Francis Hornicek, MD, PhD

Dr. Francis Hornicek is Chief of the Orthopaedic Oncology Unit at Massachusetts General Hospital and an Associate Professor in the Department of Orthopaedic Surgery at Harvard Medical School. Dr. Hornicek received his M.D. from the University of Pittsburgh School of Medicine and his Ph.D. from Georgetown University School of Medicine.

Mary McMaster, MD

Update on the Epidemiology of Chordoma: SEER Registry Data 1973-2007

Background*:* Because chordoma is rare, it has been difficult to obtain population-based data about it. In 2003, we published the first large descriptive epidemiological study of chordoma, based on data for 400 cases collected over 23 years (1973-1995) by the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute. Following expansion of the SEER registry coverage which, combined with the accumulation of nearly another decade of case reporting and follow-up, resulted in the addition of a substantial number of chordoma cases to its database (total ~800), we presented epidemiological data at the 2007 International Chordoma Workshop. Currently, an additional four years of chordoma data have accumulated in SEER. We have updated our descriptive analysis to reflect these larger numbers (n=1086), which are likely to result in more stable rate estimates.

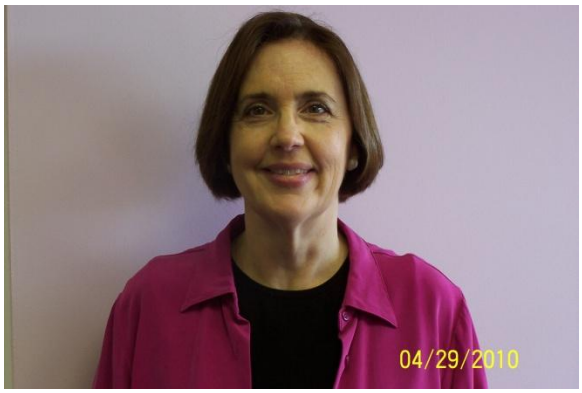
Methods*:* We used data from the 9 original SEER registries, (1973 – 2007) to calculate incidence rates of microscopically-confirmed chordoma and data from 17 registries to derive information regarding distribution of primary site and to calculate survival rates.

Results*:* The age-adjusted chordoma incidence rate (IR) has been stable over the 35-year period at 0.08 per 100,000. Incidence was age-dependent, more common in males (IR 0.11) than females (IR 0.07) and rare among patients < 40 years (IR 0.03) and blacks (IR 0.03). Within the axial skeleton, 40.3% of cases were cranial, 28.7% spinal and 30.9% sacral. Young age ($p<0.001$) and female gender ($p=0.01$) were associated with greater likelihood of cranial presentation. Overall median survival (without stratification by treatment) was 7.9 years; 5- and 10-year relative survival rates were 73.4%and 52.2%, respectively. Median cause-specific survival was 14.1 years. Survival was significantly affected by time period of diagnosis (year of diagnosis pre-1987 v. 1987 and later, $p<0.05$), age at diagnosis (age <70 v. 70+, $p<0.05$), and primary site (skull-based v. sacrum, $p<0.05$), but not by gender.

Conclusions*:* This study expands on data supporting previous estimates of incidence and survival patterns of chordoma in the U.S. Additional epidemiologic studies are required to elucidate the genetic and environmental determinants underlying this rare, distinctive neoplasm.

Notes:

Mary McMaster, MD



Dr. Mary McMaster received her B.A. in English from the University of North Carolina (UNC) and her M.D. from the Wake Forest University Bowman Gray School of Medicine. She then completed training in Internal Medicine and Medical Oncology at Vanderbilt University Medical Center. During her fellowship, she became interested in cancer genetics. She returned to North Carolina for postdoctoral training in cellular biology and genetics. She joined the National Institutes of Health in 1996. She completed a residency in Clinical Medical Genetics with the National Human Genome Research Institute before moving to the National Cancer Institute (NCI) in 1998. At NCI, she has pursued her long-term interest in cancer genetics. She is especially interested in understanding the basis for susceptibility to certain rare cancers, including chordoma. She has worked closely with Dr. Dilys Parry at NCI to investigate the epidemiology of chordoma as a means of understanding trends in chordoma incidence, treatment and survival. She has also worked with Dr. Parry to clinically evaluate many of the chordoma-prone families who have contributed to genetic studies of familial chordoma.

Petur Neilsen, MD

Associate Pathologist
Director of Bone and Soft Tissue Pathology
and Electron Microscopy Unit
Massachusetts General Hospital

Associate Professor of Pathology
Harvard Medical School

Brian Harfe, PhD

The notochord and chordoma

Brian D. Harfe, Ph.D.
University of Florida, College of Medicine and the Genetics Institute, PO Box 103610, Gainesville, Florida 32611 USA

Objective: Understand the role of notochord cells in causing chordoma.

Methods: An unfortunate consequence of aging is the eventual failure of tissues and organs, which leads to pain, loss of mobility and eventually to death. A tissue that commonly deteriorates in older vertebrates are the intervertebral discs (located between the vertebrae). Age-related changes in the intervertebral discs are thought to cause most cases of back pain. Back pain affects more than half of the people over the age of 65 and the treatment of back pain costs 50-100 billion dollars per year in the United States. Our investigation of back pain resulted in the identification of notochordal cells in mice. These are the same cells that in humans have been postulated to form chordoma. We have created a mutant mouse in which most notochord cells do not end up residing within the discs. Instead, in these mutant animals large numbers of notochord cells are scatered througout the vertebral column throughout development.

Summary of the results: Using the *ShhCre* mosue allele we have recently demonstrated that the embryonic notochord forms all cell types found in the adult nucleus pulposus (the middle part of the intervertebral disc). Using this allele we removed Hedgehog signaling from the notochord. In these mutant animals, nuclei pulposi failed to form from the notochord. Instead notochord cells were observed to scatter throughout the vertebral column. Removal of hedgehog signaling from the notochord caused the notochord sheath to disappear, possible causing the notochord to lose its rod-like structure resulting in defects in disc formation. During our notochord fate-mapping experiments in wild type mice we identified a small population of notochord cells that failed to migrate into the intervertebral discs. In humans, this cell population has been postulated to form chordomas.

Conclusion: Using the mouse model system we have begun to characterize what happens to notochord cells that do not end up residing within the nucleus pulposus in both normal and mutant mice.

Sponsoring agencies: NIA/NIH, NIAMS/NIH, Chordoma Foundation and the Liddy Shriver Sarcoma Initiative

Notes:

Brian Harfe, PhD

Dr. Harfe obtained a BS (honours) degree from the University of Glasgow in Glasgow, Scotland and a Ph.D. investigating muscle development in the nematode C. elegans in the laboratory of Dr. Andrew Fire (2006 Nobel Prize winner for his discovery of RNAi) at Johns Hopkins University. After obtaining his Ph.D. he moved to Emory University and began a postdoctoral position in the laboratory of Dr. Sue Jinks-Robertson working on DNA damage pathways in yeast. In 2000, he moved to Boston were he began a second postdoctoral position in the laboratory of Dr. Cliff Tabin at Harvard Medical School working on the molecular pathways responsible for limb formation using the mouse and chick model systems. In 2003, he became an Assistant Professor in the Molecular Genetics and Microbiology Department at the University of Florida (UF) College of Medicine in Gainesville, Florida. Currently, he is an Associate Professor (tenured) in the UF College of Medicine, Director of the Program in Developmental Genetics and a Provost Fellow. Current projects in the Harfe laboratory include investigating limb and intervertebral disc development using the mouse, chick and C. elegans model systems.

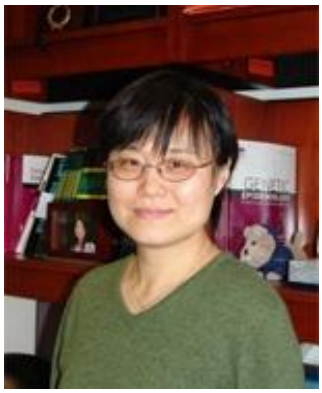
Rose Yang, PhD, MPH

Identification of a major susceptibility gene, T (brachyury), for familial chordoma using combined linkage and array-CGH approaches.

Rare copy number variants (CNVs) with high penetrance that directly cause disease susceptibility have rarely been reported in familial cancer syndromes. Using high-resolution array-CGH, we identified a duplication of a region on 6q27 that showed significant linkage to familial chordoma, but contained no disease-associated mutations of known genes by sequencing, in four multiplex chordoma families (≥3 cases). This region contains only one gene, *T* (Brachyury), which plays an important role in notochord development and is expressed in the majority of sporadic chordomas. Quantitative-PCR confirmed *T* duplication and its co-segregation with chordoma in the four families. The breakpoint junctions were amplified from three of the four families and each revealed similar tandem duplication. These findings suggest that rare CNVs may play an important role in cancer susceptibility. They also highlight the need to include screening for complex genomic rearrangements in searches for disease susceptibility genes.

Notes:

Xiaohong Rose Yang, PhD, MPH



Dr. Yang received a Ph.D. in physiology from the Lombardi Cancer Center, Georgetown University in 1999 and a M.P.H. in epidemiology from Johns Hopkins University School of Public Health in 2003. She joined the Genetic Epidemiology Branch (GEB) in 2000 as a fellow, and became a tenure track investigator in 2006. Her research interests include the genetics of familial cutaneous malignant melanoma/dysplastic nevi syndrome and molecular heterogeneity of breast cancer.

Patrick Tarpey, PhD

Whole Exome Resequencing for the Identification of Somatic Variants in Chordoma.

Patrick Tarpey[1], P Andrew Futreal[1], Peter Campbell[1], Mike Stratton[1], Adrienne Flanagan[2,3,4]

1. Wellcome Trust Sanger Institute, Cambridge, UK,
2. UCL Cancer Institute, 72 Huntley Street, London WC1 6BT, UK
3. Institute of Orthopaedics and Musculoskeletal Science, University College London, Stanmore, Middlesex HA7 4LP, UK
4. Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, Middlesex HA7 4LP, UK

We have used whole exome capture (Agilent 50Mb Exome) and Illumina re-sequencing to screen chordoma tumour, and paired normal DNA, for the presence of somatically acquired variants. In-house algorithms were used to identify substitutions and insertions/deletions, and putative somatic variants were validated by PCR and capillary or 454 sequencing.
Over 21,000 protein coding genes and 1644 miRNA's were enriched, using the solution capture technology, and these genes have yielded 143 validated somatic variants (19 truncating, 92 missense, 32 silent). An additional 153 variants are still undergoing validation (49 truncating, 66 missense, 37 silent, 1 miRNA).

A validated PTEN missense variant was identified in two chordoma tumours, but no other known cancer genes were recurrently mutated. Two novel genes (RELN, ZNF717) had non-synonymous variants in more than one tumour, however more data is required to determine the significance of these findings, and to investigate the presence of additional recurrently mutated genes.
The strategy for detection, validation and analysis of these data will be presented.

Notes:

Patrick Tarpey, PhD

Following my PhD, I qualified as a Clinical Molecular Geneticist in London and Cambridge prior to moving to the Wellcome Trust Sanger Institute in 2002 to pursue a research project on X-linked mental retardation (XLMR).

This project utilized high-throughput capillary sequencing to screen all genes on the X chromosome in a large cohort of males affected with XLMR. This study was the largest investigation conducted on a Mendelian disease, and resulted in the discovery of over 10 new XLMR genes (~10% of all known XLMR genes) in addition to novel genes underlying nystagmus and female-limited epilepsy.

I am now focused on cancer genome research, aiming to identify the somatically acquired variants which contribute to cancer progression. We have used SureSelect exome capture and Illumina sequencing on paired tumour and normal samples, to look for the recurrently mutated genes which may represent novel cancer genes. These investigations recently lead to the identification of the *PBRM1* gene in renal cancer.

Jim Mullikin, PhD

Sequencing and Analysis of the Kinome of Chordoma Tumor/Normal Pairs

Jim Mullikin[1,3], Pedro Cruz[1], Xiaomu Wei[2], Praveen Cherukuri[1], NISC Comparative Sequencing Program[1,3], Eric Green[1,3], Adrienne Flanagan[4], and Yardena Samuels[2]

[1]Genome Technology Branch, [2]Cancer Genetics Branch, [3]NIH Intramural Sequencing Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD and [4]UCL Cancer Institute, University College London, London, England

Protein kinases are encoded by the largest superfamily of conserved genes in the human genome, and represent the largest family of genes implicated in human cancer. Protein tyrosine kinases (PTKs) are frequently mutated in cancer, and since they are amenable to pharmacologic inhibition, further analysis of the PTK gene family may identify new therapeutic strategies. Leveraging off of earlier studies of the PTK gene family, also called the kinome, reagents were already available at the NIH Intramural Sequencing Center (NISC) to sequence the kinome of chordoma tumors and matched normal samples. Using these reagents and available approaches (i.e., polymerase chain reaction amplification of kinome exons followed by Sanger sequencing), we identified somatic mutations specific to 7 of the 21 tumor-normal pairs examined. Across the 86 PTK genes, sequence from the coding exons comprising the kinase domains revealed somatic mutations in the following genes: *AKT2*, *EPHA2, EPHA5, EPHA6, EPHA8, PIK3CA, PTEN, RET*. Follow-up studies are necessary to confirm these mutations along with their potential functional consequence since any of these mutations could be passenger somatic mutations without consequence.

Since this sequencing project started at NISC, technology has changed dramatically, and a new approach should be taken. We propose to use targeted enrichment of all exons of the PTK family followed by next-generation sequencing. This approach has a much lower false-positive rate, and since all exons across these genes will be targeted, we should achieve better sensitivity to the PTK mutational process of chordoma tumorgenesis.

Notes:

Jim Mullikin, PhD

Dr. Jim Mullikin develops and utilizes computer programs to analyze large data sets generated by systematic DNA sequencing projects. A highly skilled computational geneticist, he collaborates extensively with biomedical researchers, developing data analysis methods specifically tailored for each class of project.

His educational background is in Electrical Engineering and Physics, with an emphasis in image processing and pattern recognition. Dr. Mullikin first began to apply these skills to the genomics field in 1997, just as the Human Genome Project was scaling up. He has been involved in many aspects of the Human Genome Project as well as numerous other genome projects. His Sequence Search and Alignment by Hashing Algorithm (SSAHA) greatly sped up the process of SNP discovery, first for The SNP Consortium Project and later in the International HapMap Project. Dr. Mullikin also developed a whole genome assembly program, called Phusion, which most recently assembled the Kalahari Bushman Genome from GS454 next-generation sequence data (Nature, 18 February 2010). Dr. Mullikin is head of the Comparative Genomics Unit within the Genome Technology Branch of NHGRI, and Acting Director of the NIH Intramural Sequencing Center (NISC). His research group provides critical computational support and guidance for a large-scale medical sequencing (LSMS) program based at NISC. Dr. Mullikin works with collaborating investigators to generate preliminary feasibility assessments for their LSMS projects by evaluating the genomic regions that they wish to target, whether it be a specific list of genes or entire genomic intervals. His group then develops an initial design of PCR assays across the regions of interest. If a project is deemed feasible, it is then entered into the NISC sequencing pipeline which, in the end, produces a large number of DNA sequence reads. The reads are then automatically analyzed for the presence of genetic variants. The approach to LSMS is changing rapidly now that next-generation DNA sequencing machines are able to produce sequence 1,000 times less expensive per base than the Sanger-based sequencing instruments. Our most recent LSMS projects capitalize on the strengths of these new sequencing platforms in conjunction with targeted DNA capture methods, especially whole exome capture. Dr. Mullikin's group is also developing new analytical methods to accurately detect genetic variants from data generated by these next-generation instruments.

Stephen Yip, MD, PhD

Whole exome and transcriptome sequencing of chordoma at BC Cancer Agency – A work in progress

Stephen Yip[1], Sohrab Shah[2], Torsten Nielsen[3], David Huntsman[1]
Department of Pathology and Laboratory Medicine, BC Cancer Agency, Vancouver, BC, Canada[1]
BC Cancer Research Centre, BC Cancer Agency, Vancouver, BC, Canada[2]
Department of Pathology and Laboratory Medicine, Vancouver General Hospital, BC, Canada[3]

Chordoma is a tumour marked by dichotomy between histological appearance and biological behaviour. The relatively innocent histology of chordoma is easily recognizable and consists of a lobulated tumour populated by cells with vacuolated cytoplasm, a unique feature of chordoma also known as physaliferous appearance, in a mucinous background. This however doesn't translate to the rather insidious and malignant behaviour of the tumour that is characterized by local infiltration, destruction and is often recalcitrant to treatment.

We have undertaken a systematic approach to examine the genome and epigenome of chordoma using second generation sequencing on the Illumina platform. We have elected to pursue whole transcriptome shotgun sequencing (WTSS) of tumours and exome sequencing of tumour/normal pairs. WTSS, in combination with exome data, provides unique information on gene expression, single nucleotide variants (SNVs), INDELs, gene fusion events as well as information on RNA editing alterations and splicing defects. Frozen tumours and adjacent non-involved skeletal muscles represent the main source of genetic material. In addition, we are also performing deep sequencing, MeDIP/MRE-sequencing on the chordoma cell lines UCH1 and UCH2.

Preliminary WTSS results of a primary tumour show gene expressions similar to what is known previously. In our case we also found significantly elevated expressions of *EGR1, HSP90AA1, PDGFRB, ERBB2*. Gene fusion analysis shows several potential candidates of which one involves *KLF2.* There are 210 candidate SNVs generated after filtering of known dbSNP and 1000 genome variants. The absence of matched normal for this particular case makes verification of somatic changes particularly challenging. We are currently awaiting results from additional matched chordoma/normal pairs to assist in our data analysis. Lastly, we plan to perform high content screening of a kinase inhibitor library on both UCH1 and UCH2 and to correlate drug sensitivity with genomic findings.

Many challenges remain in WTSS of chordoma and the biggest hurdle remains obtaining RNA of adequate quality (RIN >7.0) and quantity (2µg) for WTSS. The poor quality of the extracted RNA thus far likely represents a combination of factors including the intrinsic nature of the tumour (paucicellular, mucinous matrix), technical issues such as prolonged ischemic time and RNA extracting and stabilizing agents, as well as other intangible factors. On the other hand, we are able to obtain adequate DNA from primary tumours for Illumina library construction. We are currently testing alternative harvesting and fixation protocols to enhance the recovery of usable RNA from primary chordoma specimens.

Notes:

Stephen Yip, MD, PhD

I obtained my combined M.D.-Ph.D. degree at University of British Columbia and was accepted into the neurosurgery residency program at Vancouver General Hospital and completed four years of surgical training before switching to the neuropathology program and obtained my FRCPC certification in 2006. Next, I completed two years of Royal College- funded research fellowship in molecular neuro-oncology at the Massachusetts General Hospital under the supervision of David Louis. My research was on the molecular characterization of recurrent glioblastomas specifically somatic mutations in the mismatch repair gene MSH6. I then completed fellowship training in molecular genetics pathology at Harvard Medical School under the supervision of John Iafrate at MGH.

I returned to Vancouver in 2009 and am physically located at BCCA Vancouver Cancer Centre. My clinical duties consist of clinical molecular diagnostics and neuropathology signouts. I am also affiliated with the Centre for Translational and Applied Genomics (CTAG) and am involved in the developments of novel molecular diagnostic assays. CTAG has a variety of advanced molecular diagnostics instruments including Affymetrix array fluidic station, NANOSTRING, access to 454FLX sequencer and soon to acquire an Ion Torrent sequencer.

My research interests are in the genomic and epigenomic profiling of cancers especially primary brain tumours, taking advantage of the local expertise and resources at the Genome Sciences Centre or GSC. Currently I am using 2nd generation sequencing technology to study oligodendroglioma, ependymoma, and chordoma. Ultimately I want to take novel genomic/epigenomic discoveries to the clinic – by developing clinical molecular assays that can be used to better prognosticate patients and to identify those that might respond to novel molecular targeting agents. I am also associated with the development of BrainCare, a local effort to develop multidisciplinary seamless care for brain tumour patients (including chordomas) in British Columbia and also in the establishment of a local neuro-oncology research network which includes the development of a brain tumour tissue bank.
I strongly believe in the integration of molecular genetics with clinical pathology and the rapid translation of cancer genomic discoveries in medicine.

Slim Sassi, PhD

I am currently working on a project to decipher complex biological systems. I will attempt to develop an approach to synergize molecular evolution and molecular biology to understand these systems. This approach will involve computational molecular evolutionary methods, high-throughput genetic screening and other classical molecular biology methods.

Wesley Hsu, MD

Role of Cancer Stem-Like Cells in Chordoma

Chordomas are the most common malignant primary tumor of the osseous spine. The mechanisms underlying the resistance of chordoma to chemotherapy and radiation therapy is unknown. The role of cancer stem-like cells in chordoma pathophysiology has not been defined. Using a novel chordoma cell line that is morphologically identical to chordoma with expression of brachyury, S100 and keratin, we have isolated and characterized chordoma sarcospheres that are self-perpetuating and exhibit higher expression of the functional stem cell marker ALDH1 compared to typical chordoma cells. Furthermore, sarcospheres can be successfully differentiated into neuroepithelial and mesodermal cell types. This data suggests that chordoma contain cancer stem-like cells that may play a role in chordoma pathophysiology.

Notes:

Wesley Hsu, MD

Paola Riva, PhD

Dysregulation of apoptotic pathway/s in chordoma: Search for prognostic markers and study of FAS and FASL involvement in zebrafish models

[1]Luca Ferrari, [2]Anna Pistocchi, [3]Nicola Boari, [3]Pietro Mortini, [2]Franco Cotelli and [1]Paola Riva
[1]Dipartimento di Biologia e Genetica per le Scienze Mediche –University of Milan, Italy
[2]Dipartimento di Biologia Sezione di Citologia e Zoologia–University of Milan, Italy
[3]Dipartimento di Neurochirurgia, San Raffaele Scientific Institute, Vita-Salute University Italy,

Chordoma is a rare malignant bone tumor arising from notochord remnants, characterized by local invasiveness and variable tendency for recurrency. Given the implication of apoptosis in notochord regression, we studied in 32 tumours the expression by RT-PCR of 8 proapoptotic genes mapping in 1p36 region, showing loss of heterozigosity in most chordomas analyzed (83%). TNFRSF8 and TNFRSF14 genes are differently expressed compared to control (nucleus pulposus, NP) in 50% tumors, while DFFA, DFFB, CASP9, TNFRSF1B, TNFRSF14 and TP73 showed occasional different pattern of expression from control. The comparison of the expression profile of each tumour with that of the control didn't reveal any overlapping. As the apoptotic pathway mediated by FAS-FASL was found to be involved in notochord regression, we studied their expression in 34 chordoma and in 3 derived cell lines to verify their possible dysregulation. Since most analyzed samples express the receptor, but not the ligand, a possible implication of FAS/FASL pathway dysfunction during tumorigenesis has been hypothesized. At this purpose we started an *in vivo* study on zebrafish (*Danio rerio*) animal model. We investigated *zfas/zfasl* expression in embryos' total RNA during different developemental stages and observed that *zfas* has maternal and ubiquitous zygotic expression, while *zfasl* has maternal and specific developmental stages expression (2-4, 64-100 cells, 48, 72, 120 hpf, 8, 10, 14 dpf, 6, 9 mm). Notochordal cells were then sorted by FACS, following the injection of a luciferase gene construct activated by a specific physalipherous cell factor, to study z*fas/zfasl* expression in notochord. A preliminary analysis at 24hpf and 48hpf revealed that *zfas* is expressed at both stages, while *zfasl* is expressed in none of them; further stages will be studied to better define z*fas/zfasl* developmental expression profile. We also observed *zfasl* expression in the notochord by *in situ hybridization* at the stage of 9mm larva, when the chondrification process of this structure begins. Further analyses will be performed to determine the expression timing of *zfas* and *zfasl* to guide functional studies aimed at silencing these two genes. Possible appearance of aberrant phenotypes during notochord regression will be studied and it will be verified whether the reactivation of *zfas/zfasl* pathway might rescue normal phenotype.

Notes:

Claudia Palena, PhD

Brachyury as a target for T-cell mediated immunotherapy

The goal of cancer vaccine approaches is to induce a long-lasting, tumor-specific T-cell mediated immune response in the patient that ultimately will reduce tumor burden. A common cancer vaccine modality consists of the active immunization of the patient against a specific molecule, designated as tumor antigen, that is either exclusively expressed in the tumor cells or that is over-expressed in cancerous versus normal tissues. We have identified the T-box transcription factor, Brachyury, as a novel human tumor antigen highly expressed in various human tumors of epithelial origin, including lung, breast, colon, ovarian, and prostate, but not in most human normal adult tissues. Our results also demonstrated that Brachyury is a regulator of the epithelial-to-mesenchymal transition (EMT) in epithelial tumor cells, a process of relevance for the initiation of metastasis of solid carcinomas. We have also demonstrated the suitability of Brachyury as a target for T-cell mediated immunotherapy of cancer by identifying a CD8 T-cell epitope of Brachyury capable of expanding Brachyury-specific T cells from the peripheral blood of cancer patients. The reports by others of high expression of Brachyury in chordoma tumors makes it a potentially attractive candidate target for T-cell mediated anti-tumor therapy for chordoma. To this end, we are currently evaluating the ability of Brachyury-specific T cells generated in our laboratory to lyse chordoma cells in vitro. We plan to focus our research on the development of Brachyury-based vaccine platforms.

Notes:

Claudia Palena, PhD

Dr. Palena is a Staff Scientist and Head of the Immunoregulation Group in the Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda. Dr. Palena received her Ph.D. in Biochemistry from the National University of Rosario, Argentina, and completed a Postdoctoral Fellowship in the Laboratory of Tumor Immunology and Biology, NCI. Dr. Palena has made significant contributions to the field of cancer immunotherapy, including the identification and characterization of novel tumor-associated antigens, and the use of costimulation for optimal activation of human T-cell responses to tumor antigens. Dr. Palena's current research is focus on the development of novel immunotherapeutic approaches aimed at targeting critical events in tumor progression with the ultimate goal of designing vaccine(s) platform(s) and combinatorial therapies for the prevention and/or treatment of metastases in human cancer.

Adrienne Flanagan, MD FRCPath PhD

In vivo and In vitro Implication of the Transcription Factor T (Brachyury) in pathogenesis of Sporadic Chordomas

Chordomas are rare malignant bone tumours that express brachyury, a gene rarely expressed in other tumours and crucial for notochord development. In a recent study of familial chordomas *brachyury* duplication was found to confer a major susceptibility in four of seven studied families. **AIM:** to investigate whether *brachyury* copy number was increased in sporadic chordomas and to study the effect of *brachyury* silencing on U-CH1 cells, a bona fide chordoma-derived cell line with which we have established a xenograft showing typical chordoma morphology and immunopehotype. **METHODS:** A combination of quantitative-PCR, FISH and array-CGH were used to investigate a series of 156 chordomas (sacro-coccygeal (n=86), skull-based (n=58) and mobile spine (n=12). A lentiviral vector expressing *brachyury*-targeting shRNA was used to knockdown *brachyury* in U-CH1 cells. **RESULTS:** 72 of 156 chordomas (46%) analysed by at least two technologies in most cases, revealed chromosomal abnormalities involving *brachyury* locus, none of which were germline. These included amplification (7/156, 4.5%, with one case showing further copy number gain on recurrence), chromosome 6 aneuploidy (49/153, 32%), and *brachyury* locus imbalance (15/138, 11%). One case showed aneuploidy in addition to insertion of the *brachyury* locus into chromosome 2q. The knockdown of *brachyury* revealed a marked decrease in cell proliferation and striking morphologic changes consistent with premature senescence.

Conclusion: Genetic abnormalities involving *brachyury* are common in sporadic chordomas and these along with our *in vivo* and *in vitro* data provide strong evidence that brachyury is crucial in the pathogenesis of this disease.

Notes:

Adrienne Flanagan, MD FRCPath PhD

I am a pathologist with a specialist in bone and soft tissue tumours. A few years ago with my team I identified that brachcyury was highly sensitive and specific for the diagnosis of chordoma. Since then we have identified that chordomas (brachyury and CK19+ tumours) also occur in soft tissue and in skeletal extra-axial sites. We are: 1. studying what effect brachyury mediates on mesenchymal stem cells 2. studying how to maintain chordoma cell lines and 3. attempting to identify if there are biomarkers for clinical progression.

David Alcorta, PhD

Bioinformatics analysis of Brachyury gene expression

David A. Alcorta[1], Smita Patel[2], Sufeng Li[1], and Michael J. Kelley[1]

[1] Dept of Medicine, and [2] Undergraduate Studies, Duke University, Durham, NC.

Chordoma is a rare cancer that grows in and on the bone processes of the axial spine from the pelvis to the base of the skull. The etiology of the cancer is unknown but phenotypic and protein expression studies suggest the cancer cells are similar to developmental precursors involved in the formation of the spine. Increased germline gene copy number of the transcription factor, Brachyury, has been shown to be associated with increased risk of chordoma development in some multiplex chordoma families. Brachyury protein is expressed mid-embyonic development and is required for the formation of the spine. To assess putative regulators and targets of Brachyury, we examined correlations of expression in 11827 Affymetrix® gene expression microarrays from different array platforms (Hg-U133 Plus 2.0 / Hg-U133 A / Hg-U133 A-2) presenting datasets covering a wide range of cells, cancers and tissues. The raw data from each array was analyzed using Affymetrix®'s MAS5 statistical algorithms which determines the absolute level of expression for each gene and the statistical certainty of its expression across all the probe pairs as designated by a present (P), absent (A) or maybe (M) call. Three hundred and eighty-four arrays expressed Brachyury as noted by a P call. To avoid platform variability, analysis was performed on only the 330 samples derived from the 5991 Hg-U133 plus 2 arrays. Absolute Brachyury expression levels within these 330 samples ranged from 46 to 15,964, with a median value of 214. Pearson correlation analysis comparing the Brachyury levels to levels of all genes on the array was performed to identified genes that showed similar up, directly correlated, expression or inverse, indirectly correlated, expression relative to Brachyury levels. Pearson correlation coefficients (R) for these comparisons ranged from 0.882 to -0.270 with 34 unique genes having $R>0.7$ ($p<10^{-45}$). The three top correlated genes are Mas-related G-coupled protein receptor (R=0.882), aggrecan I (R=0.872) and sushi domain containing 5 (R=0.871). No inversely correlated genes were found with statistical significance, consistent with the known transcriptional activation function of Brachyury. Gene ontology analysis of the top 181 probe pairs ($R>0.5$ and $p<2x10^{-22}$) revealed these genes were involved in vascular development, angiogenesis and cartilage/bone neoplasia. In vitro experimental confirmation of the role of correlated genes using Brachyury expression and suppression vectors in transformed and untransformed cells, including two chordoma lines, is underway.

Notes:

David Alcorta, PhD

I am a scientist that uses cell and molecular approaches to understanding mechanisms of cancer.

JianJian Zhu, MD, PhD

Role of Brachyury in determining cell fate of notochord progenitors

Jianjian Zhu, Susan Mackem
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Brachyury/T is the founding member of the T-box family of transcription factors and plays an essential role in primary mesoderm formation in the primitive streak, and is also expressed in the notochord. Brachyury/T expression is also found in chordomas, a type of adult notochord tumors, and has been proposed to play a role in their genesis. Understanding the normal function of Brachyury/T during notochord development will be important for gaining insight into its role in the pathogenesis of chordomas. Brachyury/T mutant mice have axial truncations below the forelimb level and die by E10.5 due to lack of allantois formation. To selectively evaluate the role of Brachyury/T in mouse notochord formation and function, as well as potential roles at other sites in later development, we used an in vivo conditional short-hairpin RNA (shRNA) approach to knock-down Brachyury expression at selective times and in specific tissues. In this conditional transgene, Cre-mediated recombination activates a floxed promoter driving shRNA expression (developed by Tyler Jacks, PNAS 2004). A number of Brachyury/T shRNAs were designed and their efficiency tested in tissue culture to identify shRNAs that decreased Brachyury/T RNA by >90%. The T-shRNA approach was validated in vivo in transgenic mice using a Cre line expressed throughout early epiblast (Adeno EIIaCre). In embryos with half the normal Brachyury gene dose (T+/-), activation of T-shRNA expression recapitulates the germline null (T-/-) phenotype. In preliminary experiments, we have used ShhCre (expressed in notochord but not primitive streak), to knock-down Brachyury selectively in notochord of wildtype (T+/+) embryos. The knock-down embryos have a markedly attenuated tailbud and subsequently the axial sacral and tail skeleton fail to form. In addition, the entire thoracic and lumbar vertebral column is malformed and attenuated, indicating a loss of normal axial signaling to somitic mesoderm. In situ hybridization with notochord markers (Shh and Noto) reveals that the notochord is disrupted in mutant embryos below the forelimb level by E10.5. Genetic lineage tracing of notochordal progenitor cells using a RosaLacZ reporter suggests that notochord progenitors can survive but change fate, producing epithelial tubular structures morphologically suggestive of neural tube. Further molecular analysis of the phenotype is ongoing. These studies indicate that Brachyury/T function is important in specifying and maintaining notochordal fate, but may not be essential for cell survival of notochord progenitors.

Notes:

Jianjian Zhu, MD, PhD

Education: 1998-2001 Ph.D. in Life Science, Peking University Health Science Center, P.R.China, Thesis advisor: Prof. Jian Tang and Guoying Zhu
1991-1998 M.D. and B.S. joint program, Shandong Medical University, P.R.China (1991-1993 Department of Biology, Shandong University, P.R. China)

Professional Experience: 2011- Staff Scientist, Mackem Laboratory, Regulation of Vertebrate Morphogenesis Section, Cancer and Developmental Biology Lab./Lab. of Pathology, CCR, NCI, NIH. 2002-2010 Postdoctoral and Research Fellow, Mackem Laboratory, Regulation of Vertebrate Morphogenesis Section, Cancer and Developmental Biology Lab./Lab. of Pathology, CCR, NCI, NIH. 2001-2002 Postdoctoral Fellow, Prof. Jian Tang Laboratory, Institute of Cardiovascular Science, Peking University Health Science Center, China.

Awards: Apr., 2009 Poster Presentation Winner, NCI-Frederick 2009 Spring Research Festival
Aug., 2006 Fellows Award for Research Excellence (FARE) 2007 Award, National Institutes of Health (competitive award given to NIH fellows for excellence in basic or clinical research accomplishments)
May, 2000 Guanghua scholarship, Peking University Health Science Center, China (competitive award given to graduate students for excellence in academic and research accomplishments)

Professional Societies and Honors: Full Member of Sigma Xi (election based on original, noteworthy scientific achievement), Active Member of American Association for Cancer Research (nominated by two senior scientists), Society for Developmental Biology (SDB), International Union Against Cancer (UICC)'s Association of UICC Fellows (AUF)

Professional Activities: Independently solicited reviewer: Developmental Dynamics, Mechanisms of Development
Ad hoc poster reviewer for Annual Biomedical Research Conference for Minority Students (ABRCMS) sponsored by American Society for Microbiology in 2007 through 2010
Chief Judge for FARE 2011 Award Competition (Gene Therapy study section)
Lead Judge for Graduate Student Award in the 7th Annual Graduate Student Research Symposium at NIH, 2011
Judge for Graduate Student Award in the 5th Annual Graduate Student Research Symposium at NIH, 2008

Fiona Wardle, PhD

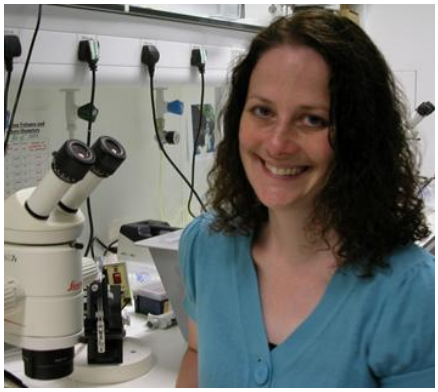
Brachyury in embryonic notochord formation and cell migration – insights into chordoma formation and cancer metastasis?

Fiona Wardle, Randall Division of Cell and Molecular Biophysics, New Hunt's House, King's College London, Guy's Campus, London, SE1 1UL.

The T-box transcription factor, Brachyury, plays several important roles during embryonic development. It is required amongst other things for differentiation of the notochord and for the morphogenetic movements of cells during gastrulation. Interestingly, given these embryonic roles, it is also involved in chordoma development and in the epithelial-mesenchyme transition and migratory cell behaviour of epithelial carcinoma cells. Thus understanding its role in notochord formation and cell migration during embryonic development has potential to shed light on its role during formation of chordoma and metastasis of cancer cells. We have recently identified putative transcriptional targets of Brachyury in different embryonic and chordoma cell types by chromatin immunopreciptation and expression studies. In this talk I will present this data and discuss it in terms of cancer development.

Notes:

Fiona Wardle, PhD



Brachyury in embryonic notochord formation and cell migration – insights into chordoma formation and cancer metastasis?

Fiona Wardle currently holds an MRC Career Development Award and a Lister Institute Fellowship at the Randall Division, King's College London, where she studies transcriptional regulation of early embryonic development. Fiona graduated with a BA (Hons) in Natural Sciences from Cambridge University, then moved to University College London to study for a PhD in Developmental Biology. Fiona gained her PhD in 1998 and in early 1999 moved to the Whitehead Institute, MIT, Cambridge, USA for postdoctoral training. She was awarded a Sokol Fellowship in 2001. In 2002 Fiona moved back to Cambridge University to the Gurdon Institute where she continued to study early embryonic development. During this time Fiona also initiated a project to investigate the transcriptional networks that pattern the early embryo and established the technique of chromatin immunoprecipitation combined with genomic microarrays (ChIP-chip) in zebrafish embryos. In 2007 Fiona moved to the Department of Physiology, Development and Neuroscience at Cambridge University to establish her own lab, before moving the lab to the Randall Division at King's College London in 2010.

David Loeb, MD, PhD

A Primary Chordoma Xenograft

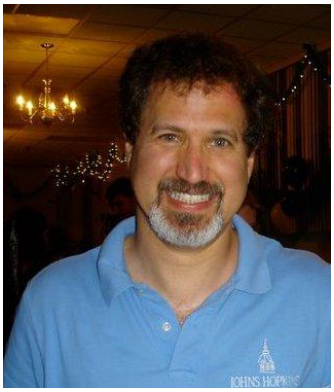
David M. Loeb, MD, PhD, Ola Awad, PhD, Preeti Shah, PhD, Naheed Gul, Paul Meltzer, MD, PhD, and Varalakshmi Katuri, PhD

Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD Center for Cancer Research, National Cancer Institute, Bethesda, MD

We report the establishment and characterization of the first primary chordoma xenograft. A tumor fragment was obtained from a patient diagnosed with conventional chordoma. Eighteen months later, the patient had a metastatic relapse, and biopsy showed dedifferentiated chordoma. A single cell suspension was prepared from the initial tumor sample, and cells were injected into NOD/SCID/interleukin 2 receptor gamma null (NOG/SCID) mice in the parasacral region. After several months a tumor grew. The tumor has undergone serial passages in NOG/SCID mice, at least 10 times. T brachyury expression was demonstrated by RT-PCR and western blotting. Immunohistochemistry confirmed T brachyury expression, but interestingly, staining was cytoplasmic, not nuclear. Molecular characterization using cDNA and comparative genome hybridization arrays was performed. A cell line was established, and cytoplasmic T brachyury expression was confirmed in the cell line as well. We isolated cells with high level aldehyde dehydrogenase (ALDH) expression from the cell line. ALDH has been shown to be a marker of stem cells in other tumor types. ALDHhigh cells were enriched for sphere forming activity compared with the ALDHlow population. Thus, from this patient's tumor, we were able to establish both a primary xenograft and a cell line, both of which express T brachyury, as expected of a chordoma. We have begun a molecular characterization of these cellular reagents and initiated studies aimed at identifying and characterizing chordoma stem cells. These tools will be invaluable in the development of novel therapies for this difficult to treat tumor.

Notes:

David Loeb, MD, PhD



Dr. Loeb received his undergraduate degree in biology from the Johns Hopkins University. He received an MD and a PhD from Columbia University College of Physicians and Surgeons, and returned to Baltimore for his pediatrics residency at Johns Hopkins. Following his residency, Dr. Loeb completed a fellowship in Pediatric Hematology/Oncology at Johns Hopkins and then joined the faculty there in 2000. His laboratory interests include studying the function and regulation of WT1 and identifying and targeting sarcoma stem cells. He is also the institutional PI for SARC (the Sarcoma Alliance for Research through Collaboration). He is also the recipient of a research grant from the Chordoma Foundation (which helped fund the work being presented at this conference) and was the proud recipient of the Justin Straus Chordoma Research Award in 2009.

Gary Gallia, MD

Dr. Gallia is an Assistant Professor of Neurosurgery and Oncology and the Director of Endoscopic and Minimally Invasive Neurosurgery at Johns Hopkins. Dr. Gallia's specialty is endoscopic endonasal surgical approaches to skull base pathologies with a focus on neurosurgical oncology. He utilizes the latest techniques in preoperative imaging, computer guided surgical navigation, intraoperative monitoring and minimally invasive and neuroendoscopic approaches in the management of patients with benign and malignant brain tumors, metastatic tumors to the brain, skull base neoplasms and pituitary tumors.

Dr. Gallia's primary research interests are in the development of novel therapeutics against malignant brain and skull base tumors, outcomes following endonasal endoscopic skull base surgery and development of next generation intraoperative endoscopic platforms.

Dr. Gallia graduated summa cum laude from the Gibbons Scholar MD/PhD program at Jefferson Medical College and Thomas Jefferson University. He completed his general surgery internship at Johns Hopkins Hospital where he was awarded surgical intern of the year. He then completed his neurological surgery residency and a postdoctoral fellowship in neuro-oncology at Johns Hopkins Hospital. Following residency, he completed a minimally invasive and endoscopic neurosurgery fellowship with Dr. Charles Teo at the Prince of Wales Private Hospital and Sydney Children's Hospital in Sydney, Australia.

Fabio Bozzi, PhD

Preclinical models for chordoma

Fabio Bozzi°, Giacomo Manenti*, Eva Tarantino°, Tiziana Negri°, Marta Orsenigo°, Marco A. Pierotti[#], Silvana Pilotti° and Elena Tamborini°.

°Laboratory of Experimental Molecular Pathology, Department of Pathology; * Department of Experimental Oncology; [#]Scientific Directorate Fondazione IRCCS, Istituto Nazionale Tumori, Milan, Via G. Venezian 1, 20133, Milano, Italy.

We have shown that chordomas express activated PDGFRB sustained by an autocrine/paracrine loop and, more recently, that EGFR may also be activated by the same mechanism, reinforced by a cross-talk between the two tyrosine kinase receptors (RTK). At downstream-signalling pathway, mTOR is activated too. In line with this, tumour responses have been observed in patients treated with imatinib, and imatinib combined with sirolimus. Taken together, our findings provided a rationale for treatments targeting chordoma-activated kinases and supported the idea that a combination of upstream antagonists (such as RTKI) and mTOR inhibitors may enhance the control of tumour growth.

However, pre-clinical models as murine xenografts, primary or stabilized chordoma cell lines, aimed to the evaluation of drugs effectiveness for chordoma treatment have not been completely elucidated. Thereby, we explored the usefulness of our chordoma xenografts and primary cell cultures as a preclinical model for chordoma treatment.

We established murine xenografts by subcutaneously implanting 7 surgical human chordoma samples into CD1 nu/nu mices. One tumor are still growing (BMA) and three grew for at least two passages. Tumors appearance was variable ranging from 2 to 4 months after transplantation. The latency time decreased after the first passage slightly, still remaining rather variable. The tumors grew very slowly reaching dimensions useful for transplantation in 4 to 8 months. After each passage, the histology and the biochemical features of the regularly growing tumors were very similar to those observed in the surgical specimens taken from patients.

Since the established chordoma xenograft (BMA) was characterized by the expression/activation of EGFR, a preliminary experiment for evaluating the chordoma response to lapatinib was performed. No evidence of pathological response, evaluated as chordoma cell depletion, was observed after the treatment even if biochemical analyses showed a reduction in EGFR activation.

We also evaluated the use of short term primary chordoma cell lines. To this end, the cells obtained from chordoma surgical specimens were seeded in complete standard medium. Five primary cell cultures were obtained. They can be propagated only for one or two passages because in all cases, chordoma cells (recognized by their expression of cytokeratin CAM 5.2 by flow cytometry) were subsequently substituted possibly by mesenchymal cells (defined by the absence of cytokeratin CAM 5.2 coupled with CD90, CD44, and CD73 expression). One of the primary cell lines was treated by metformin and, interestingly, a growth arrest of both chordoma and mesenchymal cells could be evidenced.

Conclusions: The obtained chordoma murine xenografts and primary cell cultures, mimicking the biological features of the corresponding human tumors, represent suitable models for evaluating alternative pharmacological treatments.

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Fabio Bozzi, PhD

Education:
1994: Degree in Biological Sciences at University of Milan (110/110)
2002: Master in chemistry and biochemistry at University of Milan (70/70 cum laude).

Career:
Researcher, division of pathology, Istituto Nazionale Tumori. Milan, Italy.
2001-2005: Researcher, department of medical oncology, Istituto Nazionale Tumori, Milan, Italy.
1999-2000: Researcher, department of pathology, Ospedale Niguarda, Milan Italy.
1995-1999: Researcher, Department of human genome and multifactorial disesae (CNR), Segrate, Italy.

Professional skills and major research interests:
Development of pre-clinical model for human sarcoma treatment, Development of phosflow protocols for the detection of the activation status of receptor tyrosine kinases in solid tumors, Normal haematopoietic stem cell (CD34+) and sarcoma cancer stem cells isolation and characterization, Genetic and biochemical study of receptor tyrosine kinases activation in soft tissue tumors, Flow cytometry and real time technologies for diagnosis and minimal residual disease assessment for lymphoma / leukaemia, neuroblastoma, Ewing family tumours and rhabdomiosarcoma, Genetic study of gene involved in paediatric severe combined immunodeficiency; SCID T- B+, T- B- and Omenn syndrome.

Silke Bruederlein, PhD

Establishment of chordoma cell lines

Peter Möller, Silke Brüderlein
University of Ulm, Institute of Pathology, Germany

In principle, every tumor cell that is capable of infinite growth in vivo should grow in vitro too. As everyone knows, this is not the case. In most cases the reasons are unknown. But in some cases it could just be the lack of knowledge to treat the tumor tissue to establish a cell line. In this context chordoma tumors do not pose any difficulty. In most, if not all, cases these tumor cells are growing in primary cultures. The major difficulty is that they are extremely slow-growing. The next problem is to get rid of the normal cells which are mostly faster growing than the tumor cells. Here, we show how to establish a chordoma cell line.

Notes:

Silke Bruderlein, PhD

Establishment of Chordoma Cell Lines

I studied Biology in Erlangen, Germany with a focus in cellular biology and tumor cytogenetics. I am working in the Institute of Pathology in Ulm, Germany, and I spend a significant amount of time establishing cell lines out of rare tumors.

Beate Rinner, PhD

Establishment and detailed characterisation of a novel sacral chordoma cell line: MUG-Chor1

Beate Rinner[1], Elke Verena Froehlich[2], Karin Buerger[1], Birgit Lohberger[2], Susanne Scheipl[2], Carina Fischer[1], Andreas Leithner[2], Christian Guelly[1], Slave Trajanoski[1], Karoly Szuhai[3], Bernadette Liegl-Atzwanger[4]

[1]Center for Medical Research, Medical University of Graz, Austria
[2]Department of Orthopaedic Surgery, Medical University of Graz, Austria
[3]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, the Netherlands
[4]Institute of Pathology, Medical University of Graz, Austria

Background: Chordomas are rare, low to intermediate grade malignant bone tumors of the axial skeleton. Although chordomas rarely metastasize, they show a locally destructive growth pattern, and are often not diagnosed until they have reached an advanced stage of disease. Treatment options are limited, as chordomas have been proven to be largely resistant to conventional ionizing radiation and chemotherapy. Therefore new treatment options are urgently required. Chordoma cell lines are exceedingly rare, due to the slow growth of the tumor cells under in vitro conditions. This fact has prompted us to establish a chordoma cell line for further in vitro studies.

Material and Methods: Our chordoma cell line was established from a recurrence of a sacrococcygeal chordoma of a 58 year old Caucasian female. Various culture media were tested to determine the best growth conditions. Fibroblasts were separated from the tumor tissue. The cell line has been characterized by immunohistochemistry, western blot, karyotyping using cobra fish technology and Affymetrx SNP 6.0 array. Furthermore the MMP expressions during cultivation period were detected by luminex technology and confirmed by RT-PCR.

Results: The tumor was lobulated and tumor cells were arranged in cords and sheets within an abundant myxoid matrix. In addition classic physaliphorous cells were present. The cell line expressed brachyury, S-100, Pan-Cytokeratin, CK18 and EMA by immunohistochemistry and showed a peridiploid chromosome contend and chromosomal aberrations. The CNV and LOH kinetics becomes more homogeneous during in-vitro cultivation. High MMP2 expression was analyzed.

Conclusion: Through screening of different culturing approaches we have been able to determine optimal conditions for the establishment of a chordoma cell line. Creating chordoma cell lines will have a fundamental impact in the development of new treatment options and better understanding of the molecular basis of this disease.

Clinical Relevance: MUG-Chor1 cell line and the isolated fibroblasts may serve for further studies of cell biology, cell function, and molecular genetics of tumor development and furthermore, as a model for the development of anticancer agents.

Notes:

Beate Rinner, PhD

Our mainfocus is the culturing and establishment of new chordoma cell lines. Another focus in or research is the culturing and investigation of surrounding fibroblasts. We want to establish further chordoma cell lines to test new chemotherapeutic and plant agents. We want to explore the pronounced vacuoles and vesicles in our cell line, through electron microscopy and the isolation of the vesicles. We are specialist in cell culture and flow cytometry and very intersted in any collaboration.

Christopher Austin, MD

Christopher Austin is Director of the NIH Chemical Genomics Center (NCGC), and Senior Advisor to the Director for Translational Research at the National Human Genome Research Institute (NHGRI). The NCGC, part of the NIH Roadmap Molecular Libraries initiative, develops small molecule probes for biological functions and new paradigms for high-throughput screening, chemistry, and cheminformatics. In his role as Senior Advisor for Translational Research, he initiated the Knockout Mouse Project, which is producing knockout mice for all mouse genes, and an in-depth transcriptome map of the mouse. Dr. Austin came to NIH from Merck, where he directed research programs genomics-based target discovery, pharmacogenomics, and DNA microarray technologies, with a focus on neuropsychiatric diseases. Dr. Austin received his A.B. from Princeton and M.D. from Harvard. He did clinical training in neurology at Massachusetts General Hospital, followed by a fellowship in genetics at Harvard.

Joseph H. Schwab, MD, MS

Dr. Schwab is a board certified orthopedic surgeon who received his residency training from the Mayo Clinic where he was awarded the P.J. Kelly award for outstanding basic science research. He has sub-specialty fellowship training in spine surgery from The Hospital for Special Surgery and orthopaedic oncology from Memorial Sloan Kettering Cancer Center.
Dr. Schwab earned a BA from Miami University in Oxford, Ohio majoring in Religion. He earned his MD from Chicago Medical School where he was a member of the Alpha Omega Alpha honor society, as well as a Master's degree in Clinical Pathology.

Dr. Schwab recently earned his second Master's degree from Harvard/MIT School of Health sciences and Technology as part of the Clinical Investigator Training Program (CITP). The program is designed to train young investigators in the science of translational research and clinical trials.

Dr. Schwab has an active clinical and research interest in chordomas. As part of the sarcoma service at Massachusetts General Hospital he works closely with his colleagues in orthopedic oncology, medical oncology and radiation oncology in the management of chordomas involving the sacrum and mobile spine. His research has focused on targeting Chondroitin Sulfate Proteoglycan 4 (CSPG4) in chordomas.

Soldano Ferrone, MD, PhD

Grp94-specific fully human monoclonal antibody-based immunotherapy

Soldano Ferrone , University of Pittsburgh Cancer Institute, Pittsburgh, PA

The growing evidence that tumor antigen (TA)-specific monoclonal antibodies are effective in the treatment of some hematological malignancies and solid tumors has stimulated interest in the development of TA-specific mAb-based immunotherapy for the treatment of chordoma. As reported at this meeting by Dr. J. Schwab, experiments are in progress to target Chondroitin sulphate protidoglycan 4 which is expressed in about 50% of chordoma lesions. In parallel we are investigating the usefulness of glucose regulated protein of 94kDa (Grp94) as a target of mAb-based immunotherapy. This molecule, which is the endoplasmic reticulum paralog of HSP90, is receiving significant attention as an emerging therapeutic target, since it binds a wide variety of signal transduction pathway components relevant to cell proliferation and survival. To this end we have developed a fully human mAb which has the unique specificity to recognize an extracellular epitope of Grp94. In this presentation I will describe the specificity of this mAb and its functional properties. In addition I will discuss the potential immunotherapeutic applications of this mAb.

Notes:

Soldano Ferrone, MD, PhD

Degree in medicine in 1964 from the University of Milan.
Has held faculty positions at several academic institutions.
Since 2007 he is Professor at the University

Silvia Stacchiotti, MD

Latest advances in targeted chemotherapy for chordomas

Chordoma is a very rare tumor, characterized by slow growth over several years. Surgery is the standard treatment but the sites where chordoma originates make treatment of primary disease challenging. Local relapse affects >50% of cases, with a minority of patients curable by further surgery. Furthermore, metastases occur in at least 20% of patients. High-dose radiotherapy may be indicated as alternative to surgery. However, systemic therapy is needed in patients non amenable of surgery and/or RT. Conventional cytotoxic chemotherapy is almost inactive. The recent molecular evidence of several RTKs activation in the disease provided a scientific rationale to target this pathways therapeutically. In fact, imatinib as single agent or in combination with mTOR inhibitors such as sirolimus were preliminarily shown to be active in chordoma. Even EGFR inhibitors activity was detected. We report on the final analysis of the Phase II study on imatinib in advanced chordoma, and on the two Phase II studies currently ongoing in advanced chordoma in Italy. The first one on lapatinib, a dual EGFR and Her2/neu inhibitor, the second one on imatinib plus everolimus.

Notes:

Silvia Stacchiotti, MD

Silvia Stacchiotti, MD, medical oncologist, works in the Adult Sarcoma Medical Oncology Unit, Istituto Nazionale Tumori, Milan, Italy, directed by dr Paolo G. Casali. Her clinical and research activities focus on rare tumors, especially adult sarcomas, including gastrointestinal stromal tumors (GIST), and uncommon histotypes such as chordoma, alveolar soft part sarcoma and PEComa. She is a member of the Italian Sarcoma Group, a national cooperative group for clinical and translational research on soft tissue and bone sarcomas, and is a member of the EORTC Soft Tissue & Bone Sarcoma Group. She collaborates to the Italian Network on Rare Tumors, a collaborative effort among Italian cancer centers, which tries to exploit distant patient sharing in order to improve quality of care and diminish health migration for rare solid cancers. She is a member of ESMO (European Society for Medical Oncology), Connective Tissue Oncology Society (CTOS) and of ASCO (America Society of Medical Oncology). She has authored more than 30 scientific publications on sarcoma.

Deric Park, MD

Translating chordoma in vitro growth to development of targeted therapies

Deric Minwoo Park joined the faculty of the Department of Neurological Surgery at the University of Virginia in April of 2010. He is a board certified neurologist with subspecialty training in clinical neuro-oncology.

Dr. Park received his medical degree from Loma Linda University and completed neurology residency at the University of Chicago, where he was selected by the faculty to serve as Chief Resident. He then trained in clinical neuro-oncology and performed research on paraneoplastic neurologic syndromes at the Memorial Sloan-Kettering Cancer Center with Dr. Jerome B. Posner. This was followed by five years as a Research Fellow at the National Institutes of Health.

Dr. Park provides medical care for patients with brain tumors and is the principal investigator of the Laboratory of Brain Tumor Biology in the Department of Neurological Surgery. He is a member of American Association for Cancer Research, American Society of Clinical Oncology, American Academy of Neurology, International Society for Stem Cell Research, Society for Neuro-Oncology, Society for Neuroscience, and the Scientific Advisory Board of the Chordoma Foundation.

Scott Schuetze, MD, PhD

Results of a phase II trial of dasatinib, a SRC kinase inhibitor, in patients with chordoma.

Background: Chordomas express tyrosine kinases that are targets of the drug dasatinib. Dasatinib is an oral, small molecule inhibitor of SRC, FAK and PDGFR among others and is approved for the treatment of chronic myelogenous leukemia. The Sarcoma Alliance for Research through Collaboration (SARC) conducted a phase II study of dasatinib in patients with advanced sarcoma. Patients with unresectable, recurrent or metastatic chordoma were included, and results of the subset of patients with chordoma are presented.

Trial design: To be eligible for the study, measurable chordoma by CT or MRI must have been present, and patients must not have had significant heart disease or abnormality, not been using blood thinners or anti-platelet drugs and had an ECOG performance score of $\leq$ 2. Patients were treated with 70 mg of dasatinib twice daily. Reduction in dose was allowed if significant side effects developed. Chordoma response was assessed by imaging every 2 months. The primary endpoint was lack of tumor growth after 6 months of treatment. Secondary endpoints included patient safety, progression-free survival and patient survival.

Results: Thirty-four patients with chordoma were enrolled in the trial and 32 of the patients were evaluable for the primary endpoint. One patient did not start treatment and one died of unrelated cause before completing one month of therapy. The mean patient age was 53 years (range 35-87), 27 were male and 7 female, and chordoma was locally recurrent or advanced in 10 and metastatic in 24 of the patients. Nine patients required reduction in dose of dasatinib because of adverse events, and 6 patients had a serious adverse event related to dasatinib. Twelve patients (35%) were free from chordoma progression for at least 6 months and 5 had stable disease for at least 1 year. Eight patients remain on treatment, 2 of whom have been on therapy for more than 2 years.

Conclusion: Dasatinib is tolerated in the majority of patients with chordoma and can be continued for more than a year in patients benefitting from the treatment. The high rate of stable disease at 6 months is preliminary evidence of drug activity in this disease. However, because of the variable natural history of chordoma, confirmation of dasatinib activity in chordoma will likely require a randomized clinical trial.

Notes:

Scott Schuetze, MD, PhD

Dr. Schuetze graduated from the Oregon Health and Sciences University with degrees in medicine and molecular biology/biochemistry. After residency in internal medicine at Duke University, he attended the University of Washington/Fred Hutchinson Cancer Research Center for fellowship training in medical oncology. He was an assistant professor of medicine at the University of Washington before accepting directorship of the connective tissue oncology program at the University of Michigan Comprehensive Cancer Center. He has devoted his medical career to treatment of patients with bone or soft tissue sarcomas and clinical research of drug therapy and imaging in sarcoma. He also serves as the medical director of the University of Michigan Cancer Center Clinical Trials Office.

Paul Gardner, MD

Expanded endoscopic surgery for clival chordomas: A 7 year experience

Maria Koutourousiou[1], Paul A. Gardner[1], Carl H. Snyderman[1,2], Juan C. Fernandez-Miranda[1], Stephanie L. Henry [1]

[1] Department of Neurological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[2] Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Objective: Clival chordomas represent one of the most challenging cranial base tumors to treat, given their location, invasiveness, potential extension around vital neurovascular structures and their high recurrence rate.

Methods: From April 2003 to March 2010, approximately 1100 patients underwent an endoscopic endonasal approach (EEA) for skull base lesions at the University of Pittsburgh Medical Center. Among them, 40 were histologically diagnosed as chordomas. Medical records and radiologic images of these patients were retrospectively analyzed and evaluated.

Results: Forty patients (65% male) with a median age of 41 years (range 4-76) underwent 56 EEAs for the treatment of skull base chordomas. Nineteen chordomas (47.5%) were recurrent at the time of surgery. In the group of 21 patients with primary tumors, total resection of the tumor was achieved in 15 (71.4%), near total in 4 (19.1%) and subtotal in 2 (9.5%) cases. With a mean follow-up of 19 months (range 1-68), 9 patients (42.9%) remain free of disease, 4 (19%) have stable residual tumor, and 4 (19%) show tumor progression and are being monitored closely. Of the recurrences, 3 (14.3%) underwent re-operation and 1 (4.8%) died secondary to progression of disease. In the group of 19 patients that presented with recurrent chordomas, total resection was achieved in 6 (31.6%), near total in 4 (21%) and subtotal in 9 (47.4%). During the follow-up period, 6 (31.6%) of these patients are free of disease, 5 (26.3%) have stable residual tumor and 8 (42.1%) underwent EEA for tumor regrowth. Twenty three patients received adjuvant radiation therapy. In total, 11 cases (27.5%) were complicated by cerebrospinal fluid leakage resulting in meningitis in 2 (5%) patients. Vascular injuries occurred in 2 cases, without resulting in any deficit. One patient experienced quadriparesis and neuropathy of lower cranial nerves as a result of delayed pontine hemorrhage. During the follow-up period, 2 patients died due to disease progression.

Conclusion: In our experience, disease control is related to the extent of chordoma resection. In resectable tumors, complete removal with low morbidity can be achieved with the use of EEA, in the hands of experienced surgeons. In extensively invasive or recurrent chordomas, EEA can provide satisfactory tumor reduction while minimizing complications.

Notes:

Paul Gardner, MD

Expanded endoscopic surgery for clival chordomas: A 7 year experience

Dr. Gardner is an assistant professor of neurological surgery at the University of Pittsburgh School of Medicine and co-director of the Center for Skull Base Surgery. He specializes in endoscopic endonasal skull base surgery, pituitary tumors, and vascular neurosurgery. Dr. Gardner received his medical degree from the University of Pittsburgh School of Medicine, where he also completed a two–year fellowship focusing on endoscopic endonasal pituitary and skull base surgical techniques. Dr. Gardner's research has focused on evaluating patient outcomes following these surgeries. His publications can be reviewed through the National Library of Medicine's publication database. Dr. Gardner is a member of the Alpha Omega Alpha Honor Society, American Association of Neurological Surgeons, Congress of Neurological Surgeons, and North American Skull Base Society.

Peter Rose, MD

Surgical algorithm for resection of advanced spinopelvic malignancies

Peter S. Rose, Michael J. Yaszemski, Mark B. Dekutoski, Michelle J. Clarke, Franklin H. Sim.

Mayo Clinic, Rochester, Minnesota

Approximately half of all chordomas occur in the sacral region. The majority can be resected without the need for concurrent pelvic resection or disruption of spinopelvic continuity. However, advanced chordomas and other primary sacral malignancies may require innovative and aggressive surgical strategies to allow resectability and potential cure. We present our algorithm and surgical strategy for these conditions based on 50 extended spinopelvic resections for cure.

Resections are classified by the extent of the sacral involvement and the need for an associated external hemipelvectomy into 5 categories. Each category implies a surgical approach, staging algorithm, reconstruction technique (bone and soft tissue), and expected functional outcome (ambulatory status, bowel, bladder, and sexual function).

Ambulatory function is expected in patients who have at least one L5 nerve root preserved. Index colostomy is recommended for patients with less than unilateral preservation of the S2 and S3 nerve roots.

We present this classification to aid in guiding patient selection, surgical execution, and counseling about expected outcomes for advanced sacropelvic malignancies.

Notes:

Peter Rose, MD

Dr. Rose is a spine tumor surgeon at the Mayo Clinic in Rochester, Minnesota. He is trained in orthopedic surgery, spine surgery, and tumor surgery and confines his practice to oncologic processes of the spine, pelvis, and extremities.

Tom DeLaney, MD

F-18 Misonidazole Scans to Assess Hypoxia in Patients with Chordoma: Preliminary Results

T.F. DeLaney, Y-L Chen, H. Shih, E. A. Weyman, B.K. Winrich, R. Lim, A. Trofimov, J. H. Schwab, U. Mahmood, F. J. Hornicek.

Objective: For patients requiring radiation, high radiation doses are required if permanent local control of chordomas is to be achieved. As radiation effects are mediated via oxygen free radicals and hypoxia has been shown to impair radiation response, we sought (1) to assess whether hypoxia could be detected in patients with chordomas undergoing radiation therapy, (2) whether there was any detectable change in the extent of hypoxia after 24-36 Gy of radiotherapy, and (3) whether focal areas of hypoxia might theoretically be able to undergo selective radiation dose intensification with dose-painted intensity modulated proton therapy (IMPT).

Materials/Methods: Prospective, IRB-approved clinical trial of 20 patients > age 18 to be treated with proton or combined photon/proton RT for primary chordomas or locally recurrent chordomas after surgery with a gross tumor mass larger than 1 cm. Patients were injected with 325-400 MBq of F-18 misonidazole two hours prior to PET CT scan of the chordoma with the patient in their radiation therapy treatment position/ immobilization device prior to their first radiation fraction. They were re-scanned in similar fashion after having received a radiation dose of 24-36 Gy. All voxels in the gross tumor volume (GTV) where standard uptake value (SUV) was ≥ 1.4 x SUVmean in muscle were considered to comprise the hypoxic subvolume. Patients with hypoxic subvolumes underwent a radiation planning study to evaluate the feasibility of delivering focal radiation dose intensification to the hypoxic subvolume within the GTV with dose-painted IMPT.

Results: Between 1/13/2009 and 9/28/2010, 14 patients(13 primary and 1 locally recurrent tumor) with chordomas (12 sacrococcygeal, 1 cervical, 1 lumbar) were entered on to the protocol. Per protocol, all patients underwent scans prior to the start of XRT and after 24-36 Gy. Four patients had hypoxia (as defined above) noted on both scans, 2 patients had hypoxia noted only the first scan, 3 patients had hypoxia seen on only on the second scans, 5 patients had no evidence of hypoxia on either scan. Although the largest hypoxic subvolume was 418.81 cc in a patient with a 751 cc tumor, the median hypoxic subvolume was 4.26 cc (range 0.04- 418.81 cc) Radiation treatment planning studies to evaluate the feasibility of focal radiation dose intensification to the hypoxic subvolume within the GTV with dose-painted IMPT are in progress.

Conclusions: Some patients with chordomas appear to have hypoxic subvolumes within the gross tumor, as assessed by F-18 misonidazole PET/CT scans. The clinical significance of this finding will require additional follow-up in the patients who have been scanned to date. Accrual to the protocol continues as does the dosimetry study of the feasibility of radiation dose intensification to sites of hypoxia with IMPT.

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Thomas Delaney, MD

Dr. DeLaney is a graduate of Harvard College and Harvard Medical School. Following an internship in General Surgery at Yale-New Haven Hospital, he trained in Radiation Oncology at the Massachusetts General Hospital. He then spent 6 years as a Senior Investigator at the National Cancer Institute in Bethesda MD. Since 1992, he has been on the staff of the Massachusetts General Hospital and on the faculty of Harvard Medical School. Currently, he is the Medical Director of the Francis H. Burr Proton Therapy Center at Massachusetts General Hospital, Co-Director of the Center for Sarcoma and Connective Tissue Oncology at Massachusetts General Hospital, and Professor of Radiation Oncology, Harvard Medical School. He has served as an Executive Board Member of the Connective Tissue Oncology Society, is a member of the National Comprehensive Cancer Network Soft Tissue Sarcoma Guidelines Panel, and serves as a member of the Scientific Advisory Board of the Chordoma Foundation. He is active in the Radiation Therapy Oncology Group Sarcoma Working Group. Dr. DeLaney has made contributions to the treatment of soft tissue and bone sarcomas and is actively involved in clinical research in this area as well as the use of charged particle (proton) radiation therapy. His bibliography lists 73 original reports as well as 89 reviews, book chapters, editorials, and clinical guidelines. He, along with Hanne Kooy, Ph.D. edited the book, Proton and Charged Particle Radiotherapy(Lippincott Williams and Wilkins, Philadelphia, 2007). He is on the editorial boards of Annals of Surgical Oncology, Journal of Surgical Oncology, Journal of Clinical Oncology.and UpToDate in Oncology.

Yen-lin Chen, MD

Preliminary Experience Using High Dose Proton Based Radiotherapy for Unresected Chordomas

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Purpose: To report the preliminary results of high dose proton based definitive radiotherapy for unresected spinal chordomas.

Methods: We retrospectively reviewed 19 patients with newly diagnosed, previously untreated spinal chordomas (core biopsy only; excluding recurrent disease, incisional biopsy, debulking, and partial or complete resection) treated with high dose definitive radiotherapy using protons and photons at the Massachusetts General Hospital between 1997 to 2008. Reasons for definitive radiotherapy rather than resection or combined modality treatment included 7 patients who were deemed medically inoperable due to comorbidities and 12 who declined resection due to concerns of neurological morbidity. Patients were treated with median dose of 77.4 GyRBE (relative biological effectiveness-weighted absorbed dose) with a combination of protons and photons, given in standard fractionation of 1.8 to 2 GyRBE per fraction per day.

Results: Median follow up was 43.5 months (range 21 to 155 months). Fifteen patients had sacrococcygeal chordoma (11 with superior extent at S1/2 , 1 extending cephalad to also involve L1 and 2 arising at S3/4 or below) while the remainder were in the mobile spine (2 cervical, 1 thoracic, and 1 lumbar). Median maximal tumor diameter was 8.7 cm. The three-year overall survival rate was 89% and local control rate was 89%. Two patients failed locally at 2 years and 6.33 years respectively. One patient failed distantly at one year but was locally controlled until death at 1.5 years. There were no spinal cord injuries, necrosis, or severe skin toxicities. 1 patient developed neuropathy resulting in partial leg weakness but all patients remained ambulatory. One patient developed erectile dysfunction, 3 had moderate soft tissue fibrosis, 3 had grade 2 rectal bleeding, and one patient developed sacral insufficiency fracture.

Conclusions: We have encouraging preliminary results using high dose definitive photon/proton radiotherapy in patients with mobile spine or sacrococcygeal chordomas who are medically inoperable or otherwise unresected after biopsy only. Treatment appears to be well tolerated with acceptable toxicity so far. Longer follow-up is needed to assess durability of disease control and late functional outcome with this treatment approach.

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Yen-Lin Chen, MD

Dr. Chen is a staff radiation oncologist at the Massachusetts General Hospital specializing in the treatment of soft tissue and bone sarcomas including chordomas with advanced radiation techniques including proton radiotherapy, intraoperative radiotherapy, and brachytherapy.

Josh Yamada, MD FRCPC

Very High Dose Stereotactic Radiosurgery: A New Paradigm in the Management of Chordomas of the Mobile Spine and Sacrum

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Introduction: Local disease progression of chordomas are devastating. Even with the use of high energy particle beam radiotherapy, the local control rates with conventionally fractionated radiotherapy have been disappointing. High dose single fraction radiotherapy, made possible by integrating advanced image guided technology with intensity modulated treatment planning and delivery, have significantly improved the durable local control of radioresistant tumors in the the spine and sacrum. We report the preliminary results of applying this paradigm to the management of chordomas of the mobile spine and sacrum.

Methods: Twelve patients with chordoma of the mobile spine and sacrum have undergone high dose stereotactic radiosurgery (median dose 2400cGyx1). Each patient was immobilized in a customized immobilization cradle, utilizing intensity modulated techniques to deliver highly conformal treatment to spare spinal cord and paraspinal dose sensitive organs. Treatment was performed on a linear accelerator equipped with 2D and 3D on board KV imaging to verify isocenter placement. Each patient was followed in a multidisciplinary spine clinic with MRI imaging every 3-4 months. Toxicity was scored using Common Toxicity Criteria (NCI CTC vs 3) scales.

Results: Median follow up is 10 months (8-50 months). One patient has died of progressive metastatic disease. The median age is 71 (62-80 years). Three patients had a transient grade 2 pharyngitis/esophagitis. One patient with sacral disease involving the S1 root has developed a foot drop. One patient who later underwent surgical resection had significant wound complications. One patient developed mechanical instability requiring surgical fixation. In both cases, pathologic review revealed near complete necrosis. All cases have demonstrated no evidence of disease progression, with central necrosis a hallmark of follow up imaging.

Conclusion: Hypofractionated radiotherapy has been reported in the treatment of sacral chordomas. High dose stereotactic radiosurgery represents extreme hypofractionation and appears to be a promising treatment modality for chordomas of the mobile spine and sacrum. Although results are very preliminary, both pathologic and radiographic responses suggesting a high degree of necrosis after radiosurgery are encouraging. En bloc resection remains the gold standard therapy, but the addition of an effective adjuvant to surgery is an attractive paradigm to improve local control and should be tested in a prospective controlled trial.

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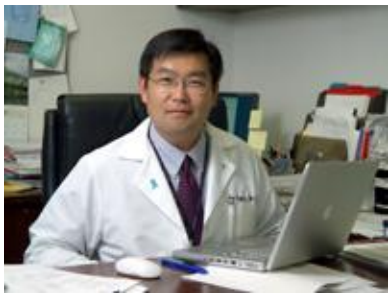
Yoshiya Yamada, MD FRCPC



I am a board-certified radiation oncologist with expertise in treating cancers with brachytherapy (radiation placed inside of tumors) and image-guided radiation (using advanced medical imaging technology to deliver precise beams of radiation to safely destroy tumors.) Brachytherapy and image-guided radiation are both very effective and safe ways to treat tumors because high doses of radiation can be given to a tumor while sparing healthy tissue and causing few side effects.

I work with a multidisciplinary team of highly skilled surgeons, radiologists, medical physicists, radiation therapists, and nurses. Through these collaborations, we are able to take advantage of the sophisticated medical technology available at Memorial Sloan-Kettering that is necessary to provide these types of treatment.

In addition, I am involved in clinical research that involves using image-guided technologies to treat tumors in all sites of the body, including adult brain and spine tumors, as well as liver tumors. I am also the primary investigator for several protocols using brachytherapy to treat cancers in the prostate.

I have been invited to speak in many scientific forums both nationally and internationally. I am currently serving on the board of directors of the American Brachytherapy Society.