**Francis Hornicek, MD, PhD**

***Overview of Chordoma and Review of NCCN Guidelines for Clinical Management***

**Massachusetts General Hospital,** Boston, MA

The presentation will be a review of the NCCN guidelines for chordoma management. This review will include the rationale and an overview of chordoma.

**Notes:**

**Rose Yang, PhD, MPH**

***Characterization of genetic variants in the T gene in familial and sporadic chordoma***

**National Cancer Institute,** Bethesda, MD

Chordoma is a rare bone tumor that is believed to originate from notochordal remnants. *T* gene, which encodes Brachyury, is essential for the proper development and maintenance of the notochord and has been implicated in the pathogenesis of chordoma. Germline duplication of the *T* gene was reported to confer major susceptibility of familial chordoma. A common genetic variant in *T* was recently associated with the increased risk of sporadic chordoma. To further investigate the role of *T* variants in chordoma susceptibility, we sequenced all exons in the *T* gene in germline DNA from 112 individuals from chordoma families (40 chordoma cases and 72 unaffected members) and 91sporadic chordoma cases. We also conducted qPCR for *T* duplication in these individuals. Results from these analyses will be presented at the chordoma conference.

**Notes:**

**Patrick Tarpey, PhD**

***Identification of somatically acquired mutations in chordoma exomes***

**Wellcome Trust Sanger Institute,** Cambridge, UK

We are interested in exploring the genomes of tumours to better understand the genes and pathways which become disrupted during cancer progression. To do this, we search for somatically acquired mutations in large numbers of samples from specific cancer types, and perform analyses to recognize the patterns of mutation indicative of dominant or recessive cancer genes. One of the cancer-types we study is bone cancer, including osteosarcoma, chondrosarcoma, and rarer bone tumours such as chordoma.

To kick-start these investigations we have analysed the coding sequence (exomes) of bone tumours including 25 chordoma tumour/normal pairs. We identified driver variants in several known cancer genes including PIK3CA, PBRM1, ARID1A and PTEN, however we did not identify any likely novel cancer genes. Our future aims are to sequence the full genomes of several chordomas to unravel the full spectrum of variation underlying these cancers.

**Notes:**

**Stephen Yip, MD, PhD, FRCPC**

***Whole genome sequencing of chordoma***

**The University of British Columbia,** Vancouver, Canada

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.

Results of ongoing whole genome sequencing of primary chordoma specimens will be discussed including preliminary findings in mutation discoveries and potential fusion events in conjunction with existing whole exome sequencing results.

**Notes:**

**Roberto Diaz, MD**

***High-resolution whole genome analysis of skull base chordomas implicates FHIT loss in chordoma pathogenesis***

**University of Toronto,** Toronto, Canada

Chordoma is a rare tumor arising in the sacrum, clivus, or vertebrae. It is often not completely resectable and shows a high incidence of recurrence and progression with shortened patient survival and impaired quality of life. Chemotherapeutic options are limited to investigational therapies at present. Therefore, adjuvant therapy for control of tumor recurrence and progression is of great interest, especially in skull base lesions where complete tumor resection is often not possible due to the proximity of cranial nerves. In order to understand the extent of genetic instability and associated chromosomal and gene losses or gains in skull base chordoma we undertook whole genome single nucleotide polymorphism microarray analysis of flash frozen surgical chordoma specimens, 21 from the clivus, and 1 from C1-C2 vertebrae. We confirm the presence of a deletion at 9p involving CDKN2A, CDKN2B, and MTAP, but at a much lower rate (22%) than previously reported for sacral chordoma. At a similar frequency (21%) we found aneuploidy of chromosome 3. Tissue microarray immunohistochemistry demonstrated absent or reduced FHIT protein expression in 98% of sacral chordomas and 67% skull base chordomas. Our data suggest that chromosome 3 aneuploidy and epigenetic regulation of *FHIT* contribute to loss of the FHIT tumor suppressor in chordoma. The finding that FHIT is lost in a majority of chordomas provides new insight into chordoma pathogenesis and points to a potential new therapeutic target for this challenging neoplasm.

**Notes:**

**Beate Rinner, PhD**

***DNA Methylation markers in chordoma cancer***

**Medical University of Graz,** Graz, Austria

**Background:** The aim of this study was to explore if DNA methylation, a well-known epigenetic marker, may play a role in chordoma development and if hypermethylation of specific CpG islands may serve as potential biomarkers correlated with SNP analyses in chordoma.
**Material and Methods:** The study was performed on tumor samples from ten chordoma patients. Affymetrix GeneChip Human Mapping SNP 6.0 arrays were performed as described in the Genome-Wide Human SNP Nsp/Sty 6.0. DNA digestion were done with methylation-sensitive restriction enzymes (MSRE). PCRs were pooled and hybridized onto the AIT-CpG360 microarray. Microarrays were scanned and intensity data extracted from images using Genepix6.0 software (AXON).
**Results:** We found significant genomic instability by Affymetrix 6.0. Interestingly all chordomas showed a loss of 3q26.32 (PIK 3CA) and 3q27.3 (BCL6) underlining the potential importance of the PI3K pathway in chordoma development. By using the AITCpG360 methylation assay we elucidated 20 genes which were hyper/hypomethylated compared to normal blood. The most promising candidates were nine hyper/hypomethylated genes C3,XIST, TACSTD2, FMR1, HIC1, RARB, DLEC1, KL, and RASSF1.
**Conclusion:** In summary, we have shown that chordomas are characterized by significant changes of the DNA methylation pattern. A multigene DNA methylation based classifier suitable to distinguish healthy blood and chordoma DNA presented here will add a new dimension for chordoma diagnosis and treatment.
**Clinical Relevance:** Although validation of results has to be conducted on additional patient sample cohorts and serum cfDNA, we think that the DNA methylation classifiers elucidated here could be useful novel biomarkers advancing diagnostic workup for patients.

**Notes:**

**Yair Benita, PhD**

***A molecular analysis identifies chondrogenesis as the driving mechanism of chordoma***

**Merck Research Labs, Informatics and Analysis**, Boston, MA

Chordoma is a rare malignant tumor thought to originate from embryonic notochord. This belief arises from the finding that chordoma is associated with alterations in T brachyury, a hallmark notochord gene, as well as similar histology between the two tissues. However, to date no molecular comparison between chordoma and notochord has been performed, leaving unresolved the questions of tissue origin as well as the identities of dysregulated pathways in chordoma. Here we perform an unbiased comparison of chordoma and notochord using gene expression profiling, revealing a striking molecular resemblance between the tissues. Furthermore, we identify a 12-gene diagnostic chordoma signature and demonstrate hyperactivation of the TGF-β:SOX9 pathway, suggesting that chondrogenesis, the process of cartilage development, may be a driving mechanism.

**Notes:**

**Zhenfeng Duan, MD, PhD**

***Role of MicroRNA-1 (miR-1) in chordoma and its therapeutic potentials***

**Massachusetts General Hospital,** Boston, MA

MicroRNAs(miRNAs, miRs) are small non-coding RNA molecules that regulate post-transcriptional gene expression by binding to complementary sequences in the specific region of multiple target mRNAs, and resulting down-regulation of gene expression. The mechanisms of chordoma development are not fully understood. However, evidence of miRs dysregulation has been reported in many human cancers, including chordoma. Not only certain miRs are consistently dysregulated across many cancers, but miRs also play critical roles in many aspects of cell growth, proliferation/differentiation, apoptosis and drug sensitivity/resistance. Recent studies from our group and others revealed that miR-1 is frequently downregulated in various types of cancer including chordoma. Through targeting multiple oncogenes and oncogenic pathways, miR-1 has been demonstrated as a tumor suppressor gene that represses cancer cell proliferation, metaststasis, and promotes apoptosis by ectopic expression. In this study, we highlight these recent findings on the aberrant expression and functional significance of miR-1 in chordoma and emphasize its significant values for cancer therapeutic potentials.

**Notes:**

**Deric Park, MD**

***DJ-1/PARK7 regulates oxidative stress in chordoma cells***

**University of Virginia,** Charlottesville, VA

Intratumoral hypoxia and its adaptive response contribute to cancer pathogenesis and resistance to therapy. Because low tissue oxygen environment may lead to generation of reactive oxygen species (ROS), cancer growth under hypoxia requires efficient mechanism to prevent excessive accumulation and enhanced clearance of these toxic substances. DJ-1/PARK7, a putative oncogene, is overexpressed in a variety of solid tumors, and its functional loss by gene mutation is responsible for a type of early onset familial Parkinson's disease through oxidative stress induced cell death. Because DJ-1/PARK7 appears to play a key role in cellular management of ROS-induced stress, we investigated its role in chordoma, a tumor characterized by hypoxia. Here we show: 1) Chordoma cells exhibit enhanced proliferation under hypoxia, a condition that leads to elevation of ROS; 2) DJ-1 is expressed in chordoma cells; 2) Hypoxia augments DJ-1 protein level; 3) Disruption of DJ-1 leads to increased ROS and diminished ability of chordoma cells to survival under hypoxic stress. These results suggest that a protein necessary for neuronal survival in conditions of oxidative stress may enhance chordoma cell tolerance to ROS. Targeting DJ-1 may provide novel therapeutic opportunities.

**Notes:**

**Sagar Shah**

***Stem cells in chordomas: delineating mediators of self-renewal and tumor-initiation***

**Johns Hopkins Medicine,** Baltimore, MD

The cancer stem cell hypothesis has provided a major paradigm breakthrough in our understanding of cancer biology. It postulates that tumor cells are hierarchically organized with respect to tumor growth initiation and maintenance. Remarkably these cells have been shown to contribute substantially to tumor recurrence and treatment failure. CSCs are strictly defined on the basis of their ability to seed tumors in animal hosts, to self-renew and to demonstrate lineage commitment towards differentiated progeny. CSCs, otherwise known as tumor initiating cells, have now been isolated from several human cancers including leukemias, breast, brain, melanoma, colon, and pancreatic cancer. Remarkably, these cells have been shown to contribute substantially to tumor recurrence and treatment failure. More recently, CSCs have been isolated from Ewing sarcoma as well as other bone sarcomas, compelling us to inquire whether such a population of cancer cells may exist in chordoma. But perhaps most intriguing, chordomas are thought to derive from transformed embryonic remnants of notochord arousing even more suspicion that this malignancy may be driven by a similar population of cancer stem cells. Microarray data from a broad range of connective tissue neoplasms indicate that at the transcriptional level, chordomas express many genes known to be involved during development. Thus, we aim to delineate developmentally-relevant genetic players and their signaling cascades that may govern the existence of these putative tumor-initiating cells.

**Notes:**

**Susanne Scheipl, MD**

***Histone Deacetylase inhibitors in chordomas: an immunohistochemical and functional analysis in MUG-chor1***

**Medical University of Graz,** Graz, Austria

**Introduction:** Chordomas are rare malignancies of the axial skeleton, behaving locally destructive. Therapeutic modalities are mainly restricted to surgery and irradiation. Additional treatment options are therefore urgently sought.

Histone deacetylases (HDACs) remove acetyl groups from amino acids on histone tails. Apart from histone modification, HDACs can influence malignant cell transformation by alteration of non-histonic proteins, as e.g. transcription factors, cytoplasmic proteins, and signaling molecules. HDAC inhibitors are tested in many clinical trials as promising new treatment options for various types of cancer.

**Objectives:** We intended to study whether HDAC inhibitors could be regarded as promising therapeutic targets for chordomas.

**Materials and Methods:** Fifty chordomas (34 primary tumors, 16 recurrences) from 44 patients (27 male, 17 females) were evaluated immunohistochemically for the expression of HDACs1-6. HDAC inhibitors Vorinostad (SAHA), Panobinostad (LBH-589), and Belinostad (PXD101) were tested in the chordoma cell line MUG_Chor1 for dose-dependent apoptotic effects. $IC_{50}$ values were determined. Apoptosis induction was investigated by caspase 3/7 activity, caspase-3 cleavage and PARP cleavage. Two-sided *P*-values below 0.05 were considered statistically significant.

**Results:** IHC: HDAC1 expressed a slight nuclear positivity (n = 5; 10%), but showed no cytoplasmic staining. Expression of HDAC2 was positive in the majority of cases (n = 36; 72%). HDACs 3 to 6 stained positive in all specimens available (n = 43; 86%). The strongest expression was observed for HDAC6.

Cell line: Caspase 3/7 activity was measured by the Caspase-Glo® 3/7 Assay in MUG-Chor1 cells after 3, 6, 24, 48, and 72 h treatment with the $IC_{50}$ of SAHA, LBH-589, and PXD101. It peaked after 48 and 72 h in SAHA and LBH-589 treated cells. However, PXD101 treatment did not lead to caspase 3/7 activity. In the cell line, cleaved caspase-3 was detected in 54.5±7.4% of SAHA treated, and in 63.1±13.2% of LBH-589 treated cells, respectively. In contrast, the control and PXD101 treated cells showed almost no cleaved caspase-3 (2.7±1.5% and 8.2±3.4% of gated cells, respectively). The percentage of cleaved caspase-3 positive cells increased significantly over time ($p$=0.0003 for SAHA, and $p$=0.0014 for LBH-589 after 72h, respectively). The apoptotic induction by SAHA and LB-589 was confirmed by PARP cleavage by Western blotting.

**Discussion:** We observed an immunohistochemical detectability of HDACs in our series, with HDAC1 showing the weakest, and HDAC6 showing the strongest staining. SAHA and LBH-589

significantly increased apoptosis of chordoma cells *in vitro*. Although sufficient data from chordomas is still lacking to date, the efficacy of various HDAC inhibitors has been shown in several types of sarcomas, *in vitro* as well as in animal models. The greatest effects were seen in combination with other anticancer therapeutics. Our results provide evidence to support further research on HDACs as potential therapeutic targets for chordoma therapy.

**Notes:**

**Anna DiGregorio, PhD**

***Control of notochord gene expression by Brachyury: lessons from a sea squirt***

**Weill Cornell Medical College,** New York, NY

Brachyury is a sequence-specific transcription factor required for proper development of the notochord. The notochord is an axial structure of mesodermal origin that provides the embryo with structural support as well as with patterning cues necessary for the formation of the vertebral bodies and numerous other structures and organs. As ossification of the vertebrae proceeds, the notochord regresses and its remnants form the *nuclei pulposi* of the intervertebral discs. Of note, notochord remnants can also give rise to chordomas, and these tumors, similarly to normal notochord cells, also express Brachyury. Our research focuses on elucidating the molecular mechanisms employed by Brachyury to control gene expression. In particular, we study how Brachyury can activate the expression of its numerous target genes during notochord formation by binding their *cis*-regulatory sequences. We carry out our studies in the model system *Ciona* (tunicate, or 'sea squirt') an invertebrate chordate, *i.e*. an animal that has a notochord but does not form vertebrae. Differently from other chordate *Brachyury* genes, *Ciona Brachyury* (*Ci-Bra*) is expressed exclusively in the notochord, and this has allowed the selective identification of several notochord-specific genes whose expression is controlled by Ci-Bra. We noticed that these genes are expressed at different times during notochord formation, which suggested that they are differentially controlled by Ci-Bra. To elucidate the molecular bases of this differential expression, we isolated notochord *cis*-regulatory modules (or enhancers) associated with several *Ciona* notochord genes and we carried out an in-depth characterization of the minimal sequences required for their activity. Our results reveal a multiplicity of mechanisms through which Ci-Bra, and possibly other Brachyury proteins, create a temporal gradient of gene expression in the notochord. Some of these mechanisms appear to be evolutionarily conserved from sea squirts to mice.

**Notes:**

**Claudia Palena, PhD**

***The Role of Brachyury in Tumor Progression***

**Center for Cancer Research,** Bethesda, MD

The T-box transcription factor Brachyury has been recently characterized as a regulator of the epithelial-mesenchymal switch of human carcinomas, a process underlying tumor metastasis. Over-expression of Brachyury in human epithelial tumor cells has been shown to promote the acquisition of mesenchymal-like features, including enhanced cell motility and invasiveness in vitro, and to promote tumor dissemination in vivo. Recent work conducted in our laboratory elucidated yet another interesting function of the Brachyury protein in human carcinomas, as tumor cells with high levels of Brachyury are more resistant to the cytotoxic effect of conventional anti-tumor therapies. Utilizing tumor cell populations with various levels of Brachyury, we have shown that the expression of this transcription factor negatively correlates with the ability of various chemotherapies and radiation to induce tumor cell death. Current studies in our laboratory are aimed at elucidating the mechanism(s) involved in Brachyury-mediated tumor resistance. Owing to the functional relevance of Brachyury in cancer cells, its tumor-restricted pattern of expression and its highly immunogenic character, a Brachyury-based yeast recombinant vaccine has been developed that is currently ongoing Phase I clinical testing in patients with advanced carcinomas.

**Notes:**

**Adrianne Flanagan, MD, FRCPath, PhD**

***The role of brachyury in chordoma pathogenesis and as a therapeutic target***

**University College London Cancer Institute,** London, UK

Brachyury is the diagnostic hallmark of chordomas and is implicated in the pathogenesis of this disease. A tandem duplication of brachyury in the germline of patients with familial chordoma has been identified as a genetic susceptibility factor. Although somatic amplification (7% of cases) and minor allelic gain (13% of cases) of the brachyury locus is present in sporadic chordomas susceptibility determinants in this cohort of patients are unknown.

To determine the genetic susceptibility variants in patients with sporadic chordomas, we performed high resolution arrayCGH on 23 sporadic chordomas to determine if a duplication of brachyury is a common event in this cohort. We also conducted a gene association study on 40 patients with chordoma and 358 ancestry-matched, unaffected individuals with replication in an independent cohort.

Whole-exome sequencing revealed that the strongest candidate genetic risk factor was a common SNP (rs2305089) in exon 4 of brachyury. This finding was validated using Sanger sequencing and Taqman genotyping resulting in an allelic odds ratio (OR) =6.1, P=4.4x10-9,CI= 3.1-12.1. rs2305089 (Gly177Asp, G>A) alters the DNA binding ability of brachyury. By using gene expression microarrays and qRT-PCR assays we show that the altered function of brachyury, caused by the SNP, is associated with different expression levels of brachyury and its downstream targets.

The targets of brachyury were identified using an integrated functional genomics approach involving shRNA-mediated brachyury knockdown, gene expression microarray, ChIP-seq experiments, and bioinformatics analysis to achieve this goal. We confirm that the T-box binding motif of human brachyury is identical to that found in mouse, Xenopus, and zebrafish development, and that brachyury acts primarily as an activator of transcription. Using human chordoma samples for validation purposes, we show that brachyury binds 99 direct targets and indirectly influences the expression of 64 other genes, thereby acting as a master regulator of an elaborate oncogenic transcriptional network encompassing diverse signalling pathways including components of the cell cycle, and extracellular matrix components.

The heritability risk conferred by common genetic variants (MAF>5%) in cancers is generally modest (~1-2 fold). The finding of a per allele OR>5 is exceptional amongst those cancers for which there is a non-Mendelian mode of inheritance. In view of this, and the dearth of functional variants, other than rs2305089, there is a strong case that this SNP not only

contributes significantly to the development of chordoma. Given the wide repertoire of its active binding and the relative specific localization of brachyury to the tumour cells, we propose that an RNA interference-based gene therapy approach is a plausible therapeutic avenue worthy of investigation.

**Notes:**

**Wesley Hsu, MD**

***Targeting Brachyury Using A Lipid-based Nanoparticle Delivery System for shRNA Inhibits Chordoma Cell Growth In Vitro***

**Wake Forest School of Medicine,** Winston-Salem, NC

**Introduction:** Chordoma is a rare malignant neoplasm arising from notochordal remnants. Recent studies demonstrate that brachyury knockdown using shRNA resulted in premature cell senescence and reduced tumor growth in vitro. Lipid nanoparticles consisting of dioleoyltrimethylammoniumpropane (DOTAP) and cholesterol has been shown to successfully deliver RNAi in vitro and in vivo, but whether these nanoparticles can deliver shRNA targeting Brachyury to chordoma cells is unknown. The aim of this study is to determine if lipid nanoparticles can be used to deliver shRNA targeting brachyury to chordoma cells and inhibit cell growth in vitro.

**Methods:** A constructed brachyury shRNA/protamine complex was coated with cationic liposomes consisting of DOTAP and cholesterol to produce liposome-polycation-DNA (LPD) nanoparticles. Agarose gel electrophoresis was used to test the efficiency of LPD formulations. Cell proliferation and apoptosis were measured by MTS and caspase 3/7 activity, respectively. Gene expressions related to epithelial-mesenchymal transition (EMT) were detected by quantitative RT-PCR.

**Results:** Agarose gel electrophoresis showed a strong binding capacity of liposome and Brachyury shRNA. LPD nanoparticles delivered brachyury shRNA into JCH7 and UCH1 chordoma cells and reduced brachyury expression at gene and protein levels after transfection with shRNA for 24hrs (80-99.9% and 61-63% reduction for JCH7 and UCH1 cells, respectively) Growth inhibition was observed in both JCH7 and UCH1 cells when transfected with nanoparticles conjugated with brachyury shRNA (64% and 37% reduction for JCH7 and UCH1 cells, respectively). Liposome-encapsulated brachyury shRNA also increased caspase 3/7 activity in above cell lines (2.9- and 1.5-fold increases for JCH7 and and UCH1 compared with control, respectively). Brachyury shRNA/nanoparticles led to upregulation of E-cadherin and downregulation of slug and snail expression, which are critical factors in EMT.

**Conclusions:** Targeting brachyury using lipid-based nanoparticles delivery system for shRNA inhibits growth, induces apoptosis and alters regulation of factors critical to EMT in chordoma cell lines. This lipid based-nanoparticle delivery system may offer a novel therapeutic strategy for treating chordoma.

**Notes:**

**Cheryle Seguin, PhD**

***Can the embryonic notochord model chordoma?***

**Western University,** Ontario, Canada

The notochord is an essential structure during embryonic development which provides the primitive axial skeleton and signals for the patterning of surrounding tissues. Despite the similarities between chordomas and the embryonic notochord, to date there is limited understanding of the biology of chordomas or the processes underlying the malignant transformation of notochord remnants. Recent studies have identified hallmarks of malignant chordoma transformation including copy number variation of the Brachyury (T) gene locus, a feature that serves to distinguish chordomas from the embryonic notochord or notochord-derived cells which populate the intervertebral disc.

Morphologically, ultrastructurally, and immunohistochemically, chordomas are reported to be identical to the embryonic notochord, suggesting that developmental programs are re-activated enabling quiescent notochordal cells to undergo malignant transformation. We hypothesize that notochordal cells – isolated from the embryonic notochord and/or postnatal intervertebral disc – can be used to model chordoma.

Our recent studies have established interesting parallels between the phenotype of mouse embryonic notochord cells and human chordoma cell lines. We are working towards evaluating the relative contribution of specific candidate factors in regulating the “stem cell” or cancer phenotype in both of these cell types using a combination of in vitro and in vivo assays.

**Notes:**

**Andreas Fritz, PhD**

***Developing a Zebrafish Chordoma Model***

**Emory University,** Atlanta, GA

Chordomas are rare malignant bone tumors that form during human development. However the pathogenic mechanisms and molecular characteristics of the tumors are largely unknown. It is thought that chordomas develop from notochordal precursor cells which retain some developmental characteristics of the notochord. Notably, Brachyury is a transcription factor involved in notochord formation and there is evidence that it plays a role in the development and pathogenesis of chordomas. A recent study has also implicated hyperactivation of the Akt/mTOR pathway in the formation of chordomas. We have recently observed that in zebrafish embryos treated with the TGF-ß/nodal-blocking drug SB-505124 tumors of the notochord can easily and highly relialbly be induced by 3 days post fertilization. Preliminary analysis has shown that these notochord tumors express markers typical of chordomas, such as notail (zebrafish Brachyury) and pS6, a marker for activated mTOR signaling. These studies suggest that zebrafish may be a useful model to study the etiology of chordomas and point to a possible novel role of nodal signaling as a pathogenic mechanism. We propose to develop a zebrafish model for chordoma formation with the long-term goal of establishing an in vivo system for high-throughput drug discovery. The zebrafish is an ideal organism for small molecule studies as embryos are easily treatable by waterborne exposure. The ability to use the whole organism integrates screening of in vivo phenotypes with animal testing. We are in the process to cellularly and molecularly characterize these tumors to further validate the zebrafish model, to address the role of TGF-ß/nodal signaling in chordoma formation, and to develop the necessary tools to achieve the long-term goal.

**Notes:**

**David Alcorta, PhD**

***Development of a genetically engineered mouse model***

**Duke University** Durham, NC

**Introduction:** Chordoma is a rare malignancy arising in the axial skeleton from notochord remnants. The T-box transcription factor, Brachyury (T), is associated with familial chordoma, is expressed in over 90% of chordomas, and is a driver of cell growth in cultured chordoma cells. Suppression of T expression or function in vitro results in down-regulation of cell growth pathways and reduction in matrix formation. To further understand the mechanisms of T's oncogenic properties, we sought to generate a murine model of constitutive, notochord-directed T expression. **Methods:** To produce elevated expression of T in the notochord-derived (ND) cells, we generated a murine transgenic animal in which a cassette (T/GFP) containing the □-actin promoter, a transcriptional stop sequence flanked by LoxP sites (FS), the murine T cDNA, an intragenic ribosomal entry sequence and a GFP cDNA was homologously recombined into the ROSA26 locus. We termed these animals FS-T/GFP mice. FS-T/GFP mice were mated to mice with Cre expression regulated by the developmentally-timed and tissue-specific control of the Sonic Hedgehog promoter (Shh-Cre mice). **Results:** Excision of the FS sequence by Cre was confirmed to result in constitutive expression of T and GFP in 293 cells prior to transgenic creation, and subsequently in cultured fibroblasts from ear punches of the transgenic animals. Mice containing homozygous FS-T/GFP at ROSA26 were healthy with no apparent physiologic or developmental defects and with the expected Mendelian ratios of sex and genotype. A cross of FS-T/GFP with Shh-Cre mice resulted in T/Cre pups that died perinatally with grossly evident heart, lung and kidney defects. In addition, these animals showed incomplete fusion of the vertebral bodies, and dysmorphic spinal disks. Elevated T protein levels and GFP co-expression in disk cells was demonstrated by immunofluorescent detection in tissue sections.

**Summary:** Constitutive expression of T in ND lineage cells results in alteration of the morphology of intervertebral disks possibly indicative of a proliferative effect of Brachyury on ND cells. Expression of T in "off-target" tissues that express Sonic Hedgehog likely results in perinatal death.

**Notes:**

**Menghang Xia, PhD**

***Identification of Repurposed Drugs for Chordoma Therapy***

**National Institute for Health,** Bethesda, MD

To identify drugs that inhibit chordoma cell growth, we screened the NCGC Pharmaceutical Collection (NPC) containing approximately 2,800 clinically approved and investigational drugs at multiple concentrations in two chordoma cell lines, U-CH1 and U-CH2. After primary screening, we identified a group of drugs that inhibited chordoma cell growth in U-CH1 cells, but less potent in U-CH2 cells. Most of these drugs also induced caspase 3/7 activity with a similar rank order as the cytotoxic effect on UCH1 cells. These drugs also showed similar inhibitory effect on cell lines and three primary chordoma cell cultures. Our results provide information useful for repurposing currently approved drugs for chordoma therapy.

**Notes:**

**Gary Gallia, MD, PhD**

***In vivo testing in a patient derived chordoma xenograft model***

**Johns Hopkins Medicine,** Baltimore, MD

Chordomas are rare primary bone tumors that occur along the neuraxis. Primary treatment is surgery, often followed by radiotherapy. Treatment options for patients with recurrence are limited and, notably, there are no FDA approved therapeutic agents. This lack of treatment options is in part due to the paucity of preclinical model systems. We have established and previously reported the initial characterization of a patient-derived chordoma xenograft model. We have utilized this xenograft model for *in vivo* drug testing of top hits identified by the NCATS at NIH in an *in vitro* screen against numerous chordoma cell lines. Additional analysis of this xenograft has identified other targets which have also been evaluated *in vivo*. The results from these studies will be presented and discussed.

**Notes:**

**Elena Tamborini, PhD**

***Analysis of RTK's and downstream signalling pathways, potential therapeutic targets***

**Instituto dei Tumori Milan,** Milan, Italy

More than 30 chordoma tumors (both naive and pretreated cases) have been analyzed for RTK expression profile with a phospho array kit and other biochemical analyses. Three groups of chordomas have been thus identified on the bases of activation/expression presence of RTK families: EGFR family, PDGFR family and TAM family. Correlation with follow up have been made and very preliminary data indicate that chordomas with activation of RTKs of EGFR family only have a distinct clinical behavior.

**Notes:**

**Adrianne Flanagan, MD, FRCPath, PhD**

**David Drewry, PhD**

***Kinase inhibitor screening***

**University College London Cancer Institute,** London, UK

Protein kinases are chemically tractable drug targets, yet less than 5% of the human kinome has been thoroughly explored with selective small molecule inhibitors against these kinases. This demonstrates that the therapeutic potential of protein kinase inhibitors is largely unexplored. Pharmacological evaluation of the ”untargeted” kinome is expected to define many new opportunities to address unmet medical needs but requires access to potent and selective chemical probes. To seed such research, a set of 367 small molecule kinase inhibitors previously published by GlaxoSmithKline, has been defined for use in the identification of chemical starting points for selective kinase probes and as a phenotypic screening set to identify potential kinases or combinations of kinases of interest.

The PKIS set is available to academic collaborators with the requirement that results be publicly accessible. The presentation will include a description of the PKIS as well as examples of results from screening the set. We hope that the results will server as a valuable resource for the purposes of guiding a rational cancer therapeutic strategy.

**Notes:**

**Silvia Stacchiotti, MD**

***Chordoma clinical trials***

**Instituto dei Tumori Milan,** Milan, Italy

Following the recent publication of the Phase 2 study on imatinib in advanced chordoma, a Phase 2 study on the EGFR-inhibitor lapatinib was reported preliminarily and will be published. Lapatinib has some anti-tumor activity in chordoma, suggesting that the clinical exploitation of EGFR-targeting in the disease deserves to be further investigated, both clinically and preclinically. A Phase 2 study on imatinib plus everolimus in advanced chordomas is ongoing in Italy. About 30 patients with PDGFRB and mTOR effectors expression/activation were included the study so far (a total of 50 patients is foreseen). We are trying to put in place an effort to evaluate trabectedin in chordoma. A European network on chordoma is under construction in collaboration with the Chordoma Foundation in the US, aimed to develop consensus processes on state-of-the-art definition, to build a dispersed clinical data base, to create the facilities to undertake prospective clinical studies in the disease, to advocate for patient access to available drugs.

**Notes:**

**Christopher Heery, MD**

***First-in-human phase 1 trial of a recombinant yeast vaccine genetically modified to express Brachyury***

**Center for Cancer Research,** Bethesda, MD

Background: A therapeutic cancer vaccine was created consisting of a whole, heat-killed recombinant Saccharomyces cerevisiae yeast engineered to express the transcription factor, Br (GI-6301). Br is a member of the T-box family of transcription factors, characterized by a highly conserved DNA-binding domain designated as the T-domain and is a master driver of the epithelial to mesenchymal transition (EMT) in human carcinoma cell lines. We have demonstrated that Br over expression in human carcinoma cell lines is able to induce down-regulation of epithelial markers (E-cadherin, Plakoglobin) and up-regulate mesenchymal markers (Fibronectin, N-cadherin, and Vimentin). Conversely, stable silencing of Br-positive cell lines resulted in down-regulation of mesenchymal markers and up-regulation of epithelial markers. Br expression in tumor cells also correlated with resistance to chemotherapy and radiation. Analysis for Br expression by RT PCR and immunohistochemistry indicated over-expression in carcinoma cells and no expression in normal tissues, except for thyroid and testes. There was a positive correlation between stage and grade of tumor and the expression of Br. The vaccine induces Br-specific T-cell responses in vivo in a mouse model and in vitro in human T-cells. In a mouse model, vaccination resulted in decreased number of metastases compared with control. We have also identified Br specific T-cells in patients (pts) vaccinated against other antigens presumably via cross presentation of antigen.

Methods: This phase I, open-label trial with sequential dose escalation cohorts of subjects (3-6 per dose cohort) receiving the Yeast-Brachyury vaccine (GI-6301). The vaccine will be administered subcutaneously at 4 sites biweekly for 7 visits, then monthly until progression, as defined by immune-related response criteria. Ten additional pts will be enrolled on the maximum tolerated dose to assess for immunologic and clinical responses. Enrollment is ongoing and will include up to 28 pts.

Results: To date, we have enrolled 8 patients on trial (3 on DL 1, 4 on DL 2, and 1 on DL 3. No patient has experienced a grade 3 or 4 adverse event as defined by Common Terminology Criteria for Adverse Events (CTCAE) v 4.0. The most common adverse event has been grade 1 or 2 injection site reaction. We are currently in the process of screening for enrollment of the final 5 patients for DL 3. After dose escalation is complete, we will enroll 10 additional patients at the MTD for further immune analysis

**Notes:**

**Chetan Bettegowda, MD, PhD**

**Saurabh Saha, MD, PhD**

***Phase I Study of a Novel Hypoxia Targeting Agent for the Treatment of Solid Tumors***

**Johns Hopkins Medicine,** Baltimore, MD

Hypoxia is a hallmark of most solid tumors. In order to exploit hypoxia for therapeutic benefit we developed a genetically attenuated bacterial strain, coined *C. novyi-NT*, that has been shown to specifically target tumors and cause dramatic therapeutic responses in pre-clinical models. A Phase I study examining the safety and efficacy of this agent is currently underway and individuals with recurrent chordomas may be eligible to participate.

**Notes:**

**Shreyaskumar Patel, MD**

***Collaborating with SARC: A Sarcoma Clinical Trials Consortium***

**MD Anderson Center,** Houston, TX

Sarcoma Alliance for Research through Collaboration (SARC) is a consortium representing laboratory and clinician scientists who have a common interest in developing new and effective diagnostic and therapeutic options for patients diagnosed with bone and soft-tissue tumors. SARC is a 501c3, non-for-profit entity incorporated in 2003 with an operations office located in Ann Arbor, MI.

SARC was established to provide the infrastructure needed for physicians with expertise in sarcoma to move forward the science and understanding of the prevention, diagnosis and treatment through clinical trials research. Physicians with sarcoma expertise provide the scientific and operational oversight to the collaborative clinical trials.

The major goal of SARC is to provide the public with clinical trial results that are based on quality data that have been collected and analyzed with rigorous standards and high integrity. SARC partners with a variety of different organizations in order to obtain and study important new agents in sarcoma. SARC has worked with the Department of Defense (DoD), the European Organization for Research and Treatment of Cancer (EORTC) as well as the Cancer Therapeutic Evaluation Program (CTEP). SARC also works collaboratively with pharmaceutical companies who often provide investigational agents and research funding. Any interested investigator is encouraged to submit a concept which is reviewed by the Concept Review Committee and the Clinical Research Committee to help strengthen the final protocol overseen by SARC. During the last 10 years, SARC has conducted 9 phase 2 and 4 phase 3 clinical trials resulting in 9 publications. SARC has the flexibility and welcomes opportunities for new and creative ways to collaborate and advance science.

**Notes:**

**Chetan Bettegowda, MD, PhD**

***AOSpine Tumor Knowledge Forum Biobank***

**Johns Hopkins Medicine,** Baltimore, MD

The AOSpine Tumor Knowledge Forum was created to improve our understanding of spine tumors. It is a collaborative effort involving high volume spine tumor centers around the world. One aim of this group is to develop a biobank consisting of high quality frozen tumor and normal tissue from primary spinal column tumors that are linked to well curated de-identified clinical data. Chordomas represent the primary tumor type that will be collected and analyzed with these efforts.

**Notes:**

**Carmen Vleggeert-Lankamp, MD, PhD**

***Prospective chordoma patient database: a European initiative***

**Leiden University Medical Center,** Leiden, Netherlands

Because of the extremely low incidence rate and limited cooperation amongst physicians of different institutions, it is very difficult to gather sufficient chordoma patients for clinical trials and prospective cohort studies. During the Amsterdam round-table conference of June 2012, a proposal was made to create a Europe-wide prospective web-based chordoma patient database. As far as we know, no such continent-wide project exists for chordoma research.

The Leiden University Medical Center sarcoma team – physicians and researchers from all disciplines involved in sarcoma treatment – in cooperation with the Chordoma Foundation, took on the task of developing a concept version of the database, which was presented during the CTOS conference in Prague in November 2012. The overall reaction to this presentation was a positive one and physicians from several institutions have agreed to cooperate with the project. A number of issues, such as funding, were also discussed and we are currently busy implementing solutions.
The database is a strong instrument to provide data for research, in particular for Comparative Effectiveness Research (CER) projects, making use of variation between centers and countries, and virtual tissuebanking. Moreover, the database could potentially serve as a quality control mechanism, allowing clinicians from different institutions to (anonymously) compare treatment outcome. The database should also contain data on value in health care, to assess the outcome and the burden for the patient, together with sustainability of care, so that edit value for the patient can also be distilled (according to Porter's Outcome Measures Hierarchy[1]). Because of important differences in legislation, it has been decided that for the time being, this will be a European database only. If in due time the database proves to be a success, extending its reach across the Atlantic to the US is a viable option.

In conclusion, this is a unique and important development in the world of chordoma research. We are keen to share our knowledge and experience and we look forward to discussing the further development with experts from around the world.

[1]Porter ME. What is value in health care? **N Engl J Med**. 2010;363(26):2477-81

**Notes:**

**Kathryn Hess, MD, MSW**

***Beyond the Surgical Treatment – Developing Novel Interactive Tools for Patient–reported Quality of Life Data Collection***

**Massachusetts General Hospital,** Boston, MA

Chordoma is a very rare slow-growing tumor of the spine. In the United States, the incidence of these tumors is only 300 per year. Given the scarcity of these cases, very little is known about the best course of clinical management for the patients. Still less is known about how patients fare after treatment.Surgery remains the mainstay treatment for most chordoma patients; nevertheless it is invasive and oftentimes results in a severe neurological deficit. Our group at the Massachusetts General Hospital Chordoma Center developed a novel interactive online tool for collecting patient-reported quality of life data to help answer these questions. We were able to use the REDCap platform – Research Electronic Data Capture –to effectively collect information about overall health, functional status, and emotional health of our patients before and after treatment. Using this never before collected data, we can help guide the decisions of our patients and their families about their health and treatment.

**Notes:**

**Josh Yamada, MD FRCPC**

***Novel mechanisms of response for stereotactic radiosurgery: food for thought for the management of Chordomas***

**Memorial Sloan-Kettering Cancer Center,** New York City, NY

Image guided technology has made the safe delivery of very high dose per fraction radiation feasible. The mechanisms of response of high dose per fraction radiation therapy is likely significantly different than conventionally fractionated radiotherapy. This is manifest in the high rates of response seen with stereotactic radiosurgery (2400cGy in a single fraction) seen for radioresistant tumors such as melanoma and sarcoma metastases to the spine in excess of 90% local control. Short term results with chordoma also suggests an equally high rate of favorable response. Preclinical models suggest that tumor endothelial apoptosis mediated by the acid sphingomylinase/ceramide pathway may play a critical role in response, a mechanism not seen in conventionally fractionated regimens. Dynamic contrast enhanced MRI seems to also demonstrate endothelial response in patients. Also, immune responses may also play an important role in response to high dose per fraction radiation. These mechanisms of response may be important for response in chordomas to high dose per fraction radiotherapy.

**Notes:**

**Tom DeLaney, MD**

***Updates on Proton Clinical Trials for Chordomas***

**Massachusetts General Hospital,** Boston, MA

Objective: High radiation therapy (RT) doses are required for RT of chordoma. As RT effects are mediated via oxygen free radicals and hypoxia impairs RT, we sought (1) to assess whether hypoxia could be detected and localized in chordoma patients (pts) undergoing RT, (2) whether there was any detectable change in hypoxia after 24-36 Gy of RT, and (3) whether focal hypoxic areas might theoretically undergo selective RT dose intensification with dose-painted intensity modulated proton therapy (IMPT).

Materials/Methods: Prospective, IRB-approved clinical trial involved 20 pts $\geq$ age 18 treated with definitive or preoperative RT (proton or combined photon/proton) for primary or locally recurrent chordoma after surgery. Pts underwent F-18 misonidazole(18FMiso) PET/CT scan before start of RT and after 24-36 Gy. For scans, pts were injected with 350-400 MBq of 18FMiso 2 hours prior to PET/CT. 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. Pts with hypoxic subvolumes underwent a RT planning study to evaluate the feasibility of delivering focal RT dose intensification to the hypoxic subvolume within the GTV with dose-painted IMPT.

Results: Between 1/13/2009 and 4/26/2010, 20 pts (19 primary and 1 locally recurrent) with chordomas (16 sacrococcygeal, 3 lumbar, 1 cervical) were enrolled to the protocol. Tumor size ranged from 17-2398 cc (median 101 cc, average 462.60 cc). Per protocol, all pts underwent scans prior to the start of RT and after 24-36 Gy. Seven pts had hypoxia (as defined above) noted on both scans, 2 pts had hypoxia noted only on the 1st scan, 3 pts had hypoxia seen only on the 2nd scan, while 8 pts had no evidence of hypoxia on either scan. Among the 19 scans showing hypoxia, hypoxic subvolume ranged from 0.04-418.81 cc (median 7.72 cc, average 57.45 cc). Among the 9 pts with hypoxia seen on the scans before RT, the % hypoxic volumes ranged from 0.0392% to 36.3% (median 4.06%, average 6.33%). Among the 10 pts with hypoxia seen after 24-36 Gy, the % hypoxic volumes ranged from 0.261% to 55.8% (median 1.73%, average 12.2%).

Conclusions: Some pts with chordomas have hypoxic subvolumes within the GTV, as assessed by 18FMiso PET/CT scans. The clinical significance of this finding will require additional follow-up in these pts. The dosimetry planning studies of the feasibility of RT dose intensification to sites of hypoxia with IMPT are in progress.

**Notes:**