



## SPEAKER ABSTRACTS

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### **Rare germline variants in BRCA2 and PALB2 in familial and sporadic chordoma**

Rose Yang

*National Cancer Institute*

Chordoma is a rare bone tumor, which is believed to originate from notochordal remnants. Chordoma is typically sporadic, however, chordoma occasionally occurs in a familial setting, usually in an autosomal dominant pattern. We previously identified germline T duplication as a major susceptibility mechanism in several chordoma families. However, genetic causes in the majority of sporadic cases and some chordoma families remain unknown. The goal of this study was to identify additional susceptibility genes in T-negative families (five families with 19 cases) and sporadic cases (N=137) using whole-exome sequencing. We focused on rare exonic variants that were present in 0.1% in the 1000 Genomes Project (1,092 subjects), ESP (6,500 subjects), 500 healthy in-house population-based controls of European ancestry, and 1 family from our in-house database of >900 cancer-prone control families (excluding chordoma families and sporadic chordoma cases). We identified a missense non-synonymous (NS) variant in PALB2 (c.1042C>A, p.Gln348Lys) that was present in all three chordoma cases and one obligate gene carrier in one chordoma family. This variant was reported in 0.00023 and 0.00015 of individuals of European ancestry in ESP and The Exome Aggregation Consortium (ExAC), respectively. Five additional rare NS variants in PALB2 were identified, each in a single sporadic case. PALB2 is a binding partner of BRCA2 and functions as a tumor suppressor gene. One of the top biological processes involving BRCA2 is chordate embryonic development whose specific outcome is the progression of the embryo over time, from zygote formation through a stage including a notochord and neural tube until birth or egg hatching. Interestingly, we also identified 11 rare variants in BRCA2 in 10 sporadic cases. Among these variants, one was a stopgain, one was a frameshift, and the others were NS missense variants. Two of these variants were classified as DM (disease-causing mutation) and one variant was classified as uncertain DM in the Human Gene Mutation Database for breast cancer and/or ovarian cancer. Results from the rare variant burden test showed that chordoma cases had a significantly higher number of rare exonic variants in PALB2 (P=0.028) and BRCA2 (P=0.025) compared to 500 population controls. We plan to validate all promising genes/variants using targeted sequencing and design experimental assays to evaluate the functional relevance of these variants.



## **The genomic landscape of chordoma**

Patrick Tarpey

*Sanger Institute*

We have performed a detailed investigation of the genomic variation responsible for chordoma utilising whole genome, whole exome or targeted resequencing in over 100 tumours. We performed analyses to identify variants implicated in chordoma development, and identified drivers in multiple known cancer genes including those responsible for chromatin remodelling and PI3-kinase signalling. A targeted analysis to detect Brachyury (T) amplification identified mutations in > 20% of cases. Inactivating mutations in the lysosomal trafficking regulator, LYST were identified in multiple cases, suggesting LYST may be a novel cancer gene in chordoma development.



## **Epigenetic profiling reveals a unique histone code in chordoma**

Nelson Moussazadeh

*Memorial Sloan Kettering*

### **Purpose/Objectives**

Pathognomonic genetic “driver” lesions to explain the brachyury transcriptional program have not been described in chordoma. Herein we characterize the epigenetic histone and somatic chromatin-modifying genotypic (CMG) landscape of chordoma.

### **Materials/Methods**

Six chordomas with matched germline tissue representing a spectrum of location (sacral, clival, mobile spine), histopathology (classical and dedifferentiated) and stage (primary and post-irradiation recurrences) underwent mass spectrometric histone profiling. Genotype was obtained via exome sequencing.

### **Results**

Histone marks were highly conserved across chordoma samples regardless of site of origin or histopathology. Pathognomonic histone marks included hypermethylated lysine 27 on H3.1 (H3.1K27) compared to a cohort of 5 cancer cell and 1 normal cell lines ( $p = 4.8e-10$ ), marks associated with transcriptional repression. Other significant alterations included H3K4 hypermethylation, predicted on the basis of brachyury overexpression. Genotype further predicted some degree of variability across chordoma samples, with CMG alterations including lysine demethylase loss at the predicted residues.

### **Conclusions**

In this first utilization of mass spectrometric analysis of a solid tumor, chordoma harbors a histone code distinct from other profiled neoplastic and normal tissues.



## **Emerging microRNAs (miRs) roles and strategies in chordoma**

Zhenfeng Duan

*Massachusetts General Hospital*

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 several miRs including miR-1 and miR-155 are frequently deregulated in various types of cancer including chordoma. Through targeting multiple oncogenes or tumor suppressor genes pathways, these miRs have been demonstrated play important roles in chordoma cell growth and proliferation. In this presentation, we highlight these recent findings on the aberrant expression and functional significance of miRs in chordoma and emphasize their significant values for chordoma therapeutic potentials.



## **Establishment and characterization of chordoma cell lines as cell model systems**

Thomas Barth

*Institute of Pathology, Ulm, Germany*

Chordoma cell lines are very important systems for research in this rare disease. Up to now, several cell lines originating from primary tumors have been established to create cell culture models of this orphan disease. Due to the rarity and the slow cellular growth of this tumour entity the generation of stable cell lines is a challenging task. A very close cooperation of an interdisciplinary team of oncologists, surgeons, pathologists and specialized cell culture scientists is essential to overcome these problems. In this way we have established 9 chordoma cell lines. Here, we present three novel cell lines. U-CH11R was set up from a recurrent tumor of the same patient of which the cell line U-CH11 was established 3 years earlier. U-CH17KM and U-CH17DJ were derived from material of two metastases of a 38 year old chordoma patient from different locations (a cutaneous and soft tissue metastasis, respectively). All three lines show typical chordoma features with physaliphorous cell morphology and protein expression of Brachyury, S-100, EMA, and CD24. Corresponding to the slow growth of the primary tumors, most chordoma cell lines show very long population doubling times. The metastases cell lines U-CH17KM and U-CH17DJ contrariwise grow fast indicating substantial differences compared to lines derived from the primary tumors. To determine relevant pathways and genomic abnormalities of tumor progression and evolution, we performed array-based expression analyses and comparative genomic hybridization. Hence, we established three true new chordoma cell lines as model systems for chordoma recurrence and metastasis to enhance the understanding in chordoma progression and evolution over time.



## **Establishment of patient derived chordoma xenograft models for in vivo testing: opportunities and challenges**

Gary Gallia

*Johns Hopkins University*

Accurate model systems are essential in the preclinical evaluation of therapeutics. Cell line-based and patient derived xenograft (PDX) models have recently been described in chordoma. In this talk, we will summarize our experience in the generation of chordoma PDXs, highlight in vivo therapeutic analysis using these model systems, and describe limitations of these models.



## **START-Chordoma Foundation preclinical collaboration: Establishment and evaluation of patient derived xenograft (PDX) and cell-based chordoma models**

Michael Wick

*South Texas Accelerated Research Therapeutics (START)*

Chordoma is a rare cancer originating from the notochord and presenting in the skull and spine. Treatment options for chordoma include resection and local radiation therapy; however, recurrence rates following treatment are high and no proven effective treatment options are available. Recent studies have identified actionable molecular targets of potential importance in the pathogenesis and progression of chordoma; however, lack of validated preclinical chordoma models has limited detailed analysis.

To address this need, START and the Chordoma Foundation have formed a unique collaboration to establish, develop and characterize preclinical chordoma tumor models. START has previously established over 1300 human tumor xenograft models from patient samples and cancer cell lines including uncommon and rare cancers and the Chordoma Foundation has created a network of medical professionals, researchers, and patient advocates to facilitate donation of chordoma tissue and fund model development and characterization.

As of May, 2016, twenty one samples have been engrafted from twenty chordoma patients. Of these, four have been established as *in vivo* models and six additional implants are reporting sustained growth. In addition two chordoma models have been recapitulated from START and from Chordoma Foundation collaborators and two others established from cell lines. Three of these models have been tested *in vivo* against a panel of approved and investigational therapies.

Overall through this collaboration we have implanted over twenty chordoma tumor samples in less than twelve months and have an establishment rate of at least 20% with potential increase up to 50% depending on implant growth. In addition we have tested several anti-tumor agents in PDX and cell-based chordoma models and are developing a preclinical platform to allow for efficient evaluation of therapies for the treatment of chordoma.



## **Lineage-directed expression of Brachyury induces chordoma in mice**

Michael J Kelley

*Duke University, Durham VA Hospital*

**Background:** Chordoma is a slow growing cancer of the bony structures of the axial skeleton. Expression of Brachyury (T) is a defining characteristic. Brachyury has been implicated in chordomagenesis by genetic association of one or more additional gene copies and high penetrance of familial chordoma. Furthermore, inhibiting T expression or function inhibits chordoma cell lines. However, T does not have transforming activity in vitro.

**Methods:** We created a murine transgenic that allows Cre recombinase induced activation of T expression in a tissue specific manner. The transgene is a tandem construct of the chicken beta-actin promoter, a transcriptional stop sequence flanked by lox sites, T, IRES sequence and the green fluorescent protein (GFP) inserted by homologous recombination at the ROSA26 locus of the R1 (129Sv) strain. In this mouse (FS-T, Flox-stopped-T), Cre results in excision of the transcriptional stop sequence and constitutive transcription of T and GFP from the beta-actin promoter.

**Results:** FS-T mice were healthy and the transgene segregated with the expected Mendelian frequency. Activation of T in embryonic notochord was accomplished by mating FS-T to mice containing Cre-recombinase downstream of the native sonic hedgehog promoter (Shh-Cre). Shh-Cre/FS-T pups died at birth with heart, lung, kidney and vertebral defects suggesting expression of T in Shh-expressing tissues outside the notochord resulting in altered development of one or more of these organs. Next, we used a Noto-Cre strain containing a developmentally regulated promoter known to be involved in spinal development. Dual heterozygous Noto-Cre/FS-T pups were viable, segregated at the expected Mendelian frequency and had no abnormal phenotype to adulthood. Among 40 mice aged to at least 18 months, two female mice developed small tumors on their tails. Histologically, these tumors showed a characteristic physaliferous pattern by light microscopy and T and keratin expression by immunohistochemistry similar to human chordoma. Triple transgenic Noto-Cre/FS-T/floxed-p53 mice did not develop tumors at 1 year.

**Conclusion:** This Noto-Cre/FS-T transgenic strain further supports the role of Brachyury in genesis of chordoma. A shorter tumor latency period is being pursued by crossing in additional transgenes.





## Seeing cancer where it starts: modeling chordoma in zebrafish

Alexa Burger

*Institute of Molecular Life Sciences, University of Zurich*

The earliest steps of chordoma formation from notochord cells remain unclear, as the tumor has not been observed at its earliest stages when it arises. The goal of our project is to model, observe, and modulate the initial phases of chordoma formation in the first animal model for chordoma in zebrafish (*Danio rerio*). Our work aims to provide a modular genetic system to test tumor-causing genes to clarify the unknown tumorigenic mechanisms in chordoma and to uncover novel therapeutic approaches.

To study chordoma and its gene-regulatory program *in vivo*, we use our recently established first animal model for chordoma in zebrafish. The zebrafish embryo provides a potent chordoma model: the genetically malleable and transparent embryos form their notochord within 24 hours post-fertilization, providing an ideal readout for chemical compounds and genetic tests. Our zebrafish chordomas rapidly form within 3-5 days in developing notochord cells that mimic constitutive receptor tyrosine kinase (RTK) pathway activation by transgenic HRAS<sup>V12</sup> expression. The resulting zebrafish tumors share extensive histological characteristics with human chordoma and can be observed and imaged in the developing zebrafish embryo. The zebrafish chordoma cells respond to treatment with the mTOR inhibitor rapamycin, establishing a proof-of-principle for the screening and *in vivo* testing of novel therapeutic compounds.

Based on our tumor-modeling and developmental work, we hypothesize that early chordoma initiation combines constitutive receptor tyrosine kinase signaling, such as mediated by the EGF or FGF pathways, with co-activation developmental regulatory genes involved in notochord formation. Using latest transgenic tools for zebrafish, we are applying oncogene overexpression and CRISPR-Cas9-based tumor suppressor mutagenesis to for the first time validate chordoma candidate genes for their tumor-forming potential.



**Defects in HLA class I antigen processing machinery in chordoma. Potential clinical implications.**

Soldano Ferrone

*Departments of Surgery and of Orthopedic Surgery, MGH, Boston, MA*

The revival of interest in the role of immune surveillance in the control of tumor growth and the impressive clinical responses in patients treated with immunotherapy have prompted us to analyze the expression of immunologically relevant molecules in chordoma. In this presentation we describe defects in the expression of HLA class I antigen processing machinery components in chordoma. In addition we discuss the potential clinical implications of these findings.



## **Anti-PD-1 therapy for metastatic chordoma**

Michael Lim

*Johns Hopkins University*

The refractoriness of metastatic chordoma mandate new approaches. Immunotherapy offers the ability to provide a durable and specific anti tumor immune response. We will discuss the preclinical results of anti-PD-1 in a relevant preclinical model and a planned Phase I clinical trial using anti-PD-1 in patients with metastatic chordoma.



## **Immunogenic modulation of chordoma cells results in enhanced immune cell killing: Foundation for combination therapy clinical trials**

James W. Hodge

*Laboratory of Tumor Immunology and Biology  
Center for Cancer Research, National Cancer Institute*

Chordoma, a rare bone tumor derived from the notochord, has been shown to be resistant to conventional therapies. Our laboratory conducts studies to provide the foundation for combining immunotherapy to induce NK cells or tumor antigen-specific T cells with standard-of-care therapies. We have recently defined “immunogenic modulation,” whereby tumor cells surviving conventional therapy nonetheless become more susceptible to immune mediated lysis. Within the context of chordoma, we will discuss the rationale for combining immunotherapy with either a) proton radiation, or b) a novel anti-PD-L1 monoclonal antibody. As chordoma tumors are frequently treated with proton radiation, we examined the effect of proton radiation on the viability and induction of immunogenic modulation. There, proton and photon radiation induced comparable upregulation of surface molecules involved in immune recognition (HLA, ICAM-1, and the tumor-associated antigens CEA and MUC-1). In addition, proton radiation mediated calreticulin cell-surface expression, increasing sensitivity to cytotoxic T-lymphocyte killing of tumor cells.

Checkpoint inhibition has shown great promise in immune-mediated therapy of diverse cancers. The anti-PD-L1 mAb avelumab is unique among checkpoint inhibitors in that it is a fully human IgG1 capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC) of PD-L1-expressing tumor cells. We investigated avelumab as a potential therapy for chordoma. We examined 4 chordoma cell lines, first for expression of PD-L1, and *in vitro* for ADCC killing using NK cells and avelumab. PD-L1 expression was markedly upregulated by IFN- $\gamma$  in all 4 chordoma cell lines, which significantly increased sensitivity to ADCC. Brachyury is a transcription factor that is uniformly expressed in chordoma. Clinical trials are ongoing in which chordoma patients are treated with brachyury-specific vaccines. Co-incubating chordoma cells with brachyury-specific CD8<sup>+</sup> T cells resulted in significant upregulation of PD-L1 on the tumor cells, mediated by the CD8<sup>+</sup> T cells’ IFN- $\gamma$  production, and increased sensitivity of chordoma cells to avelumab-mediated ADCC. Residential cancer stem cell subpopulations of chordoma cells were also killed by avelumab-mediated ADCC to the same degree as non-cancer stem cell populations. These findings offer a rationale for the use of select standard-of-care or emerging immunotherapeutics in combination with immunotherapy.



## **Brachyury expression and function in human carcinomas**

Duane Hamilton, PhD

*Center for Cancer Research, National Cancer Institute*

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 effects of conventional anti-tumor therapies. Recently, we have developed a highly specific rabbit monoclonal anti-brachyury antibody, and have used it to characterize brachyury expression in several thousand tumor samples comprising multiple tumor types, where we observed high levels of nuclear brachyury expression in chordoma, germ cell tumors and small cell lung cancer. Furthermore, using this antibody, we consistently observe a short brachyury isoform in several human carcinoma cell lines, and are currently assessing the potential biological role for this brachyury isoform in human cancers.



## **Characterization of protein-protein interactions for brachyury**

Ernest Radovani

*University of Toronto*

Brachyury is a TF (transcription factor) that has been closely associated with the occurrence of chordoma. Targeting of Brachyury could be an important therapeutic treatment for the disease. However, TFs have proven to be nearly impossible to inhibit directly with small molecule drugs. TFs generally bind other proteins to control the transcription of various genes, and the functions of these other proteins may be more readily disrupted with small molecules as they contain active sites. In order to have the ability to target transcription pathways regulated by Brachyury, we have undertaken an effort to identify and functionally characterize protein-protein interactions for this TF. Using affinity purification coupled to mass spectrometry, among other proteins, we have identified BRCA2 and ZMYM4 as putative cofactors of Brachyury. Consistent with its role in DNA stability, BRCA2 mutations have been widely linked to many cancers. Furthermore, BRCA2 has also been linked to transcription regulation through recruitment of the histone acetyltransferase PCAF. On the other hand, ZMYM4's role in transcription is poorly characterized. However, we show that in addition to Brachyury, ZMYM4 co-purifies with 19 other TFs, as well as with other proteins involved in epigenetic modification of chromatin, such as the acetyl reader BAZ2A as well as RCOR2, a member of the KDM1A histone demethylase complex. Altogether these interactions implicate ZMYM4 in transcription regulation. Given their physical interactions with Brachyury and their association with gene expression regulation, BRCA2, ZMYM4 and their associated proteins could represent drug targets in chordoma. Currently, we are in the process of confirming these interactions in various chordoma cell lines.



## **T-Brachyury reporter system for high-throughput screening**

Slim Sassi

*Massachusetts General Hospital*

A T-Brachyury transcriptional reporter was used to construct chordoma based stable cell lines for high-throughput screening. The system was used to perform an overexpression screen of a cDNA library. The screen identified activators and some inhibitors of T Brachyury. This effort is directed towards a better understanding of the T Brachyury pathway in chordoma and identification of practical drug targets. Further, we adapted the system for high-throughput small molecule screens. An FDA approved panel and a novel library was screened. A small number of hit compounds were further validated.



## **Systematic identification of chordoma vulnerabilities**

Tanaz Sharifnia

*Broad Institute*

This presentation will describe our efforts to systematically identify chordoma dependencies through the application of genetic, epigenetic, and small-molecule sensitivity profiling approaches.





## **Developing therapies for transcriptionally addicted cancers**

Nathanael Gray

*Dana Farber Cancer Institute*

Tumour oncogenes include transcription factors that co-opt the general transcriptional machinery to sustain the oncogenic state, but direct pharmacological inhibition of transcription factors has so far proven difficult. However, the transcriptional machinery contains various enzymatic cofactors that can be targeted for the development of new therapeutic candidates, including cyclin-dependent kinases (CDKs). Here we present the discovery and characterization of a covalent CDK7 inhibitor, THZ1, which has the unprecedented ability to target a remote cysteine residue located outside of the canonical kinase domain, providing an unanticipated means of achieving selectivity for CDK7. Cancer cell-line profiling indicates that a subset of cancer cell lines, including human T-cell acute lymphoblastic leukaemia (T-ALL), have exceptional sensitivity to THZ1. Genome-wide analysis in Jurkat T-ALL cells shows that THZ1 disproportionately affects transcription of RUNX1 and suggests that sensitivity to THZ1 may be due to vulnerability conferred by the RUNX1 super-enhancer and the key role of RUNX1 in the core transcriptional regulatory circuitry of these tumour cells. Pharmacological modulation of CDK7 kinase activity may thus provide an approach to identify and treat tumor types that are dependent on transcription for maintenance of the oncogenic state.



## **The role of TGFb3 in the pathogenesis of chordoma**

Wei Chen

*CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, CAS*

To identify the important pathway in the formation of chordoma, we conducted miRNA and mRNA sequencing in two chordoma tissues and two fetal notochords, which were seen as normal control tissue of chordoma. We found the differentially expressed mRNA-miRNA pairs were enriched in TGFB pathway. And the chordoma up-regulated miR-29b-3p and its down-regulated target gene TGFB3 were further validated in additional 8 chordoma tissues and 8 notochords. There is a significant negative correlation between the expression of TGFB3 and miR-29 in chordoma samples ( $r^2=0.87$ ). Lacking of such a correlation in notochords suggests the down-regulation of TGFB3 in chordoma were mainly caused by up-regulation of miR-29. We further compared the copy number of miR-29 gene loci between chordoma tissues and their paired blood samples. Among the 8 chordoma patients, 5 had somatic copy number gain at miR-29b1 and/or miR-29b2 loci. In addition, in seven chordoma samples with enough blood controls, somatic copy number loss of TGFB3 were observed in 4/7 samples. The upregulation of miR-29 and downregulation of TGF $\beta$ 3 were also found in a chordoma cell line UM-Chor1 and proliferation inhibition was observed by TGF $\beta$ 3 treatment. Moreover, in a zebrafish model in which TGF $\beta$ 3 was knocked down by morpholino-oligomers, chordoma formation was revealed by H&E staining and immunohistochemistry in larvae. By whole exome sequencing of 4 chordoma tissues and paired blood samples, several somatic mutations were also found to affect genes related to TGFB signaling pathway. These results demonstrate, for the first time, that the dramatic inhibition of TGF $\beta$ 3 signaling by multiple somatic changes is a major cause of chordoma development.



## **The CDK4/CDK6 pathway as a target for growth inhibition of chordomas cell lines**

Thomas Barth

*Institute of Pathology, Ulm, Germany*

Although chordomas are rare in incidence, they are often deadly owing to slow growth and a lack of effective therapeutic options. In this study, we addressed the need for chordoma cell systems that can be used to identify therapeutic targets and empower testing of candidate pharmacologic drugs. Eight human chordoma cell lines that we established exhibited cytology, genomics, mRNA, and protein profiles that were characteristic of primary chordomas. Candidate responder profiles were identified through an immunohistochemical analysis of a chordoma tissue bank of 43 patients. Notably, all cells exhibited a loss of CDKN2A and p16, resulting in universal activation of the CDK4/6 and Rb pathways. Therefore, we investigated the CDK4/6 pathway and responses to the CDK4/6-specific inhibitor palbociclib. In the newly validated system, palbociclib treatment efficiently inhibited tumor cell growth in vitro and a drug responder versus non-responder molecular signature was defined on the basis of immunohistochemical expression of CDK4/6/pRb (S780). Overall, our work offers a valuable new tool for chordoma studies including the development of novel biomarkers and molecular targeting strategies.

Cancer Res. 2015 Sep 15;75(18):3823-31.



## **Novel protein phosphatase 2A inhibitor, LB100, sensitizes chordoma cells to irradiation**

Shuyu Hao

*National Cancer Institute*

**INTRODUCTION:** Patients with chordoma, a rare bone cancer of the axial skeleton, face limited therapeutic options. Although patients are offered surgical resection often followed by irradiation, there are no effective medical therapies. Protein phosphatase 2A (PP2A) is a ubiquitously expressed serine/threonine phosphatase involved in signal transduction, cell cycle, cell differentiation, and DNA repair. LB100, a small molecule inhibitor of PP2A, has been shown to sensitize cancer cells to DNA damage from chemotherapy and irradiation. Here, we investigated the radiation sensitizing potential of LB100 in chordoma.

**METHODS:** Four patient derived chordoma cell lines, UCH1, UCH2, JHC7, and UM-Chor1 were used in this study. Proliferation assay assessed cell viability at various doses of LB100. Quantitative  $\gamma$ -H2AX immunofluorescence and immunoblot evaluated LB100 effect on radiation-induced cytotoxicity. Cell cycle analysis was conducted with flow cytometry. In vivo xenograft model was established to determine potential clinical utility of adding LB100 to irradiation.

**RESULTS:** LB100 demonstrated dose-dependent in vitro growth inhibition of chordoma cells 48 hours after treatment. LB100 enhanced radiation-induced DNA double-strand breaks. LB100 combined with radiation induced cell cycle progression to G2/M phase. Cell cycle analysis revealed G2/M phase arrest. Animals treated with the combination of LB100 and irradiation demonstrated striking tumor growth delay compared to those receiving irradiation or LB100 alone.

**CONCLUSION:** Combining LB100 to irradiation enhanced DNA damage and cell death, and delayed tumor growth in mice. Addition of LB100 may improve effectiveness of irradiation in chordoma.



## **Afatinib: a promising therapeutic approach in chordoma**

Paola Magnaghi

*Nerviano Medical Sciences, Nerviano, Italy*

Chordomas are rare, slowly growing bone tumors that arise from notocord remnants, for which no active standard medical therapy is available. Beside “Brachyury” transcription factor, a known major driver, chordomas were shown to express several tyrosine kinase receptors. The wide expression of MET, PDGFR and EGFR in chordomas prompted different attempts to treat these tumours with tyrosine kinase inhibitor drugs. Although clinical responses have been reported, particularly with imatinib, these treatments cannot cure the disease. Sporadic clinical responses have been reported also to EGFR inhibitors, but a clinical study with lapatinib could not prove a clear benefit.

We found that a panel of 7 chordoma cell lines was insensitive to Met and PDGFR inhibitors in vitro. We then tested the four registered EGFR inhibitor drugs, including lapatinib, and other EGFR inhibitors in clinical development, and found that U-CH1 and UM-Chor1 were exquisitely sensitive to all inhibitors, while the remaining cell lines were insensitive. Afatinib was the only EGFR inhibitor drug active across the chordoma cell line panel. Afatinib also displayed strong antitumor efficacy in U-CH1 xenograft and in a PDX chordoma model in vivo.

These data confirm that a subset of chordomas may be particularly sensitive to EGFR inhibition and that afatinib may be superior to other EGFR inhibitors in the treatment of chordomas, providing a strong rationale to explore its efficacy in the clinical setting.



## **Use of circulating tumor DNA as a biomarker for individuals with chordoma**

Chetan Bettegowda

Johns Hopkins Medicine

A hallmark of cancers is the presence of somatic mutations that drive the neoplastic process. These somatic mutations are harbored within the DNA of tumor cells and can be used for both diagnostic and therapeutic purposes. We, and others, have shown that many cancers shed molecules of cell free DNA into the bloodstream. Tumor derived cell free DNA (ct-DNA) contain mutations that can be used as a personalized biomarker of disease burden.

We set out to determine whether spinal chordomas shed ct-DNA into the circulation. Our pilot study suggests that many spinal chordomas do shed appreciable amounts of ct-DNA in the bloodstream but more longitudinal samples need to be analyzed to better understand the relationship between ct-DNA levels and disease burden. We will present our initial experience in analyzing ct-DNA levels in plasma samples derived from individuals with chordoma.



## **Computing cancer: Applications for mathematical and computational modeling in cancer**

Kimberly Luddy

*Moffitt Cancer Center*

The role of mathematical and computational biology in basic and clinical cancer research is expanding. This is due in part to the fact that recent scientific advances are highlighting the true complexity of this disease. In silico experiments can be used to test new treatment options, inform trial design, and gather a better understanding of trial outcomes. Mathematical biology combines experimental and clinical data to build models that allow us to test our current biological assumptions beyond what is currently possible in the lab or clinic. As researchers and clinicians it is imperative that we integrate more quantitative methods to navigate the wealth of new information and provide better outcomes for patients.