%), including 1 in field and 2 out field. **CONCLUSIONS:** Primary diagnostic and therapeutic nerve sparing retroperitoneal lymph node dissection still has a role in the primary management of clinical stage I nonseminomatous germ cell tumor. Surgery is associated with low morbidity and patient followup is easy and cost-effective due to the concentration on extraretroperitoneal locations. Primary nerve sparing retroperitoneal lymph node dissection is curative in about 70% of clinical stage I nonseminoma cases with a maximum of 3 positive lymph nodes.

Heidenreich, A. and J. W. Moul (2002). "Contralateral testicular biopsy procedure in patients with unilateral testis cancer: is it indicated?" *Semin Urol Oncol* **20**(4): 234-8.

About 5% of all patients with unilateral testis cancer harbor testicular intraepithelial neoplasia (TIN) in their contralateral testicle, which will progress into an invasive germ-cell tumor over time. Accurate diagnosis of TIN by a random surgical testis biopsy examination and effective therapy by local radiation has led to the concept of a contralateral screening biopsy procedure in all testis cancer patients. Screening and preventive treatment, however, only are indicated if (1) therapeutic outcome of the screened population is improved and (2) physiologic function of the affected organ might be maintained. Based on a critical review of the literature, some drawbacks of this policy have to be considered and the routine indication for contralateral testis biopsy procedure has to be questioned: (1) all TIN-negative patients still have to undergo meticulous follow-up evaluation for metachronous testis cancer owing to a false-negative biopsy diagnosis rate of 0.3%; (2) testis biopsy procedure is associated with a 15% to 20% complication rate, which might have a negative impact on endocrine and exocrine testicular function; (3) local radiation of TIN results in irreversible infertility owing to eradication of spermatogenesis; (4) local

radiation of TIN results in an impairment of endocrine Leydig cell function in 25% of patients; (5) therapeutic outcome and prognosis will not be improved in irradiated patients as compared with patients on surveillance; (6) local tumor resection for the management of metachronous testicular cancer represents an effective and viable option. The current literature does not support the strategy to perform contralateral testis biopsy procedures in all patients with unilateral testicular germ-cell tumors. Testis biopsy procedures might, however, be offered to high-risk (34%) patients for contralateral TIN with a testicular volume less than 12 mL, a history of cryptorchidism, and an age less than 30 years.

Hindley, R. G., A. Chandra, et al. (2003). "Impalpable testis cancer." *BJU Int* **92**(6): 572-4.

**OBJECTIVE:** To assess the significance of ultrasonographically detected hypoechoic lesions of the testis when the clinical examination is normal, and to highlight the management difficulties thereafter. **PATIENTS AND METHODS:** Over a 2-year period four patients underwent radical orchidectomy where the sole indication for surgery was a hypoechoic lesion detected on ultrasonography (US). The indications for US were persistent scrotal discomfort in two men, contralateral orchitis, and the follow-up of testicular microlithiasis. The lesions were 4-11 mm in size and one man had several. None of the lesions were palpable; the tumour markers were normal in all patients. **RESULTS:** Three of the testes contained seminoma; in one there were two foci of seminoma and in all intratubular germ cell neoplasia was also identified. The remaining case was a Leydig-cell tumour. All tumours were staged as pT1 after radical inguinal orchidectomy. **CONCLUSION:** Impalpable lesions of the testis are likely to be malignant if they are hypoechoic on US and should be considered as seminoma until proved otherwise. The management thereafter is not straightforward, but must ensure an adequate histological diagnosis if the US appearances do not resolve.

Hoepfner, L. H., J. A. Dubovsky, et al. (2006). "Humoral immune responses to testis antigens in sera from patients with prostate cancer." *Cancer Immun* **6**: 1.

Tumor vaccines represent one type of molecularly targeted therapy being investigated for the treatment of prostate cancer. Although many prostate-specific proteins are being tested as target antigens for prostate cancer vaccines, most are not natural targets of an immune response in patients with cancer. Using sera from cancer patients, several research groups have identified a large family of immunologically recognized proteins whose



expression is normally confined to immune-privileged testis tissue but which may be expressed in cancers of different histological origins. These proteins, so-called cancer-testis (CT) antigens, are appealing targets for immune-based therapies because they are essentially tumor-restricted antigens and there is less risk of preexisting immune tolerance. In addition, specifically targeting these proteins by means of vaccines should reduce the risk of potential autoimmune reactions to normal tissues. In the current study, we hypothesize that prostate CT antigens can be identified using a SEREX screening method with sera from patients with prostate cancer and probing with a human testis cDNA expression library. We have identified several potential prostate cancer antigens with predominantly testis-specific expression in normal tissues, including MAD-CT-1 (protamine 2) and MAD-CT-2. Each was independently identified from different subjects with prostate cancer. Antigens identified by these studies can be investigated further as potential prostate cancer tumor antigens.

Hofmann, O., O. L. Caballero, et al. (2008). "Genome-wide analysis of cancer/testis gene expression." *Proc Natl Acad Sci U S A* **105**(51): 20422-7.

Cancer/Testis (CT) genes, normally expressed in germ line cells but also activated in a wide range of cancer types, often encode antigens that are immunogenic in cancer patients, and present potential for use as biomarkers and targets for immunotherapy. Using multiple in silico gene expression analysis technologies, including twice the number of expressed sequence tags used in previous studies, we have performed a comprehensive genome-wide survey of expression for a set of 153 previously described CT genes in normal and cancer expression libraries. We find that although they are generally highly expressed in testis, these genes exhibit heterogeneous gene expression profiles, allowing their classification into testis-restricted (39), testis/brain-restricted (14), and a testis-selective (85) group of genes that show additional expression in somatic tissues. The chromosomal distribution of these genes confirmed the previously observed dominance of X chromosome location, with CT-X genes being significantly more testis-restricted than non-X CT. Applying this core classification in a genome-wide survey we identified >30 CT candidate genes; 3 of them, PEPP-2, OTOA, and AKAP4, were confirmed as testis-restricted or testis-selective using RT-PCR, with variable expression frequencies observed in a panel of cancer cell lines. Our classification provides an objective ranking for potential CT genes, which is useful in guiding further identification and

characterization of these potentially important diagnostic and therapeutic targets.

Hogan, K. T., M. A. Coppola, et al. (2004). "Identification of novel and widely expressed cancer/testis gene isoforms that elicit spontaneous cytotoxic T-lymphocyte reactivity to melanoma." *Cancer Res* **64**(3): 1157-63.

Multiple isoforms (TAG-1, TAG-2a, TAG-2b, and TAG-2c) of a novel cancer/testis antigen gene have been identified and are expressed in 84-88% of melanoma cell lines tested. The tumor antigen (TAG) genes are also expressed in K562, a myelogenous leukemia cell line, and they have homology to two chronic myelogenous leukemia-derived clones and a hepatocellular carcinoma clone in the human expressed sequence tags (EST) database, thus indicating that their expression is not restricted to melanomas. In contrast to the fact that many cancer/testis antigens are poorly immunogenic, the TAG-derived peptide, RLSNRLLLR, is recognized by HLA-A3-restricted, melanoma-specific CTLs that were obtained from a melanoma patient with spontaneous reactivity to the peptide. Unlike most cancer/testis antigen genes which are located on the X chromosome, the TAG genes are located on chromosome 5. The genes have the additional unusual features of being coded for in an open reading frame that is initiated by one of three nonstandard initiation codons, and the sequence coding the RLSNRLLLR peptide crosses an exon-exon boundary. The properties of the TAG antigens indicate that they are excellent vaccine candidates for the treatment of melanoma and perhaps other cancers.

Hong, J. A., Y. Kang, et al. (2005). "Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells." *Cancer Res* **65**(17): 7763-74.

Regulatory sequences recognized by the unique pair of paralogous factors, CTCF and BORIS, have been implicated in epigenetic regulation of imprinting and X chromosome inactivation. Lung cancers exhibit genome-wide demethylation associated with derepression of a specific class of genes encoding cancer-testis (CT) antigens such as NY-ESO-1. CT genes are normally expressed in BORIS-positive male germ cells deficient in CTCF and meCpG contents, but are strictly silenced in somatic cells. The present study was undertaken to ascertain if aberrant activation of BORIS contributes to derepression of NY-ESO-1 during pulmonary carcinogenesis. Preliminary experiments indicated that NY-ESO-1 expression coincided with derepression of BORIS in cultured lung cancer cells.

Quantitative reverse transcription-PCR analysis revealed robust, coincident induction of BORIS and NY-ESO-1 expression in lung cancer cells, but not normal human bronchial epithelial cells following 5-aza-2'-deoxycytidine (5-azadC), Depsipeptide FK228 (DP), or sequential 5-azadC/DP exposure under clinically relevant conditions. Bisulfite sequencing, methylation-specific PCR, and chromatin immunoprecipitation (ChIP) experiments showed that induction of BORIS coincided with direct modulation of chromatin structure within a CpG island in the 5'-flanking noncoding region of this gene. Cotransfection experiments using promoter-reporter constructs confirmed that BORIS modulates NY-ESO-1 expression in lung cancer cells. Gel shift and ChIP experiments revealed a novel CTCF/BORIS-binding site in the NY-ESO-1 promoter, which unlike such sites in the H19-imprinting control region and X chromosome, is insensitive to CpG methylation in vitro. In vivo occupancy of this site by CTCF was associated with silencing of the NY-ESO-1 promoter, whereas switching from CTCF to BORIS occupancy coincided with derepression of NY-ESO-1. Collectively, these data indicate that reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter mediates epigenetic regulation of this CT gene in lung cancer cells, and suggest that induction of BORIS may be a novel strategy to augment immunogenicity of pulmonary carcinomas.

Huyghe, E., A. Muller, et al. (2007). "Impact of diagnostic delay in testis cancer: results of a large population-based study." *Eur Urol* **52**(6): 1710-6.

**OBJECTIVE:** Testis cancer is the most common cancer in young men, and its incidence continues to rise. Even if prognosis is considered as good, a group with bad prognosis still remains. Diagnostic delay (DD), defined as the time elapsing from the onset of tumour symptoms to the day of diagnosis, is a way to evaluate the rapidity of diagnosis. We assessed the relationship between DD, disease stage, and survival rate. **METHODS:** A series of 542 patients diagnosed with a germ cell tumour between 1983 and 2002 at health facilities in the Midi-Pyrenees region, southwest France, were asked about DD. We analysed DD together with data regarding the disease (histologic type, stage), its treatments, and prognosis (impact on survival). **RESULTS:** Mean DD was longer in seminoma (4.9+/-6.1 mo) than in non-seminomatous germ cell tumour (NSGCT; 2.8+/-4.0 mo). DD was correlated with disease stage for the whole population ( $p=0.014$ ) and for NSGCT ( $p=0.0009$ ), but not for seminoma. DD had a significant impact on the 5-yr survival rate in the overall population ( $p=0.001$ ) and in the NSGCT group ( $p=0.001$ ), but not in the seminoma group.

Global trends in mean DD did not change over the 20-yr study period, but we observed a slight decrease during the last decade. **CONCLUSIONS:** DD is highly correlated with stage and survival in NSGCT. Urologists should promote programmes to enhance awareness and knowledge of testis cancer, so the diagnosis can be made more rapidly.

Ishikawa, N., A. Takano, et al. (2007). "Cancer-testis antigen lymphocyte antigen 6 complex locus K is a serologic biomarker and a therapeutic target for lung and esophageal carcinomas." *Cancer Res* **67**(24): 11601-11.

Gene expression profile analyses of non-small cell lung carcinomas (NSCLC) and esophageal squamous cell carcinomas (ESCC) revealed that lymphocyte antigen 6 complex locus K (LY6K) was specifically expressed in testis and transactivated in a majority of NSCLCs and ESCCs. Immunohistochemical staining using 406 NSCLC and 265 ESCC specimens confirmed that LY6K overexpression was associated with poor prognosis for patients with NSCLC ( $P = 0.0003$ ), as well as ESCC ( $P = 0.0278$ ), and multivariate analysis confirmed its independent prognostic value for NSCLC ( $P = 0.0035$ ). We established an ELISA to measure serum LY6K and found that the proportion of the serum LY6K-positive cases was 38 of 112 (33.9%) NSCLC and 26 of 81 (32.1%) ESCC, whereas only 3 of 74 (4.1%) healthy volunteers were falsely diagnosed. In most cases, there was no correlation between serum LY6K and conventional tumor markers of carcinoembryonic antigen (CEA) and cytokeratin 19-fragment (CYFRA 21-1) values. A combined ELISA for both LY6K and CEA classified 64.7% of lung adenocarcinoma patients as positive, and the use of both LY6K and CYFRA 21-1 increased sensitivity in the detection of lung squamous cell carcinomas and ESCCs up to 70.4% and 52.5%, respectively, whereas the false positive rate was 6.8% to 9.5%. In addition, knocked down of LY6K expression with small interfering RNAs resulted in growth suppression of the lung and esophageal cancer cells. Our data imply that a cancer-testis antigen, LY6K, should be useful as a new type of tumor biomarker and probably as a target for the development of new molecular therapies for cancer treatment.

Iwata, T., T. Fujita, et al. (2005). "Frequent immune responses to a cancer/testis antigen, CAGE, in patients with microsatellite instability-positive endometrial cancer." *Clin Cancer Res* **11**(10): 3949-57.

**PURPOSE:** Identification of cancer/testis antigens useful for diagnosis or immunotherapy of cancers was attempted by cDNA expression cloning with patients' sera (SEREX). **EXPERIMENTAL**

DESIGN: cDNA expression libraries made from testis or endometrial cancer cell lines were screened using sera from patients with endometrial cancer or melanoma patients immunized with dendritic cells pulsed with autologous tum or lysates. Tissue-specific expression by RT-PCR and immunogenicity by Western blotting of the bacterial recombinant antigen with sera from cancer patients were evaluated. RESULTS: A cancer/testis antigen, CAGE, was isolated by two independently performed SEREX. CAGE was expressed in various cancer cell lines including endometrial cancer, colon cancer, and melanoma in 7 of 10 endometrial cancer tissues and in 1 of 3 atypical endometrial hyperplasia, but not in normal tissues including the endometrium and testis. The protein expression on cancer cells was confirmed by Western blot analysis with the recombinant CAGE protein, anti-CAGE IgG antibody was detected in sera from 5 of 45 endometrial cancer, 2 of 24 melanoma, and 2 of 33 colon cancer patients, but not in sera from healthy individuals. By ELISA analysis, anti-CAGE antibody was detected in 12 of 45 endometrial cancer, 2 of 20 melanoma, and 4 of 33 colon cancer patients. Intriguingly, anti-CAGE antibody was highly positive in 7 of the 13 (53.8%) microsatellite instability (MSI)-H patients with endometrial cancer, but negative in 20 non-MSI-H patients (P = 0.001). CONCLUSION: CAGE may be useful for immunotherapy and diagnosis of various cancers particularly MSI-positive endometrial cancer.

Jack, R. H., E. A. Davies, et al. (2007). "Testis and prostate cancer incidence in ethnic groups in South East England." *Int J Androl* 30(4): 215-20; discussion 220-1.

International variations in the incidence of testis and prostate cancer are well established. Data from the USA have also shown differences between White and Black men; however, there has been little work on ethnicity and cancer incidence in the UK, due to incomplete ethnicity information in cancer registries. The Hospital Episode Statistics (HES) dataset has more complete information on self-assigned ethnicity for inpatients of English NHS hospitals. Data on 194 590 male patients resident in South East England diagnosed with cancer between 1998 and 2003 were extracted from the Thames Cancer Registry (TCR). Of these, ethnicity information from HES was obtained for 123 507 (63%), ethnicity information from TCR was available for a further 5909 (3%), and no ethnicity was available for 65 174 (33%). Compared with 'All White' men, testis cancer incidence was significantly lower in Indian, Pakistani, Bangladeshi, Other Asian, Black Caribbean, Black African, Other Black and Chinese men. Prostate cancer incidence was

significantly increased in Black Caribbean, Black African, Other Black, Indian, Pakistani, Mixed White and Black Caribbean and Mixed White and Black African groups compared with 'All White' men. Bangladeshi and Chinese men had a significantly decreased incidence of prostate cancer. The incidence of prostate cancer in Indian and Pakistani men showed convergence towards the rates in the white population, suggesting the existence of modifiable risk factors in these men. Most other variations in these data are consistent with international comparisons, and indicate that genetic variations in susceptibility are very influential.

Jager, D., M. Unkelbach, et al. (2002). "Identification of tumor-restricted antigens NY-BR-1, SCP-1, and a new cancer/testis-like antigen NW-BR-3 by serological screening of a testicular library with breast cancer serum." *Cancer Immun* 2: 5.

Serological analysis of recombinant cDNA expression libraries (SEREX) has led to the identification of several categories of new tumor antigens. We analyzed a testicular cDNA expression library with serum obtained from a breast cancer patient and isolated 13 genes designated NW-BR-1 through NW-BR-13. Of these, 3 showed tumor-restricted expression (NW-BR-1, -2 and -3), the others being expressed ubiquitously. NW-BR-3, representing 9 of 24 primary clones, showed tissue-restricted mRNA expression, being expressed in normal testis but not in 15 other normal tissues tested by Northern blotting. RT-PCR analysis showed strong NW-BR-3 expression in normal testis, weak expression in brain, kidney, trachea, uterus and normal prostate, and was negative in liver, heart, lung, colon, small intestine, bone marrow, breast, thymus, muscle, spleen, and stomach. NW-BR-3 mRNA expression was found in different tumor tissues and tumor cell lines by RT-PCR, thus showing a 'cancer/testis' (CT)-like mRNA expression pattern. NW-BR-3 shares 71% nucleotide and amino acid homology to a mouse gene cloned from mouse testicular tissue. Based on the mRNA expression pattern, NW-BR-3 represents a new candidate target gene for cancer immunotherapy. NW-BR-1 and NW-BR-2 also showed tumor-restricted mRNA expression. NW-BR-1 is a partial clone of the breast differentiation antigen NY-BR-1 previously identified by SEREX. NY-BR-1 is expressed in normal breast, testis and 80% of breast cancers. NW-BR-2 is identical to the CT antigen SCP-1, initially isolated by SEREX analysis of renal cancer. This study provides further evidence that SEREX is a powerful tool to identify new tumor antigens potentially relevant for immunotherapy approaches.

Jewett, M. A., A. Grabowski, et al. (2003). "Management of recurrence and follow-up strategies for patients with nonseminoma testis cancer." *Urol Clin North Am* **30**(4): 819-30.

This review serves as an outline of the clinical features and management options for the majority of recurrence situations in NSGCTs. The combination of reliable serum tumor markers, improved imaging techniques, effective cisplatin chemotherapy regimens, and application of meticulous surgical techniques has resulted in dramatic improvements in cure rates in NSGCT. These factors have caused the incidence of recurrent NSGCT to decline substantially in the past 20 years. This rarity of recurrence in combination with the low incidence of NSGCT prevents the practicing clinician from accumulating experience in this challenging patient population. Therefore, to ensure improvement in salvage rates, patients are best managed in centers with extensive experience in NSGCT.

John, T., O. L. Caballero, et al. (2008). "ECSA/DPPA2 is an embryo-cancer antigen that is coexpressed with cancer-testis antigens in non-small cell lung cancer." *Clin Cancer Res* **14**(11): 3291-8.

**PURPOSE:** Cancer cells recapitulate many behaviors of pluripotent embryonic cells such as unlimited proliferation, and the capacity to self-renew and to migrate. Embryo-cancer sequence A (ECSA), later named developmental pluripotency associated-2 (DPPA2), is an embryonic gene initially isolated from pluripotent human preimplantation embryos. We hypothesized that ECSA/DPPA2 would be quiescent in most normal tissues but expressed in cancers and may therefore be a useful target for immunotherapy. **EXPERIMENTAL DESIGN:** ECSA/DPPA2 expression was examined in a panel of normal and tumor tissue by reverse transcription PCR, quantitative real-time PCR, and immunohistochemistry. A panel of 110 non-small cell lung cancers (NSCLC) were further investigated for the presence of ECSA/DPPA2 transcripts and several cancer testis antigens (CTA). Sera from 104 patients were analyzed for spontaneous ECSA/DPPA2 antibody production by ELISA and Western blot. **RESULTS:** ECSA/DPPA2 transcripts were limited to normal testis, placenta, bone marrow, thymus, and kidney but expressed in a variety of tumors most notably in 30% of NSCLC. Enrichment for CTAs in ECSA/DPPA2-positive NSCLC was observed. Immunohistochemistry confirmed nuclear and cytoplasmic localization in subpopulations of cells with coexpression of the CTA MAGE-A3. Antibodies to recombinant ECSA/DPPA2 protein were detected in the sera of 4 of 104 patients with NSCLC but not in healthy controls. **CONCLUSIONS:** The restricted

expression in normal tissues, expression in tumors with coexpression of CTAs, and spontaneous immunogenicity indicate that ECSA/DPPA2 is a promising target for antigen-specific immunotherapy in NSCLC.

Jungbluth, A. A., S. Ely, et al. (2005). "The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation." *Blood* **106**(1): 167-74.

Multiple myeloma is a malignancy of plasma cells. Vaccine immunotherapy is among the novel therapeutic strategies under investigation for this disease. To identify myeloma-associated antigens as potential targets for vaccine immunotherapy, we surveyed a comprehensive panel of bone marrow specimens from patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma for expression of cancer-testis (CT) antigens. Immunohistochemistry (IHC) demonstrated that 82% of stage-III myeloma specimens expressed the CT antigen CT7 (also known as melanoma antigen C1 [MAGE-C1]) and 70% expressed MAGE-A3/6. Messenger RNA for CT7 and MAGE-A family members was detected in 87% and 100% of stage-III samples, respectively. CT7 protein expression increased with advanced stage of disease. Higher levels of CT7 and MAGE-A3/6 proteins also correlated with elevated plasma-cell proliferation. These results show that CT7 and MAGE-A3/6 are promising myeloma-associated antigens for application in vaccine immunotherapy. Furthermore, the common expression and correlation with proliferation suggest a possible pathogenic role for these proteins in myeloma.

Jungbluth, A. A., W. A. Silva, Jr., et al. (2007). "Expression of cancer-testis (CT) antigens in placenta." *Cancer Immun* **7**: 15.

Besides their variable presence in fetal and adult germ cells, CT antigens have occasionally been detected in placental tissue. However, these data are scarce and solely based on mRNA analyses; nothing is known about their presence at the protein level. Here, we analyzed the expression of various CT antigens in placental tissues from gestational age week 5 to week 42 using monoclonal antibodies to various antigens of the MAGE-A and -C families, NY-ESO-1, as well as GAGE. We show that CT antigen expression in placenta varies widely for the various antigens, ranging from completely negative to abundant. Since little is known about the function and biology of CT antigens, interpretation of this highly variable expression pattern is purely speculative. However, our

data indicate that the various CT antigens have different functions during placental development.

Juretic, A., G. C. Spagnoli, et al. (2003). "Cancer/testis tumour-associated antigens: immunohistochemical detection with monoclonal antibodies." *Lancet Oncol* 4(2): 104-9.

Cancer/testis tumour-associated antigens (C/T TAA) were the first human tumour-associated antigens to be characterised at the molecular level. Specific genes are expressed in the testis and in tumours of varying histological origin. The tissue expression pattern supports the notion that these antigens could be targets for active specific immunotherapy. Specific serological reagents have been developed and have helped to clarify biochemical characteristics of C/T TAA and to assess their distribution within clinical tumour samples. We review immunohistochemical evidence of the expression of C/T TAA known to be recognised by specific cytotoxic T lymphocytes. The emerging picture is consistent with a mostly heterogeneous expression in human cancers. These findings support the concept of multiantigenic tumour vaccine preparations. Moreover, the wide range of tumours in which C/T TAA have been detected urges further efforts to develop effective specific immunotherapeutic procedures.

Kalejs, M. and J. Erenpreisa (2005). "Cancer/testis antigens and gametogenesis: a review and "brainstorming" session." *Cancer Cell Int* 5(1): 4.

Genes expressed both in normal testis and in malignancies (Cancer/ Testis associated genes - CTA) have become the most extensively studied antigen group in the field of tumour immunology. Despite this, many fundamentally important questions remain unanswered: what is the connection between germ-cell specific genes and tumours? Is the expression of these genes yet another proof for the importance of genome destabilisation in the process of tumorigenesis?, or maybe activation of these genes is not quite random but instead related to some programme giving tumours a survival advantage? This review collates most of the recent information available about CTAs expression, function, and regulation. The data suggests a programme related to ontogenesis, mostly to gametogenesis. In the "brainstorming" part, facts in conflict with the hypothesis of random CTA gene activation are discussed. We propose a programme borrowed from organisms phylogenetically much older than humans, which existed before the differentiation of sexes. It is a programme that has served as a life cycle with prominent ploidy changes, and from which, as we know, the germ-cell ploidy cycle - meiosis - has

evolved. Further work may show whether this hypothesis can lead to a novel anti-tumour strategy.

Kanehira, M., T. Katagiri, et al. (2007). "Oncogenic role of MPHOSPH1, a cancer-testis antigen specific to human bladder cancer." *Cancer Res* 67(7): 3276-85.

To disclose the molecular mechanism of bladder cancer, the second most common genitourinary tumor, we had previously done genome-wide expression profile analysis of 26 bladder cancers by means of cDNA microarray representing 27,648 genes. Among genes that were significantly up-regulated in the majority of bladder cancers, we here report identification of M-phase phosphoprotein 1 (MPHOSPH1) as a candidate molecule for drug development for bladder cancer. Northern blot analyses using mRNAs of normal human organs and cancer cell lines indicated this molecule to be a novel cancer-testis antigen. Introduction of MPHOSPH1 into NIH3T3 cells significantly enhanced cell growth at in vitro and in vivo conditions. We subsequently found an interaction between MPHOSPH1 and protein regulator of cytokinesis 1 (PRC1), which was also up-regulated in bladder cancer cells. Immunocytochemical analysis revealed colocalization of endogenous MPHOSPH1 and PRC1 proteins in bladder cancer cells. Interestingly, knockdown of either MPHOSPH1 or PRC1 expression with specific small interfering RNAs caused a significant increase of multinuclear cells and subsequent cell death of bladder cancer cells. Our results imply that the MPHOSPH1/PRC1 complex is likely to play a crucial role in bladder carcinogenesis and that inhibition of the MPHOSPH1/PRC1 expression or their interaction should be novel therapeutic targets for bladder cancers.

Kelnar, C. J., C. McKinnell, et al. (2002). "Testicular changes during infantile 'quiescence' in the marmoset and their gonadotrophin dependence: a model for investigating susceptibility of the prepubertal human testis to cancer therapy?" *Hum Reprod* 17(5): 1367-78.

**BACKGROUND:** Inexplicably, boys treated with some therapies for cancer at age 2-10 years, a time of supposed 'testicular quiescence', are at risk of low sperm counts/infertility in adulthood. Our aims were to use the marmoset as a surrogate for man to establish testicular cell function/activity during 'quiescence' between the neonatal period and puberty, and to test if any cell activity could be suppressed by prior treatment with a GnRH antagonist. **METHODS AND RESULTS:** Based on immunoexpression studies, functional development of Sertoli cells (SGP-2, androgen receptor) and Leydig cells (3 beta-hydroxysteroid dehydrogenase) was detectable at an

age (35 weeks) when the testis is considered to be quiescent, and in advance of the pubertal rise in blood testosterone levels (50-60 weeks). Other changes at 35 weeks were the appearance of focal seminiferous tubule lumens and proliferating germ cells [indicated by immunoperoxidase staining of proliferating cell nuclear antigen (PCNA)]. Treatment from 25 to 35 weeks with GnRH antagonist largely (>85%) prevented these changes. However, the PCNA-labelling index of spermatogonia in GnRH antagonist-treated animals did not differ from controls (41.3 versus 43.6%) though total spermatogonia volume per testis was reduced by 41%. Some protein markers (inhibin-alpha, estrogen receptor-beta) showed little change with age or treatment. Beyond 35 weeks, GnRH antagonist-treated animals showed a delay in the pubertal rise in plasma testosterone levels. CONCLUSIONS: These findings reinforce the view that the 'childhood' testis is not quiescent. This may explain the damaging effects of some cancer therapies on subsequent fertility of boys and raises the issue of protective intervention. The present studies suggest that GnRH antagonist-based intervention might be only partially successful. Identification of the factors regulating spermatogonial development in the infant marmoset may aid in the design of such strategies.

Kim, S. H., S. Lee, et al. (2009). "Expression of cancer-testis antigens MAGE-A3/6 and NY-ESO-1 in non-small-cell lung carcinomas and their relationship with immune cell infiltration." *Lung* **187**(6): 401-11.

Cancer-testis antigens (CTAs) are expressed only in many cancers and limited immunoprivileged sites such as the testis and placenta. Dendritic cells (DCs) and CD8+ T lymphocytes (CTLs) play roles in the immune responses to tumor growth and may affect the prognosis of cancers. This study was designed to investigate the clinicopathologic significance of CTA expression in non-small-cell lung carcinomas (NSCLCs) and its relationship with immune cells. Immunohistochemical staining to CTAs such as MAGE-A3/6 and NY-ESO-1 was performed using paraffin blocks from 132 cases of NSCLCs, including 75 cases of squamous cell carcinoma (SqCC) and 57 cases of adenocarcinoma (AdC), and the results were evaluated to correlate with tumor-infiltrating DCs and CTLs and clinicopathologic features. MAGE-A3/6 and NY-ESO-1 were expressed in 50.0% (66/132) and 18.2% (24/132) of NSCLCs, respectively. MAGE-A3/6 was expressed more frequently in SqCC than in AdC, but the expression of NY-ESO-1 showed no difference in both types. CTAs revealed a higher expression in male than in female. In advanced stage III, NY-ESO-1-positive patients showed poorer survival than NY-ESO-1-negative patients. Otherwise, the CTA expression did not correlate with

clinicopathologic parameters. No relationship was found between DC and CTL infiltration in all NSCLCs. Regarding DC infiltration, the group showing negative expression to CTAs displayed an even higher number of infiltrating DCs than those showing positivity to one or the other or both CTAs. Although the aberrant expression of MAGE-A3/6 and NY-ESO-1 in NSCLC did not directly influence clinical prognostic factors, the higher expression of MAGE-A3/6 in SqCC suggests its value as a potential target for immunotherapy in this type of NSCLC. The inverse relationship between DCs and CTA expression may indicate that CTA-positive tumor cells would be akin to tumor stem cells escaping host immune response.

Kirkin, A. F., K. N. Dzhandzhugazyan, et al. (2002). "Cancer/testis antigens: structural and immunobiological properties." *Cancer Invest* **20**(2): 222-36.

Characterization of tumor-associated antigens recognized by cytotoxic T lymphocytes which has evolved during recent years opens new possibilities for specific anti-cancer immunotherapy. Among different groups of tumor-associated antigens, cancer/testis (CT) antigens (expressed in many tumors and among normal tissues only in testes) represent the most perspective antigens for immunotherapy because of their broad tumor-specific expression. More than 50 CT antigens have been described so far and, for many of them, epitopes recognized by T lymphocytes have been identified. The most studied group of CT antigens is the MAGE proteins, which form the so-called MAGE superfamily, together with some MAGE-like proteins that have a different distribution than classical CT antigens. The MAGE superfamily includes five families: MAGE-A, MAGE-B, MAGE-C, MAGE-D, and necdin. Comparison of the structure of members of MAGE superfamily points to the existence of a domain organization of these proteins. The central, core domain (second domain) is highly conservative. The first domain is homologous among MAGE family members with a CT expression, but unique for each member of the MAGE-D and necdin families. In addition to the homology of the central domain, the third domain is also homologous among all members of MAGE superfamily, but to a much lesser extent. The MAGE-D proteins contain an additional, fourth domain, which in the case of MAGE-D3 coincides with trophinin, a separate molecule described previously as an adhesion molecule that participates in embryo implantation. The structural classification of the members of MAGE superfamily might help in the future to understand the biological function of MAGE proteins. One important property of the CT antigens is the up-regulation of

their expression by DNA demethylating agents, indicating a possible mechanism for their re-expression in tumors. One of the implications of this particular property could be that a combination of immunotherapy targeting CT antigens with chemotherapy inducing up-regulation of CT antigens might result in more efficient tumor eradication.

Kondo, T., X. Zhu, et al. (2007). "The cancer/testis antigen melanoma-associated antigen-A3/A6 is a novel target of fibroblast growth factor receptor 2-IIIb through histone H3 modifications in thyroid cancer." *Clin Cancer Res* **13**(16): 4713-20.

**PURPOSE:** Fibroblast growth factor (FGF) signals play fundamental roles in development and tumorigenesis. Thyroid cancer is an example of a tumor with nonoverlapping genetic mutations that up-regulate mitogen-activated protein kinase. We reported recently that FGF receptor 2 (FGFR2) is down-regulated through extensive DNA promoter methylation in thyroid cancer. Reexpression of the FGFR2-IIIb isoform impedes signaling upstream of the BRAF/mitogen-activated protein kinase pathway to interrupt tumor progression. In this analysis, we examined a novel target of FGFR2-IIIb signaling, melanoma-associated antigen-A3 and A6 (MAGE-A3/6). **EXPERIMENTAL DESIGN:** cDNA microarray analysis was done on human WRO thyroid cancer cells transfected with FGFR2-IIIb or empty vector. Identified gene target was confirmed by reverse transcription-PCR and Western blotting. Gene regulation was examined by treatment of WRO cells with the methylation inhibitor 5'-azacytidine followed by methylation-specific PCR and reverse transcription-PCR and by chromatin immunoprecipitation. **RESULTS:** Gene expression profiling identified the cancer/testis antigen MAGE-A3/6 as a novel target of FGFR2-IIIb signaling. MAGE-A3/6 regulation was mediated through DNA methylation and chromatin modifications. In particular, FGF7/FGFR2-IIIb activation resulted in histone 3 methylation and deacetylation associated with the MAGE-A3/6 promoter to down-regulate gene expression. **CONCLUSIONS:** These data unmask a complex repertoire of epigenetically controlled signals that govern FGFR2-IIIb and MAGE-A3/6 expression. Our findings provide insights into the interrelationship between novel tumor markers that may also represent overlapping therapeutic targets.

Kono, K., Y. Mizukami, et al. (2009). "Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer." *Cancer Sci* **100**(8): 1502-9.

We previously identified three novel HLA-A24-restricted epitope peptides, which were derived from three cancer-testis antigens, TTK protein kinase (TTK), lymphocyte antigen 6 complex locus K (LY6K), and insulin-like growth factor (IGF)-II mRNA binding protein 3 (IMP-3), as targets for cancer vaccination against esophageal squamous cell carcinoma (ESCC). To examine the safety, immunogenicity, and antitumor effect of vaccine treatment using a combination of these three peptides, 10 HLA-A2402-positive advanced ESCC patients who failed to standard therapy were enrolled in a phase I clinical trial. Each of the three peptides (1 mg each) was intradermally administered with 1 mL of incomplete Freund's adjuvant to the neck in three separate regions weekly for 5 weeks. The cancer vaccination therapy was well tolerated without any treatment-associated adverse events of grade 3 or 4. The TTK-, LY6K-, and/or IMP-3-specific T-cell immune responses were observed by enzyme-linked immunospot assay in peripheral blood lymphocytes obtained from nine of the 10 ESCC patients after their vaccination. The median survival time after the vaccination was 6.6 months. The vaccination could induce good clinical responses in 50% of the 10 patients. One patient experienced a complete response in hepatic metastasis lasting 7 months, one showed objective responses in all lung metastasis lesions, and three patients revealed a stable disease condition for at least 2.5 months. The cancer vaccine therapy using these three peptides demonstrated satisfactory safety and good immunogenicity as well as promising disease control rate, and therefore warrants further clinical studies.

Konstantinos, S., A. Alevizos, et al. (2006). "Association between testicular microlithiasis, testicular cancer, cryptorchidism and history of ascending testis." *Int Braz J Urol* **32**(4): 434-8; discussion 439.

**OBJECTIVE:** To prospectively determine the prevalence of testicular microlithiasis in symptomatic patients who were referred for scrotal ultrasound examination and to evaluate the possible association of testicular microlithiasis with testicular cancer and other conditions such as cryptorchidism or history of ascending testis. **MATERIALS AND METHODS:** 391 men who were referred to our institutions between July 2002 and May 2005 for any type of symptoms from the testicles, underwent physical and scrotal ultrasound examination. The presence of testicular microlithiasis, the number of lesions and the involvement of both testicles in relation to the symptoms as well as the coexistence of other lesions were studied. **RESULTS:** Eighteen (4.6%) of 391 men enrolled into the study had

testicular microlithiasis. Two out of the eighteen patients (11%) had concomitant testicular cancer, which was confirmed by pathological evaluation of the orchidectomy specimen. One of the patients with testicular microlithiasis presented a rising in biochemical tumor markers (LDH, and HCG) and underwent orchidectomy one year later. Five of the remaining 373 (1.3%) patients without microlithiasis were diagnosed with testicular cancer. Thirty six men reported having a history of ascending testis, but none of them was found with testicular cancer. Two cases of testicular torsion in a cryptorchid position had testicular microlithiasis, but the orchidectomy specimen (after surgery) was negative for testicular cancer. The correlation between testicular cancer and testicular microlithiasis found in our study was statistically significant ( $p < 0.05$ ). CONCLUSION: There seems to be an association between testicular microlithiasis and testicular cancer.

Kouprina, N., M. Mullokandov, et al. (2004). "The SPANX gene family of cancer/testis-specific antigens: rapid evolution and amplification in African great apes and hominids." *Proc Natl Acad Sci U S A* **101**(9): 3077-82.

Human sperm protein associated with the nucleus on the X chromosome (SPANX) genes comprise a gene family with five known members (SPANX-A1, -A2, -B, -C, and -D), encoding cancer/testis-specific antigens that are potential targets for cancer immunotherapy. These highly similar paralogous genes cluster on the X chromosome at Xq27. We isolated and sequenced primate genomic clones homologous to human SPANX. Analysis of these clones and search of the human genome sequence revealed an uncharacterized group of genes, SPANX-N, which are present in all primates as well as in mouse and rat. In humans, four SPANX-N genes comprise a series of tandem duplicates at Xq27; a fifth member of this subfamily is located at Xp11. Similarly to SPANX-A/D, human SPANX-N genes are expressed in normal testis and some melanoma cell lines; testis-specific expression of SPANX is also conserved in mouse. Analysis of the taxonomic distribution of the long and short forms of the intron indicates that SPANX-N is the ancestral form, from which the SPANX-A/D subfamily evolved in the common ancestor of the hominoid lineage. Strikingly, the coding sequences of the SPANX genes evolved much faster than the intron and the 5' untranslated region. There is a strong correlation between the rates of evolution of synonymous and nonsynonymous codon positions, both of which are accelerated 2-fold or more compared to the noncoding sequences. Thus, evolution of the SPANX family appears to have involved positive selection that affected not only the

protein sequence but also the synonymous sites in the coding sequence.

Kratzik, C., G. Schatzl, et al. (2006). "Transcutaneous high-intensity focused ultrasonography can cure testicular cancer in solitary testis." *Urology* **67**(6): 1269-73.

OBJECTIVES: To report the long-term results in 7 patients (including the 5-year results in 3 patients) after high-intensity focused ultrasonography (HIFU) combined with irradiation to treat testicular tumors in a solitary testis. METHODS: Transcutaneous HIFU ablation of testicular tumors is based on a technique using a piezoceramic transducer operating at 4.0 MHz with a site intensity of 1600 to 2000 W/cm<sup>2</sup>. In a Phase II trial, 7 patients with the typical sonographic pattern of a tumor in a solitary testis were treated with transcutaneous HIFU, as a minimally invasive organ-preserving approach, followed 6 weeks later by prophylactic testicular irradiation (range 18 to 20 Gy). The aim was to ablate the entire cancer in a single therapeutic HIFU session. In all 7 patients, the contralateral testis had previously been removed because of testicular cancer. RESULTS: One patient received two cycles of chemotherapy for a single suspicious retroperitoneal lymph node diagnosed 6 months after HIFU. The other 6 protocol-treated patients remained tumor free at a mean follow-up of 42 months (range 3 to 93). One patient, who had refused postoperative irradiation, developed a recurrent tumor within 6 months. No patient showed any signs of clinical hypogonadism, and the International Index of Erectile Function score was normal for all patients. No androgen substitution was necessary. The only adverse effect noted was a small thermal lesion of the scrotum in 1 patient. CONCLUSIONS: Despite the lack of tumor histologic examination, transcutaneous HIFU followed by irradiation permits a minimally invasive, organ-preserving, curative treatment for tumors in a solitary testis.

Kruger, S., V. Ola, et al. (2007). "Expression of cancer-testis antigen CT7 (MAGE-C1) in breast cancer: an immunohistochemical study with emphasis on prognostic utility." *Pathol Oncol Res* **13**(2): 91-6.

High expression of the cancer-testis antigen CT7, also referred to as MAGE-C1, has been recently described in a variety of malignant tumors, including breast carcinoma. To our knowledge, no data concerning the prognostic utility of CT7 expression in breast cancer are available. In this retrospective study, we evaluated the relationship between CT7 immunoreactivity and clinicopathological parameters as well as relapse-free survival (RFS) and metastasis-free survival (MFS) of 124 women with invasive



breast cancer. A positive CT7 status, defined as immunoreactivity in more than 50% of tumor cells, was found in 18% of cases and correlated significantly with high tumor grade ( $p=0.004$ ), but with no other clinicopathological parameter. In a univariate analysis, CT7 status showed an association with RFS by trend ( $p=0.107$ ; relative risk [RR]: 1.85) and a significant association with MFS ( $p=0.043$ ; RR: 2.02). In a multivariate analysis, tumor grade, stage, nodal status, angioinvasion, HER2 expression as well as estrogen and progesterone receptor expression were identified as significant independent prognostic factors of RFS and/or MFS. In this respect, CT7 expression showed a weak, statistically not significant trend towards an independent prognostic relevance concerning prediction of MFS ( $p=0.147$ ; RR: 1.95). Our data suggest that estimation of CT7 immunoreactivity is of limited prognostic usefulness in breast cancer. It may provide additional information concerning assessment of MFS in selected cases.

Lee, M. H., E. I. Son, et al. (2008). "Expression of cancer-testis genes in brain tumors." J Korean Neurosurg Soc **43**(4): 190-3.

**OBJECTIVE:** Cancer-testis (CT) genes are considered promising candidates for immunotherapeutic approaches. The aim of this study was to investigate which CT genes should be targeted in immunotherapy for brain tumors. **METHODS:** We investigated the expression of 6 CT genes (MAGE-E1, SOX-6, SCP-1, SSX-2, SSX-4, and HOM-TE85) using reverse-transcription polymerase chain reaction in 26 meningiomas and 32 other various brain tumor specimens, obtained from the patients during tumor surgery from 2000 to 2005. **RESULTS:** The most frequently expressed CT genes of meningiomas were MAGE-E1, which were found in 22/26 (85%) meningioma samples, followed by SOX-6 (9/26 or 35%). Glioblastomas were most frequently expressed SOX-6 (6/7 or 86%), MAGE-E1 (5/7 or 71%), followed by SSX-2 (2/7 or 29%) and SCP-1 (1/7 or 14%). However, 4 astrocytomas, 3 anaplastic astrocytomas, and 3 oligodendroglial tumors only expressed MAGE-E1 and SOX-6. Schwannomas also expressed SOX-6 (5/6 or 83%), MAGE-E1 (4/6 or 67%), and SCP-1 (2/6 or 33%). **CONCLUSION:** The data presented here suggest that MAGE-E1 and SOX-6 genes are expressed in a high percentage of human central nervous system tumors, which implies the CT genes could be the potential targets of immunotherapy for human central nervous system tumors.

Lee, S. Y., B. Williamson, et al. (2004). "Identification of the gonad-specific anion transporter SLCO6A1 as a cancer/testis (CT) antigen expressed in human lung cancer." Cancer Immun **4**: 13.

Serological analysis of recombinant cDNA expression libraries (SEREX) has led to the identification of many of the antigens recognized by the immune system of cancer patients, which are collectively referred to as the cancer immunome. We used SEREX to screen a testicular cDNA expression library with sera obtained from non-small cell lung cancer patients and isolated cDNA clones for 82 antigens. These included a total of 31 antigens previously identified by SEREX, and 51 that did not match entries in the Cancer Immunome Database and were considered newly identified antigens. Overall, the antigens comprised 62 known proteins and 20 uncharacterized gene products. Six antigens (NY-TLU-6, -37, -39, -57, -70, -75) were identified as putative cell surface proteins that are potential targets for monoclonal antibody-based immunotherapy. Of these, the gonad-specific anion transport protein SLCO6A1 (NY-TLU-57) was shown to be tissue-restricted. RT-PCR showed it to be expressed strongly only in normal testis, and weakly in spleen, brain, fetal brain, and placenta. In addition, NY-TLU-57 mRNA was found in lung tumor samples (5/10) and lung cancer cell lines (6/11), as well as bladder (5/12) and esophageal (5/12) tumor samples. These data suggest that SLCO6A1 is a putative cancer/testis (CT) cell surface antigen of potential utility as a target for antibody-based therapy for a variety of tumor types. The analysis also permits us to estimate the eventual size of the SEREX-defined cancer immunome at around 4000 genes. This emphasizes the importance of continued SEREX screening to define the cancer immunome.

Leisinger, H. J. and J. P. Donohue (2002). "The role of retroperitoneal surgery in testis cancer." Crit Rev Oncol Hematol **44**(1): 71-80.

Testis cancer is today a curable malignancy. But controversy remains about the appropriate management of patients presenting different stages. There is an increasing interest in surveillance rather than in primary retroperitoneal lymph node dissection (RPLND) for stage I non-seminomatous germ cell tumors (NSGCT). Adjuvant chemotherapy has become an efficient treatment option for high risk non-seminomatous germ cell testis cancer, however, biological and histologic risk factors of the primary tumor are not yet precisely defined. To determine the appropriate management of patients with testicular cancer, postoperative morbidity after RPLND and risk of chemotherapy-induced morbidity must be balanced. Whoever reviews the literature must take into consideration that the excellent postoperative results after RPLND depend on high volume and large experience with testis cancer. As treatment morbidity and its intensity have a major impact on testis cancer

patient quality of life, the choice of management must be based on the patient's social situation, his personal needs, and the doctor's experience and resources.

Li, M., Y. H. Yuan, et al. (2005). "Expression profile of cancer-testis genes in 121 human colorectal cancer tissue and adjacent normal tissue." *Clin Cancer Res* **11**(5): 1809-14.

**PURPOSE:** Among tumor antigens identified to date, cancer-testis (CT) antigens, which are coded by CT genes, are identified as a group of highly attractive targets for cancer vaccines. This study is the first to analyze the mRNA expression and possible correlation with pathologic characteristics of multiple CT genes in a large cohort of colorectal cancer (CRC) patients. **EXPERIMENTAL DESIGN:** The expression of 10 individual CT genes in 121 CRC and adjacent tissues were analyzed by RT-PCR method. The presence of autologous antibodies against NY-ESO-1 was examined in serum samples by ELISA. To confirm the protein expression, immunohistochemistry was done for detecting the NY-ESO-1 antigen in mRNA-positive CRC tissues. **RESULTS:** The CT genes were detected with various frequencies in CRC tissue, SCP-1, 1.7%; SSX-2, 2.5%; SSX-4, 2.5%; SSX-1, 5.0%; CT10, 6.6%; NY-ESO-1, 9.9%; MAGE-1, 11.6%; LAGE-1, 15.7%; MAGE-4, 22.3%; and MAGE-3, 27.3%. In 56.2% of tumor tissues examined in this study, at least one CT gene was detected. In contrast, no CT gene expression was found in cancer adjacent tissues. Among 10 CT genes investigated, NY-ESO-1 and LAGE-1 are of particular interest because their mRNA expression in CRC was rarely reported before. In our study, NY-ESO-1 mRNA was found to express in 9.9% of the samples, and also correlated significantly with stages ( $P = 0.041$ ) and local lymph node metastasis ( $P = 0.002$ ). In addition, we also identified one NY-ESO-1 antibody-positive serum sample. MAGE-4 mRNA was expressed at a high frequency in tumor tissues with vessel emboli samples ( $P = 0.025$ ). **CONCLUSIONS:** These results suggested that CT genes, especially NY-ESO-1 and LAGE-1, do express in CRC. More than 50% of the CRC patients in this study express at least one CT gene, making them eligible for CT vaccination. NY-ESO-1 gene may serve as a marker for local metastasis and advanced disease. MAGE-4 gene is significantly associated with the vessel emboli.

Li, Z., W. Li, et al. (2007). "A yeast two-hybrid system using Sp17 identified Ropporin as a novel cancer-testis antigen in hematologic malignancies." *Int J Cancer* **121**(7): 1507-11.

Since most intracellular proteins are expressed with their ligands, ligands of cancer-testis

(CT) antigens may also be CT in their distribution. Applying Sperm protein 17 (Sp17) as the bait in a yeast 2-hybrid system of a testicular cDNA library, 17 interacting clones were isolated and all encoded Ropporin, a spermatogenic cell-specific protein that serves as an anchoring protein for the A-kinase anchoring protein, AKAP110. Ropporin showed a very restricted normal tissue gene expression, detected only in testis and fetal liver. Ropporin mRNA could also be detected in tumor cells from patients with multiple myeloma, chronic lymphocytic leukemia and acute myeloid leukemia. Interestingly, expression of Sp17 did not necessarily predict for the expression of Ropporin suggesting that their coexpression in these tumor cells was random rather than coordinated. Ropporin gene expression in tumor cells is associated with the presence of high titer IgG antibodies against Ropporin, suggesting the *in vivo* translation of the mRNA into protein and the immunogenicity of the protein to the autologous hosts. Using a CT antigen as the bait in a yeast 2-hybrid system may, therefore, identify novel tumor antigen. Our results also suggest that Ropporin is a novel CT antigen in hematologic malignancies.

Liang, S. and M. Fan (2009). "Immunotherapy for adenoid cystic carcinoma of salivary glands: cancer/testis antigens and 5-aza-2'-deoxycytidine." *Med Hypotheses* **73**(5): 768-9.

Adenoid cystic carcinoma of salivary glands is the epithelial tumor. There are amount of malignant occurrences of adenoid cystic carcinoma of salivary glands in the head and neck area. Cancer/testis antigens can be found in various malignant tumors, normal adult testis and occasionally placenta, but not in the other normal adult tissues. This characteristic makes Cancer/testis antigens as potential markers to be applied in immunotherapeutic strategies against cancer. It has been shown that in different tumors, the expression of certain Cancer/testis antigens is activated treated with 5-aza-CdR via the demethylation of their promoter CpG islands. It is logical that multiple Cancer/testis antigens may correlate with the clinicopathologic factors of adenoid cystic carcinoma of salivary glands and be the potential markers of prognosis treated with 5-aza-CdR. So the hypothesis will provide the new direction that we can use Cancer/testis antigens as candidate antigens for adenoid cystic carcinoma of salivary glands immunotherapy due to the high expression rate activated with 5-aza-CdR.

Liggins, A. P., P. J. Brown, et al. (2004). "A novel diffuse large B-cell lymphoma-associated cancer testis antigen encoding a PAS domain protein." *Br J Cancer* **91**(1): 141-9.

Here we report that the OX-TES-1 SEREX antigen, which showed immunological reactivity with serum from four out of 10 diffuse large B-cell lymphoma (DLBCL) patients, is encoded by a novel gene, PAS domain containing 1 (PASD1). PASD1\_v1 cDNA encodes a 639 amino-acid (aa) protein product, while an alternatively spliced variant (PASD1\_v2), lacking intron 14, encodes a 773 aa protein, the first 638 aa of which are common to both proteins. The PASD1-predicted protein contains a PAS domain that, together with a putative leucine zipper and nuclear localisation signal, suggests it encodes a transcription factor. The expression of PASD1\_v1 mRNA was confirmed by RT-PCR in seven DLBCL-derived cell lines, while PASD1\_v2 mRNA appears to be preferentially expressed in cell lines derived from non-germinal centre DLBCL. Immunophenotyping studies of de novo DLBCL patients' tumours with antibodies to CD10, BCL-6 and MUM1 indicated that two patients mounting an immune response to PASD1 were of a poor prognosis non-germinal centre subtype. Expression of PASD1 mRNA was restricted to normal testis, while frequent expression was observed in solid tumours (25 out of 68), thus fulfilling the criteria for a novel cancer testis antigen. PASD1 has potential for lymphoma vaccine development that may also be widely applicable to other tumour types.

Lim, E., J. Browning, et al. (2006). "Desmoplastic melanoma: comparison of expression of differentiation antigens and cancer testis antigens." *Melanoma Res* **16**(4): 347-55.

Desmoplastic melanoma is a diagnostic and therapeutic challenge. Immunohistochemical analysis with antibodies to melanoma antigens can complement morphologic evaluation. Although staining for S100 protein is generally positive, staining for other melanoma differentiation antigens, particularly gp100, Melan-A/MART1 and tyrosinase, is often negative despite being commonly positive in other melanoma types. A high clinical index of suspicion and better diagnostic techniques are essential as atypical features and incorrect diagnosis can lead to poor clinical outcomes. Antigens associated with melanoma, such as the melanocyte differentiation and cancer testis antigen, may become important targets for immune therapies. We characterized the patterns of antigen expression of desmoplastic melanoma from 32 patients, including gp100, Melan-A/MART-1, tyrosinase, MAGE-A1, MAGE-A4 and NY-ESO-1. Consistent positive staining with S100 was observed. Differentiation antigens were expressed more frequently than cancer testis antigens regardless of the histological subtype of desmoplastic melanoma. When present, cancer testis antigen expression correlated to positive staining with

differentiation antigens. The diagnostic yield of desmoplastic melanoma did not increase with the addition of cancer testis antigen typing. Low levels of expression of cancer testis antigen may indicate that they are suboptimal targets for vaccine development in desmoplastic melanoma.

Lim, J. H., S. P. Kim, et al. (2005). "Activation of human cancer/testis antigen gene, XAGE-1, in tumor cells is correlated with CpG island hypomethylation." *Int J Cancer* **116**(2): 200-6.

Expression of the XAGE-1 antigen is restricted to germ cells of the testis and a variety of neoplastic tissues. To date, the molecular mechanism for regulating expression of this cancer/testis antigen gene has been unknown. To evaluate methylation as a potential mechanism for regulating expression of this gene, we first correlated gene methylation status (measured by sequencing of bisulfide-modified DNA and COBRA) to expression of XAGE-1 mRNA in normal and cancerous cells. This analysis revealed dense methylation of the CpG island in the XAGE-1 gene promoter for the normal and cancerous cells that do not express this gene but loss of this methylation in normal testis, cancer cell lines and the primary gastric cancers where the gene is highly expressed. Further supporting the role of methylation in regulating expression of XAGE-1 were observations that treatment of 2 heavily methylated cell lines, SNU620 and HT29, with 5'-aza-deoxycytidine resulted in demethylation of XAGE-1 promoter and corresponding expression of this gene. Finally, we cloned various segments of the CpG-rich XAGE-1 gene promoter linked to a luciferase reporter construct and transiently transfected this construct into HCT116 cells. These experiments confirmed transcriptional regulatory activity for the promoter region that incorporates the CpG island and demonstrated that in vitro methylation of this island results in loss of promoter activity. Collectively, these studies indicate that XAGE-1 expression in normal and cancerous tissues is regulated by methylation of the CpG island in the gene promoter.

Liu, X., M. S. Cicek, et al. (2007). "Association of testis derived transcript gene variants and prostate cancer risk." *J Urol* **177**(3): 894-8.

PURPOSE: The testis derived transcript gene has been suggested as a tumor suppressor gene for prostate cancer at 7q31. To investigate this concept we evaluated the effects of 7 tagging single nucleotide polymorphisms that comprehensively captured the common genetic variants in TES on aggressive prostate cancer in a case-control study. MATERIALS AND METHODS: A total of 506 cases diagnosed with aggressive prostate cancer, and an equal number

of age, institute and ethnicity matched controls, were recruited from the major medical institutions in Cleveland, Ohio. A logistic regression model was used to evaluate the association between SNPs/multimarker haplotypes and prostate cancer. RESULTS: When looking at all study subjects and white men only, no statistically significant associations were observed between any variants and more aggressive disease. However, 3 variants showed inverse associations with disease in black men (178), including 2 intronic SNPs (rs2402056, rs1004109) and 1 SNP close to the 3' untranslated region (rs4730721) with ORs of 0.57 (95% CI 0.36-0.90, under an additive mode of inheritance), 0.57 (95% CI 0.36-0.91, under an additive mode of inheritance) and 0.45 (95% CI 0.21-0.98, under a dominant mode of inheritance), respectively. Variants rs2402056 and rs1004109 are in tight linkage disequilibrium ( $r^2=0.8$ ) and the reconstructed haplotype did not provide any additional evidence for association than their genotype level results. CONCLUSIONS: Our findings suggest that the variants in TES, or in a nearby gene, may be associated with prostate cancer in black men.

Lohmann, C. M., W. J. Hwu, et al. (2003). "Primary malignant melanoma of the oesophagus: a clinical and pathological study with emphasis on the immunophenotype of the tumours for melanocyte differentiation markers and cancer/testis antigens." *Melanoma Res* **13**(6): 595-601.

SUMMARY: Primary malignant melanomas of the oesophageal squamous mucosa are exceedingly rare. We present here the clinical and pathological findings of 10 patients (mean age 64 years) with primary oesophageal melanoma, with emphasis on the immunophenotype of the tumours. The majority of melanomas were located in the mid to distal oesophagus and were large (mean tumour size at the time of diagnosis 6.2 cm; mean depth of invasion 1.86 cm). All but two of the melanomas were associated with an extensive in situ component. Half of the tumours were amelanotic. The histological spectrum was wide, including appearances mimicking lymphoma, poorly differentiated adenocarcinoma or sarcoma. Immunohistochemical studies were performed on six tumours using monoclonal antibodies (MAb) to S100 protein, tyrosinase (MAb T311), Melan-A (MAb A103), and gp100 (MAb HMB-45), as well as antibodies to five cancer/testis (CT) antigens (MAb CT7-33 to CT7/MAGE-C1, MAb ESO121 to NY-ESO-1, MAb 57B to MAGE-A4, MAb MA454 to MAGE-A1, and MAb M3H67 to MAGE-A3). Seven patients had metastatic disease at the time of presentation. All but one patient underwent resection of the tumour with negative surgical margins. Survival was poor, with a mean

survival of 19.8 months. One patient, however, whose tumour was limited to the submucosa, is still alive 108 months post-oesophagectomy. All six melanomas examined by immunohistochemistry were positive for all the melanocyte differentiation markers tested. In addition, they were all positive for CT antigens, with MA454 being the most commonly found, suggesting that CT antigens may be a promising immunotherapeutic target for oesophageal melanomas.

Lonn, M. E., C. Hudemann, et al. (2008). "Expression pattern of human glutaredoxin 2 isoforms: identification and characterization of two testis/cancer cell-specific isoforms." *Antioxid Redox Signal* **10**(3): 547-57.

The cellular redox state is associated with major cellular processes including differentiation, transformation, and apoptosis. Glutaredoxin 2 (Grx2) is a mitochondrial oxidoreductase suggested to play a critical role in protection against apoptotic stimuli. An alternative Grx2 transcript variant encoding a nonmitochondrial protein (Grx2b) was proposed before, but no data was available on the expression of this isoform. We have systematically investigated the expression of Grx2 transcript variants in human tissues and transformed cell lines. The transcript variant encoding mitochondrial Grx2 (Grx2a) was found to be ubiquitously expressed, emphasizing the general importance of the protein for mitochondrial redox homeostasis. In addition, we confirmed the previously suggested isoform Grx2b and identified a new third isoform (Grx2c) derived from alternative splicing of the Grx2b-encoding transcript. In normal tissue expression of both Grx2b and Grx2c was restricted to testes, but additionally we were able to demonstrate transcripts in various cancer cell lines. Both Grx2b and Grx2c are enzymatically active, but only Grx2c can complex the regulatory iron-sulfur cluster described for Grx2a. Expression of GFP fusion proteins suggested a cytosolic and nuclear localization of both Grx2b and Grx2c. Our findings provide the first evidence for functions of Grx2 outside mitochondria.

Luftl, M., G. Schuler, et al. (2004). "Melanoma or not? Cancer testis antigens may help." *Br J Dermatol* **151**(6): 1213-8.

BACKGROUND: Cancer testis antigens (CTAs) are expressed in a variety of malignant tumours. No CTA expression is found in normal adult tissues, except in male germ cells and occasionally placenta. To date, more than 20 CTAs or antigen families have been identified. OBJECTIVES: Owing to their tumour-associated expression pattern, CTAs may be useful for making a distinction between

benign and malignant neoplasms. The present study was done to analyse the value of CTAs for the discrimination of cutaneous melanoma and naevi. PATIENTS AND METHODS: Primary melanomas (38) and 19 naevi were analysed for their expression of CTAs by immunohistochemistry using the following monoclonal antibodies (mAb) to the following antigens (mAb/antigen): MA454/MAGE-A1, 57B/MAGE-A4, ES121/NY-ESO-1. In a subset of melanomas (n = 26), the CTA panel was extended to three additional CTAs (mAb/antigen): CT7-33/MAGE-C1, M3H67/MAGE-A3 and GAGE/GAGE. RESULTS: All 19 naevi were negative for the mAbs MA454, M3H67, 57B, ES121, CT7-33 and GAGE. In melanoma, the immunoreactivity was as follows: MA454: 8/38 (21%), 57B 11/38 (29%), ES121 9/38 (24%). However, 19/38 (50%) were positive for at least one CTA. When 26 melanomas were tested for the expression of six different CTAs 20/26 (77%) were positive for at least one CTA. CONCLUSIONS: CTAs may be useful in the determination of suspected malignancy in cutaneous melanomas. The low incidence of particular CTAs can be overcome by increasing the number of CTAs analysed.

Luo, C., X. Xiao, et al. (2007). "CABYR is a novel cancer-testis antigen in lung cancer." *Clin Cancer Res* **13**(4): 1288-97.

PURPOSE: Cancer-testis (CT) antigens are often expressed in a proportion of tumors of various types. Their restricted normal tissue expression and immunogenicity make them potential targets for immunotherapy. CABYR is a calcium-binding tyrosine phosphorylation-regulated fibrous sheath protein initially reported to be testis specific and subsequently shown to be present in brain tumors. This study was to determine whether CABYR is a novel CT antigen in lung cancer. EXPERIMENTAL DESIGN: mRNA expression of CABYR-a/b (combination of CABYR-a and CABYR-b) and CABYR-c was examined in 36 lung cancer specimens, 14 cancer cell lines, and 1 normal cell line by conventional and real-time reverse transcription-PCR. Protein expression of CABYR was analyzed in 50 lung cancer tissues by immunohistochemistry. Antibodies specific to CABYR were analyzed in sera from 174 lung cancer patients and 60 healthy donors by ELISA and Western blot. RESULTS: mRNA expression of CABYR-a/b and CABYR-c was observed, respectively, in 13 and 15 of 36 lung cancer tissues as well as in 3 and 5 of 14 cancer cell lines, whereas neither of them was observed in adjacent noncancerous tissues or the normal cell line. Protein expression of CABYR-a/b and CABYR-c was observed, respectively, in 20 and 19 of 50 lung cancer

tissues. IgG antibodies specific to CABYR-a/b and CABYR-c were detected, respectively, in 11% and 9% of sera from lung cancer patients but not from the 60 healthy donors. CONCLUSION: CABYR is a novel CT antigen in lung cancer and may be a promising target for immunotherapy for lung cancer patients.

Luo, G., S. Huang, et al. (2002). "Expression of cancer-testis genes in human hepatocellular carcinomas." *Cancer Immun* **2**: 11.

Cancer-testis (CT) genes are expressed in a variety of human cancers, but not in normal tissues except for testis, and represent promising targets for immunotherapy and gene therapy. We investigated the expression of 10 CT genes (MAGE-1, MAGE-3, MAGE-4, GAGE, NY-ESO-1, SSX-1, HOM-MEL-40/SSX-2, SSX-4, HOM-TES-14/SCP-1, and HOM-TES-85) in 21 hepatocellular carcinoma (HCC) biopsy specimens. The most frequently expressed CT genes were SSX-1 and GAGE, which were found in 8/21 (38%) HCC samples, followed by HOM-TES-14/SCP-1 (6/21 or 29%), MAGE-3 (5/21 or 24%), HOM-TES-85 and MAGE-1 (4/21 or 19% each), whereas SSX-4 and HOM-MEL-40/SSX-2 were only expressed in 2/21 cases each, MAGE-4 in one case, and NY-ESO-1 not at all. Of the 21 HCC cases investigated, only four did not express any of the CT genes tested, 17 (81%) expressed at least one, 9 (43%) coexpressed two, four (19%) coexpressed four, three (14%) coexpressed five and one coexpressed 8 of the 10 CT genes tested. We conclude that a majority of HCC cases might be amenable to specific immunotherapeutic interventions. However, the identification of additional tumor-specific antigens with a frequent expression in HCCs is warranted to develop widely applicable, polyvalent HCC vaccines.

Madsen, B., M. Tarsounas, et al. (2003). "PLU-1, a transcriptional repressor and putative testis-cancer antigen, has a specific expression and localisation pattern during meiosis." *Chromosoma* **112**(3): 124-32.

PLU-1, a large multi-domain nuclear protein with strong transcriptional repression activity, is a member of the ARID family of DNA binding proteins. In previous studies, high levels of expression of Plu-1 mRNA and PLU-1 protein were detected in breast cancers, while expression in normal adult tissues was detected only in the testis, ovary and transiently in the mammary gland of the pregnant female. Due to its high levels of expression in the testis and to its specific relationship to cancer, PLU-1 has been proposed to belong to the family of testis-cancer antigens. In this study we attempted to determine putative functions for PLU-1 during spermatogenesis. To address this, we analysed the

pattern of expression and localisation of this protein in mouse testicular cells during postnatal development and adulthood. Using in situ hybridisation and immunostaining of testis sections we show that Plu-1 mRNA and PLU-1 protein are both highly expressed in the mitotic spermatogonia. Expression is reduced dramatically in the early prophase I stages (zygotene, leptotene), but reappears at pachytene and is still detectable in diplotene cells. We also found that PLU-1 localises diffusely over the nucleus, which indicates a potential chromatin binding ability of this protein. Consistent with this notion, we found that PLU-1 is present in the chromatin fraction in biochemical cell fractionation experiments using both somatic and meiotic cells. Our data point to a role for PLU-1 in meiotic transcription, which may be restricted to certain meiotic stages and may be mediated by the ability of this protein to associate with the chromatin.

Maio, M., S. Coral, et al. (2003). "Analysis of cancer/testis antigens in sporadic medullary thyroid carcinoma: expression and humoral response to NY-ESO-1." *J Clin Endocrinol Metab* **88**(2): 748-54.

Cancer/testis antigens (CTA) are tumor-associated antigens expressed during ontogenesis, in a number of solid tumors but not in normal tissues except testis. Most of these CTA are highly immunogenic, eliciting a humoral and cellular response in the patients with advanced cancer, and are useful for tumor-specific immunotherapy. Medullary thyroid carcinoma (MTC) is a neoplasm derived from the parafollicular cells of the thyroid and occurs in either a sporadic or a familial form. In the present study, we examined by RT-PCR the expression of a number of genes encoding CTA in 23 surgical samples of sporadic MTC. Among the 11 cDNA antigens examined, RAGE, MAGE-4, and GAGE 1-2, were not expressed in any of the tissues. SSX 2 was present only in one tissue, whereas BAGE, GAGE 1-6, MAGE-1, MAGE-2, MAGE-3, and SSX 1-5 were detected in two to five samples. NY-ESO-1 cDNA was the most frequent, being detected in 15 of 23 examined samples (65.2%). Six (26.1%) tissues did not express any CTA-specific mRNA, whereas 10 tumors expressed only one gene (43.5%), 3 (21.4%) expressed 2 genes, and 4 displayed a broad CTA gene expression. NY-ESO-1 expression in primary MTC tissues significantly correlated with tumor recurrence. The presence of specific anti-NY-ESO-1 antibodies was searched in the sera of MTC-affected patients examined by ELISA using recombinant NY-ESO-1 protein. A humoral response against this CTA was detected in 6 of 11 NY-ESO-1 expressing patients (54.5%), and in 1 of 6 patients with NY-ESO-1-negative tumor. No anti-NY-ESO-1 antibodies were detected in healthy subjects (n = 17). The presence of

anti-NY-ESO-1 antibodies was searched also in the sera of MTC affected patients whose tissues were not available for CTA analysis. Anti-NY-ESO-1 antibodies were present in 15 of 42 sera (35.7%), demonstrating that MTC is a neoplasm frequently associated with humoral immune response to NY-ESO-1. Serological survey may be useful as a way to identify patients with humoral immune response to NY-ESO-1 that provide a new attractive target for vaccine-based immunotherapy of MTC.

Maldonado-Valadez, R., D. Schilling, et al. (2007). "Post-chemotherapy laparoscopic retroperitoneal lymph-node dissection in testis cancer patients." *J Endourol* **21**(12): 1501-4.

**BACKGROUND AND PURPOSE:** Retroperitoneal lymph node dissection (RPLND) is still the most sensitive and specific method for the detection of malignant tumor and mature teratoma in stage II nonseminomatous testicular carcinoma after chemotherapy. Acceptance of this operation, however, has decreased because of the morbidity associated with the open approach. To reduce the morbidity and to improve the acceptance of RPLND, laparoscopy has been introduced. In this study, we describe our experiences with laparoscopic RPLND for stage II testicular carcinoma after chemotherapy. **METHODS:** Sixteen patients underwent 17 laparoscopic RPLND after chemotherapy for clinical stage IIA-III nonseminomatous testicular cancer. Patients with post-chemotherapy residual masses >1 cm and normalization of tumor markers were considered for the procedure. Our dissection field included the resection of the residual tumor as well as the ipsilateral template. **RESULTS:** Laparoscopic RPLND was completed in all patients. Operative time ranged from 125 to 370 minutes (mean 240 +/- 56 min). No transfusions were required, and no intra- or postoperative complications occurred because of the procedure. A bleomycin-induced interstitial pneumonia developed in one patient. After a mean follow-up period of 26 +/- 11 months (range 4 to 38), two disease recurrences were observed. **CONCLUSION:** Laparoscopic RPLND after chemotherapy is a feasible and oncologically safe procedure. However, the technique is challenging and should only be performed in selected patients with low residual tumor volume.

Meklat, F., Z. Li, et al. (2007). "Cancer-testis antigens in haematological malignancies." *Br J Haematol* **136**(6): 769-76.

Immunotherapy is an attractive therapeutic option for patients with haematological malignancies. Until recently, the progress in the development of tumour vaccines for haematological malignancies had

been slow due to the lack of suitable targets. Cancer-testis (CT) antigens are potentially suitable molecules for tumour vaccines of haematological malignancies because of their high immunogenicity in vivo and their relatively restricted normal tissue distribution. This review evaluates the properties and potential functions of CT antigens. We discuss the expression of CT antigens in patient with haematological malignancies and provide evidence in support of their immunogenicity in vivo in these patients. We also address the role of 'epigenetic' regulation of CT antigens in haematological malignancies and how hypomethylating agents could induce the expression of some of these antigens in tumour cells to overcome the problem of heterogeneity of expression of the antigen within individual tumour specimens. Data implicating the interaction of the promoter genes of some of these CT antigens with the MeCP2 protein also suggest the potential role of the histone deacetylase inhibitors in inducing antigen expression in tumour cells. Finally, we discuss the direction of future research in advancing the development of tumour vaccines for haematological malignancies.

Meklat, F., Y. Zhang, et al. (2009). "Identification of protamine 1 as a novel cancer-testis antigen in early chronic lymphocytic leukaemia." *Br J Haematol* **144**(5): 660-6.

Early chronic lymphocytic leukaemia (CLL) is an ideal disease for immunotherapy. We previously showed that SEMG 1 is a cancer-testis (CT) antigen in CLL. In this study, SEMG 1 was applied as the bait in a yeast two-hybrid system of a testicular cDNA library. Seven clones were isolated and Protamine (Prm) 1 was identified as a novel CT antigen in early CLL. PRM1 transcripts were detected in 11/41 (26.8%) patients. Prm 1 protein was also expressed but heterogeneously within individual patients. Of the 11 patients expressing Prm 1, four expressed Zap 70 protein and seven did not. These results, therefore, indicate that Prm 1 could potentially be a suitable target for the design of tumour vaccine for patients with early CLL, including for those with poor risk CLL. High titres of Prm 1 IgG antibodies could be detected in 20 of these 41 CLL patients but not in any of the 20 healthy donors ( $P = 0.0001$ ), suggesting the presence of Prm 1-reactive immune responses within the immune repertoire of patients with early CLL. Further work is warranted, especially in approaches to upregulate Prm 1 expression, and to determine the role of Prm 1 as an immunotherapeutic target for early CLL.

Melloni, G., A. J. Ferreri, et al. (2004). "Prognostic significance of cancer-testis gene expression in

resected non-small cell lung cancer patients." *Oncol Rep* **12**(1): 145-51.

MAGE, BAGE and GAGE genes encode T cell-defined tumor-associated antigens (TAA), which are expressed by various human tumors and are silent in normal tissues. Because of their expression pattern these TAA have received attention as potential targets for active immunotherapy and as molecular tumor markers. Both of these features are potentially useful in improving treatment of non-small cell lung cancer (NSCLC). We analyzed the expression of some members of the MAGE, BAGE and GAGE gene families by reverse transcription polymerase chain reaction (RT-PCR) in a cohort of 46 NSCLC patients who underwent complete resection and were followed-up for a median period of 41 months. A substantial proportion (range, 25-41%) of NSCLC expressed MAGE-A1, -A2, -A3, GAGE-1, -2, -8 and MAGE-B2 genes. On the contrary, BAGE and MAGE-B1 were expressed less frequently (17% and 11%, respectively). Overall, 59% of NSCLC patients expressed at least one gene and therefore could be eligible for tumor-specific immunotherapy protocols. Moreover, while MAGE-A, BAGE and MAGE-B genes did not provide any prognostic information, GAGE expression was associated with a worse survival ( $p=0.05$ ). Multivariate analysis confirmed this association, which is independent of TNM stage and other clinicopathologic variables. In conclusion, the detection of GAGE gene expression by RT-PCR appears to be an independent survival predictor in completely resected NSCLC patients.

Mesak, F. M., N. Osada, et al. (2003). "Molecular cloning, genomic characterization and over-expression of a novel gene, XRR1, identified from human colorectal cancer cell HCT116Clone2\_XRR and macaque testis." *BMC Genomics* **4**(1): 32.

**BACKGROUND:** As part of our investigation into the genetic basis of tumor cell radioresponse, we have isolated several clones with a wide range of responses to X-radiation (XR) from an unirradiated human colorectal tumor cell line, HCT116. Using human cDNA microarrays, we recently identified a novel gene that was down-regulated by two-fold in an XR-resistant cell clone, HCT116Clone2\_XRR. We have named this gene as X-ray radiation resistance associated 1 (XRR1) (GenBank BK000541). Here, we present the first report on the molecular cloning, genomic characterization and over-expression of the XRR1 gene. **RESULTS:** We found that XRR1 was expressed predominantly in testis of both human and macaque. cDNA microarray analysis showed three-fold higher expression of XRR1 in macaque testis relative to other tissues. We further cloned the

macaque XRRRA1 cDNA (GenBank AB072776) and a human XRRRA1 splice variant from HCT116Clone2\_XRR (GenBank AY163836). In silico analysis revealed the full-length human XRRRA1, mouse, rat and bovine Xrra1 cDNAs. The XRRRA1 gene comprises 11 exons and spans 64 kb on chromosome 11q13.3. Human and macaque cDNAs share 96% homology. Human XRRRA1 cDNA is 1987 nt long and encodes a protein of 559 aa. XRRRA1 protein is highly conserved in human, macaque, mouse, rat, pig, and bovine. GFP-XRRRA1 fusion protein was detected in both the nucleus and cytoplasm of HCT116 clones and COS-7 cells. Interestingly, we found evidence that COS-7 cells which over-expressed XRRRA1 lacked Ku86 (Ku80, XRCC5), a non-homologous end joining (NHEJ) DNA repair molecule, in the nucleus. RT-PCR analysis showed differential expression of XRRRA1 after XR in HCT116 clones manifesting significantly different XR responses. Further, we found that XRRRA1 was expressed in most tumor cell types. Surprisingly, mouse Xrra1 was detected in mouse embryonic stem cells R1. CONCLUSIONS: Both XRRRA1 cDNA and protein are highly conserved among mammals, suggesting that XRRRA1 may have similar functions. Our results also suggest that the genetic modulation of XRRRA1 may affect the XR responses of HCT116 clones and that XRRRA1 may have a role in the response of human tumor and normal cells to XR. XRRRA1 might be correlated with cancer development and might also be an early expressed gene.

Mikhaylova, I. N., D. A. Kovalevsky, et al. (2008). "Cancer/testis genes expression in human melanoma cell lines." *Melanoma Res* **18**(5): 303-13.

We analyzed the expression of 15 cancer/testis and four melanoma differentiation antigens in 21 metastatic melanoma cell lines using reverse transcriptase-polymerase chain reaction (RT-PCR) assay. On the basis of morphological characteristics, tumor cell lines were divided into three groups with high, moderate, and low grade of differentiation. Evaluation of gene expression and melanoma cell morphology has revealed a correlation between increased expression of cancer/testis genes and differentiation grade of cancer cells. The gene expression pattern for lymph node metastases and primary tumors exhibits the distribution of expression level and frequency similar to that found for established cell lines. Nevertheless, only 60% lymph node metastases or primary tumor tissue of randomly selected patients show marked expression of the most prominent cancer/testis genes, and almost 90% lesion tissue expresses at least one of 15 cancer/testis genes.

Mischo, A., B. Kubuschok, et al. (2006). "Prospective study on the expression of cancer testis genes and antibody responses in 100 consecutive patients with primary breast cancer." *Int J Cancer* **118**(3): 696-703.

To determine the expression of cancer testis (CT) genes and antibody responses in a nonselected population of patients with primary breast cancer, we investigated the composite expression of 11 CT genes by RT-PCR in fresh biopsies of 100 consecutive cases of primary breast carcinoma and by immunohistology in selected RT-PCR-positive cases. Antibody responses against 7 CT antigens were analyzed using recombinant antigen expression on yeast surface. In 98 evaluable cases, SCP-1 and SSX-4 were expressed most frequently (both 65%), followed by HOM-TEST-85/CT-8 (47%), GAGE (26%), SSX-1 (20%), NY-ESO-1 (13%), MAGE-3 (11%), SSX-2 (8%), CT-10 (7%), MAGE-4 (4%) and CT-7 (1%). One CT gene was expressed by 90% of the cases; 79% expressed > or =2, 48% > or =3, 29% > or =4, 12% > or =5, 6% > or =6, 3% > or =7, 2% > or =8 and one case coexpressed 9 antigens. Of 100 serum samples screened for CT antigen-specific antibodies, antibodies against NY-ESO-1 were detected in 4 patients, against SCP-1 in 6 patients and against SSX-2 in 1 patient, while no antibodies were detected against MAGE-3, CT-7 and CT-10. Expression of CT genes or antibody responses was not correlated with clinical parameters (menopausal status, tumor size, nodal involvement, grading, histology and estrogen receptor status) or the demonstration of CT gene expression at the protein level, by immunohistology. Our results show that breast carcinomas are among the tumors with the most frequent expression of CT antigens, rendering many patients potential candidates for vaccine trials.

Mizukami, Y., K. Kono, et al. (2008). "Detection of novel cancer-testis antigen-specific T-cell responses in TIL, regional lymph nodes, and PBL in patients with esophageal squamous cell carcinoma." *Cancer Sci* **99**(7): 1448-54.

We recently identified three HLA-A2402-restricted epitope peptides derived from cancer-testis antigens (CTA), TTK protein kinase (TTK), lymphocyte antigen 6 complex locus K (LY6K), and insulin-like growth factor (IGF)-II mRNA binding protein 3 (IMP-3) for the development of immunotherapies against esophageal squamous cell carcinoma (ESCC). In order to evaluate their immunotherapeutic potential in ESCC patients, we estimated by ELISPOT assay the TTK-, LY6K-, or IMP-3-specific T-cell immune responses in tumor-infiltrating lymphocytes (TIL), regional lymph node lymphocytes (RLNL), and peripheral blood lymphocytes (PBL) expanded from 20HLA-A2402



(+) ESCC patients, and correlated their immune activity with the expression levels of TTK, LY6K, and IMP-3, and MHC class I in the tumors. Induction of TTK-antigen specific T-cell response in TIL to the peptide-pulsed target cells was detected in 14 out of 20 (70%) cases, while LY6K or IMP-3 specific T-cell activity was observed in 11 of 20 (55%) or in eight of 20 (40%) cases, respectively. Furthermore, T-cell activity in RLNL and PBL was detectable in the similar proportion of the 20 ESCC patients. Interestingly, CTA-specific T-cell immune response was found in 13 of 14 (93%) TIL obtained from ESCC tumors with strong MHC class I expression, while it could be observed only in two of six (33%) TIL from ESCC tumors with weak MHC class I expression. These results strongly suggest the pre-existence of specific T-cell responses to HLA-A24-restricted epitope peptides from TTK, LY6K, and IMP-3 in ESCC patients. Monitoring antigen-specific T-cell responses, as well as the expression levels of MHC class I and epitope CTA in tumors, should be a selection index for application of cancer vaccine therapies to the patients who are likely to show good immune response.

Monji, M., T. Nakatsura, et al. (2004). "Identification of a novel human cancer/testis antigen, KM-HN-1, recognized by cellular and humoral immune responses." *Clin Cancer Res* **10**(18 Pt 1): 6047-57.

**PURPOSE:** We used serologic screening of a cDNA expression library of human testis to identify novel cancer/testis antigens that elicit both humoral and cellular immune responses in cancer patients. **Experimental Design and RESULTS:** We identified a novel gene designated KM-HN-1 the expression of which is testis-specific among normal tissues; it contains coiled coil domains and a leucine zipper motif and encodes a putative protein consisting of 833 amino acids. KM-HN-1 expression was observed in various cancer tissues and cancer cell lines at both mRNA and protein levels. Immunofluorescence staining of an esophageal cancer cell line revealed that KM-HN-1 protein was present exclusively in the nucleus during mitosis. Recombinant KM-HN-1 protein was produced, and used for ELISA to quantitate levels of IgG antibody specific to KM-HN-1. Higher levels of IgG antibodies specific to KM-HN-1 were detected in many types and numbers of cancer patients but not in healthy donors. The CTL lines specific to KM-HN-1, generated from HLA-A\*2402-positive healthy donors and cancer patients, killed human leukocyte antigen (HLA)-A24-positive cancer cells expressing KM-HN-1 but not cell lines that did not express either KM-HN-1 or HLA-A24. **CONCLUSIONS:** We identified a novel cancer/testis antigen, KM-HN-1, which elicited humoral immune

responses in patients with various types of cancer. Furthermore, KM-HN-1-specific CTLs could be generated from both healthy donors and cancer patients, which indicated that KM-HN-1 can be a candidate for an ideal target for cancer immunotherapy.

Monk, M., M. Hitchins, et al. (2008). "Differential expression of the embryo/cancer gene ECSA(DPPA2), the cancer/testis gene BORIS and the pluripotency structural gene OCT4, in human preimplantation development." *Mol Hum Reprod* **14**(6): 347-55.

In this paper, we examine the expression profiles of two new putative pluripotent stem cell genes, the embryo/cancer sequence A gene (ECSA) and the cancer/testis gene Brother Of the Regulator of Imprinted Sites (BORIS), in human oocytes, preimplantation embryos, primordial germ cells (PGCs) and embryo stem (ES) cells. Their expression profiles are compared with that of the well-known pluripotency gene, OCT4, using a primer design that avoids amplification of the multiple OCT4 pseudogenes. As expected, OCT4 is high in human oocytes, down-regulated in early cleavage stages and then expressed de novo in human blastocysts and PGCs. BORIS and ECSA show distinct profiles of expression in that BORIS is predominantly expressed in the early stages of preimplantation development, in oocytes and 4-cell embryos, whereas ECSA is predominantly expressed in the later stages, blastocysts and PGCs. BORIS is not detected in blastocysts, PGCs or other fetal and adult somatic tissue tested. Thus, BORIS and ECSA may be involved in two different aspects of reprogramming in development, viz., in late gametogenesis, and at the time of formation of the ES cells (inner cell mass (ICM) and PGC), respectively. However, in human ES cells, where a deprogrammed stem cell state is stably established in culture, an immunofluorescence study shows that all three genes are co-expressed at the protein level. Thus, following their derivation from ICM cells, ES cells may undergo further transformation in culture to express a number of embryo and germ line stem cell functions, which, in normal development, show different temporal and spatial specificity of expression.

Mosharafa, A. A., R. S. Foster, et al. (2004). "Complications of post-chemotherapy retroperitoneal lymph node dissection for testis cancer." *J Urol* **171**(5): 1839-41.

**PURPOSE:** Post-chemotherapy retroperitoneal lymph node dissection (PC RPLND) is a tool in the management of testis cancer. Our impression has been that the short-term morbidity of

standard PC RPLND has diminished with time. Therefore, we attempted to verify this hypothesis by evaluating the morbidity of the procedure in 2 comparable groups of patients from 2 different periods. **MATERIALS AND METHODS:** We compared 150 patients who underwent post-chemotherapy RPLND between July 2000 and July 2002 to 79 patients who underwent the same procedure between 1990 to 1992. All patients had clinical stage II-III testis cancer and had received 3 to 4 courses of standard platinum based chemotherapy before surgery. We compared surgical morbidity and postoperative complications in both groups. We also assessed a number of factors (patient characteristics, mass size, pathological features and surgical aspects) that could impact the rate of complications. **RESULTS:** The 2 groups were comparable regarding preoperative clinical stage, patient characteristics and postoperative pathological findings. PC RPLND procedures were performed using the same technique. Compared to patients in the 1990 to 1992 group, the patients from the 2000 to 2002 group had fewer intraoperative complications and additional procedures (44 [29.3%] of 150 versus 41 [51.9%] of 79,  $p = 0.0008$ ), a trend toward a lower postoperative complication rate (10 [6.7%] compared to 11 [13.9%],  $p = 0.07$ ) and shorter hospital stay (average 5.6 versus 8.4 days [ $p < 0.0001$ ]). **CONCLUSIONS:** With time morbidity and hospital stay after standard PC RPLND have decreased. This finding probably reflects differences in patterns of care rather than changes in surgical technique. Therefore, comparing newer surgical techniques to historical controls is inappropriate since differences may not actually represent the technical advances of the newer procedure.

Muehleisen, B., L. Schaerer, et al. (2007). "Cancer/testis antigen MAGE-A4 expression pattern differs in epithelial skin tumors of organ-transplant recipients and immunocompetent patients." *J Cutan Pathol* **34**(1): 1-6.

**BACKGROUND:** Lifetime risk for squamous cell carcinoma (SCC) of the skin is 1:30. Risk in organ-transplant recipients (OTR) is increased over 60-fold through long-term drug-induced immunosuppression. MAGE family-derived peptides are cancer/testis antigens recognized by specific CD8(+) T cells and employed for immunotherapy. We were interested in the frequency and distribution of MAGE-A4 in epithelial skin tumors of OTR and immunocompetent patients. **METHODS:** mAb 57B predominantly recognizing MAGE-A4 was used to stain 119 formalin-fixed, paraffin-embedded epithelial skin tumors (actinic keratosis, bowenoid actinic keratosis, Bowen's disease, and SCC;  $n = 17, 25, 61,$

16, respectively) in immunocompetent patients ( $n = 84$ ) and OTR ( $n = 35$ ). **RESULTS:** All four epithelial skin tumors showed comparable immunoreactivity ranging from (25-71%,  $p = 0.361$ ). Scattered immunoreactivity pattern was more frequent in OTR ( $p = 0.025$ ). SCC showed polarized immunoreactivity basally ( $p = 0.002$ ). **CONCLUSION:** MAGE-A4 was expressed in a large part of epithelial skin tumors with predominantly scattered immunoreactivity pattern in OTR. The difference in immunoreactivity pattern for immune status was limited, suggesting important non-immunosuppressor-mediated mechanisms for increased skin carcinogenesis in OTR. mAb 57B may be a helpful tool for immunohistochemistry and micrographic surgery using formalin-fixed paraffin-embedded tissue.

Nadano, D., T. Notsu, et al. (2002). "A human gene encoding a protein homologous to ribosomal protein L39 is normally expressed in the testis and derepressed in multiple cancer cells." *Biochim Biophys Acta* **1577**(3): 430-6.

We identified and characterized a gene encoding a protein that was 92% identical to human ribosomal protein L39. This gene was located on the long arm of chromosome 3, and was composed of three exons and two long introns. Analysis of mRNA expression in 16 types of normal human tissues showed that this gene was expressed specifically in the testis, in sharp contrast to the ubiquitous expression of the ribosomal protein L39 gene. Surprisingly, the new gene was expressed in 19 out of 24 human cancer samples of various tissue origins. When the new gene was expressed in the cell, a translated product was observed by immunofluorescence microscopy in the nucleus, especially strongly in the nucleolus, and in the cytoplasm. Association of this protein with the large subunit of cytoplasmic ribosomes was detected by polyacrylamide-agarose composite gel electrophoresis followed by immunodetection. These immunochemical data suggest a relationship between the new gene and the ribosome.

Nakamura, Y., F. Tanaka, et al. (2007). "Opa interacting protein 5 (OIP5) is a novel cancer-testis specific gene in gastric cancer." *Ann Surg Oncol* **14**(2): 885-92.

**BACKGROUND:** Identification of novel cancer-specific antigens is important for the advancement of immunotherapy. Our aim was to identify cancer-specific genes in gastric cancer. **METHODS:** Using cDNA microarray analysis, we detected genes overexpressed specifically in gastric cancer cells. The expression levels of selected genes, including OIP5, was confirmed by real time RT-PCR

analysis in tumor/normal paired bulk samples of 58 clinical cases. The expression levels of selected genes in normal tissues were also determined with a human total RNA master panel. We also compared the expression status of OIP5 with that of the other known cancer-testis specific genes. RESULTS: Twenty-two genes were determined to be upregulated in gastric cancer cells. Among these, three genes (CDC6, Exo1, and OIP5) were selected and confirmed to be upregulated in the tumor tissue compared to normal tissue. A human total RNA master panel demonstrated that OIP5, but not Exo1 or CDC6, showed high specificity in testis. Thus OIP5 may be considered a cancer-testis specific gene. In 58 clinical cases of gastric cancer examined, we found OIP5 gene expression in 27 cases (47%). Thirteen of these 27 cases showed no expression of the known cancer specific genes such as MAGE-1, MAGE-3 or NY-ESO-1. CONCLUSIONS: Using a combination of LMD and microarray, we identified OIP5 as a cancer-testis specific gene. Further expression analysis in a set of clinical cases revealed that OIP5 may be a novel immunotherapy target for patients with gastric cancer.

Nangia, A. K., O. Hill, et al. (2007). "Testicular maturation arrest to testis cancer: spectrum of expression of the vitamin D receptor and vitamin D treatment in vitro." *J Urol* **178**(3 Pt 1): 1092-6.

PURPOSE: We investigated the qualitative distribution of vitamin D receptor in human testis pathologies and performed an in vitro study of vitamin D receptor expression in a human testis cancer cell line model. MATERIALS AND METHODS: Qualitative immunohistochemical analysis of vitamin D receptor in testis tumors, normal testis and specimens from infertile patients was performed. The human embryonal carcinoma cell line NT2/D1 (American Type Culture Collection, Manassas, Virginia) was cultured. Vitamin D receptor expression was examined by Western immunoblot analysis after incubating the cells with 250 to 800 nM vitamin D, 10 to 70 nM testosterone, 2 nM calcium or a combination of the 3 products. RESULTS: Negative controls, syncytiotrophoblasts and interstitial stroma did not stain positive for vitamin D receptor. Spermatogenic, Sertoli's, Leydig and tumor cells stained positive in all specimens. Embryonal carcinoma demonstrated more nuclear and cytoplasmic staining than other tumors. Vitamin D receptor expression was seen at 50 kDa in the cell line. Sequential concentrations of vitamin D increased vitamin D receptor expression intensity. Simultaneous addition of vitamin D and testosterone decreased the vitamin D receptor signal, as did testosterone alone. Delayed administration of vitamin D 5 hours after testosterone showed the return of vitamin D receptor expression. A combination of

calcium, testosterone and vitamin D showed decreased or no vitamin D receptor expression. Calcium alone increased vitamin D receptor expression at later passages. CONCLUSIONS: To our knowledge this is the first description of vitamin D receptor in different primary testis pathologies and in an embryonal carcinoma cell line. The in vitro model showed that vitamin D receptor is an active receptor and it is inducible with the addition of vitamin D. Testosterone may be important for vitamin D receptor down-regulation. Calcium may be an important co-factor in vitamin D receptor expression.

Nelson, P. T., P. J. Zhang, et al. (2007). "Cancer/testis (CT) antigens are expressed in fetal ovary." *Cancer Immun* **7**: 1.

Cancer/testis (CT) antigens are named after their expression pattern as they are typically present in various types of tumors and in the germ cells of normal adult testis. Adult ovarian tissue is usually reported to be CT antigen negative. Based on the differences in female versus male gonadal development, the ovarian counterpart of the most predominant CT antigen positive testicular germ cells are not prevalent in the adult ovary. Hence, we analyzed the protein expression of several CT antigens in fetal ovary by immunohistochemistry with various monoclonal antibodies (mAbs) previously generated by our group. The mAbs used were: MA454 (MAGE-A1), M3H67 (MAGE-A3), 57B (MAGE-A4), CT7-33 (CT7/MAGE-C1), and ES121 (NY-ESO-1). All mAbs showed some immunopositivity in fetal ovarian germ cells. The most intense staining was seen with mAbs M3H67, 57B, and CT7-33 during weeks 16-23 of gestation. The most prevalent cells stained were oogonia, with only focal staining of oocytes of the primordial follicle. We conclude that CT antigens are regularly expressed in fetal ovarian germ cells and might play an important role in male and female germ cell biology.

Neumann, F., C. Wagner, et al. (2004). "Identification of an antigenic peptide derived from the cancer-testis antigen NY-ESO-1 binding to a broad range of HLA-DR subtypes." *Cancer Immunol Immunother* **53**(7): 589-99.

NY-ESO-1 is a SEREX-defined cancer-testis antigen of which several MHC I, but only few MHC II-restricted epitopes have been identified. Searching for highly promiscuous MHC II-restricted peptides that might be suitable as a CD4+ stimulating vaccine for many patients, we used the SYFPEITHI algorithm and identified an NY-ESO-1-derived pentadecamer epitope (p134-148) that induced specific CD4+ T-cell responses restricted to the HLA-DRB1 subtypes

\*0101, \*0301, \*0401, and \*0701 that have a cumulative prevalence of 40% in the Caucasian population. The DR restriction of the p134-148 pentadecamer was demonstrated by inhibition with an HLA-DR antibody and a functional peptide displacement titration assay with the pan-DR-binding T-helper epitope PADRE as the competitor. The natural processing and presentation of this epitope was demonstrated by recognition of an NY-ESO-1+ melanoma cell line by T cells with specificity for p134-148. The pentadecamer p134-148 was able to induce CD4+ responses in 4/38 cancer patients tested. However, no strict correlation was found between CD4+ T-cell responses against p134-148 reactivity and anti-NY-ESO-1 antibody titers in the serum of patients, suggesting that CD4+ and B-cell responses are regulated independently. In conclusion, p134-148 holds promise as a broadly applicable peptide vaccine for patients with NY-ESO-1-positive neoplasms.

Neumann, F., C. Wagner, et al. (2005). "Identification of an epitope derived from the cancer testis antigen HOM-TES-14/SCP1 and presented by dendritic cells to circulating CD4+ T cells." *Blood* **106**(9): 3105-13.

Because of their frequent expression in a wide spectrum of malignant tumors but not in normal tissue except testis, cancer testis antigens are promising targets. However, except for HOM-TES-14/SCP1, their expression in malignant lymphomas is rare. SCP1 (synaptonemal complex protein 1) has been shown to elicit antibody responses in the autologous host, but no T-cell responses against HOM-TES-14/SCP1 have been reported. Using the SYFPEITHI algorithm, we selected peptides with a high binding affinity to major histocompatibility complex class 2 (MHC 2) molecules. The pentadecamer epitope p635-649 induced specific CD4+ T-cell responses that were shown to be restricted by HLA-DRB1\*1401. The responses could be blocked by preincubation of T cells with anti-CD4 and antigen-presenting cells with anti-HLA-DR, respectively, proving the HLA-DR-restricted presentation of p635-649 and a CD4+ T-cell-mediated effector response. Responding CD4+ cells did not secrete interleukin-5 (IL-5), indicating that they belong to the T(H)1 subtype. The natural processing and presentation of p635-649 were demonstrated by pulsing autologous and allogeneic dendritic cells with a protein fragment covering p635-649. Thus, p635-649 is the first HOM-TES-14/SCP1-derived epitope to fulfill all prerequisites for use as a peptide vaccine in patients with HOM-TES-14/SCP1-expressing tumors, which is the case in two thirds of peripheral T-cell lymphomas.

Neumann, F., C. Wagner, et al. (2004). "Identification of an HLA-DR-restricted peptide epitope with a promiscuous binding pattern derived from the cancer testis antigen HOM-MEL-40/SSX2." *Int J Cancer* **112**(4): 661-8.

The SSX2 gene encodes the tumor-specific antigen HOM-MEL-40/SSX2 expressed in a broad spectrum of tumors of different origin, against which humoral and CD8+ T-cell-mediated MHC-I-restricted responses have been demonstrated. Searching for promiscuous MHC-II-restricted peptides that might be suitable as a CD4+ stimulating vaccine for many patients, we used the SYFPEITHI algorithm and identified a HOM-MEL-40/SSX2-derived pentadecamer epitope (p45-59) that induced specific CD4+ T-cell responses restricted by the HLA-DRB1 subtypes \*0701, \*1101 and \*1302 that have a cumulative prevalence of approximately 25% in the Caucasian population. The CD4+-mediated response against p45-59 and its DR restriction was demonstrated by inhibition with anti-CD4 and HLA-DR antibodies, respectively, and by blocking experiments using HLA-specific antibodies. The natural processing and presentation of p45-59 was demonstrated by recognition of the SSX2+ melanoma cell line Me 275 as well as autologous and allogeneic dendritic cells pulsed with whole-protein SSX2 by T cells with specificity for p45-59. p45-59 was able to induce responses in 3/6 breast cancer patients and 1/5 healthy controls. No correlation was found between CD4+ T-cell responses against p45-59 reactivity and anti-SSX2 antibody titers in the serum of patients, suggesting that CD4+ and B-cell responses are regulated independently. p45-59 holds promise as a broadly applicable peptide vaccine for patients with SSX2-positive neoplasms.

Nurmio, M., J. Toppari, et al. (2009). "Functional in vitro model to examine cancer therapy cytotoxicity in maturing rat testis." *Reprod Toxicol* **27**(1): 28-34.

Childhood cancer treatment can lead to infertility. Organ culture of early postnatal testicular tissue might provide a valuable approach to the study of acute testicular toxicity. The aim of the present study was to develop a functional in vitro organ culture method, in order to identify sensitive target cells to doxorubicin-induced cytotoxicity in immature rat testis during germ cell migration prior initiation of the first wave of spermatogenesis. Testicular tissue fragments from 5-day-old Sprague-Dawley rats were cultured in the absence or presence of doxorubicin (40 and 100ng/ml) and morphology, apoptosis, proliferation and testosterone secretion was analyzed. Postnatal testicular development proceeded normally in control samples for 48h in vitro. In these untreated culture conditions germ and Sertoli cell numbers and

germ cell migration were comparable to in vivo. Germ cells were the primary, most sensitive targets for in vitro-induced doxorubicin (100ng/ml) toxicity and their death was not associated with any morphological defects in the Sertoli cells. Organ culture which reduces the need of animal experimentation can be used to study the cytotoxic effects of doxorubicin on the immature testis.

Oba-Shinjo, S. M., O. L. Caballero, et al. (2008). "Cancer-testis (CT) antigen expression in medulloblastoma." *Cancer Immun* 8: 7.

Medulloblastoma is the most common childhood malignant tumor of the central nervous system. Treatment of medulloblastoma requires harmful therapy and nevertheless carries a poor prognosis. Due to their presence in various cancers and their limited expression in normal tissues, CT antigens are ideal vaccine targets for tumor immunotherapy. CT antigens, such as MAGE and NY-ESO-1, have been employed in clinical trials in various malignancies but little is known about their presence in medulloblastoma. We analyzed 25 medulloblastomas for the expression of a panel of CT antigens by RT-PCR and immunohistochemistry. Messenger RNA expression in the samples was as follows: GAGE 64%, MAGEA3/6 56%, SYCP1 44%, SLCO6A1 32%, MAGEC1 28%, MAGEC2 28%, MAGEA4 28%, NY-ESO-1 20%, MAGEA1 16%, and TPTE 0%. All cases except one (96%) were positive for mRNA expression of at least one CT gene. However, CT antigen expression was scarce on a protein level. Immunoreaction to monoclonal antibody E978 (NY-ESO-1) was negative in all cases; MA454 (MAGEA1), 57B (MAGEA4), M3H67 (MAGEA3/6), CT10#5 (MAGEC2) and #23 (GAGE) were each positive in 1 case, while the highest incidence of positive immunostaining, albeit heterogeneous, was seen with CT7-33 (MAGEC1) in 3 out of the 25 cases. The absence of correlation between mRNA and protein expression in medulloblastoma has not been observed in other tumors and further studies addressing the biology of CT antigens are necessary to investigate the present discrepant results.

Odunsi, K., A. A. Jungbluth, et al. (2003). "NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer." *Cancer Res* 63(18): 6076-83.

Cancer-testis (CT) antigens are expressed in a variety of cancers, but not in normal adult tissues, except for germ cells of the testis, and hence appear to be ideal targets for immunotherapy. In an effort to examine the potential of NY-ESO-1 and LAGE-1 CT antigens for immunotherapy in epithelial ovarian

cancer (EOC), we examined the expression of these antigens by reverse transcription-PCR (RT-PCR) and immunohistochemistry (IHC) in a large panel of EOC tissues and cell lines. Sera from a subgroup of the patients were tested for NY-ESO-1/LAGE-1 antibody by ELISA. The data indicated that four ovarian cancer cell lines were positive for one or both CT antigens. Expression of NY-ESO-1 in EOC was demonstrated by RT-PCR and/or IHC in 82 of 190 (43%) specimens. NY-ESO-1 expression by IHC ranged from homogeneous to heterogeneous pattern. LAGE-1 mRNA expression was present in 22 of 107 (21%) tumor tissues. Overall, the expression of either NY-ESO-1 or LAGE-1 mRNA was present in 42 of 107 (40%) EOC specimens and coexpression of both antigens was demonstrated in 11% of specimens. Antibody to NY-ESO-1/LAGE-1 was present in 11 of 37 (30%) patients whose tumors expressed either NY-ESO-1 or LAGE-1. Detectable antibodies were present for up to 3 years after initial diagnosis. Although there was no statistically significant relation between expression of NY-ESO-1/LAGE-1 antigen and survival, the data showed aberrant expression of NY-ESO-1 and LAGE-1 by IHC/RT-PCR in a significant proportion of EOC patients. These findings indicate that NY-ESO-1 and LAGE-1 are attractive targets for antigen-specific immunotherapy in EOC.

Okada, T., M. Akada, et al. (2006). "A novel cancer testis antigen that is frequently expressed in pancreatic, lung, and endometrial cancers." *Clin Cancer Res* 12(1): 191-7.

PURPOSE: To isolate cancer testis antigens that are expressed in pancreatic cancers and may be useful in clinical applications. EXPERIMENTAL DESIGN: To efficiently isolate cancer testis antigens, a testis cDNA library was immunoscreened (SEREX) with serum from a patient with pancreatic ductal adenocarcinoma. The expression of isolated antigens in various cancer cell lines and tissues was evaluated by reverse transcription-PCR and Northern blot analyses. The immunogenicity of the antigen in cancer patients was evaluated by detection of the IgG antibody in sera from patients with various cancers. RESULTS: Of the three clones isolated through screening of a total of  $2 \times 10^6$  cDNA library clones, one clone (KU-CT-1) was found to be expressed in various cancers but only in testis among normal tissues, indicating that it was a novel cancer testis antigen. The KU-CT-1 gene is located on chromosome 10p12 and produces two splice variants, which encode proteins of 397 and 872 amino acids, respectively. KU-CT-1 was expressed in pancreatic cancer tissues (3 of 9, 33%), lung cancer tissues (9 of 24, 38%), and endometrial cancer tissues (7 of 11, 64%). Specific serum IgG antibodies were detected in

3 of 20 pancreatic cancer patients, 2 of 12 endometrial cancer patients, 1 of 18 colon cancer patients, and 1 of 10 prostate cancer patients but not detected in 30 healthy individuals. CONCLUSIONS: KU-CT-1 is a new cancer testis antigen that is expressed in pancreatic, lung, and endometrial cancers and may be useful for diagnosis and immunotherapy for patients with various cancers.

Oliver, R. T., J. Ong, et al. (2003). "Testis conserving chemotherapy in germ cell cancer: its potential to increase understanding of the biology and treatment of carcinoma-in-situ." *Apmis* **111**(1): 86-91; discussion 91-2.

Prompted by recognition of the potential of chemotherapy to increase the success of testis conserving surgery in patients with germ cell cancer, background and outcome data are reviewed and their contribution to the ongoing debate about how germ cell cancer develops discussed. The review is based on three previous studies of: a) time trends in tumour size in 578 personal series of all stages of testis cancer treated since 1978; b) impact of chemotherapy on actuarial risk of tumours in contralateral testis examined on 1221 patients treated in trials through the Anglian Germ Cell Cancer Consortium; and c) testes conservation attempted using chemotherapy in 78 patients. Since 1978 tumour size has decreased from 4.8 to 3.0 cms while cure has gone from 77 to 97%. There was no overall long term reduction in second cancers beyond 10 years in stage 1 patients after orchidectomy alone compared to stage 1 or metastatic disease patients receiving chemotherapy though the incidence was non significantly lower up to 10 years particularly in those patients receiving etoposide based combination. Testis conservation was initially successful in 28 of 78 (36%). An additional 25 (32%) had no viable cancer in orchidectomy specimen. In the 28 primary tumours cured by chemotherapy there was a 26% late relapse rate between 5 and 10 years (all cured by orchidectomy) compared to less than 5% in those cured with established metastases. In conclusion, testis conservation with chemotherapy is safe and feasible, though relapse is too frequent for routine service use. Confirmation of the high frequency of late relapse by others has raised the question whether these recurrences are due to post pubertal events reinducing CIS in intrauterine oestrogen primed germ cells and highlights the potential of testes conservation studies to better understand germ cell cancer development.

Ortmann, C. A., L. Eisele, et al. (2008). "Aberrant hypomethylation of the cancer-testis antigen PRAME correlates with PRAME expression in acute myeloid leukemia." *Ann Hematol* **87**(10): 809-18.

PRAME is a tumor-associated antigen, which belongs to the family of cancer-testis antigens (CTA). The expression of CTA is mainly restricted to the testis and various tumors. In contrast to other CTA, PRAME expression is also frequently detected in acute and chronic leukemias. Due to this expression pattern, PRAME has attracted great interest as a prognostic tumor marker that can be used for the detection of minimal residual disease and as a potential target for immunotherapy. In acute myeloid leukemia (AML), PRAME expression has been observed in 30-64% of cases. To evaluate whether epigenetic mechanisms contribute to PRAME activation in AML, we studied DNA methylation of 15 CpG dinucleotides within a CpG-rich region located in the intron 1 of the PRAME gene. DNA methylation was determined by sequence analysis of cloned PCR products generated from bisulfite-treated genomic DNA. Methylation patterns were correlated with PRAME mRNA levels as determined by microarray analysis and real-time PCR. We found almost complete methylation in mononuclear blood cells from two healthy donors and in bone marrow cells of four PRAME-negative AML patients. In contrast, the degree of PRAME methylation was clearly reduced in four PRAME-positive AML bone marrow samples. In particular, these samples were characterized by the presence of clones, which were completely devoid of methylation. The significant inverse correlation between the degree of methylation and PRAME expression suggests a causal role of DNA methylation in PRAME regulation. Such a role is further supported by the observation that treatment of PRAME-negative cell lines U-937 and THP-1 with the demethylating agent 5'-Aza-2'dC resulted in a dose-related upregulation of PRAME expression.

Park, J. H., M. L. Lin, et al. (2006). "PDZ-binding kinase/T-LAK cell-originated protein kinase, a putative cancer/testis antigen with an oncogenic activity in breast cancer." *Cancer Res* **66**(18): 9186-95.

Breast cancer is one of the most common cancers among women. To discover molecular targets that are applicable for development of novel breast cancer therapy, we previously did genome-wide expression profile analysis of 81 breast cancers and found dozens of genes that were highly and commonly up-regulated in breast cancer cells. Among them, we here focused on one gene that encodes PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK), including a kinase domain. Northern blot analyses using mRNAs of normal human organs, breast cancer tissues, and cancer cell lines indicated this molecule to be a novel cancer/testis antigen. Reduction of PBK/TOPK expression by small

interfering RNA resulted in significant suppression of cell growth probably due to dysfunction in the cytokinetic process. Immunocytochemical analysis with anti-PBK/TOPK antibody implicated a critical role of PBK/TOPK in an early step of mitosis. PBK/TOPK could phosphorylate histone H3 at Ser10 in vitro and in vivo, and mediated its growth-promoting effect through histone H3 modification. Because PBK/TOPK is the cancer/testis antigen and its kinase function is likely to be related to its oncogenic activity, we suggest PBK/TOPK to be a promising molecular target for breast cancer therapy.

Park, S., Y. Lim, et al. (2003). "Identification and characterization of a novel cancer/testis antigen gene CAGE-1." *Biochim Biophys Acta* **1625**(2): 173-82.

Serological analysis of cDNA expression library (SEREX) was employed to identify cancer-associated genes. By screening cDNA expression libraries with sera of patients with lung cancers, we identified a total of 49 genes that specifically reacted with the sera of patients with lung cancers. Among these, we characterized a novel gene with expression pattern similar to that of cancer/testis antigens. Its open reading frame is 1920 bp in size and encodes for putative protein composed of 639 amino acids. Southern blot analysis reveals that this gene exists as single copy. In vitro transcription/translation and Western blot analysis confirm that this gene encodes a protein of 73 kDa in size. The comparison of cDNA and genomic sequences reveals that it is composed of 11 exons and 10 introns. This gene displays testis-specific expression among normal tissues, and wide expression among various cancer tissues and cancer cell lines. A study using GFP fusion construct reveals mainly nuclear localization of CAGE-1 protein. The expression of this clone is relatively higher in cancer tissues compared with their surrounding non-cancerous tissues. This suggests that overexpression of CAGE-1 may be associated with the progression of tumor. Because of its association with cancer, this gene was named cancer-associated gene-1 (CAGE-1). Given the fact that several cancer/testis antigens reportedly induce cytolytic T lymphocyte (CTL) reactions, it is reasonable that this gene will be a valuable target for cancer immunotherapy. The exact functional role of CAGE-1 in tumorigenesis remains to be seen.

Parmigiani, R. B., F. Bettoni, et al. (2008). "Antibodies against the cancer-testis antigen CTSP-1 are frequently found in prostate cancer patients and are an independent prognostic factor for biochemical-recurrence." *Int J Cancer* **122**(10): 2385-90.

Cancer-testis (CT) antigens are immunogenic proteins expressed in normal gametogenic tissues and

in different types of tumors. CTSP-1 is a CT antigen frequently expressed in prostate tumors, and capable of eliciting humoral response in prostate cancer patients. Here, we analyzed the presence of anti-CTSP-1 antibodies in 147 patients with localized prostate cancer and determined its prognostic value for predicting biochemical-recurrence after radical prostatectomy. Anti-CTSP-1 antibodies were detected in 25% of the patients and a significant correlation ( $p = 0.017$ ) between CTSP-1 protein expression and the presence of specific humoral response was observed. No association was found between the presence of antibodies and the pathological variables analyzed. On univariate analysis, patients without antibodies against CTSP-1 had a lower biochemical-recurrence free survival than did those with anti-CTSP-1 antibodies, although the difference between the groups was not statistically significant (57 vs. 75%,  $p = 0.075$ ). However, the presence of antibodies against CTSP-1 was significantly associated with a better prognosis in patients with higher Gleason score (36 vs. 80%,  $p = 0.028$ ). On multivariate analysis, antibodies against CTSP-1 were associated with a better prognosis (Hazard ratio = 0.41, 95% IC 0.18-0.90  $p = 0.039$ ), being the third most powerful prognostic factor among Gleason score and preoperative PSA levels. CTSP-1 should be considered a promising candidate for prostate cancer immunotherapy, since it is frequently expressed in prostate tumors and capable of eliciting humoral immune response in prostate cancer patients. Our results also suggest that humoral response against CTSP-1 could be used as a prognostic marker, especially among patients with a high Gleason score.

Parmigiani, R. B., F. Bettoni, et al. (2006). "Characterization of a cancer/testis (CT) antigen gene family capable of eliciting humoral response in cancer patients." *Proc Natl Acad Sci U S A* **103**(48): 18066-71.

Cancer/testis (CT) antigens are immunogenic proteins expressed in normal gametogenic tissues and in different types of tumors. CT antigens are promising candidates for cancer immunotherapy, and the identification of novel CT antigens is a prerequisite for the development of cancer vaccines. We have identified a CT antigen, named CTSP-1, with partial similarity to the breast differentiation antigen NY-BR-1. CTSP-1 presents several splicing and polyadenylation variants and has a very restricted expression pattern among normal tissues. CTSP-1 is exclusively expressed in normal testis and is aberrantly expressed in 47.6% (10 of 21) of tumor cell lines and in 44.4% (75 of 169) of tumors from different histological types. The highest percentages of positive expression were observed in melanomas

(59.0%) followed by prostate (58.0%) and lung (57.0%) tumors. CTSP-1 is part of a highly conserved gene family, and members of this family also have a restricted expression pattern and similar protein structure. Antibodies against members of this gene family were detected in 10% (14 of 141) of plasma samples from patients with a wide spectrum of tumors. The highest percentages of antibody response were observed in patients with prostate (20.8%), thyroid (20.0%), and breast (16.6%) tumors. Because of its very restricted expression pattern in normal tissues and immunogenicity in different types of tumors, CTSP-1 should be considered a promising candidate for cancer immunotherapy.

Peng, J. R., H. S. Chen, et al. (2005). "Expression of cancer/testis (CT) antigens in Chinese hepatocellular carcinoma and its correlation with clinical parameters." *Cancer Lett* **219**(2): 223-32.

For investigating the expression of cancer/testis (CT) antigens in patients with hepatocellular carcinoma (HCC) in China, and evaluating the correlations between the expression of these CT antigens and clinical parameters, we collected tumors and adjacent non-cancerous tissues of 43 HCC patients from Beijing and 30 HCC patients from Guangxi province. Expression of the mRNA of 14 CT antigens was evaluated by reverse transcription PCR (RT-PCR). The average age of the HCC patients bearing CT antigen positive tumors was higher than that of the HCC patients bearing CT antigen negative tumors. The expression of MAGE-A3, SSX-1, SSX-2, SSX-4, MAGE-B2, MAGE-C1, and MAGE-C2 correlated significantly with older age ( $P < 0.05$ ). Moreover, the expressions of MAGE-A4 and SCP-1 were related to alpha-fetoprotein abnormality ( $P < 0.05$ ), and the expression of NY-ESO-1 was related to early tumor stage ( $P < 0.05$ ). There was no correlation observed between the expression of CT antigens and the sex, HBV infection or tumor size.

Perez, D., T. Herrmann, et al. (2008). "Cancer testis antigen expression in gastrointestinal stromal tumors: new markers for early recurrence." *Int J Cancer* **123**(7): 1551-5.

Cancer testis antigens (CTAs) are expressed in a variety of malignant tumors but not in any normal adult tissues except germ cells and occasionally placenta. Because of this tumor-associated pattern of expression, CTAs are regarded as potential vaccine targets. The expression of CTAs in gastrointestinal stromal tumors (GIST) has not been analyzed systematically previously. The present study was performed to analyze the expression of CTA in GIST and to determine if CTA expression correlates with prognosis. High-risk GISTs which stained positive for

at least 1 CTA, recurred in 100% ( $n = 25$ ) of the cases. This is the first study analyzing CTA expression in GIST and its prognostic value for recurrence. The CTA staining could add information to the individual patient prognosis and represent an interesting target for future treatment strategies.

Perrotti, M., M. Ankem, et al. (2004). "Prospective metastatic risk assignment in clinical stage I nonseminomatous germ cell testis cancer: a single institution pilot study." *Urol Oncol* **22**(3): 174-7.

OBJECTIVE: To gain initial experience with a histopathologic model to assign metastatic risk in patients with clinical stage I nonseminomatous germ cell testis cancer (CSI NSGCTC). MATERIALS AND METHODS: Histopathologic factors were recorded prospectively, and metastatic risk assigned according to the proposed model. In the model tested, percentage of embryonal carcinoma (%EMB)  $\geq$  80% and/or vascular invasion (+VI) denoted high ( $> 50\%$ ) occult disease risk, while %EMB  $< 80\%$  plus absence of VI denoted low ( $\leq 10\%$ ) risk. Risk stratification was correlated with outcome and assessed statistically. RESULTS: There were 54 patients with CSI testis cancer evaluated during the study period. Patients with pure seminoma ( $n = 30$ ), Sertoli cell tumor ( $n = 1$ ), and Leydig cell tumor ( $n = 1$ ) were excluded from analysis. Twenty-two patients had CSI NSGCTC and comprise the pilot study cohort. The median follow-up duration from the time of study entry is 31 months (range, 20-61 months). Utilizing the model tested, a statistically significant higher likelihood of occult disease in the high risk cohort compared to the low risk cohort was observed (67% vs. 0%; Fisher's exact test,  $P = 0.005$ ). CONCLUSIONS: The results of the present pilot study are encouraging, particularly in the potential of identifying a cohort at low metastatic risk. In the appropriate setting, such a patient might be considered for surveillance alone following orchiectomy. High risk assignment was associated with a positive predictive value (PPV) of 67%. This level of risk is superior to single factor PPV, and if confirmed, could influence clinical decision making. Further experience with this model in an expanded setting is required to establish its reproducibility and predictive value.

Pettersson, A., L. Richiardi, et al. (2007). "Age at surgery for undescended testis and risk of testicular cancer." *N Engl J Med* **356**(18): 1835-41.

BACKGROUND: Undescended testis, which is a risk factor for testicular cancer, is usually treated surgically, but whether the age at treatment has any effect on the risk is unclear. We studied the relation between the age at treatment for undescended testis and the risk of testicular cancer. METHODS: We



identified men who underwent orchiopexy for undescended testis in Sweden between 1964 and 1999. Cohort subjects were identified in the Swedish Hospital Discharge Register and followed for the occurrence of testicular cancer through the Swedish Cancer Registry. Vital statistics and data on migration status were taken from the Register of Population and Population Changes for the years 1965 through 2000. We estimated the relative risk of testicular cancer using Poisson regression of standardized incidence ratios, comparing the risk in the cohort with that in the general population. We also analyzed the data by means of Cox regression, using internal comparison groups. RESULTS: The cohort consisted of 16,983 men who were surgically treated for undescended testis and followed for a total of 209,984 person-years. We identified 56 cases of testicular cancer during follow-up. The relative risk of testicular cancer among those who underwent orchiopexy before reaching 13 years of age was 2.23 (95% confidence interval [CI], 1.58 to 3.06), as compared with the Swedish general population; for those treated at 13 years of age or older, the relative risk was 5.40 (95% CI, 3.20 to 8.53). The effect of age at orchiopexy on the risk of testicular cancer was similar in comparisons within the cohort. CONCLUSIONS: Treatment for undescended testis before puberty decreases the risk of testicular cancer.

Piatesi, A., S. W. Howland, et al. (2006). "Directed evolution for improved secretion of cancer-testis antigen NY-ESO-1 from yeast." *Protein Expr Purif* **48**(2): 232-42.

NY-ESO-1 is a highly immunogenic tumor antigen and a promising vaccine candidate in cancer immunotherapy. Access to purified protein both for vaccine formulations and for monitoring antigen-specific immune responses is vital to vaccine development. Currently available recombinant *Escherichia coli*-derived NY-ESO-1 is isolated from inclusion bodies as a complex protein mixture and efforts to improve the purity of this antigen are required, especially for later-stage clinical trials. Using yeast cell surface display and fluorescence activated cell sorting techniques, we have engineered an NY-ESO-1 variant (NY-ESO-L5; C(75)A C(76)A C(78)A L(153)H) with a 100x improved display level on yeast compared to the wild-type protein. This mutant can be effectively produced as an Aga2p-fusion and purified in soluble form directly from the yeast cell wall. In the process, we have identified the epitope recognized by anti-NY-ESO-1 mAb E978 (79-87, GARGPESRL). The availability of an alternative expression host for this important antigen will help avoid artifactual false positive tests of

patient immune response due to reaction against expression-host-specific contaminants.

Rassweiler, J. J., W. Scheitlin, et al. (2008). "Laparoscopic retroperitoneal lymph node dissection: does it still have a role in the management of clinical stage I nonseminomatous testis cancer? A European perspective." *Eur Urol* **54**(5): 1004-15.

CONTEXT: Laparoscopic retroperitoneal lymph node dissection (L-RPLND) is not recommended as standard tool in European Association of Urology (EAU) guidelines. OBJECTIVE: To update the role of L-RPLND in patients with clinical stage I nonseminomatous germ cell tumour (NSGCT) compared to open retroperitoneal lymph node dissection (O-RPLND). EVIDENCE ACQUISITION: A systematic literature search from 1992 to 2008 was performed in Medline, EMBASE, and Cochrane. The largest series from each group was considered. Comparative analysis was based on raw data of series published in 2000 and later. EVIDENCE SYNTHESIS: Results of >800 patients treated by L-RPLND reported in 34 articles were analyzed. Lymph node dissection (LND) was based on modified templates, removing an average of 16 (5-36) lymph nodes. At experienced centres, complication rates were 15.6% (9.4-25.7), including 2% (0-5) retrograde ejaculation and 1.7% (0-6) reintervention. Operating room times are longer compared to O-RPLND (204 vs 186min). Five publications with a follow-up of 63 (36-89) mo include 557 patients. One hundred twenty-six of 140 (90%) patients with positive nodes (25%, range: 17-38) received adjuvant chemotherapy, resulting in a local relapse rate of 1.4% (0.7-2.3) with no in-field recurrence; rate of distant relapses was 3.3% (1.8-4.6), including one port-site metastasis; and rate of biochemical failure was 0.9% (0.7-2.3). Two of 14 patients with positive nodes (pN1) who did not receive adjuvant chemotherapy relapsed, both 8 mo after surgery, and were salvaged by chemotherapy. Compared with O-RPLND, there was no difference in relapse rates, percentage of patients receiving chemotherapy (29% vs 31%), chemotherapy (CTx) cycles per cohort (0.6), rate of salvage surgery (1.2% vs 1.5%), and patients with no evidence of disease (NED; 100% vs 99.7%). CONCLUSIONS: L-RPLND offers similar staging accuracy and long-term outcome to O-RPLND. In a late series of experienced L-RPLND centres, there was a trend towards fewer complications. L-RPLND represents a valuable tool for experienced laparoscopic surgeons. Further studies must focus on the curative potential of the procedure in pathologic stage IIA.

Renaud, S., E. M. Pugacheva, et al. (2007). "Expression of the CTCF-paralogous cancer-testis gene, brother of the regulator of imprinted sites (BORIS), is regulated by three alternative promoters modulated by CpG methylation and by CTCF and p53 transcription factors." *Nucleic Acids Res* **35**(21): 7372-88.

BORIS, like other members of the 'cancer/testis antigen' family, is normally expressed in testicular germ cells and repressed in somatic cells, but is aberrantly activated in cancers. To understand regulatory mechanisms governing human We found that DNA methylation and functional p53 contributes to the negative regulation of each promoter. Moreover, reduction of CTCF in normally BORIS-negative human fibroblasts resulted in derepression of BORIS promoters. These results provide a mechanistic basis for understanding cancer-related associations between haploinsufficiency of CTCF and BORIS derepression, and between the lack of functional p53 and aberrant activation of BORIS.

Resnick, M. B., E. Sabo, et al. (2002). "Cancer-testis antigen expression in uterine malignancies with an emphasis on carcinosarcomas and papillary serous carcinomas." *Int J Cancer* **101**(2): 190-5.

The cancer testis (CT) family of antigens are expressed in certain malignant neoplasms and are silent in normal adult tissues, except for the testis. Expression of 2 members of this family, MAGE-A4 and NY-ESO-1, has been described recently in germ cell tumors, malignant melanomas, certain carcinomas and sarcomas. Our study is the first to describe the expression pattern of CT antigens in uterine neoplasms. Ninety-eight cases of uterine neoplasms, including 41 endometrioid, 19 papillary serous and 7 clear cell carcinomas, 22 carcinosarcomas and 9 endometrial stromal sarcomas were studied. Immunohistochemistry was carried out with the 57B monoclonal antibody that recognizes predominantly the MAGE-A4 antigen in paraffinized tissues and the D8.38 antibody that recognizes NY-ESO-1. MAGE-A4 expression was found to be present in 12% of the endometrioid adenocarcinomas, 63% of the papillary serous carcinomas and 91% of the carcinosarcomas. Within the tumor population the extent of MAGE-A4 expression was highest in the carcinosarcomas. In 12 of 22 positively staining carcinosarcomas more than 50% of the tumor cells expressed MAGE-A4. NY-ESO-1 expression was seen in 19% of the endometrioid adenocarcinomas, 32% of the papillary serous carcinomas and in 45% of the carcinosarcomas. CT antigen immunoreactivity was observed in both the carcinomatous and sarcomatous components of the carcinosarcomas and strong correlation between MAGE-A4 and NY-ESO-1 expression was present in

individual cases. In summary, strong MAGE-A4 expression and to a lesser degree NY-ESO-1 expression is characteristic of the vast majority of uterine carcinosarcomas and a major subset of papillary serous carcinomas. These results suggest that CT antigen expression by these tumors may represent a novel target for immunotherapy.

Riener, M. O., P. J. Wild, et al. (2009). "Frequent expression of the novel cancer testis antigen MAGE-C2/CT-10 in hepatocellular carcinoma." *Int J Cancer* **124**(2): 352-7.

Cancer testis (CT) antigens are attractive targets for immunotherapy in cancer patients. Immunohistochemistry was used to study the expression of the CT antigens MAGE-C2/CT-10, MAGE-C1/CT-7, GAGE, MAGE-A4 and NY-ESO-1 in 146 hepatocellular carcinomas, 13 intrahepatic cholangiocarcinomas, 37 extrahepatic cholangiocarcinomas and 32 gallbladder carcinomas. Immunopositivity was correlated with clinicopathological parameters, MHC Class 1 expression, intratumoral CD4+, CD8+ and FOXP3+ T cells and CD163+ antigen-presenting cells. Of the 146 hepatocellular carcinomas, 34% were positive for MAGE-C2/CT-10, 12% for MAGE-C1/CT-7, 11% for GAGE and 2% for NY-ESO-1, respectively. MHC Class 1 coexpression was identified in almost all CT antigen-positive tumors. The number of intratumoral FOXP3+ regulatory T cells was increased in CT antigen-positive hepatocellular carcinomas ( $p < 0.004$ ), suggesting inhibition of immune response in such tumors. Furthermore, MAGE-C1/CT-7 and GAGE positivity was correlated with reduced overall survival in patients with hepatocellular carcinoma ( $p = 0.03$  and  $0.01$ , respectively). Four (13%) gallbladder carcinomas stained positive for MAGE-C2/CT-10, of which 1 tumor (3%) was also positive for NY-ESO-1 and GAGE. CT antigens were not expressed in intra- and extrahepatic cholangiocarcinomas. Our results suggest that MAGE-C2/CT-10 may be a good candidate for peptide vaccination in patients with hepatocellular carcinoma.

Risinger, J. I., G. V. Chandramouli, et al. (2007). "Global expression analysis of cancer/testis genes in uterine cancers reveals a high incidence of BORIS expression." *Clin Cancer Res* **13**(6): 1713-9.

PURPOSE: Cancer/testis (CT) genes predominantly expressed in the testis (germ cells) and generally not in other normal tissues are aberrantly expressed in human cancers. This highly restricted expression provides a unique opportunity to use these CT genes for diagnostics, immunotherapeutic, or other targeted therapies. The purpose of this study was to identify those CT genes with the greatest incidence of

expression in uterine cancers. **EXPERIMENTAL DESIGN:** We queried the expression of known and putative CT gene transcripts (representing 79 gene loci) using whole genome gene expression arrays. Specifically, the global gene expressions of uterine cancers (n = 122) and normal uteri (n = 10) were determined using expression data from the Affymetrix HG-U133A and HG-U133B chips. Additionally, we also examined the brother of the regulator of imprinted sites (BORIS) transcript by reverse transcription-PCR and quantitative PCR because its transcript was not represented on the array. **RESULTS:** Global microarray analysis detected many CT genes expressed in various uterine cancers; however, no individual CT gene was expressed in more than 25% of all cancers. The expression of the two most commonly expressed CT genes on the arrays, MAGEA9 (24 of 122 cancers and 0 of 10 normal tissues) and Down syndrome critical region 8 (DSCR8)/MMA1 (16 of 122 cancers and 0 of 10 normal tissues), was confirmed by reverse transcription-PCR methods, validating the array screening approach. In contrast to the relatively low incidence of expression of the other CT genes, BORIS expression was detected in 73 of 95 (77%) endometrial cancers and 24 of 31 (77%) uterine mixed mesodermal tumors. **CONCLUSIONS:** These data provide the first extensive survey of multiple CT genes in uterine cancers. Importantly, we detected a high frequency of BORIS expression in uterine cancers, suggesting its potential as an immunologic or diagnostic target for these cancers. Given the high incidence of BORIS expression and its possible regulatory role, an examination of BORIS function in the etiology of these cancers is warranted.

Roman-Gomez, J., A. Jimenez-Velasco, et al. (2007). "Epigenetic regulation of human cancer/testis antigen gene, HAGE, in chronic myeloid leukemia." *Haematologica* **92**(2): 153-62.

**BACKGROUND AND OBJECTIVES:** Cancer testis antigens (CTA) provide attractive targets for cancer-specific immunotherapy. Although CTA genes are expressed in some normal tissues, such as the testis, this immunologically protected site lacks MHC I expression and as such, does not present self antigens to T cells. To date, CTA genes have been shown to be expressed in a range of solid tumors via demethylation of their promoter CpG islands, but rarely in chronic myeloid leukemia (CML) or other hematologic malignancies. **DESIGN AND METHODS:** In this study, the methylation status of the HAGE CTA gene promoter was analyzed by quantitative methylation-specific polymerase chain reaction (MSP) and sequencing in four Philadelphia-positive cell lines (TCC-S, K562, KU812 and KYO-1)

and in CML samples taken from patients in chronic phase (CP n=215) or blast crisis (BC n=47). HAGE expression was assessed by quantitative reverse transcriptase-polymerase chain reaction. **RESULTS:** The TCC-S cell line showed demethylation of HAGE that was associated with over-expression of this gene. HAGE hypomethylation was significantly more frequent in BC (46%) than in CP (22%) (p=0.01) and was correlated with high expression levels of HAGE transcripts (p<0.0001). Of note, in CP-CML, extensive HAGE hypomethylation was associated with poorer prognosis in terms of cytogenetic response to interferon (p=0.01) or imatinib (p=0.01), molecular response to imatinib (p=0.003) and progression-free survival (p=0.05). **INTERPRETATIONS AND CONCLUSION:** The methylation status of the HAGE promoter directly correlates with its expression in both CML cell lines and patients and is associated with advanced disease and poor outcome.

Ruault, M., P. van der Bruggen, et al. (2002). "New BAGE (B melanoma antigen) genes mapping to the juxtacentromeric regions of human chromosomes 13 and 21 have a cancer/testis expression profile." *Eur J Hum Genet* **10**(12): 833-40.

A first BAGE (B melanoma antigen) gene, BAGE1, was identified because it encodes a human tumour antigen recognised by a cytolytic T lymphocyte. Here, we characterised five new BAGE genes mapping to the juxtacentromeric regions of human chromosomes 13 and 21 and nine BAGE gene fragments mapping to the juxtacentromeric regions of chromosomes 9, 13, 18, and 21. Genes and gene fragments share extensive regions of 90-99% nucleotide identity. We analysed the expression of BAGE genes on 215 tumours of various histological types and on nine normal tissues. Similar to BAGE1, the new BAGE genes are expressed in melanomas, bladder and lung carcinomas and in a few tumours of other histological types. All the normal tissues were negative, with the exception of testis. Our results show that human juxtacentromeric regions harbour genes, which are transcribed and translated, in addition to gene fragments that are generally not expressed. We suggest that the pattern of expression restricted to cancer/testis is a feature of the few genes mapping to juxtacentromeric regions.

Rustin, G. J., G. M. Mead, et al. (2007). "Randomized trial of two or five computed tomography scans in the surveillance of patients with stage I nonseminomatous germ cell tumors of the testis: Medical Research Council Trial TE08, ISRCTN56475197--the National Cancer Research Institute Testis Cancer Clinical Studies Group." *J Clin Oncol* **25**(11): 1310-5.

**PURPOSE:** Surveillance is a standard management approach for stage I nonseminomatous germ cell tumors (NSGCT). A randomized trial of two versus five computed tomography (CT) scans was performed to determine whether the number of scans influenced the proportion of patients relapsing with intermediate- or poor-prognosis disease at relapse. **METHODS:** Patients with clinical stage I NSGCT opting for surveillance were randomly assigned to chest and abdominal CT scans at either 3 and 12 or 3, 6, 9, 12, and 24 months, with all other investigations identical in the two arms. Three of five patients were allocated to the two-scan schedule. Four hundred patients were required. **RESULTS:** Two hundred forty-seven patients were allocated to a two-scan and 167 to five-scan policy. With a median follow-up of 40 months, 37 relapses (15%) have occurred in the two-scan arm and 33 (20%) in the five-scan arm. No patients had poor prognosis at relapse, but two (0.8%) of those relapsing in the two-scan arm had intermediate prognosis compared with 1 (0.6%) in the five-scan arm, a difference of 0.2% (90% CI, -1.2% to 1.6%). No deaths have been reported. **CONCLUSION:** This study can rule out with 95% probability an increase in the proportion of patients relapsing with intermediate- or poor-prognosis disease of more than 1.6% if they have two rather than five CT scans as part of their surveillance protocol. CT scans at 3 and 12 months after orchidectomy should be considered a reasonable option in low-risk patients.

Sarcevic, B., G. C. Spagnoli, et al. (2003). "Expression of cancer/testis tumor associated antigens in cervical squamous cell carcinoma." *Oncology* **64**(4): 443-9.

We investigated the expression of tumor-associated antigens (TAA) of the cancer/testis (C/T) gene family in cervical squamous cell carcinomas. First, we focused on the HeLa cervical cancer derived cell line, and we found that it expresses MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A12, GAGE-3/6, LAGE-1, and PRAME genes, encoding defined C/T TAA. In contrast, no expression of MAGE-A10, BAGE, GAGE-1/2, or NY-ESO-1 genes was observed. Corresponding gene products could also be detected by immunoblotting and immunocytochemistry, taking advantage of monoclonal antibodies recognizing discrete TAA. Capitalizing on these data, a monoclonal antibody predominantly recognizing MAGE-A4 TAA in paraffin-embedded sections (57B) was used to investigate the C/T gene expression in clinical tumor samples. A group of 60 patients was studied, and 57B positivity was detectable to different extents in 33% of the cases (20/60). In 13 of them (21%), staining of over 50% of the tumor cells was evident, whereas

healthy cells always scored negative. Remarkably, MAGE-A4 expression was significantly ( $p < 0.05$ ) more frequently detectable in poorly differentiated tumors (8/13) than in well-differentiated or moderately differentiated cancers (3/15 and 9/32, respectively) and in stage FIGO II as compared with stage FIGO Ib tumors (12/23 and 5/24, respectively,  $p = 0.04$ ). Interestingly, staining was mostly nuclear in well-differentiated tumors, but involved both nuclei and cytoplasm in less differentiated cancers. Positivities of comparable frequency were also detectable in a smaller series of specimens upon staining with MAGE-A1- or NY-ESO-1/LAGE-1-specific reagents. Considering the high tumor specificity of C/T TAA, our data provide the rationale for the design of immunotherapy procedures targeting these antigens in cervical cancers.

Sasao, T., N. Itoh, et al. (2004). "The protein encoded by cancer/testis gene D40/AF15q14 is localized in spermatocytes, acrosomes of spermatids and ejaculated spermatozoa." *Reproduction* **128**(6): 709-16.

We have previously identified and cloned a human gene, D40, that is preferentially expressed in testis among normal organs, while it is widely expressed in various human tumor cell lines and primary tumors derived from different organs. In this report, we have examined the expression and localization of this protein in human testis with an antibody specific to D40 protein. In Western analyses, the anti-D40 antibody recognized a major band with a molecular mass of 300 kDa and a minor band of 250 kDa. These bands were not observed in the testis lysates from patients with Sertoli-cell-only syndrome and with Klinefelter syndrome, who lack germ cells of the testis, indicating that D40 protein is expressed in the germ cells of normal testis. Immunohistochemical studies have revealed that D40 protein is highly expressed in spermatocytes and in the pre-acrosome of round spermatids. In the acrosome, D40 protein expression is observed not inside but outside the acrosome membrane. This is consistent with the finding that the amino-acid sequence at the amino terminal of the D40 protein lacks a hydrophobic signal peptide that is required for proteins to translocate to the membrane. Expression of D40 protein is observed in the acrosome of ejaculated spermatozoa as well, although the level is low compared with that in the pre-acrosome of spermatids. These results suggest that D40 protein plays important roles in spermatogenesis, especially in the formation and maintenance of the acrosome.

Satie, A. P., E. Rajpert-De Meyts, et al. (2002). "The cancer-testis gene, NY-ESO-1, is expressed in normal

fetal and adult testes and in spermatocytic seminomas and testicular carcinoma in situ." *Lab Invest* **82**(6): 775-80.

Cancer/testis genes are potential targets for therapeutic genetic and immunologic approaches, and are highly expressed in a large variety of human cancers. However, they are not expressed in normal tissues, with the exception of the testis. The NY-ESO-1 gene is the most recently identified member of the cancer/testis family and its product is one of the most immunogenic tumor antigens. We used immunohistochemistry to investigate the expression of NY-ESO-1 in healthy human prenatal and adult testes and in 59 human testicular tumors of different subtypes. We found that NY-ESO-1 was expressed from 18 weeks until birth in human fetal testes. In the adult testis, NY-ESO-1 was strongly expressed in spermatogonia and in primary spermatocytes, but not in post-meiotic cells or in testicular somatic cells. NY-ESO-1 was not expressed in the Sertoli cells, Leydig cells, classical seminomas, or nonseminomatous germ cells in the 59 testicular tumors. In contrast, NY-ESO-1 was expressed both in carcinomas in situ, which are the earliest stage of testicular tumors (7 of 15 cases), and in spermatocytic seminomas, which are believed to be derived from spermatogonia or primary spermatocytes (8 of 16 cases). We conclude that NY-ESO-1 is a marker that can be used to follow the early progression of testicular tumorigenesis when the tumors present a similar pattern of expression to the cells from which they originated, although the later tumors cease to express NY-ESO-1.

Scanlan, M. J., C. M. Gordon, et al. (2002). "Identification of cancer/testis genes by database mining and mRNA expression analysis." *Int J Cancer* **98**(4): 485-92.

Cancer/testis (CT) antigens are immunogenic proteins expressed predominantly in gametogenic tissue and cancer; they are considered promising target molecules for cancer vaccines. The identification of new CT genes is essential to the development of polyvalent cancer vaccines designed to overcome tumor heterogeneity and antigen loss. In the current study, a search for new CT genes was conducted by mining the Unigene database for gene clusters that contain expressed sequence tags derived solely from both normal testis and tumor-derived cDNA libraries. This search identified 1,325 different cancer/testis-associated Unigene clusters. The mRNA expression pattern of 73 cancer/testis-associated Unigene clusters was assessed by reverse transcriptase polymerase chain reaction. Three gene products, CT15/Hs.177959, CT16/Hs.245431 and CT17/Hs.178062, were detected only in testis and in tumor tissue. CT15 is equivalent to ADAM2/fertilin-

beta. CT16, an uncharacterized gene product, has homology (30-50%) to members of the GAGE gene family and is 89% identical to CT16.2/Hs.293317, indicating that CT16 and CT16.2 are members of a new GAGE gene family. The uncharacterized gene product, CT17, has homology (30%) to phospholipase A1. RT-PCR analysis showed that CT15 is expressed exclusively in renal cancer, whereas CT16 and CT17 are expressed in a range of human cancers. Real-time RT-PCR analysis of newly defined CT genes and the prototype CT antigens, MAGE-3 and NY-ESO-1, revealed low levels (less than 3% of the level detected in testis) of CT15, CT16 and NY-ESO-1 in a limited range of normal, non-gametogenic tissues. This study demonstrates the merits of database mining with respect to the identification of tissue-restricted gene products expressed in cancer.

Scanlan, M. J., A. O. Gure, et al. (2002). "Cancer/testis antigens: an expanding family of targets for cancer immunotherapy." *Immunol Rev* **188**: 22-32.

Cancer/testis (CT) antigens are a category of tumor antigens with normal expression restricted to male germ cells in the testis but not in adult somatic tissues. In some cases, CT antigens are also expressed in ovary and in trophoblast. In malignancy, this gene regulation is disrupted, resulting in CT antigen expression in a proportion of tumors of various types. Since their initial identification by T-cell epitope cloning, the list of CT antigens has been greatly expanded through serological expression cloning (SEREX) and differential mRNA expression analysis, and approximately 20 CT antigens or antigen families have been identified to date. Characteristics commonly shared by CT antigens, aside from the highly tissue-restricted expression profile, include existence as multigene families, frequent mapping to chromosome X, heterogeneous protein expression in cancer, likely correlation with tumor progression, induction of expression by hypomethylation and/or histone acetylation, and immunogenicity in cancer patients. Spontaneous humoral and cell-mediated immune responses have been demonstrated against several CT antigens, including NY-ESO-1, MAGE-A, and SSX antigens. Since CT antigens are immunogenic and highly restricted to tumors, their discovery has led directly to the development of antigen-specific cancer vaccines, and clinical trials with MAGE-A and NY-ESO-1 are in progress.

Scanlan, M. J., A. J. Simpson, et al. (2004). "The cancer/testis genes: review, standardization, and commentary." *Cancer Immun* **4**: 1.

Cancer/testis (CT) antigens are immunogenic in cancer patients, exhibit highly tissue-restricted

expression, and are considered promising target molecules for cancer vaccines. To date, 44 CT gene families have been identified and their expression studied in numerous cancer types. For example, bladder cancer, non-small cell lung cancer, and melanoma are high CT gene expressors, with 11/20 (55%), 17/33 (51%) and 17/32 (53%) of the CT transcripts examined by RT-PCR detected in 20% or more of the specimens examined, respectively. Breast and prostate cancer can be considered moderate CT gene expressors, with 12/32 (37%) and 6/20 (30%) CT transcripts having an expression frequency >20%, respectively, while renal and colon cancer are low CT gene expressors, with only 3/33 (9%) and 4/25 (16%) CT transcripts having an expression frequency >20%, respectively. In normal tissues, standardized RT-PCR experiments showed that 19/43 CT genes were testis-restricted, 10/43 CT genes were tissue-restricted (mRNA detected in 2 or fewer non-gametogenic tissues), 9/43 CT genes were differentially expressed (mRNA detected in 3-6 non-gametogenic tissues), and 5/43 CT genes were ubiquitously expressed. With the exception of testis-restricted CT transcripts, all remaining CT transcripts were expressed in normal pancreas. In terms of immunogenicity, 14/29 testis/tissue-restricted CT gene families have been shown to induce a cellular and/or humoral immune response in humans. In view of the expanding list of CT genes, a CT gene database was created to standardize CT nomenclature and accumulate relevant data regarding their expression profiles, immunogenicity, function (where known), gene structure and location, and orthologous groups.

Schrader, A. J., C. H. Ohlmann, et al. (2006). "Impact of evidence-based interdisciplinary guidelines on testis cancer management." *Cancer* **106**(2): 313-9.

**BACKGROUND:** In 1999, interdisciplinary evidence-based guidelines were elaborated for treatment of germ cell tumors in Germany. The aims of the current study were to analyze failures in diagnosis and therapy and to demonstrate the influence of guidelines on individual therapeutic approaches and clinical outcome. Therefore, patient collectives treated before the introduction of guidelines (Group A, 1990-1999, n = 234) and those thereafter (Group B, 2000-2002, n = 84) were compared for recurrence and survival. **METHODS:** In both groups, medical and/or surgical treatment and clinical outcome were evaluated for therapeutic mistakes and violations of guidelines. These were analyzed for their clinical consequences. **RESULTS:** There was no significant difference between groups concerning median age of patients or clinical stage before therapy. Altogether, 27.8% and 8.3% of all patients in Group A and B, respectively, displayed

therapeutic mistakes ( $P < 0.005$ ); 63% of these patients in Group A and 100% of these patients in Group B received an overtreatment. In Group A, 19/234 (8.1%) patients relapsed and 53% of these patients had been treated insufficiently ( $P < 0.005$ ). Advanced disease caused the death of 3/234 patients in this study. As of this writing, only 3 of 84 (3.6%) patients in Group B have relapsed, and no patient has died because of tumor or consecutive treatment. **CONCLUSIONS:** The integration of interdisciplinary evidence-based guidelines for treatment of testicular germ cell tumors has led to significant reduction of both overtreatment and treatment failure and/or relapse that were due to inappropriate primary therapy. Evidence-based guidelines should serve as internal quality controls in all institutions treating patients with testicular germ cell tumors.

Segal, N. H., N. E. Blachere, et al. (2005). "Identification of cancer-testis genes expressed by melanoma and soft tissue sarcoma using bioinformatics." *Cancer Immun* **5**: 2.

Cancer-testis or germ cell antigens (GCAs) are a category of tumor antigens expressed by male germ cells and by cancers of diverse histological origin, but not usually by normal adult somatic tissue. These antigens include products encoded by the MAGE, BAGE, GAGE, SSX, and LAGE/NY-ESO-1 families that encode antigenic peptides recognized by T lymphocytes. In this study, we exploit oligonucleotide technology to identify genes in melanoma and soft tissue sarcoma (STS) that display a cancer-testis/GCA expression profile. We identified 59 such genes, including GCAs we knew to be recognized by T lymphocytes. Among our findings are the expression of PRAME in monophasic synovial sarcoma, PRAME and NY-ESO-1 in myxoid/round cell liposarcoma, and SSX2 and members of the GAGE family in malignant fibrous histiocytoma. Furthermore, the proto-oncogene DBL/MCF2 was identified as encoding a novel candidate GCA expressed by clear cell sarcoma/melanoma of soft parts (MSP). DBL/MCF2 peptides that are bound to HLA-A\*0201 were identified and recognized by T lymphocytes. These results show the utility of high-throughput expression analysis in the efficient screening and identification of GCA candidates in cancer, and its application to the discovery of candidate targets for T cell immunity against GCAs expressed by cancer.

Serefoglu, E. C. and M. D. Balbay (2009). "Slow zoledronic acid releasing testis prostheses in the treatment of prostate cancer patients with bone metastases." *Med Hypotheses* **73**(3): 387-8.

Surgical castration is still considered the 'gold standard' for androgen deprivation therapy which have become the mainstay for the management of advanced prostate cancer. The main drawback of this safe operation is that it may have a negative psychological effect, thus, in recent years, a decline in the utilization of bilateral orchiectomy which is the most cost-effective form of androgen deprivation therapy can be witnessed. Testicular prostheses have been shown to reduce the psychological impact resulting from loss or absence of a testicle in those patients. Besides, patients with advanced prostate cancer are at risk of skeletal complications and bisphosphonates are used in treatment. Zoledronic acid is the only bisphosphonate agent demonstrated to effectively reduce skeletal related events in patients with advanced prostate cancer metastatic to bone. Therefore, zoledronic acid releasing testicular prostheses can be used in the treatment of prostate cancer patients with bone metastases after bilateral orchiectomy. This technology has the potential to become the preferred clinical management tool for prostate cancer patients with bone metastases after bilateral orchiectomy.

Sexton, W. J., C. G. Wood, et al. (2003). "Repeat retroperitoneal lymph node dissection for metastatic testis cancer." *J Urol* **169**(4): 1353-6.

**PURPOSE:** We report pathological results, perioperative complications and patient outcome in 21 men after repeat retroperitoneal lymph node dissection for metastatic testis cancer. **MATERIALS AND METHODS:** We reviewed an institutional tumor registry at our cancer center and identified 417 patients who underwent retroperitoneal lymph node dissection for testis cancer during a 21-year period. Of these 417 patients 21 underwent repeat retroperitoneal lymph node dissection. We reviewed preoperative patient characteristics, operative data and pathological findings from repeat lymphadenectomy, and determined patient disease status, morbidity and mortality after surgery. **RESULTS:** We identified viable germ cell tumor in 5 patients (24%), teratoma in 14 (67%) and fibrosis or necrosis only in 5 (24%). Intraoperatively subadventitial dissection of the aorta occurred in 2 cases, which was severe enough in 1 to require an aortic graft. The most common postoperative complications were prolonged ileus or partial bowel obstruction and chylous ascites in 6 and 3 patients, respectively. Six patients died, including 5 of disease progression and 1 of postoperative pulmonary embolus. At a mean followup of 4.7 years (range 0.1 to 14) 15 patients (71%) were alive and 14 (67%) were disease-free. **CONCLUSIONS:** Repeat retroperitoneal lymph node dissection is safe and effective in the majority of patients with recurrent or

residual retroperitoneal masses after initial multimodality treatments for metastatic testis cancer. Overall perioperative morbidity and mortality are low and yet the potential for significant vascular complications warrants careful preoperative planning and intraoperative judgment.

Sharma, P., Y. Shen, et al. (2006). "Cancer-testis antigens: expression and correlation with survival in human urothelial carcinoma." *Clin Cancer Res* **12**(18): 5442-7.

**PURPOSE:** Vaccination against human cancer is a promising therapeutic approach but the optimal antigen or antigens remain undefined. Cancer-testis antigens (CTA), a family of tumor-associated antigens, have both potent immunogenicity and restricted expression patterns in normal adult tissues, highly desirable characteristics for targets of anticancer vaccines. These antigens were evaluated for both the degree of expression and prognostic value in cancer of the urothelium. **EXPERIMENTAL DESIGN:** The expression patterns of nine CTAs (NY-ESO-1, LAGE-1, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10, CT7, CT10, and GAGE) were examined by immunohistochemistry and reverse transcription-PCR in a panel of high-grade urothelial carcinomas of the urinary bladder. Also assessed were correlations between the expression of CTAs by immunohistochemistry and both disease-free and overall survival. **RESULTS:** At least one CTA was expressed in 77% of samples and 61% of these tumors expressed more than one CTA. Additionally, patients with CT10-positive tumors had an improved disease-free survival ( $P=0.008$ ) and overall survival ( $P=0.037$ ) compared with patients with CT10-negative tumors. **CONCLUSIONS:** These findings establish CTAs as potential prognostic markers and as target candidates for vaccine development for patients with urothelial carcinoma.

Sigalotti, L., E. Fratta, et al. (2004). "Intratumor heterogeneity of cancer/testis antigens expression in human cutaneous melanoma is methylation-regulated and functionally reverted by 5-aza-2'-deoxycytidine." *Cancer Res* **64**(24): 9167-71.

Cancer/testis antigens (CTA) are suitable targets for immunotherapy of human malignancies, and clinical trials are mainly focusing on MAGE-A3. However, the heterogeneous intratumor expression of CTA may hamper the effectiveness of CTA-directed vaccination through the emergence of CTA-negative neoplastic clones. We investigated the intratumor heterogeneity of CTA in human melanoma and the underlying molecular mechanism(s) at clonal level using 14 single cell clones generated from the melanoma lesion Mel 313. Reverse transcription-PCR

revealed a highly heterogeneous expression of MAGE-A1, -A2, -A3, -A4, -A6, GAGE 1-6, SSX 1-5, and PRAME among melanoma clones. Only nine clones expressed MAGE-A3 and competitive reverse transcription-PCR identified relative differences in the number of mRNA molecules of up to 130-fold between clones 5 and 14. This clonal heterogeneity of MAGE-A3 expression correlated with the methylation status of specific CpG dinucleotides in MAGE-A3 promoter: i.e., hypomethylated CpG dinucleotides at positions -321, -151, -19, -16, -5, -2, +21, and +42 were found in clones expressing high but not low levels of MAGE-A3. Supporting the role of DNA methylation in generating the intratumor heterogeneity of CTA, the DNA hypomethylating agent 5-aza-2'-deoxycytidine (5-AZA-dCyd) invariably induced their expression in all CTA-negative clones. Furthermore, 5-AZA-dCyd-treatment reduced to 6 folds the differential expression of MAGE-A3 between clones 5 and 14, which became recognized to a similar extent by T cells specific for a MAGE-A-encoded peptide. These findings identify promoter methylation as directly responsible for the intratumoral heterogeneity of therapeutic CTA in melanoma and foresee the use of 5-AZA-dCyd to overcome the limitations set by their intratumor heterogeneous expression to CTA-based vaccine therapy.

Stang, A., W. Ahrens, et al. (2001). "Undescended testis and the risk of testicular cancer: importance of source and classification of exposure information." *Int J Epidemiol* **30**(5): 1050-6.

**BACKGROUND:** The strength of the association between undescended testis and testicular cancer varies considerably across studies. Here we report the effect of various classifications of self-reported history of undescended testis and different data sources on the estimates of the risk of testicular cancer from a case-control study. **METHODS:** We performed a population-based case-control study including 269 testicular cancer cases and 797 controls matched on age and region. Medical history was assessed by interviews (index persons) and mailed questionnaires (mothers). We used conditional logistic regression to calculate odds ratios (OR) and kappa coefficients to assess agreement between different sources of information. **RESULTS:** Odds ratios for testicular cancer ranged between 2.4 and 5.4 based on the sons' self-reports and between 1.1 and 1.9 based on the mothers' reports. The agreement between the sons and mothers on undescended, gliding or retractile testis was fair (kappa 0.53) and was good when these conditions were treated by surgery (kappa 0.89). The rating of a history of undescended testis by two urologists was fair (kappa 0.54). **CONCLUSIONS:**

The questionnaire design, the classifications of undescended testis and data sources have an important impact on the OR for the association of undescended testis and testicular cancer. These factors may partially explain the heterogeneity of the OR for this association in case-control studies relying on self-reports.

Stevenson, B. J., C. Iseli, et al. (2007). "Rapid evolution of cancer/testis genes on the X chromosome." *BMC Genomics* **8**: 129.

**BACKGROUND:** Cancer/testis (CT) genes are normally expressed only in germ cells, but can be activated in the cancer state. This unusual property, together with the finding that many CT proteins elicit an antigenic response in cancer patients, has established a role for this class of genes as targets in immunotherapy regimens. Many families of CT genes have been identified in the human genome, but their biological function for the most part remains unclear. While it has been shown that some CT genes are under diversifying selection, this question has not been addressed before for the class as a whole. **RESULTS:** To shed more light on this interesting group of genes, we exploited the generation of a draft chimpanzee (*Pan troglodytes*) genomic sequence to examine CT genes in an organism that is closely related to human, and generated a high-quality, manually curated set of human:chimpanzee CT gene alignments. We find that the chimpanzee genome contains homologues to most of the human CT families, and that the genes are located on the same chromosome and at a similar copy number to those in human. Comparison of putative human:chimpanzee orthologues indicates that CT genes located on chromosome X are diverging faster and are undergoing stronger diversifying selection than those on the autosomes or than a set of control genes on either chromosome X or autosomes. **CONCLUSION:** Given their high level of diversifying selection, we suggest that CT genes are primarily responsible for the observed rapid evolution of protein-coding genes on the X chromosome.

Sugita, M., M. Geraci, et al. (2002). "Combined use of oligonucleotide and tissue microarrays identifies cancer/testis antigens as biomarkers in lung carcinoma." *Cancer Res* **62**(14): 3971-9.

High density oligonucleotide microarrays (OMAs) have been used recently to profile gene expression in lung carcinoma tissue homogenates. The length of the lists of potentially interesting genes generated by these studies is daunting, and biological and clinical relevance of these lists remains to be validated. Moreover, specific identification of individual biomarkers that might be used for early



detection and surveillance has not been the objective of these early studies. We have developed a schema for combining the data derived from the OMA analysis of a few lung cancer cell lines with immunohistochemical testing of tissue microarrays to rapidly identify biomarkers of potential clinical relevance. Initially, we profiled gene expression in lung tumor cell lines using the Affymetrix HG-U95Av2 OMA. RNA from 2 non-small cell lung cancer (NSCLC) cell lines (A549 and H647) and 2 small cell lung cancer (SCLC) cell lines (SHP-77 and UMC-19) were tested. Cells from 1 histologically and cytogenetically normal bronchial epithelial primary culture from a volunteer who had never smoked and 10 samples of histologically unremarkable lung tissue from resection specimens served as normalization controls. Array results were analyzed with Gene Spring software. Results were confirmed by reverse transcription-PCR in an expanded number of cell lines. We then validated the cell line data by immunohistochemical testing for protein using a tissue microarray containing 187 NSCLC clinical samples. Of the 20 most highly expressed genes in the tumor lines, 6 were members of the cancer/testis antigen (CTAG) gene group including 5 MAGE-A subfamily members and NY-ESO-1. SCLC lines strongly expressed all of the MAGE-A genes as well as NY-ESO-1, whereas NSCLC lines expressed a subset of MAGE-A genes at a lower level of intensity and failed to express NY-ESO-1. Reverse transcription-PCR of an extended series of 25 lung cancer cell lines including 13 SCLC, 9 NSCLC, and 3 mesothelioma lines indicated that MAGE-A10 and NY-ESO-1 were expressed only by SCLC, and that MAGE-A1, 3, 6, 12, and 4b were expressed by both SCLC and NSCLC. By immunohistochemistry using the monoclonal antibody 6C1 that recognizes several MAGE-A gene subfamily members, 44% of NSCLC clearly expressed MAGE-A proteins in cytoplasm and/or nucleus. Expression of MAGE-A genes did not correlate with survival but did correlate with histological classification with squamous carcinomas more frequently MAGE-A positive than other NSCLC types ( $P < 0.00002$ ). We conclude that expression of CTAG gene products, whereas apparently not of prognostic importance, may be useful for early detection and surveillance because of a high level of specificity for central airway squamous and small cell carcinomas.

Suri, A. (2006). "Cancer testis antigens--their importance in immunotherapy and in the early detection of cancer." *Expert Opin Biol Ther* 6(4): 379-89.

The development of successful immunotherapeutic strategies requires the

identification and characterisation of immunogenic cancer antigens that will be recognised by the host immune system, leading to tumour rejection. The concept of immunotherapy is based on the assumption that antigenic structures expressed in tumours can be used for therapeutic approaches employing the autologous immune system or by the application of immunotherapeutic reagents. Based on this concept, there is a great need to gain profound knowledge of the actual protein/antigen expression and its distribution pattern within normal tissues and cancerous tissues. Cancer testis (CT) antigens represent a unique class of tumour antigens, which are expressed in a variety of cancerous tissues and are silent in normal tissues, except for the testis. Owing to their restricted gene expression in the testis and various malignancies, CT antigens represent potential defined targets for antigen-based vaccination and antigen-directed immunotherapy to control cancer growth. Moreover, the analysis of humoral and cellular immune responses to CT antigens has proved useful for identifying novel cancer serum biomarkers with potential implications in early diagnosis of cancer.

Tajima, K., Y. Obata, et al. (2003). "Expression of cancer/testis (CT) antigens in lung cancer." *Lung Cancer* 42(1): 23-33.

Cancer/testis (CT) antigens are considered promising candidates for vaccine-based immunotherapy. The aim of this study was to investigate which CT antigens should be targeted in immunotherapy of Japanese lung cancer. To determine the expression of 12 CT antigens in Japanese primary lung cancers and cell lines, a reverse-transcription polymerase chain reaction (RT-PCR) analysis was performed. Among 46 primary lung cancers, high expression rates were found for MAGE-3 (41%, 19/46), and SSX-4 (35%, 16/46). A similar pattern of CT antigen expression was observed in 29 lung cancer cell lines. The expression frequency of a certain CT antigen, namely NY-ESO-1, in Japanese cases was drastically different from that in Caucasians. Polyvalent CT antigen vaccine may be effective to increase the number of lung cancer patients eligible for cancer-specific immunotherapy. Vaccination with MAGE-3 and SSX-1 would cover 57% of all patients, with three antigens, MAGE-3, SSX-1, and MAGE-4, would cover 65%, and with four antigens, MAGE-3, SSX-1, MAGE-4 and SSX-4, would cover 70%. Simultaneous expression of two or more CT antigens was observed in 25/46 (54%) primary lung cancers and 18/29 (62%) lung cancer cell lines. Polyvalent CT antigen vaccines may be also effective to reduce a chance of emergence of antigen loss variants, thus preventing tumors from escaping

from the immune system. For this purpose, vaccination with combinations of MAGE-3 with MAGE-6, SSX-4, MAGE-1 or BAGE may be effective for a quarter of Japanese lung cancer patients. In addition, in silico surveys of dbEST database were used for identification of new CT antigens. We identified a novel gene, TES101RP, expressed only in some small cell lung cancers (SCLC) and in testis, as confirmed by RT-PCR analysis.

Takimoto, M., G. Wei, et al. (2002). "Frequent expression of new cancer/testis gene D40/AF15q14 in lung cancers of smokers." *Br J Cancer* **86**(11): 1757-62.

We found a significant correlation between lung cancer in smokers and the expression of a human gene, D40, predominantly expressed in testis and cancers. In an attempt to clone a novel human gene, we screened a cDNA library derived from a human B cell line and obtained a cDNA clone that we refer to as D40. A search for public databases for sequence homologies showed that the D40 gene is identical to AF15q14. D40 mRNA is predominantly expressed in normal testis tissue. However, this gene is also expressed in various human tumour cell lines and primary tumours derived from various organs and tissues, such as lung cancer. We examined the relationship between D40 expression and clinicopathological characteristics of tumours in primary lung cancer. D40 expression did not significantly correlate with either histological type or pathological tumour stage. However, D40 expression was observed more frequently in poorly differentiated tumours than in well or moderately differentiated ones. Furthermore, the incidence of D40 expression was significantly higher in tumours from patients who smoke than in those from non-smokers. D40/AF15q14 is the first gene in the cancer/testis family for which expression is related to the smoking habits of cancer patients.

Tammela, J., A. A. Jungbluth, et al. (2004). "SCP-1 cancer/testis antigen is a prognostic indicator and a candidate target for immunotherapy in epithelial ovarian cancer." *Cancer Immun* **4**: 10.

SCP-1 is a novel tumor antigen that belongs to the growing family of cancer/testis (CT) antigens, and it is a potential target for immunotherapy. In an effort to determine the expression of SCP-1 in epithelial ovarian cancer (EOC), one-step RT-PCR was performed with RNA from epithelial ovarian tumor tissues and with two normal ovarian surface epithelial cell lines. We used immunohistochemistry (IHC) to investigate SCP-1 expression in paraffin-fixed EOC samples and ELISA to test sera from a

subgroup of patients for SCP-1 antibody. SCP-1 was expressed in 15 out of 100 (15%) primary tumors, as determined by RT-PCR. The normal ovarian surface epithelial cell lines were negative for SCP-1 expression, as were a panel of other normal tissues. None of the patients whose tumors were determined to be SCP-1 positive by RT-PCR expressed the antigen by IHC or demonstrated a humoral immune response by ELISA. Tumors that expressed SCP-1 mRNA tended to have a higher grade than those that did not ( $P = 0.03$ ). There was a significant decrease in survival time ( $P = 0.004$ ) for patients with SCP-1 mRNA-positive tumors compared to those with SCP-1 mRNA-negative tumors [median 25 mo, 95% confidence interval (CI) 0-56 mo; and median 97 mo, CI 32-162 mo, respectively]. The present study shows that SCP-1 mRNA expression in patients with EOC is associated with a poorer chance of survival. These findings imply that further evaluation of SCP-1 as a potential target for vaccine therapy in EOC is warranted.

Taylor, B. J., T. Reiman, et al. (2005). "SSX cancer testis antigens are expressed in most multiple myeloma patients: co-expression of SSX1, 2, 4, and 5 correlates with adverse prognosis and high frequencies of SSX-positive PCs." *J Immunother* **28**(6): 564-75.

Cancer testis antigens (CTAs) are tumor-specific antigens that may be useful targets for cancer vaccines. Here, CTA expression was examined in multiple myeloma (MM), a B-cell cancer characterized by malignant plasma cells (PCs) in the bone marrow (BM), and monoclonal gammopathy of undetermined significance (MGUS), a condition that can progress to MM. We screened a panel of patient BMs at different stages of malignancy for CTA expression by reverse transcription polymerase chain reaction RT-PCR. Here, SSX (synovial sarcoma, X chromosome) emerged as a promising candidate for an MM vaccine, having a profile similar to currently studied CTA, NY-ESO-1, and MAGE. SSX1, 2, 4, and 5 expression was studied further in 114 MM (total SSX, 61% of patients; SSX1, 42%; SSX2, 23%; SSX4, 38%; SSX5, 35%), 45 MGUS (total SSX, 24% of patients; SSX1, 9%; SSX4, 20%), and 12 control (0/12, 0%) subjects. Several expression patterns were observed, the most predominant being co-expression of SSX1, 2, 4, and 5 (called group A expression, in 20% of MM), which correlated with reduced survival ( $P=0.0006$ ). Of the four genes, SSX2 had the strongest association with reduced survival ( $P=0.0001$ ). SSX protein expression ranged from 13.5% of PCs in an SSX1/SSX4 co-expressor to as high as 88% of PCs in group A expressor, exceeding reported frequencies of NY-ESO-1 and MAGE in MM. In single PCs from

group A patients, we detected variable degrees of SSX co-expression, emphasizing the heterogeneity of CTA expression within tumor cell populations. These results demonstrate that SSX is a frequently expressed CTA in MM and highlight its potential as an MM vaccine candidate.

Taylor, M., L. M. Bolton, et al. (2007). "Breast cancer is a promising target for vaccination using cancer-testis antigens known to elicit immune responses." *Breast Cancer Res* 9(4): R46.

**INTRODUCTION:** Cancer-testis antigens (CTAGs) are expressed solely in germ cells and in malignant tissues. They are targets of immune responses mediated by cytotoxic T cells in some cancers, and there is much interest in developing vaccines that induce these responses. The purpose of the present study was to ascertain the frequency of expression of CTAGs in breast cancer. **METHODS:** Breast tumours were collected sequentially in the Southampton Tumour Bank from donors who had given written informed consent. Stored samples where there was sufficient material were sampled in sequence. An initial series of 42 tumours was screened for expression of 17 different CTAGs. A second panel of 40 tumours was screened for the expression of those antigens present in the first panel. **RESULTS:** Ninety-three per cent of tumours in the first series expressed at least one CTAG, and 62% expressed the single antigen CTAG1. Eighty per cent of tumours in the second series expressed at least one CTAG, 50% expressing CTAG1. Tumours exhibiting higher risk features tended to express more CTAGs. **CONCLUSION:** More than two-thirds of breast cancers would be covered by a vaccine directed against just three CTAGs - CTAG1, BAGE1, and MAGEA10 - all of which are known to be targets of cytotoxic-T-lymphocyte responses.

Tfelt-Hansen, J. (2008). "The role of calcium-sensing receptor and signalling pathways in the pathophysiology in two in vitro models of malignant hypercalcemia: the rat rice H-500 Leydig testis cancer and prostate cancer (PC-3) cells. Expression and regulation of pituitary tumor transforming gene in Leydig testis cancer and astrocyte and astrocytoma cells." *Dan Med Bull* 55(1): 17-46.

The calcium-sensing receptor (CaR) is a seven transmembrane receptor incorporated into the cell membrane that is sensitive to extracellular calcium and other cations. The finding that the CaR is expressed on cancer cells has opened the door to a new understanding of the role of extracellular calcium as a promalignant stimulus through the CaR and its signaling apparatus as demonstrated in this thesis. I found, in a model of humoral hypercalcemia of

malignancy (HHM), that stimulation of the CaR worsens the promalignant features of the testicular H-500 Leydig cancer cells that were used in my studies. The CaR upregulated the release of parathyroid hormone-related peptide (PTHrP), the main mediator of hypercalcemia in HHM. The growth rate of the tumor was also increased by stimulation of the CaR, as DNA synthesis and protection against apoptosis were enhanced. The oncogene, pituitary tumor-transforming gene (PTTG), was found to be upregulated by the CaR in the H-500 cells, whereas calcium had no effect on PTTG expression in the U-87 astrocytoma cell line, but other proliferative agents did upregulate PTTG in the U-87 cells. This makes PTTG a potential marker of malignancy and a therapeutic target in cancer, where the CaR is promalignant. Nitric oxide synthase (NOS) exists in three isoforms, and I found that the CaR upregulated the inducible NOS but not the two other isoforms. This upregulation was accompanied by an increased production of NO. NO has been shown to be potentially promalignant, although such a role was not established in the H-500 cells. Therefore, the CaR stimulates several promalignant features in the H-500 cells. In turn, blocking these effects by targeting a proximal downstream signaling molecule of the CaR may be a future clinical approach, since blocking the CaR might have too many adverse effects on calcium homeostasis. In conclusion, the CaR plays diverse roles in cancer-acting as an inhibitor of cell proliferation in the colon crypt cells giving rise to colon cancer but as a promalignant receptor in most other cancer types, including Leydig cell cancers.

Utsunomiya, T., H. Inoue, et al. (2004). "Expression of cancer-testis antigen (CTA) genes in intrahepatic cholangiocarcinoma." *Ann Surg Oncol* 11(10): 934-40.

**BACKGROUND:** Cancer-testis antigens (CTA), such as MAGE, are selectively expressed in various types of human neoplasms but not in normal tissues other than testis. This characteristic feature of CTA makes them promising antigens for cancer-specific immunotherapy. **METHODS:** We investigated the expression of five genes, including MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4, in 20 surgical samples of intrahepatic cholangiocarcinomas (IHCC) using reverse transcription-polymerase chain reaction. To visualize the localization of MAGE proteins, we performed immunohistochemical studies. Furthermore, the correlation between the CTA expression and DNA methylation status was studied in three bile duct cancer cell lines. **RESULTS:** Expression of MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4 was recognized in 4, 4, 2, 6, and 3 of all 20 cases,

respectively. In contrast, the expressions of five genes were not recognized at all in the corresponding normal tissues. In 10 cases (50%), the tumors expressed at least one of the five CTA. An immunohistochemical analysis of MAGE proteins demonstrated homogenous or focal distributions in cytoplasm of the IHCC. Using a demethylating agent, MAGE-1, NY-ESO-1, SCP-1, and SSSX-4 were induced in two of three cell lines, whereas MAGE-3 was not. CONCLUSIONS: Half of the tumor tissues of IHCC expressed at least one of the CTA. Some of the patients with IHCC, therefore, should be candidates for potentially useful cancer-specific immunotherapy.

Vatolin, S., Z. Abdullaev, et al. (2005). "Conditional expression of the CTCF-paralogous transcriptional factor BORIS in normal cells results in demethylation and derepression of MAGE-A1 and reactivation of other cancer-testis genes." *Cancer Res* **65**(17): 7751-62.

Brother of the Regulator of Imprinted Sites (BORIS) is a mammalian CTCF paralog with the same central 11Zn fingers (11ZF) that mediate specific interactions with varying approximately 50-bp target sites. Regulated in vivo occupancy of such sites may yield structurally and functionally distinct CTCF/DNA complexes involved in various aspects of gene regulation, including epigenetic control of gene imprinting and X chromosome inactivation. The latter functions are mediated by mCpG-sensitive 11ZF binding. Because CTCF is normally present in all somatic cells, whereas BORIS is active only in CTCF- and 5-methylcytosine-deficient adult male germ cells, switching DNA occupancy from CTCF to BORIS was suggested to regulate site specificity and timing of epigenetic reprogramming. In addition to 11ZF-binding paternal imprinting control regions, cancer-testis gene promoters also undergo remethylation during CTCF/BORIS switching in germ cells. Only promoters of cancer testis genes are normally silenced in all somatic cells but activated during spermatogenesis when demethylated in BORIS-positive germ cells and are found aberrantly derepressed in various tumors. We show here that BORIS is also expressed in multiple cancers and is thus itself a cancer-testis gene and that conditional expression of BORIS in normal fibroblasts activates cancer-testis genes selectively. We tested if replacement of CTCF by BORIS on regulatory DNA occurs in vivo on activation of a prototype cancer-testis gene, MAGE-A1. Transition from a hypermethylated/silenced to a hypomethylated/activated status induced in normal cells by 5-aza-2'-deoxycytidine (5-azadC) was mimicked by conditional input of BORIS and is associated with complete switching from CTCF to

BORIS occupancy at a single 11ZF target. This site manifested a novel type of CTCF/BORIS 11ZF binding insensitive to CpG methylation. Whereas 5-azadC induction of BORIS takes only few hours, derepression of MAGE-A1 occurred 1 to 2 days later, suggesting that BORIS mediates cancer-testis gene activation by 5-azadC. Indeed, infection of normal fibroblasts with anti-BORIS short hairpin RNA retroviruses before treatment with 5-azadC blocked reactivation of MAGE-A1. We suggest that BORIS is likely tethering epigenetic machinery to a novel class of CTCF/BORIS 11ZF target sequences that mediate induction of cancer-testis genes.

Vaughan, H. A., S. Svobodova, et al. (2004). "Immunohistochemical and molecular analysis of human melanomas for expression of the human cancer-testis antigens NY-ESO-1 and LAGE-1." *Clin Cancer Res* **10**(24): 8396-404.

PURPOSE: NY-ESO-1 and LAGE-1 are homologous cancer-testis antigens, which are expressed in many different cancers. It is essential to type tumors accurately to assess patient suitability for clinical trials which target these. This study evaluates typing strategies used to distinguish these two homologous but distinct antigens and to characterize and quantitate expression of each in clinical samples. EXPERIMENTAL DESIGN: We typed 120 malignant melanomas for the expression of NY-ESO-1 and LAGE-1 with immunohistochemistry, reverse transcription-PCR (RT-PCR), and quantitative real-time (qRT-PCR), which was also used to explore the relationship between NY-ESO-1 and LAGE expression. RESULTS: The two monoclonal antibodies ES121 and E978 had very similar immunohistochemistry reactivities. Both were specific for NY-ESO-1 because neither bound to homologous LAGE-1 peptides despite 84% overall amino acid homology. Of 120 melanomas tested by immunohistochemistry, NY-ESO-1 was expressed in >50% of cells in 23 melanomas (19%), between 11 and 50% cells in 15 (12.5%), <11% cells in 16 (13.5%), and negative in 66 (55%). Although specific for both antigens, the PCR methods did not provide this information about microheterogeneity. Polymorphisms in the LAGE-1 gene resulted in false negative LAGE-1 typing by qRT-PCR by inhibiting binding of oligonucleotide primers, thereby showing the exquisite specificity of qRT-PCR as a typing method. CONCLUSIONS: For NY-ESO-1 typing, immunohistochemistry compared favorably with the RT-PCR, with the added advantage of being able to characterize heterogeneity of antigen expression. Because neither mAb bound LAGE and because there was no coordinate expression LAGE and NY-ESO-1, separate typing for each is required.

Velazquez, E. F., A. A. Jungbluth, et al. (2007). "Expression of the cancer/testis antigen NY-ESO-1 in primary and metastatic malignant melanoma (MM)--correlation with prognostic factors." *Cancer Immun* 7: 11.

Cancer/testis (CT) antigens are potential targets for cancer immunotherapy, with NY-ESO-1 being among the most immunogenic. In several clinical trials in malignant melanoma (MM) patients, NY-ESO-1 protein/peptides showed clear evidence of inducing specific immunity. However, little is known about NY-ESO-1 expression in primary and metastatic MM and its relationship to disease progression. We analyzed NY-ESO-1 expression immunohistochemically in a series of primary and metastatic MMs and its relation to prognostic parameters and survival. We studied 61 primary and 63 metastatic MM specimens (from 61 and 56 patients, respectively). The prevalence of NY-ESO-1 expression was significantly higher in metastatic versus primary tumors [18/56 (32%) versus 8/61 (13%),  $P = 0.015$ ]. There was a significant association between initial stage at presentation and NY-ESO-1 expression [stage I (3.45%), stage II (9.52%) and stage III (45.45%),  $P = 0.0014$ ]. Primary MMs expressing NY-ESO-1 were significantly thicker than NY-ESO-1 negative cases (median thickness 4.7 mm versus 1.53 mm respectively,  $P = 0.03$ ). No significant difference was seen in overall survival. In conclusion, NY-ESO-1 is more frequently expressed in metastatic than in primary MM and its expression is associated with thicker primary lesions and a higher frequency of metastatic disease, indicative of a worse prognosis. Our study suggests that patients with metastatic MM who express NY-ESO-1 may benefit from NY-ESO-1-based immunotherapy.

Wadle, A., B. Kubuschok, et al. (2006). "Serological immune response to cancer testis antigens in patients with pancreatic cancer." *Int J Cancer* 119(1): 117-25.

Serological screening approaches have allowed for the identification of a large number of potentially relevant tumor antigens in cancer patients. Within this group, cancer testis antigens represent promising targets for cancer immunotherapy, since they are widely expressed in a variety of human cancer entities. In pancreatic cancer, however, there are only few data available about the expression pattern and serological response to cancer testis antigens and other serological-defined tumor antigens. Therefore, we investigated the IgG antibody response against 11 cancer testis antigens (SCP-1, GAGE, LAGE-1a,-1b, CT-7, NY-ESO-1, SSX-1-5) recombinantly expressed on yeast surface (RAYS) in patients with pancreatic cancer ( $n = 96$ ), chronic

pancreatitis ( $n = 18$ ) and healthy donors ( $n = 48$ ). We found in 14% of all patients antibody responses to SCP-1, but not to other cancer testis antigens (GAGE, LAGE-1a,-1b, CT-7, NY-ESO-1, SSX-1-5). Antibody response correlated with the expression of SCP-1 in the primary tumor of the respective patient as shown by RT-PCR, immunohistochemistry and Western blot. In contrast, no serological response to cancer testis antigens was observed in healthy donors. The humoral immune response against SCP-1 was associated with the size of tumor, but not with other clinicopathological parameters such as histology, stage, presence of lymph node metastases, grading, age, gender or gemcitabine treatment. In conclusion, antibody response to cancer testis antigen SCP-1 is found in a proportion of pancreatic carcinoma patients. These results indicate that identification of additional tumor antigens by serological screening of tumor cDNA expression libraries by RAYS is a promising goal in pancreatic cancer.

Wagner, C., F. Neumann, et al. (2003). "Identification of an HLA-A\*02 restricted immunogenic peptide derived from the cancer testis antigen HOM-MEL-40/SSX2." *Cancer Immun* 3: 18.

HOM-MEL-40/SSX2 is a SEREX-defined cancer testis antigen with frequent expression in various human neoplasms. To search for HLA-A\*0201 restricted peptides that induce HOM-MEL-40/SSX2-specific CD8+ responses in breast cancer patients, we used the SYFPEITHI algorithm to identify three HOM-MEL-40/SSX2-derived nonamers with high binding affinity for HLA-A\*0201, which has a prevalence of 40% in the Caucasian population. Of the three peptides, p41-49 and p103-111 but not p167-175 had been shown to be processed by the proteasome. Only stimulation with p103-111 induced HOM-MEL-40-specific CTLs in 5/7 patients with HOM-MEL-40/SSX2 positive breast cancers and in 6/11 healthy controls. HLA-A\*0201 restriction of p103-111 was demonstrated by blocking with specific antibodies. The natural processing and presentation of p103-111 was demonstrated by the recognition of the HOM-MEL-40/SSX2 positive cell line SK-MEL-37 and of COS7/A2 cells transfected with HOM-MEL-40/SSX2 by p103-111 specific CD8+ cells. No correlation was found between CD8+ T-cell responses against p103-111 and anti-HOM-MEL-40/SSX2 antibody titers in the serum of patients, suggesting that CD8+ and B-cell responses against HOM-MEL-40/SSX2 are regulated independently. p103-111 holds promise as a broadly applicable peptide vaccine for patients with HOM-MEL-40/SSX2 positive neoplasms.

Wang, Y., X. J. Wu, et al. (2004). "Cancer/testis antigen expression and autologous humoral immunity to NY-ESO-1 in gastric cancer." *Cancer Immun* 4: 11.

Gastric cancer has the highest mortality rate and the second-highest morbidity rate of all malignant tumors in China. Since cancer/testis (CT) antigens are expressed in various types of human tumors but generally not in normal tissue except for testis, they are promising antigens for cancer immunotherapy. NY-ESO-1, in particular, is the most immunogenic of the CT antigens. To study the feasibility of developing a CT antigen vaccine for gastric cancer, 101 gastric cancer samples were analyzed for the presence of NY-ESO-1 mRNA and that of 10 other CT antigen genes. Twelve out of 101 samples (11.9%) were found to be NY-ESO-1 mRNA-positive, 11 of them from advanced stage patients. In 7 of the 12 NY-ESO-1 mRNA-positive samples, the NY-ESO-1 protein was also detected by immunohistochemistry. An autologous humoral immune response to NY-ESO-1 was detected in 6 of 12 advanced stage NY-ESO-1 mRNA-positive patients, indicating that NY-ESO-1 is immunogenic in advanced stage gastric cancer. The serum from a patient with an NY-ESO-1 negative but LAGE-1 positive tumor was also found to be NY-ESO-1 antibody positive, possibly due to cross-reactivity between NY-ESO-1 and LAGE-1. All NY-ESO-1 mRNA-positive gastric cancer samples also expressed one to seven additional CT genes, revealing a tendency toward a clustered expression pattern, regardless of disease stage. About 74% of the samples expressed at least one CT antigen, most frequently MAGE-3 (41.6%). NY-ESO-1 and MAGE-3 are thus potential targets for a multivalent CT antigen vaccine.

Wang, Z., Y. Zhang, et al. (2004). "The spermatozoa protein, SLLP1, is a novel cancer-testis antigen in hematologic malignancies." *Clin Cancer Res* 10(19): 6544-50.

**PURPOSE:** Neoplastic cells often aberrantly express normal testicular proteins. Because these proteins have a very restricted normal tissue expression, they may be suitable targets for immunotherapy. SLLP1 is an intra-acrosomal, nonbacteriolytic, c lysozyme-like protein recently isolated from human spermatozoa. In this study, we determined whether SLLP1 is a novel cancer-testis antigen in hematologic malignancies  
**EXPERIMENTAL DESIGN:** SLLP1 expression in hematologic tumor cells and normal tissues was determined using a combination of reverse transcription-PCR, real-time PCR, and Western blot analysis. The presence of antibodies against SLLP1 was determined by ELISA analysis. **RESULTS:** SLLP1 was aberrantly expressed in the tumor cells from 2 of 9 acute myeloid leukemia, 3 of 11 chronic

lymphocytic leukemia, 4 of 14 chronic myeloid leukemia, and 6 of 17 multiple myeloma. In contrast, they were not detected in corresponding specimens from any healthy donors. SLLP1 exhibited a very restricted normal tissue expression, being found only in testis/spermatozoa. SLLP1 was expressed in some tumor cells at a level of >25%. High titer IgG antibodies against SLLP1 were also detected in the sera of some of these patients. **CONCLUSIONS:** SLLP1 is a novel cancer-testis antigen in hematologic malignancies and is capable of eliciting B-cell immune responses in vivo in cancer-bearing individuals. Our results, therefore, support SLLP1 as a protein target appropriate for additional in vitro study to define its suitability for immunotherapy.

Watanabe, Y., S. LePage, et al. (2006). "Characterization of preexisting humoral immunity specific for two cancer-testis antigens overexpressed at the mRNA level in non-small cell lung cancer." *Cancer Immun* 6: 3.

In order to establish a rationale for immunotherapy for lung cancer, we have investigated immunological characteristics of tumor-associated antigens (TAAs) discovered through molecular approaches. Preexisting Abs specific to these predicted TAAs were examined using specimens of lung pleural effusions (LPEs) and sera in non-small cell lung cancer (NSCLC) patients. The novel cancer-testis (CT) antigens L514S and L552S were highly expressed in approximately half of the NSCLC tissues and established cell lines examined. When lung cancer patients in the USA and Japan were screened, 13%, 17%, and 5% were found to have Abs specific to recombinant L514S, L552S, and NY-ESO-1 proteins, respectively, whereas 48 normal donors had no Abs to these three CT antigens. The Ab titers specific to recombinant L552S and L514S proteins were similar to, and slightly lower than, Abs specific to NY-ESO-1 in stage IV NSCLC patients. To further characterize the preexisting specific Abs, the epitopes were analyzed using 20-aa length peptides entirely covering both antigens. An epitope common to the patients' L514S-specific Abs was identified as aa 85-100 and multiple epitopes, including a major epitope (aa 141-160), were identified for L552S-specific Abs. The Ab epitopes thus identified are not found in human, animal, or bacterial proteins, other than L514S, L552S, or XAGE-1. These data clearly demonstrate that both molecularly defined CT antigens L514S and L552S are immunogenic, at least in terms of humoral responses, suggesting that both CT antigens are promising candidates for immunotherapy.

Westbrook, V. A., A. B. Diekman, et al. (2001). "Differential nuclear localization of the cancer/testis-

associated protein, SPAN-X/CTp11, in transfected cells and in 50% of human spermatozoa." *Biol Reprod* **64**(1): 345-58.

Cancer-testis antigens (CTAs) represent potential targets for cancer immunotherapy because these proteins are widely distributed in tumors but not in normal tissues, except testes. In this paper, we identify homology of the CTA CTp11 with SPAN-X (sperm protein associated with the nucleus mapped to the X chromosome). On two-dimensional Western blots of human sperm extracts, SPAN-X antibodies recognized 19 spots ranging from 20 to 23 kDa with isoelectric points from 5.0 to 5.5. Differential extraction of spermatozoa demonstrated that the SPAN-X protein is highly insoluble. Only 50% of ejaculated spermatozoa exhibited SPAN-X immunofluorescent staining. Dual localization of the sex chromosomes and the SPAN-X protein demonstrated that an equal number of X- and Y-bearing spermatozoa exhibited SPAN-X staining. In transfected mammalian CV1 cells, the SPAN-Xa and SPAN-Xb proteins were localized to the nucleus and cytoplasm, respectively, by indirect immunofluorescence. On immunoblots of CV1 cells, the SPAN-Xa protein migrated at 15-20 kDa, whereas the SPAN-Xb protein migrated at a higher molecular weight of 21-22 kDa. The SPAN-X protein was ultrastructurally associated with nuclear vacuoles and the redundant nuclear envelope. SPAN-X is the first protein specifically localized to these poorly characterized structures of the mammalian sperm nucleus and provides a unique biochemical marker for investigation of their function in spermatozoa as well as the role of SPAN-X/CTp11 in human tumors.

Westbrook, V. A., P. D. Schoppee, et al. (2004). "Genomic organization, incidence, and localization of the SPAN-x family of cancer-testis antigens in melanoma tumors and cell lines." *Clin Cancer Res* **10**(1 Pt 1): 101-12.

**PURPOSE:** Members of the SPAN-X (sperm protein associated with the nucleus mapped to the X chromosome) family of cancer-testis antigens are promising targets for tumor immunotherapy because they are normally expressed exclusively during spermiogenesis on the adluminal side of the blood-testis barrier, an immune privileged compartment. **Experimental Design and Results:** This study analyzed the human SPANX genomic organization, as well as SPAN-X mRNA and protein expression in somatic and cancer cells. The SPANX family consists of five genes, one of which is duplicated, all located in a gene cluster at Xq27.1. From the centromere, the arrangement of the five SPANX genes mapped on one contiguous sequence is SPANXB, -C, -A1, -A2, and -D. Reverse transcription-PCR analyses demonstrated

expression of SPAN-X mRNA in melanoma and ovarian cell lines, and virtual Northern analysis established SPANX gene expression in numerous cancer cell lines. Immunoblot analysis using polyclonal antisera raised against recombinant SPAN-X confirmed the translation of SPAN-X proteins in melanoma and ovarian tumor cell lines. The immunoreactive proteins migrated between M(r) 15,000 and M(r) 20,000 similar to those observed in spermatozoa. Immunoperoxidase labeling of melanoma cells and tissue sections demonstrated SPAN-X protein localization in the nucleus, cytoplasm, or both. Ultrastructurally, in melanoma cells with nuclear SPAN-X, the protein was associated with the nuclear envelope, a localization similar to that observed in human spermatids and spermatozoa. Significantly, the incidence of SPAN-X-positive immunostaining was greatest in the more aggressive skin tumors, particularly in distant, nonlymphatic metastatic melanomas. **CONCLUSIONS:** The data herein suggest that the SPAN-X protein may be a useful target in cancer immunotherapy.

Williams, S. B., G. S. Steele, et al. (2009). "Primary retroperitoneal lymph node dissection in patients with clinical stage IS testis cancer." *J Urol* **182**(6): 2716-20.

**PURPOSE:** Initial management for clinical stage IS (persistently increased tumor markers) nonseminomatous germ cell tumor has evolved from primary retroperitoneal lymph node dissection to induction chemotherapy at most medical centers. We analyzed the outcome in patients treated with primary retroperitoneal lymph node dissection. **MATERIALS AND METHODS:** We reviewed the charts of patients who underwent retroperitoneal lymph node dissection at Brigham and Women's Hospital, and Dana Farber Cancer Center from 1993 to 2008. All patients with clinical stage IS were identified and perioperative data were obtained. **RESULTS:** A total of 280 patients who underwent retroperitoneal lymph node dissection were identified, of whom 24 identified with clinical stage IS underwent primary dissection. Median followup was 2.9 years. Histopathology revealed an embryonal carcinoma component in 24 orchiectomy specimens (100%) with associated teratoma in 15 (63%). Positive lymph nodes were identified at retroperitoneal lymph node dissection in 9 patients (38%), including pure embryonal carcinoma in 6 (67%), combined embryonal carcinoma and teratoma in 1, embryonal carcinoma, choriocarcinoma and teratoma in 1, and only teratoma in 1. Of the patients who underwent primary retroperitoneal lymph node dissection 5 (21%) also received chemotherapy postoperatively, which was due to persistently increased tumor markers in 3 (13%). No

retroperitoneal recurrence was noted on followup imaging. At surgery estimated blood loss was 175 cc, operative time was 3.1 hours and hospital stay was 3.9 days. There were no deaths. **CONCLUSIONS:** Patients with clinical stage IS are at significant risk for metastatic disease and can be successfully treated with primary retroperitoneal lymph node dissection, thereby sparing chemotherapy in most of them. Retroperitoneal recurrence is essentially eliminated when retroperitoneal lymph node dissection is performed in this select patient group.

Wu, L. Q., Y. Lu, et al. (2006). "Expression of cancer-testis antigen (CTA) in tumor tissues and peripheral blood of Chinese patients with hepatocellular carcinoma." *Life Sci* **79**(8): 744-8.

To investigate the expression of cancer-testis antigen (CTA) in Chinese patients with hepatocellular carcinoma (HCC), and the relationship between CTA gene expression and clinical indexes, we used one-step reverse transcription polymerase chain reaction (RT-PCR). The expression of the CTA mRNA was investigated in the tissues of HCC and corresponding peripheral blood of 37 patients with HCC. Fifteen samples of cirrhotic tissues and 15 normal tissues were examined with the same method. Two kinds of CTA (SSX-2 and SSX-5) showed high-specific and high-frequent expression in HCC tissues, but neither of them could be detected in adjacent non-HCC tissues. In corresponding peripheral blood of HCC tissues, the positive expression rate of the SSX-2 and SSX-5 mRNA was not very high. No relationship was found between the expression of CTA and clinical indicators such as age, sex, tumor size, TNM staging, serum AFP level and infection with hepatitis virus. In 15 patients with cirrhosis and 15 other non-tumor patients, none of the SSX-2 and SSX-5 mRNA was detected in liver tissue or peripheral blood. High frequency and specificity of CTAs in HCC indicates that their products may be new potential promising targets for antigen-specific immunotherapy of HCC. High frequent co-expression of the two genes in HCC provides a possibility of polyvalent vaccinations for HCC. Specific expression of CTAs was observed in AFP-negative HCC, suggested applying their mRNA as tumor markers to detect circulating HCC cells as adjuvant diagnostic tool and as indicators of recurrence and prognosis.

Xie, X., H. H. Wacker, et al. (2003). "Differential expression of cancer testis genes in histological subtypes of non-Hodgkin's lymphomas." *Clin Cancer Res* **9**(1): 167-73.

**PURPOSE:** The purpose of this study was to determine the potential of cancer testis (CT) antigens as vaccines for non-Hodgkin's lymphomas (NHLs).

**EXPERIMENTAL DESIGN:** Ninety-three specimens of NHLs were analyzed for their composite expression of eight CT genes (MAGE-3, MAGE-4, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TEST-14/SCP-1, and HOM-TEST-85). Thirty-nine of these specimens were also analyzed for their NY-ESO-1 expression. **RESULTS:** Only 1 of 7 cases of chronic lymphocytic leukemia expressed a CT gene (HOM-TEST-14/SCP-1), and 10 follicular lymphomas were negative for all of the CT genes tested. In B-cell lymphomas, the most frequent expression of CT genes was observed in diffuse large-cell lymphomas (HOM-TEST-14/SCP-1: 7 of 28; SSX-1: 5 of 28; CT-7: 2 of 28; and HOM-MEL-40/SSX-2 and HOM-TEST-85: 1 of 28 positive cases). Only 1 of 8 Burkitt's and 1 of 7 lymphoblastic lymphomas expressed a CT gene (CT7 and HOM-TEST-14/SCP-1, respectively). A majority (9 of 15) of T- NHLs (9 peripheral T-cell lymphomas, 2 lymphoblastic T-cell lymphomas, and 4 cases of ALLD) expressed HOM-TEST-14/SCP-1. **CONCLUSIONS:** HOM-TEST-14/SCP-1, and to some degree SSX-1 and CT-7 might be candidates for lymphoma vaccine development. However, the identification of additional tumor-specific antigens with a frequent expression in lymphomas is warranted to allow for the development of widely applicable polyvalent lymphoma vaccines.

Xing, Q., X. W. Pang, et al. (2008). "Identification of new cytotoxic T-lymphocyte epitopes from cancer testis antigen HCA587." *Biochem Biophys Res Commun* **372**(2): 331-5.

The cancer testis (CT) antigen HCA587 is highly expressed in human hepatocellular carcinoma (HCC) and induces specific T-cell responses in a significant proportion of HCC patients. To explore its potential in cancer immunotherapy, a reverse immunology approach was adopted to identify HCA587-derived HLA-A( \*)0201-restricted epitopes. Multiple peptides with a top ranking in various prediction programs were thus synthesized and three of them-p248-256, p140-149 and p144-152-were found to bind to HLA-A(\*)0201 molecules with a high affinity and effectively induced a recall response of CD8+ T cells, which were either primed in vitro with the HCA587 antigen or directly isolated from HCC patients bearing HCA587+ tumors. Notably, these peptide-specific CD8+ T cells exhibited potent cytotoxic activity over HCA587+ tumor cells. Taken together, the present study has identified three new HLA-A(\*)0201-restricted cytotoxic T cell epitopes in the CT antigen HCA587, which may serve as targets for peptide-based immunotherapy for HCC patients.

Yakirevich, E., E. Sabo, et al. (2003). "Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens



in serous ovarian neoplasms." *Clin Cancer Res* **9**(17): 6453-60.

**PURPOSE:** The cancer-testis (CT) family of antigens is expressed in a variety of malignant neoplasms and is silent in normal tissues, except for the testis. Expression of two members of this family, MAGE-A4 and NY-ESO-1, has been described in melanomas, germ cell tumors, certain carcinomas and sarcomas, and more recently in uterine neoplasms. The objective of this study was to evaluate the extent and prognostic significance of CT antigen expression in ovarian serous neoplasms. **EXPERIMENTAL DESIGN:** Seventy-four patients with ovarian neoplasms, including 10 with serous cystadenomas, 11 with serous tumors of borderline malignancy, and 53 with serous carcinomas, were studied. Immunohistochemistry was performed with the 57B monoclonal antibody, which recognizes predominantly the MAGE-A4 antigen and the D8.38 antibody that recognizes NY-ESO-1. **RESULTS:** MAGE-A4 expression was found to be present in 57% of the serous carcinomas and only in 9% of the serous tumors of borderline malignancy. No staining was detected in serous cystadenomas or in the normal ovary. In 8 of 30 positively stained serous carcinomas, >50% of the tumor cells expressed MAGE-A4. NY-ESO-1 expression was seen in 19% of the serous carcinomas, whereas serous tumors of borderline malignancy and cystadenomas were negative. A significant inverse correlation was found between MAGE-A4 expression and patient survival ( $P = 0.016$ ). Multivariate analysis revealed that both tumor stage and MAGE-A4 expression were independent predictors of patient survival ( $P = 0.022$  and  $P = 0.013$ , respectively). **CONCLUSIONS:** Cancer-testis antigen expression in ovarian serous neoplasms correlates directly with their degree of malignancy. MAGE-A4 expression, and to a lesser degree NY-ESO-1 expression, is characteristic of the majority of serous carcinomas. Determining the degree of MAGE-A4 expression in these tumors may provide important prognostic information. Finally, MAGE-A4 may represent a novel target for immunotherapy in serous ovarian neoplasms.

Yamaguchi, H., F. Tanaka, et al. (2004). "Identification of HLA-A24-restricted CTL epitope from cancer-testis antigen, NY-ESO-1, and induction of a specific antitumor immune response." *Clin Cancer Res* **10**(3): 890-6.

**PURPOSE:** For the development of peptide-based, cancer-specific immunotherapy, the identification of CTL epitopes from additional tumor antigens is very important. NY-ESO-1, a cancer-testis antigen, is considered to be a promising target of tumor-specific immunotherapy. Because HLA-A24-

expressing individuals cover >60% in the population of Japan, we aim at identifying NY-ESO-1-encoded peptide presented by HLA-A24. **EXPERIMENTAL DESIGN:** In our study, a HLA-A24-restricted CTL epitope was identified by using the following four-step procedure: (a) computer-based epitope prediction from the amino acid sequence of NY-ESO-1 antigen; (b) peptide-binding assay to determine the affinity of the predicted peptide with HLA-A24 molecule; (c) stimulation of primary T-cell response against the predicted peptides in vitro; and (d) testing of the induced CTLs toward various carcinoma cells expressing NY-ESO-1 antigen and HLA-A24. **RESULTS:** Of the tested peptides, effectors induced by a peptide of NY-ESO-1 at residue position 158-166 lysed three kinds of carcinoma cells expressing both NY-ESO-1 and HLA-A24. Our results indicate that peptide NY-ESO-1 (158-166) (LLMWITQCF) is a new HLA-A24-restricted CTL epitope capable of inducing NY-ESO-1-specific CTLs in vitro mediating HLA class I-restricted manner. **CONCLUSIONS:** We identified a novel HLA-A24-restricted NY-ESO-1-derived epitope peptide (LLMWITQCF) that could induce specific CTLs from the peripheral blood mononuclear cells of HLA-A24(+) healthy donors. This peptide would be useful in further evaluating the clinical utility of peptide-based, cancer-specific immunotherapy against various histological tumors.

Yang, X. A., X. Y. Dong, et al. (2004). "Purification and refolding of a novel cancer/testis antigen BJ-HCC-2 expressed in the inclusion bodies of *Escherichia coli*." *Protein Expr Purif* **33**(2): 332-8.

BJ-HCC-2 is one of the cancer/testis antigens that may be the most promising targets for tumor immunotherapy. To investigate the expression of BJ-HCC-2 protein in tumor cells and its capacity to elicit CTL response, the recombinant protein of BJ-HCC-2 was expressed in the inclusion bodies in *Escherichia coli*. The inclusion bodies were solubilized effectively with 0.3% N-lauroyl sarcosine in alkaline buffer. Under this denatured form, the BJ-HCC-2 protein carrying 6x histidine tag was purified with Ni-NTA affinity chromatography in a single step with a purity of over 97%. The yield of the purified protein was about 78%. The purified recombinant protein was refolded in a simple way. The correct refolding of the recombinant protein was verified in the recovery of its secondary and tertiary structures as assessed by circular dichroism and fluorescence emission spectra. The recovery rate of refolded protein was 92.1%. The renatured protein displayed its immunoreactivity with the antibodies to BJ-HCC-2 protein by Western blotting. This method of protein purification and refolding is easy to manipulate and may be applicable

to the hydrophobic proteins that are unable to be purified by other methods.

Yin, Y. H., Y. Y. Li, et al. (2005). "TSPY is a cancer testis antigen expressed in human hepatocellular carcinoma." *Br J Cancer* **93**(4): 458-63.

In search for genes associated with hepatocellular carcinoma (HCC) by cDNA microarray, we found that the transcription of TSPY, 'testis-specific protein Y-encoded', was upregulated in HCC. Investigation of a broad spectrum of normal and malignant tissues by RT-PCR revealed the TSPY transcript selectively expressed in normal testis, different histological types of human neoplastic tissues, and tumour cell lines. The expression of TSPY in cancer cells was further confirmed by in situ hybridisation. Indirect immunofluorescence microscopy analysis showed that TSPY was localised mainly in the cytoplasm of transiently transfected cells. Testis-specific protein Y-encoded was detected in 50% (16 of 32) of well- and moderately differentiated HCC patients, in 16% (four of 25) of poorly differentiated HCC patients, and in 5% (one of 19) of renal cell cancer patients. A serological survey revealed that 6.6% (seven of 106) HCC patients had anti-TSPY antibody response, demonstrating the immunogenicity of TSPY in humans. In conclusion, these data suggest that TSPY is a novel cancer/testis (CT) antigen and may be a potential candidate in vaccine strategy for immunotherapy in HCC patients.

Yokoe, T., F. Tanaka, et al. (2008). "Efficient identification of a novel cancer/testis antigen for immunotherapy using three-step microarray analysis." *Cancer Res* **68**(4): 1074-82.

Advanced technology in molecular biology has provided us powerful tools for the diagnosis and treatment for cancer. We herein adopted a new methodology to identify a novel cancer/testis (CT) antigen with high frequency of expression in colorectal cancer as follows: (a) combining laser microdissection and cDNA microarray was used to analyze the gene expression profile of colorectal cancer cells; (b) genes overexpressed in testis and underexpressed in normal colon epithelium were analyzed using cDNA microarray; and (c) the gene expression profile of colorectal cancer cells was compared with that of normal testis. Using this methodology, we selected 38 candidates for CT antigen. Among these genes, we identified a novel CT antigen, serine/threonine kinase 31 (STK31), which was previously reported as a gene expressed in spermatogonia. Reverse transcription-PCR analysis showed that STK31 gene expression levels in cancer samples were significantly higher ( $P < 0.0001$ ) than those in normal samples. The STK31 gene was

frequently expressed not only in colorectal cancer but also in gastric and esophageal cancer. Moreover, STK31 peptide was able to elicit specific CTLs and induced CTLs lysed either peptide-loading or endogenously STK31-expressing target cells. These results showed that the new methodology in this study facilitated identification of CT antigens and that STK31 may be a candidate for cancer immunotherapy against gastrointestinal cancer.

Yoshida, N., H. Abe, et al. (2006). "Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens and T cell infiltration in non-small cell lung carcinoma and their prognostic significance." *Int J Oncol* **28**(5): 1089-98.

Cancer-testis (CT) antigens were identified as a group of highly attractive targets for cancer immunotherapy because of their expression in a variety of malignant tumors but solely in the testis among the normal adult tissues. To evaluate the potential of two members of this family, MAGE-A4 and NY-ESO-1 antigens, for cancer vaccine in non-small cell lung carcinoma (NSCLC), we examined the expression of these antigens and T cell infiltration in tumor tissue, and evaluated their prognostic significance. One hundred fifty-seven patients with NSCLC were studied. Reverse transcription-PCR was performed to evaluate MAGE-A4 and NY-ESO-1 expression. Immunohistochemistry was performed for NY-ESO-1 expression and T cell infiltration into the tumor site. Survival analysis was also performed. MAGE-A4 and NY-ESO-1 were expressed in 40 of 141 (28.4%) and 13 of 157 (8.3%) NSCLC respectively. Both CT antigens were more frequently expressed in squamous cell carcinoma (SCC) than in adenocarcinoma. An inverse correlation was found between MAGE-A4 expression and patient survival in advanced stage cancers. Combined infiltration of both CD4+ and CD8+ T cells into tumor nest predicted better survival. There was no correlation, however, between lymphocyte infiltration and antigen expression in the tumor. MAGE-A4 expression in advanced group and T cell infiltration may provide prognostic information. Lastly, these CT antigens, especially MAGE-A4, may represent potential targets for cancer immunotherapy in patients with NSCLC.

Yuasa, T., K. Okamoto, et al. (2001). "Expression patterns of cancer testis antigens in testicular germ cell tumors and adjacent testicular tissue." *J Urol* **165**(5): 1790-4.

**PURPOSE:** The human cancer-testis antigens (CTAs) are a group of tumor specific antigens recognized by cytotoxic T lymphocytes whose expression occurs in human malignancies as well as in normal testicular tissue. We studied a series of CTA

gene transcripts in testicular germ cell tumors of various histological types to test the hypothesis that the expression of CTA in testicular germ cell tumors reflects developmental stages of tumorigenesis rather than constitutive tumor antigens recognized by cytotoxic T lymphocytes. **MATERIALS AND METHODS:** Total RNA was obtained from 31 primary and 3 metastatic testicular germ cell tumors, and 11 parenchymal tissues adjacent to the testicular germ cell tumors. We performed an expression study of the CTA genes MAGE-A, MAGE-B, GAGE, PAGE-1, HOM-MEL-40 (SSX2), NY-ESO-1, LAGE-1 and SCP-1 in these samples using reverse transcriptase-polymerase chain reaction. **RESULTS:** The results showed that expression patterns of CTA genes depended on the histological differentiation of the testicular germ cell tumors. Overall CTA expression was more common in seminomas than in nonseminomatous germ cell tumors. Specifically all 13 seminomas (100%) demonstrated the positive expression of MAGE-B1 and MAGE-B2, while 3 of 17 nonseminomatous germ cell tumor samples (18%) showed positive expression of these genes. All 5 teratomatous elements (100%) had homogenous null expression with regard to all CTA genes examined. In addition, we detected deficiencies in CTA expression in 7 of 11 parenchymal tissues adjacent to the testicular germ cell tumors (64%). **CONCLUSIONS:** These data support the idea that CTA transcripts in testicular germ cell tumors serve as developmental footprints of testicular germ cell tumors rather than as constitutive tumor antigens recognized by cytotoxic T lymphocytes.

Zendman, A. J., N. J. de Wit, et al. (2001). "Expression profile of genes coding for melanoma differentiation antigens and cancer/testis antigens in metastatic lesions of human cutaneous melanoma." *Melanoma Res* **11**(5): 451-9.

Vaccination-based therapy of melanoma has so far mainly focused on monovalent approaches using either melanoma differentiation antigens or cancer/testis antigens. To study the complementarity of expression from these two families of antigens recognized by T-cells, we screened 47 metastatic lesions of cutaneous melanoma for the expression of three melanoma differentiation antigens and eight cancer/testis antigens using reverse transcription-polymerase chain reaction (RT-PCR). The melanoma differentiation antigens were expressed in a somewhat higher percentage of lesions (94% positive for at least one marker) than the cancer/testis antigens (91% positive for at least one marker). Nearly all the melanoma metastases (98%) expressed at least one of the markers tested. One melanoma metastasis was negative for all the markers. Two out of 47 lesions did

not express any of the three differentiation markers but expressed one or more of the cancer/testis antigens, indicating some additional potential for these antigens compared with the melanoma differentiation antigens. Therefore, we conclude that polyvalent immunotherapy using multiple epitopes from both families of antigens might increase the eligibility of melanoma patients and the efficacy of the treatment.

Zendman, A. J., D. J. Ruiter, et al. (2003). "Cancer/testis-associated genes: identification, expression profile, and putative function." *J Cell Physiol* **194**(3): 272-88.

Cancer/testis-associated genes (CTAs) are a subgroup of tumor antigens with a restricted expression in testis and malignancies. During the last decade, many of these immunotherapy candidate genes have been discovered using various approaches. Most of these genes are localized on the X-chromosome, often as multigene families. Methylation status seems to be the main, but not the only regulator of their specific expression pattern. In testis, CTAs are exclusively present in cells of the germ cell lineage, though there is a lot of variation in the moment of expression during different stages of sperm development. Likewise, there is also a lot of heterogeneity in the expression of CTAs in melanoma samples. Clues regarding functionality of CTAs for many of these proteins point to a role in cell cycle regulation or transcriptional control. Better insights in the function of these genes may shed light on the link between spermatogenesis and tumor growth and could be of use in anti-tumor therapies. This review outlines the CTA family and focuses on their expression and putative function during male germ cell development and melanocytic tumor progression.

Zendman, A. J., A. A. van Kraats, et al. (2002). "Characterization of XAGE-1b, a short major transcript of cancer/testis-associated gene XAGE-1, induced in melanoma metastasis." *Int J Cancer* **97**(2): 195-204.

Suppression subtractive hybridization, comparing mRNA expression profiles of common nevocellular nevi and melanoma metastases, was used to identify potential markers of melanoma progression. From the metastases we isolated XAGE-1b, a 470 bp transcript of the XAGE-1 gene. In general, expression of XAGE-1b was much more prominent than expression of the longer XAGE-1 transcript, isolated from Ewing's sarcoma. The XAGE-1b open-reading frame codes for a putative protein of 81 amino acids, harboring a functional bipartite nuclear localization signal and a C-terminal acidic transcription-activation-like domain. On the

nucleotide level, XAGE-1b has a 50% homology with members of the GAGE family. However, homology between the corresponding proteins is weak. Expression of XAGE-1b in normal tissues was mainly restricted to testis, while placenta and brain were sporadically positive. In human tumor cell lines as well as in human tumor lesions, expression was most frequently found in melanocytic tumors and Ewing's sarcoma. In the different stages of melanocytic tumor progression, expression was exclusively seen in melanoma metastases (38%; n = 61), while all tested common and atypical nevi (n = 10) as well as primary melanomas (n = 8) were negative. Upregulation of expression after treatment with demethylating agent 5-aza-2'-deoxycytidine was detected in 1 of 4 human melanoma cell lines tested. The XAGE-1 gene consists of 4 exons and is located on chromosome Xp11.21-Xp11.22. After transfection into COS cells, the corresponding protein can direct the coupled fluorescent protein to the nucleus, showing a distinct speckled staining aspect. Our data imply the nuclear cancer/testis-associated XAGE-1b to be a marker for late melanocytic tumor progression.

Zendman, A. J., A. A. Van Kraats, et al. (2002). "The XAGE family of cancer/testis-associated genes: alignment and expression profile in normal tissues, melanoma lesions and Ewing's sarcoma." *Int J Cancer* **99**(3): 361-9.

The existence of XAGE genes was first reported after database homology searches for PAGE-like sequences identified 3 XAGE EST clusters. One of these clusters, XAGE-1, has in later studies been identified as a cancer/testis-associated gene. Here, we report the expression profiles of all 3 reported XAGE genes, as well as several splice variants of XAGE-1, in normal human tissues, Ewing's sarcoma and melanocytic tumors. We also provide the genetic structure of the corresponding genes. Moreover, by searching the databases for XAGE homologues, we identified 3 additional GAGE-like genes. RT-PCR studies showed frequent expression in melanoma metastases and Ewing's sarcoma for 2 XAGE-1-derived transcripts. XAGE-2 was expressed at lower frequency in these tissues, while XAGE-3 was seen only in normal placenta. Due to a frameshift, the largest XAGE-1 putative protein is far less homologous to GAGE-like proteins than the other XAGEs. Interestingly, all GAGE-like genes contain a large secondary open reading frame, coding for putative proteins homologues to the XAGE-1 primary protein. The XAGE family of cancer/testis-associated genes is located on chromosome Xp11.21-Xp11.22. The data outline a superfamily of GAGE-like cancer/testis antigens, consisting of at least 19 genes.

Zhang, C., T. Kawakami, et al. (2005). "Distinctive epigenetic phenotype of cancer testis antigen genes among seminomatous and nonseminomatous testicular germ-cell tumors." *Genes Chromosomes Cancer* **43**(1): 104-12.

Testicular germ-cell tumors (TGCTs) are pluripotent and display protean histology from the germ-cell stage until embryonal and somatic-cell differentiation. These properties make TGCT a fascinating model for studying germ-cell development and gametogenesis. Methylation patterns specific to cell type (stem cells, germ cells, and somatic tissues) occur throughout the normal development of mice. To shed light on the epigenetic phenotypes among histological subtypes of TGCTs, we investigated the methylation and expression of several cancer testis antigen (CTA) genes (MAGEA1, MAGEA3, and SYCP1) in TGCTs. In the current study, we showed that the 5' ends of MAGEA1 and MAGEA3 on the X chromosome are unmethylated in seminomatous TGCTs, regardless of whether MAGEA1 and MAGEA3 are expressed and are methylated in nonseminomatous TGCTs when expression is absent. These distinctive epigenetic phenotypes of MAGEA1 and MAGEA3 also were observed in pure seminomas and in the seminomatous elements of mixed-type TGCTs. In contrast, the 5' end of SYCP1, on chromosome 1, remained predominantly unmethylated, regardless of expression, in both seminomatous and nonseminomatous TGCTs. This pattern of transcriptional regulation of SYCP1 is similar to that observed for XIST in TGCTs. On the basis of the epigenetic phenotypes of CTA genes, we concluded that, first, consistent unmethylated DNA profiles in seminomatous TGCTs imply that methylation may not be the primary control mechanism of programmed gene expression in seminomatous TGCTs, and, second, that nonseminomatous TGCTs might be midway between seminomatous TGCTs and somatic tissues because gene expression in nonseminomatous TGCTs is regulated by methylation in some genes (MAGEA1 and MAGEA3) but not others (SYCP1 and XIST).

Zhang, Y., Z. Wang, et al. (2004). "Combined real time PCR and immunohistochemical evaluation of sperm protein 17 as a cancer-testis antigen." *Eur J Haematol* **73**(4): 280-4.

We previously identified sperm protein 17 (Sp17) as a normal testicular protein aberrantly expressed in a proportion of multiple myeloma (MM). However, recent studies have generated controversies on the normal tissue expression of Sp17 and whether or not it is a suitable target for immunotherapy. In this study, we have used a combination of real time polymerase chain reaction and immunohistochemistry

on a large panel of normal tissues. Although Sp17 transcripts could be detected in some normal tissues, the levels of expression were <2% of those in normal testis. In contrast, Sp17+ myeloma cells expressed 3-18% of normal testis levels of Sp17 transcript. Immunohistochemistry using two Sp17 murine monoclonal antibodies, each directed at a non-overlapping B-cell epitope, showed Sp17 protein to be expressed only in testis and not any other normal tissues. Specificity of binding of the antibodies to testis was also confirmed in competitive binding assays. Our results therefore further suggest Sp17 as a cancer-testis antigen in MM and support its suitability as a target for immunotherapy.

Zhao, L., D. C. Mou, et al. (2004). "Expression of cancer-testis antigens in hepatocellular carcinoma." *World J Gastroenterol* **10**(14): 2034-8.

**AIM:** To investigate the expression of cancer-testis (CT) antigens MAGE-1, SSX-1,CTp11 and HCA587 genes in hepatocellular carcinoma (HCC) and the possibility of applying these antigens as targets for specific immunotherapy for HCC. **METHODS:** Expression levels of MAGE-1, SSX-1, CTp11 and HCA587 mRNA were detected with reverse transcription polymerase chain reaction (RT-PCR) in HCC tissues and corresponding adjacent non-cancerous tissues from 105 HCC patients, 40 samples of cirrhosis and normal liver tissues. Genes of five samples with positive PCR results were sequenced. **RESULTS:** Of 105 HCC tissues, MAGE1, SSX-1,CTp11 and HCA587 mRNA expressions were detectable in 75.2%(79/105), 72.4%(76/105), 62.9%(66/105) and 56.2%(59/105) of HCC samples, respectively. About 93.3%(98/105), 72.4%(76/105), 48.6%(51/105) and 37.1%(39/105) of HCC tissues positively expressed at least one, two, three, and four members of CT antigens, respectively. Conversely, only SSX-1 could be detectable in 2.9%(3/105) of the corresponding adjacent non-HCC tissues in which no metastatic lesion was found. Of the latter 3 patients, biopsy samples far from tumor were obtained in 2 patients and RT-PCR indicated no expression of SSX-1 mRNA in these two samples. In addition, none of 40 samples of cirrhotic and normal liver tissues expressed CT antigen gene mRNA. DNA sequences confirmed that the RT-PCR products were true target cDNA. No relationship was found between expression of CT antigens and clinico pathological indicators such as age, gender, tumor size, degree of tumor differentiation, serum alpha-fetoprotein level and infection of hepatitis B virus or hepatitis C virus ( $P>0.05$ ). **CONCLUSION:** CT antigens genes (MAGE-1, SSX-1, CTp11 and HCA587) are expressed with high percentage and specificity in HCC and their products are promising targets for

antigen-specific immunotherapy of HCC. High frequent co-expression of multiple members of CT antigens in HCC provides possibility of polyvalent vaccinations for HCC.

Zhuang, R., Y. Zhu, et al. (2006). "Generation of monoclonal antibodies to cancer/testis (CT) antigen CT10/MAGE-C2." *Cancer Immun* **6**: 7.

CT10/MAGE-C2 is a recently identified antigen that, typically of cancer/testis (CT) antigens, can be found in various malignant tumors and in normal adult testis. As with many other CT antigens, our knowledge is based mainly on mRNA expression data. In the present study, we describe the generation of mAbs to CT10/MAGE-C2 for the analysis of its protein expression. Newly generated clones were chosen based on their reactivity in ELISA, immunoblotting, and immunohistochemistry (IHC). Emphasis was put on the reactivity of newly generated reagents on formalin-fixed, paraffin-embedded tissue to ensure their applicability to archival material. Eventually we selected two clones, LX-CT10.5 and LX-CT10.9, that showed intense reactivity to CT10/MAGE-C2 protein and CT10/MAGE-C2 mRNA-positive cell lines, but no cross-reactivity with other CT antigens. Both mAbs show superior staining characteristics in IHC and are applicable to frozen and paraffin sections. In testis, CT10/MAGE-C2 displays the typical CT pattern with regard to staining of germ cells, which is intense during the early maturation stages. In tumors, we analyzed a limited number of cases displaying the typical heterogeneous CT expression pattern. Interestingly, immunoreactivity was seen solely in the nucleus: No staining was seen in the cytoplasm of tumor cells.

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