Emerging Therapeutic Targets in Chordomas: A Review of the Literature in the Genomic Era

Corey M. Gill, BS, BA *
Mary Fowkes, MD, PhD ‡
Raj K. Shrivastava, MD *

*Department of Neurosurgery, Mount Sinai Medical Center, Icahn School of Medicine at Mount Sinai, New York, New York; ‡Department of Pathology, Mount Sinai Medical Center, Icahn School of Medicine at Mount Sinai, New York, New York

Correspondence:
Corey M. Gill, BS, BA,
Department of Neurosurgery,
Mount Sinai Medical Center,
Icahn School of Medicine at Mount Sinai,
1 Gustave L. Levy Place,
New York, NY 10029.
Email: corey.gill@icahn.mssm.edu

Received, March 21, 2019.
Accepted, June 13, 2019.
Published Online, August 27, 2019.

Chordomas are rare primary malignant tumors of the bones that occur along the skull base, spine, and sacrum. Long-term survival and neurological outcome continue to be challenging with continued low percentages of long-term survival. Recent studies have used genome, exome, transcriptome, and proteome sequencing to assess the mutational profile of chordomas. Most notably, Brachyury, or T-protein, has been shown to be an early mutational event in chordoma evolution. Clinically actionable mutations, including in the PI3K pathway, were identified. Preliminary evidence suggests that there may be mutational differences associated with primary tumor location. In this study, we review the therapeutic landscape of chordomas and discuss emerging targets in the genomic era.

KEY WORDS: *Brachyury*, Chordoma, Genomic, Sacral, Sequencing, Skull base, Targeted therapy

Neurosurgery 86:E118–E123, 2020 DOI:10.1093/neuros/nyz342 www.neurosurgery-online.com

Chordomas are midline rare primary bone tumors of the notochord with an age-adjusted incidence rate of 0.08 per 100 000. Approximately 32% of cases present within the skull base, 32.8% along the mobile spine, and 29.2% are sacral.[1] Such anatomic locations involve eloquent neurological structures that interfere with maximal surgical resection, which is especially problematic given the chemoradiotherapeutic-resistant clinical phenotype that is seen in chordomas.[2] Survival is dismal with 5- and 10-yr survival rates of approximately 67.6% and 39.9%, respectively. Recurrence rates are high; efficacy of surgery or reirradiation in the recurrent setting is limited.[3] Therefore, there is an unmet need to identify more efficacious treatment options for patients with chordomas. Emerging advances in genomics and immunotherapeutics may offer additional treatments regimens in the near future.

BRACHYURY

The *Brachyury* gene, located on chromosome 6q27, is a T-box transcription factor necessary for specification of the mesoderm from the epithelium, a key regulator of notochord formation from which chordomas arise.[4-6] In 2006, *Brachyury* was expressed in all 53 analyzed chordomas and was able to discriminate chordoma from chondrosarcoma.[7] Subsequently, *Brachyury* was identified as a major susceptibility gene in familial chordoma across 4 families, each of which having greater than or equal to 3 cases of chordoma.[8] Next, *Brachyury* expression was confirmed in a patient-derived cell line, JHC7, from a sacral chordoma. Silencing of *Brachyury* in the cell line using shRNA led to growth arrest.[9,10]

Brachyury acts as a master regulator of a transcriptional network involved in oncogenesis.[11] Shah and colleagues[12] recently found that *Brachyury* provides direct transcriptional activation of Yes-associated protein (YAP), which is a well-known effector of the Hippo pathway and essential for organ development. Moreover, in a cohort of 27 chordoma samples, those with high expression of *Brachyury* had upregulation of genes associated with the PI3K/Akt pathway.[13] Indeed, patients with higher *Brachyury* expression had shorter progression-free survival (5 mo compared to

ABBREVIATIONS: CDKN2A, cyclin-dependent kinase inhibitor 2A; **EGFR,** epidermal growth factor receptor; **HR,** hazard ratio; **IHC,** immunohistochemistry; **PDGFR,** platelet-derived growth factor receptor; **PD-L1,** programmed death-1 ligand; **SNP,** single nucleotide polymorphism; **YAP,** Yes-associated protein

TABLE. Chordoma Clinical Trials in Progress

NCT Number	Title	Phase	Class
02383498	A randomized, double-blind, phase 2 trial of GI-6301 (yeast-Brachyury vaccine) versus placebo in combination with standard of care definitive radiotherapy in locally advanced, unresectable, chordoma	2	Vaccine
03595228	A phase 2 trial of BN-Brachyury and radiation therapy in patients with advanced chordoma	2	Vaccine
03083678	A phase 2, single arm, European multi-center trial evaluating the efficacy of afatinib as first-line or later-line treatment in advanced chordoma	2	Tyrosine kinase inhibitor
01407198	Phase I study of nilotinib given with radiation for patients with high-risk chordoma	1	Tyrosine kinase inhibitor
03110744	CDK4/6 inhibition in locally advanced/metastatic chordoma	2	CDK inhibitor
02989636	Phase I safety study of stereotactic radiosurgery with concurrent and adjuvant PD-1 antibody nivolumab in subjects with recurrent or advanced chordoma	1	Immunotherapy
02834013	DART: dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors	2	Immunotherapy
03173950	Phase II trial of the immune checkpoint inhibitor nivolumab in patients with select rare CNS cancers	2	Immunotherapy
03623854	A signal finding phase 2 study of nivolumab (anti-PD-1; BMS-936558; ONO-4538) and relatlimab (anti-LAG-3 monoclonal antibody; BMS-986016) in patients with advanced chordoma	2	Immunotherapy

13 mo). These findings support a collision in chordomas between a regulator of embryogenesis through *Brachyury* and well-established cancer pathways.

Additional analysis of germline DNA from 40 individuals with chordoma ancestry-matched to 358 controls revealed that single nucleotide polymorphism (SNP) rs2305089 within *Brachyury* confers an increased risk of 6.1 for sporadic chordomas.[14] Others verified that SNP rs2305089 conferred an increased risk for the development of sporadic chordoma, OR 2.85, and also with familial chordoma OR 2.6.[15] To determine if SNP rs2305089 was associated with clinical outcomes, analysis of archived tissue from 109 spinal chordomas revealed that 93.6% (102/109) of specimens harbored the variant.[16] Patients with the SNP rs2305089 variant had significantly improved survival compared to those without the variant (median survival 7.6 yr compared to 3.8 yr).

To evaluate whether *Brachyury* could be a targetable alteration in patients with chordoma, a phase I trial evaluated GI-6301, a yeast-Brachyury vaccine, in 7 patients with advanced chordoma (NCT01519817).[17] Of the 7 patients, 5 had progressive disease at the start of the study, resulting in one partial response, one patient with stable disease, and 3 patients with progressive disease at day 141 restaging. Analysis confirmed safety of the vaccine with corresponding immune response. Such promising results led to conducting a phase II trial of GI-6301, which is currently underway (NCT02383498) (Table).

A second phase I trial evaluated the safety of a Modified Vaccinia Ankara vector-based vaccine that expresses the transgenes for Brachyury as well as B7.1, ICAM-1, and LFA-3 (NCT02179515)[18] in 38 patients with advanced chordoma. T-cell responses were seen at all dose levels, and there were no reported dose-limiting toxicities. These findings resulted in a phase II trial in combination with radiation in patients with advanced chordoma, which is currently underway (NCT03595228).

GENOME AND EXOME VARIANTS

Comprehensive genomic evaluation of 104 cases of chordoma revealed recurrent somatic duplication of *Brachyury* in 27% of cases.[19] Additionally, 17% of samples had alterations in chromatin modeling genes, including *ARID1A*, *PBRM1*, and *SETD2*, as well as 16% in PI3K signaling genes, including *PIK3CA*, *PIK3R1*, and *PTEN*. Interestingly, recurrent alterations in *LYST* were identified in 10% of samples, a finding that may indicate a novel cancer gene in chordomas. Moreover, no fusion events were identified. Although these results are encouraging to inform the tumor biology of chordomas, the authors make a point to identify that 47/104, or 45.2%, of tumors did not have a single plausible driver variant. Such a finding strongly suggests that alterations beyond the genome and exome support chordoma oncogenesis. Likely, multiplatform analyses will be

required to elucidate chordoma oncogenesis, including epigenetic changes.

Indeed, one informative region of the genome outside of the coding exome region is the promoter region. The promoter region of *TERT*, telomerase reverse transcriptase, is frequently mutated in cancer and has prognostic significance.[20,21] Mutations within the *TERT* promoter result in increased transcription and maintain telomere length in dividing cancer cells.[22] In meningiomas, *TERT* promoter mutations have been associated with higher-grade lesions and shorter time to progression[23]; in glioblastoma, there is shorter overall survival, independent of *IDH* status.[24]

On the other hand, in a cohort of 92 spinal chordomas, a C228T or C250T *TERT* promoter mutation was observed in 8.7% of samples.[25] Ten-year survival for chordoma patients with a *TERT* promoter mutation was 100% (n = 8), compared to 67% (n = 56) of patients without the mutation. Given the favorable clinical outcome for chordomas patients with *TERT* promoter mutations, compared to a more aggressive clinical course that is seen in other cancers, interrogation of the clinical implication of *TERT* promoter status in chordomas in a validation cohort is advised.

The current clinical trial landscape of EGFR, PDGFR, and CDKN2A inhibitors in chordomas is presented here.

EGFR

The epidermal growth factor receptor (EGFR) is frequently altered in cancer,[26] especially in nonsmall cell lung cancer.[27] Within chordomas, several studies found varying degrees of EGFR expression.[28-30] In a study of 160 patients, 69% of cases harbored EGFR expression using immunohistochemistry (IHC). Notably, however, EGFR mutation status from sequencing is a stronger predictor of response to EGFR inhibitors than is evaluation of EGFR expression with immunohistochemistry, especially in lung cancer.[27,31,32]

Additionally, *in vitro* inhibition of EGFR using a tyrphostin EGFR inhibitor in the well-established U-CH1 chordoma cell line resulted in inhibited proliferation.[30] Such a finding was recapitulated in an *in vivo* patient-derived chordoma xenograft with erlotinib, an EGFR small molecule inhibitor.[33]

Given the above data, several case reports evaluating EGFR inhibitors in chordoma patients found meaningful responses using several EGFR inhibitors including cetuximab, gefitinib, and erlotinib.[34-38] A phase II study of lapatinib in 18 EGFR-positive patients with progressive chordomas reported stable disease as the best response outcome; median progression-free survival was 8 mo.[39] As such, a phase II trial of afatinib in patients with advanced chordoma with confirmed EGFR expression is currently underway (NCT03083678).

PDGFR

Platelet-derived growth factor receptor (PDGFR) is a second targetable alteration implicated in chordoma genomics. In one series, PDGFRB expression was seen in all 18 analyzed chordomas, and to a lesser extent PDGFRA, and KIT, too.[40] Increased PDGFRB expression has also been associated with increased dural penetration in clival chordomas, as well as worse prognosis.[41] Because of the evidence suggesting increased PDGFRB expression in chordomas, 6 patients with advanced chordoma underwent treatment with imatinib mesylate, an inhibitor of BCR-ABL, KIT, PDGFRA, and PDGFRB, which demonstrated evidence of antitumor activity.[42] Efficacy of imatinib was evaluated in a phase II trial (NCT00150072/CSTI571BIT15). With intention-to-treat analysis of 56 patients, median overall survival was 34.9 mo and median progression-free survival was 9.2 mo. A total of 32.4% of patients had clinical benefit; however, the objective response rate was 0%.

Beyond imatinib, in a phase I trial of nilotinib, another tyrosine kinase inhibitor that targets PDGFRB, the safety profile is currently under study when in use with combination radiation therapy in patients with high-risk chordoma (NCT01407198).

CDKN2A

Cyclin-dependent kinase inhibitor 2A (CDKN2A) encodes for p16, which is a known tumor suppressor in cancer, and is frequently altered in chordomas.[43] In 100% of established chordoma cell lines (n = 8), loss of CDKN2A was evident and palbociclib, a CDK4/6-specific inhibitor, resulted in tumor cell growth inhibition.[44] The efficacy of palbociclib is currently being tested in a phase II trial in patients with locally advanced/metastatic chordoma (NCT03110744).

TRANSCRIPTOME

The advent of RNA-Seq allows for comprehensive characterization of the transcriptome, thereby allowing for a better understanding of the functional landscape of the genome.[45] Bell and colleagues[46] reported a cohort of 14 skull base chordomas, and analysis using RNA-Seq revealed 222 cancer-related transcripts, including upregulation of *T, LMX1A, ZIC4, LHX4*, and *HOXA1*, genes known to be related to development. Interestingly, additional work by this group using RNA-Seq revealed location specific transcripts unique to spinal and skull base chordomas.[47] As confirmed by IHC, *LMX1A* was enriched in skull base chordomas, compared to *SALL3* in spinal chordomas. Indeed, *Brachyury* expression was common amongst both chordoma subtypes.

Comparative RNA expression analysis from 3 clival chordoma and 9 sacral chordoma cell lines revealed no or very low levels of HOXA10 protein in the clival cell lines, compared to strong expression in the sacral cell lines,[48] an important aforementioned regulator of development. This evidence suggests unique tumor biology may be associated with chordoma location from skull base, to spinal, and sacral. Future work ought to address this hypothesis and evaluate impact on clinical outcomes and treatment resistance.

PROTEOME

In addition to exome and transcriptome alterations in chordomas, with the use of mass spectroscopy,[49] investigators compared the proteomic profile of a primary and recurrent chordoma sample.[50] The recurrent chordoma sample had 359 proteins that had unique protein expression. Notably, the recurrent sample had increased expression of activated leukocyte cell adhesion molecule (ALCAM or CD166), which was confirmed using IHC. Others identified 14 upregulated and 5 downregulated proteins in chordomas with higher expression of alpha enolase (ENO1), pyruvate kinase M2 (PKM2), and gp96 in recurrent compared to primary samples.[51] Although univariate analyses demonstrated an association with ENO1 and PKM2 with disease-free survival, these markers were not significant in multivariate analysis with known prognostic clinical factors. Potentially, such analysis with larger cohorts could yield promising results to support a clinically informative protein biomarker, especially in the recurrent setting.

EPIGENETIC BIOMARKERS

Genomic alterations in cancer are common in genes with known epigenetic mechanisms, including the SWI/SNF chromatin-remodeling complex and histone modification. Therefore, systematic interrogation of epigenetic biomarkers within chordomas could provide novel therapeutic targets for which targeted therapies are available or in development.[52-54] Epigenetic mechanisms, including DNA methylation and post-transcriptional gene regulation by noncoding RNA (miRNA), have been recently shown to play a role in chordoma.

DNA Methylation

Hypermethylation of promoter regions of tumor suppressor regions results in silencing of transcription and facilitates a mechanism to support definitive oncogenesis across cancers.[55] Rinner and colleagues[56] explored DNA methylation in 10 chordoma samples, identifying 9 regions that were hyper/hypomethylated: *C3*, *XIST*, *TACSTD2*, *FMR1*, *HIC1*, *RARB*, *DLEC1*, *KL*, and *RASSF1*. DNA methylation of an additional 26 chordomas matched to normal nucleus pulposus samples confirmed areas of cancer-specific hyper/hypomethylation.[57] The *MGMT* promoter was always unmethylated in clival chordomas without recurrence (n = 15) but was frequently methylated in a portion of recurrent clival chordomas (4/15, 26.7%).[58] Thus, future work ought to focus on how methylation status may play a role as a biomarker for prognostication in sufficiently powered cohorts.

miRNA

Expression levels of several miRNA candidates that regulate gene function have been associated with clinical outcomes in chordomas: miR-219-5p with tumor extent and recurrence[59]; miR-1273-3p with tumor invasion and recurrence-free survival[60]; miR-155 with disease stage, presence of metastasis, and clinical outcome[61]; and miR-140-3p with recurrence and tumor invasion.[62]

Although the aforementioned miRNA candidates ought to be externally validated, there has been agreement of the potential interaction between miRNA and c-MET, a tyrosine kinase of the hepatocyte growth factor and its receptor,[63] in chordomas.[64] Bayrak and colleagues[65] performed functional analysis of miR-31, revealing an apoptotic effect on chordoma cells. Expression correlated with downregulation of c-MET expression. Others confirmed these findings that miR-31-5p overexpression was associated with decreased c-MET expression and even activation of the PI3K/AKT signaling pathway.[66]

Another group found that miR-1 expression correlated with clinical prognosis in chordomas, and was inversely associated with MET expression.[67] Furthermore, a third group identified that miR-34a inversely correlated with MET expression.[68] Given these converging data that support a role of microRNA expression in chordoma with known pathways that support oncogenesis, future work should attempt to better elucidate how relative miRNA expression affects clinical outcomes, especially across chordoma samples from the skull base, spine, and sacrum.

CHECKPOINT INHIBITORS

The success of checkpoint inhibitors in diseases with previously dismal prognosis such as metastatic melanoma and small cell lung cancer has led to an interest in potential biomarkers that portend successful response to immunotherapy. One such biomarker is programmed death-1 ligand (PD-L1).[69] A study of 54 spinal chordomas had 68.5% of samples with PD-L1 positivity using IHC. PD-L1 expression only in tumor-infiltrating lymphocytes was a favorable independent prognostic marker for local recurrence-free survival (HR = 0.298) and overall survival (HR = 0.188). Analysis by another group revealed PD-L1 positivity in 94.9% of 78 chordoma samples from a tissue microarray.[70] In a small series, 2 chordoma patients were treated with a checkpoint inhibitor.[71] One patient with a C3 vertebral chordoma was treated with pembrolizumab, which resulted in rapid clinical improvement within 6 wk of treatment and 6 mo of disease control. A second patient had a petroclival chordoma who was treated with nivolumab and who similarly experienced rapid clinical improvement and 9 mo of disease control.

Given these findings, there are currently 3 open clinical trials evaluating the role of checkpoint inhibitors in patients with chordomas. NCT02989636 is a phase I trial evaluating nivolumab with or without stereotactic radiosurgery in the recurrent setting. Additionally, NCT02834013 is testing the role of nivolumab and ipilimumab and NCT03173950 is testing nivolumab in patients with chordomas. An additional phase II trial, NCT03623854, will evaluate the role of nivolumab and relatlimab (an anti-LAG-3 monoclonal antibody) in patients with chordoma, and is soon to open for enrollment.

CONCLUSION

Knowledge of the genomic drivers of chordomas has advanced greatly. Despite such advances, however, there is still much to learn. There remains a paucity of information to guide how alterations in the genome, exome, transcriptome, proteome, and even epigenetic changes may influence clinical presentation, management, and survival outcomes of chordoma patients. Moreover, preliminary evidence suggests that unique tumor biology may be associated with tumor location in chordoma, from the skull base to the spine and finally to the sacrum. Larger, consortium-based, focused efforts to address such differences and their clinical implications are warranted to help support neurosurgical management.

Disclosures

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

1. McMaster M, Goldstein A, Bromley C, Ishibe N, Parry D. Chordoma: incidence and survival patterns in the United States, 1973–1995. *Cancer Causes Control*. 2001;12(1):1-11.
2. Walcott BP, Nahed BV, Mohyeldin A, Coumans J-V, Kahle KT, Ferreira MJ. Chordoma: current concepts, management, and future directions. *Lancet Oncol*. 2012;13(2):e69-e76.
3. Catton C, O'Sullivan B, Bell R, et al. Chordoma: long-term follow-up after radical photon irradiation. *Radiother Oncol*. 1996;41(1):67-72.
4. Showell C, Binder O, Conlon FL. T-box genes in early embryogenesis. *Dev Dyn*. 2004;229(1):201-218.
5. Zhu J, Kwan KM, Mackem S. Putative oncogene *Brachyury* (*T*) is essential to specify cell fate but dispensable for notochord progenitor proliferation and EMT. *Proc Natl Acad Sci USA*. 2016;113(14):3820-3825.
6. Kispert A, Koschorz B, Herrmann BG. The T protein encoded by Brachyury is a tissue-specific transcription factor. *EMBO J*. 1995;14(19):4763-4772.
7. Vujovic S, Henderson S, Presneau N, et al. Brachyury, a crucial regulator of notochordal development, is a novel biomarker for chordomas. *J Pathol*. 2006;209(2):157-165.
8. Yang XR, Ng D, Alcorta DA, et al. T (Brachyury) gene duplication confers major susceptibility to familial chordoma. *Nat Genet*. 2009;41(11):1176-1178.
9. Hsu W, Mohyeldin A, Shah SR, et al. Generation of chordoma cell line JHC7 and the identification of Brachyury as a novel molecular target. *J Neurosurg*. 2011;115(4):760-769.
10. Hu Y, Singh R, Deng Z, Mintz A, Hsu W. Liposome-protamine-DNA nanoparticle-mediated delivery of short hairpin RNA targeting Brachyury inhibits chordoma cell growth. *J Biomed Nanotechnol*. 2016;12(10):1952-1961.
11. Nelson AC, Pillay N, Henderson S, et al. An integrated functional genomics approach identifies the regulatory network directed by Brachyury (*T*) in chordoma: gene targets of Brachyury (*T*) in chordoma. *J Pathol*. 2012;228(3):274-285.
12. Shah SR, David JM, Tippens ND, et al. Brachyury-YAP regulatory axis drives stemness and growth in cancer. *Cell Rep*. 2017;21(2):495-507.
13. Otani R, Mukasa A, Shin M, et al. Brachyury gene copy number gain and activation of the PI3K/Akt pathway: association with upregulation of oncogenic Brachyury expression in skull base chordoma. *J Neurosurg*. 2018;128(5):1428-1437.
14. Pillay N, Plagnol V, Tarpey PS, et al. A common single-nucleotide variant in T is strongly associated with chordoma. *Nat Genet*. 2012;44(11):1185-1187.
15. Kelley MJ, Shi J, Ballew B, et al. Characterization of T gene sequence variants and germline duplications in familial and sporadic chordoma. *Hum Genet*. 2014;133(10):1289-1297.
16. Bettegowda C, Yip S, Lo S-FL, et al. Spinal column chordoma: prognostic significance of clinical variables and *T (Brachyury)* gene SNP rs2305089 for local recurrence and overall survival. *Neuro Oncol*. 2017;19(3):405-413.
17. Heery CR, Singh H, Marte JL, et al. NCI experience using yeast-Brachyury vaccine (GI-6301) in patients (pts) with advanced chordoma. *J Clin Oncol*. 2014;32(15_suppl):3081-3081.
18. Heery CR, Palena C, McMahon S, et al. Phase I study of a poxviral TRICOM-based vaccine directed against the transcription factor Brachyury. *Clin Cancer Res*. 2017;23(22):6833-6845.
19. Tarpey PS, Behjati S, Young MD, et al. The driver landscape of sporadic chordoma. *Nat Commun*. 2017;8(1). (doi:10.1038/s41467-017-01026-0).
20. Vinagre J, Almeida A, Pópulo H, et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun*. 2013;4(1). (doi:10.1038/ncomms3185).
21. Koelsche C, Sahm F, Capper D, et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. *Acta Neuropathol*. 2013;126(6):907-915.
22. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959-961.
23. Sahm F, Schrimpf D, Olar A, et al. TERT promoter mutations and risk of recurrence in meningioma. *J Natl Cancer Inst*. 2016;108(5):djv377.
24. Labussiere M, Boisselier B, Mokhtari K, et al. Combined analysis of TERT, EGFR, and IDH status defines distinct prognostic glioblastoma classes. *Neurology*. 2014;83(13):1200-1206.
25. Bettegowda C, Yip S, Jiang B, et al. Prognostic significance of hTERT (human telomerase reverse transcriptase) promoter region mutations C228T and C250T for overall survival in spinal chordomas. *Neuro Oncol*. 2019;21(8):1005-1015.
26. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene*. 2007;26(22):3291-3310.
27. Paez JG. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497-1500.
28. Ptaszyński K, Szumera-Ciećkiewicz A, Owczarek J, et al. Epidermal growth factor receptor (EGFR) status in chordoma. *Pol J Pathol*. 2009;60(2):81-87.
29. Horbinski C, Oakley GJ, Cieply K, et al. The prognostic value of Ki-67, p53, epidermal growth factor receptor, 1p36, 9p21, 10q23, and 17p13 in skull base chordomas. *Arch Pathol Lab Med*. 2010;134(8):7.
30. Shalaby A, Presneau N, Ye H, et al. The role of epidermal growth factor receptor in chordoma pathogenesis: a potential therapeutic target. *J Pathol*. 2011;223(3):336-346.
31. Bailey R, Kris M, Wolf M, et al. O-242 Gefitinib ('Iressa', ZD1839) monotherapy for pretreated advanced non-small-cell lung cancer in IDEAL 1 and 2: tumor response is not clinically relevantly predictable from tumor EGFR membrane staining alone. *Lung Cancer*. 2003;41:S71.
32. Sholl LM, Xiao Y, Joshi V, et al. EGFR mutation is a better predictor of response to tyrosine kinase inhibitors in non-small cell lung carcinoma than FISH, CISH, and immunohistochemistry. *Am J Clin Pathol*. 2010;133(6):922-934.
33. Siu I-M, Ruzevick J, Zhao Q, et al. Erlotinib inhibits growth of a patient-derived chordoma xenograft. rutteman GR, ed. *PLoS ONE*. 2013;8(11):e78895.
34. Aleksic T, Browning L, Woodward M, et al. Durable response of spinal chordoma to combined inhibition of IGF-1R and EGFR. *Front Oncol*. 2016;6. (doi:10.3389/fonc.2016.00098).
35. Hof H, Welzel T, Debus J. Effectiveness of cetuximab/gefitinib in the therapy of a sacral chordoma. *Oncol Res Treat*. 2006;29(12):572-574.
36. Lindèn O, Stenberg L, Kjellén E. Regression of cervical spinal cord compression in a patient with chordoma following treatment with cetuximab and gefitinib. *Acta Oncol*. 2009;48(1):158-159.
37. Singhal N, Kotasek D, Parnis FX. Response to erlotinib in a patient with treatment refractory chordoma: *Anticancer Drugs*. 2009;20(10):953-955.
38. Launay SG, Chetaille B, Medina F, et al. Efficacy of epidermal growth factor receptor targeting in advanced chordoma: case report and literature review. *BMC Cancer*. 2011;11(1). (doi:10.1186/1471-2407-11-423).
39. Stacchiotti S, Tamborini E, Lo Vullo S, et al. Phase II study on lapatinib in advanced EGFR-positive chordoma. *Ann Oncol*. 2013;24(7):1931-1936.
40. Tamborini E, Miselli F, Negri T, et al. Molecular and biochemical analyses of platelet-derived growth factor receptor (PDGFR) B, PDGFRA, and KIT receptors in chordomas. *Clinical Cancer Res*. 2006;12(23):6920-6928.
41. Zhai Y, Bai J, Wang S, et al. Differences in dural penetration of clival chordomas are associated with different prognosis and expression of platelet-derived growth factor receptor-β. *World Neurosurg*. 2017;98:288-295.

42. Casali PG, Messina A, Stacchiotti S, et al. Imatinib mesylate in chordoma. *Cancer*. 2004;101(9):2086-2097.
43. Choy E, MacConaill LE, Cote GM, et al. Genotyping cancer-associated genes in chordoma identifies mutations in oncogenes and areas of chromosomal loss involving CDKN2A, PTEN, and SMARCB1. *PLoS ONE*. 2014;9(7):e101283.
44. von Witzleben A, Goerttler LT, Marienfeld R, et al. Preclinical characterization of novel chordoma cell systems and their targeting by pharmocological inhibitors of the CDK4/6 Cell-Cycle pathway. *Cancer Res*. 2015;75(18):3823-3831.
45. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 2009;10(1):57-63.
46. Bell D, Raza SM, Bell AH, Fuller GN, DeMonte F. Whole-transcriptome analysis of chordoma of the skull base. *Virchows Archiv*. 2016;469(4):439-449.
47. Bell AH, DeMonte F, Raza SM, et al. Transcriptome comparison identifies potential biomarkers of spine and skull base chordomas. *Virchows Archiv*. 2018;472(3):489-497.
48. Jäger D, Barth TFE, Brüderlein S, et al. HOXA7, HOXA9, and HOXA10 are differentially expressed in clival and sacral chordomas. *Sci Rep*. 2017;7(1). (doi:10.1038/s41598-017-02174-5).
49. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature*. 2003;422(6928):198-207.
50. Chen S, Xu W, Jiao J, et al. Differential proteomic profiling of primary and recurrent chordomas. *Oncol Rep*. 2015;33(5):2207-2218.
51. Zhou H, Chen C, Lan J, et al. Differential proteomic profiling of chordomas and analysis of prognostic factors. *J Surg Oncol*. 2010;102(7):720-727.
52. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell*. 2012;150(1):12-27.
53. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. 2003;33(S3):245-254.
54. Baylin SB, Jones PA. A decade of exploring the cancer epigenome—biological and translational implications. *Nat Rev Cancer*. 2011;11(10):726-734.
55. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med*. 2003;349(21):2042-2054.
56. Rinner B, Weinhaeusel A, Lohberger B, et al. Chordoma characterization of significant changes of the DNA methylation pattern. *PLoS ONE*. 2013;8(3):e56609.
57. Alholle A, Brini AT, Bauer J, et al. Genome-wide DNA methylation profiling of recurrent and non-recurrent chordomas. *Epigenetics*. 2015;10(3):213-220.
58. Marucci G, Morandi L, Mazzatenta D, Frank G, Pasquini E, Foschini MP. MGMT promoter methylation status in clival chordoma. *J Neurooncol*. 2014;118(2):271-276.
59. Wei W, Zhang Q, Wang Z, Yan B, Feng Y, Li P. miR-219-5p inhibits proliferation and clonogenicity in chordoma cells and is associated with tumor recurrence. *Oncol Lett*. 2016;12(6):4568-4576.
60. Zou M, Huang W, Wang X, et al. Reduced expression of miRNA-1237-3p associated with poor survival of spinal chordoma patients. *Eur Spine J*. 2015;24(8):1738-1746.
61. Osaka E, Kelly AD, Spentzos D, et al. MicroRNA-155 expression is independently predictive of outcome in chordoma. *Oncotarget*. 2015;6(11). (doi:10.18632/oncotarget.3273).
62. Zou M-X, Huang W, Wang X-B, Lv G-H, Li J, Deng Y-W. Identification of miR-140-3p as a marker associated with poor prognosis in spinal chordoma. *Int J Clin Exp Pathol*. 2014;7(8):4877-4885.
63. Gherardi E, Birchmeier W, Birchmeier C, Woude GV. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer*. 2012;12(2):89-103.
64. Walter BA, Begnami M, Valera VA, Santi M, Rushing EJ, Quezado M. Gain of chromosome 7 by chromogenic in situ hybridization (CISH) in chordomas is correlated to c-MET expression. *J Neurooncol*. 2011;101(2):199-206.
65. Bayrak OF, Gulluoglu S, Aydemir E, et al. MicroRNA expression profiling reveals the potential function of microRNA-31 in chordomas. *J Neurooncol*. 2013;115(2):143-151.
66. Wang W, Tang L, Li Q, et al. Overexpression of miR-31-5p inhibits human chordoma cells proliferation and invasion by targeting the oncogene c-Met through suppression of AKT/PI3K signaling pathway. *Int J Clin Exp Pathol*. 2017:10(7):8000-8009.
67. Duan Z, Shen J, Yang X, et al. Prognostic significance of miRNA-1 (miR-1) expression in patients with chordoma. *J Orthop Res*. 2014;32(5):695-701.
68. Zhang Y, Schiff D, Park D, Abounader R. MicroRNA-608 and microRNA-34a regulate chordoma malignancy by targeting EGFR, Bcl-xL and MET. *PLoS ONE*. 2014;9(3):e91546.
69. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci USA*. 2002;99(19):12293-12297.
70. Feng Y, Shen J, Gao Y, et al. Expression of programmed cell death ligand 1 (PD-L1) and prevalence of tumor-infiltrating lymphocytes (TILs) in chordoma. *Oncotarget*. 2015;6(13):11139-11149.
71. Migliorini D, Mach N, Aguiar D, et al. First report of clinical responses to immunotherapy in 3 relapsing cases of chordoma after failure of standard therapies. *Oncoimmunology*. 2017;6(8):e1338235.

COMMENT

This review of the potential emerging therapeutic targets in chordomas is a nice touchstone for those interested in furthering these investigations. The amount of work being done with chordoma signals the grim realization that despite improving surgical techniques and extents of resection and sophisticated radiation planning we are simply not curing the majority of these patients. Recurrences are common and each recurrence makes it less and less likely that further surgical or radiotherapeutic interventions will convey any meaningful outcome benefit. Perhaps one of the most significant findings impacting the construction of future therapeutic trials for chordoma are the distinct location-specific transcripts identified by tumor location. Sacral chordomas are not the same as clival chordomas and therapeutic trials will likely need to be location specific.

Franco DeMonte
Houston, Texas