# Molecular Diagnostics in Sarcoma Pathology

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**Venus Brady:**

Although malignant tumors of bone and soft tissues are rare, they represent an outsized diagnostic challenge. This difficulty stems in part from the impressive diversity of these neoplasms, which are comprised of at least 80 distinct types, notably a significant number of sarcomas harbor a characteristic gene fusion. Increasingly, pathologists employ molecular testing as an aid in sarcoma diagnosis. In this CAPcast featuring Dr. Matthew Hiemenez and Dr. Damon Olson we'll discuss molecular diagnostics in sarcoma pathology. Welcome to the CAPcast Dr. Hiemenez, please introduce yourself.

**Dr. Matthew Hiemenez:**

Great. Thank you so much. My name is Matt Hiemenez, I'm a pathologist at Foundation Medicine, and I'm particularly interested in molecular diagnostics of sarcomas and molecular pathology of pediatric tumors.

**Venus Brady:**

And Dr. Olson.

**Dr. Damon Olson:**

Thank you, Venus. I am the Associate Medical Director of Anatomic Pathology and the Molecular Pathology Program Director in the Department of Pathology and Laboratory Medicine at Children's Minnesota, located in Minneapolis in St. Paul Minnesota. And obviously I have an interest as well in sarcomas and in particular pediatric sarcomas and glad to be with you and Dr. Hiemenez today.

**Venus Brady:**

Thanks so much. Dr. Hiemenez, our first question is for you. How has the integration of molecular testing into sarcoma diagnosis progressed over the last few years?

**Dr. Matthew Hiemenez:**

Yes. So just a quick bit of background, and thank you for that introduction. About a quarter of sarcomas have these characteristic gene fusion, and that's often what we're trying to capture with the molecular testing methods. But sarcomas overall are quite rare. I would say three things for the integration of molecular testing. One, we're just seeing more testing overall and testing across different methods. So FISH, array like PCR more traditional methods, and then more and more capturing fusions and other sarcoma alterations with next generation sequencing.

The second thing I would say about progression is what we're seeing, I think more and more is that next generation sequencing is often substituting or coming in as a frontline test for other molecular tests, because it's able to characterize so many fusions just with one assay. And then the third thing I would say is that anatomic pathologists doing sarcoma pathology, what we're seeing is that they're increasingly integrating the molecular information into their reports often through addendums but sometimes through even issuing new integrated reports following the molecular testing.

**Venus Brady:**

I see. So what benefit does molecular diagnosis bring to pathology that traditional IHC in morphology does not?

**Dr. Matthew Hiemenez:**

Yes. So I should probably back up a little bit before I answer. So I'd see in morphology as all pathologists know, they're really the bedrock of the specialty of anatomic pathology. There really are the foundational methods and in the WHO Blue Book for sarcomas, they explicitly state that these are the central methods, and molecular testing is more of a ancillary diagnostic.

An additional benefit in morphology and IHC is that all pathologists who do anatomic pathology are familiar with these methods. And so that's their central importance. But I would say in terms of limitations for morphology, sometimes morphology won't be a slam dunk, it will rather than give you a single diagnosis will yield a differential diagnosis, so several possible entities, and then they'll need to be further studies with IHC. And then a limitation of IHC is often specificity. So many of the IHC antigens that are used for sarcoma diagnosis aren't truly exclusive to particular sarcomas, but they can often be seen across multiple different tumor types.

So when we look at molecular testing, really two big advantages, one is specificity. So if we're looking at these fusions with Next-Generation Sequencing or PCR, we can identify this fusion very specifically if it's present in a sarcoma sample and that can be used to help diagnosis. And then a second thing is that molecular confirmation of fusions is becoming more and more important because it helps to define approved pharmaceutical indications. For example, detecting NTRK fusions with an approved molecular test is important for giving a NTRK targeted therapy or the sarcomas that harbor these fusions.

**Venus Brady:**

Dr. Olson, this question is for you. What are some of the molecular diagnostics you are using most often for this purpose?

**Dr. Damon Olson:**

So in our practice, we have historically used mostly FISH probes, especially Break-Apart probes. We do continue to use them with regularity since they are well established and have a relatively fast turnaround time. However, our use of more advanced molecular diagnostics specifically Next-Generation Sequencing continues to grow. Of particular relevance to sarcoma testing is the ability to sequence for gene fusions in a fusion gene partner agnostic fashion. That is, we know what the primary gene is, but don't necessarily know what the fusion partner is, which we have largely achieved via RNA sequencing NGS assays.

Although our particular practice doesn't use them very often, other institutions might also use fusion specific reverse transcript dates PCR assays. And I would also mention finally that, although we haven't yet used it in sarcoma diagnosis, methylation testing has been very useful in brain tumor diagnosis and I fully expect it will prove itself to be similarly helpful in sarcomas.

**Venus Brady:**

So it sounds as if molecular diagnostics are starting to make meaningful contributions in sarcoma pathology as a method to bring specificity to the diagnosis, as Dr. Hiemenez mentioned. Dr. Olson, has this been your experience in practicing pathology too?

**Dr. Damon Olson:**

Thanks Venus, yes it has. As Dr. Hiemenez and you have already alluded to sarcoma diagnosis can be quite challenging due to the morphologic similarities between various entities on the differential diagnosis, often non-specific or in completely diagnostic immunophenotypes, that is the phenotype that's expressed by immunohistochemical patterns and a growing recognition of new tumor subtypes, or completely new tumor types, altogether.

For example, in my world of pediatric pathology, sarcomas composed of mostly undifferentiated, small round cells, the so-called small round blue cell tumors frequently enter the differential diagnosis. While the immunophenotype can be helpful, several entities will need molecular confirmation to ensure the correct diagnosis. As I mentioned before, one method to achieve this has been with FISH probes, which can help guide the clinical team in making initial treatment decisions. However, there are instances where the FISH result may not work for technical reasons, or is insufficient to complete the diagnosis.

As an example, I can remember a case where a small biopsy showed a small round cell tumor that had an immunophenotype characteristically matching Ewing sarcoma, but due to a series of technical challenges, FISH for EWSR break apart could not be completed. Sequencing ended up identifying in HEY1, NCOA2 fusion, HEY1, NCOA2 fusion that in this context was diagnostic for Mesenchymal Chondrosarcoma. In this case, the sequencing prevented a misdiagnosis that would have resulted in an improper treatment regimen.

There was another similar case where the FISH showed no evidence of EWSR break apart, but sequencing identified a variant EWSR fusion with a rare fusion partner, confirming the Ewing sarcoma diagnosis and giving the treatment team, the patient and their family comfort in having a definitive diagnosis and being able to establish proper treatment.

There are other sarcomas where a FISH probe is simply not commercially available, and sequencing is the only way to identify a diagnostic fusion, or maybe the most economical approach when several entities are being considered. Even if the tumor type is already known, there are some tumor types where it may be important to know the fusion partner because of potential prognostic relevance. Although the evidence is still evolving about this, an example here is FOXO1 translocation positive Rhabdomyosarcomas, usually of Alveolar Histology, where those cases fused with Pax7 have been shown to have a better prognosis than tumors where the FOXO1 fusion partner is Pax3. Knowing the fusion partner gives our oncologists the ability to more thoroughly counsel the patient and family and modify clinical monitoring.

One more point here is that sequencing not only shows utility and diagnosis and probably in prognosis, but there is additionally relevance in determining treatment options with the advent of targeted therapies, such as small molecule inhibitors or immunotherapy options, our oncologists are often seeking additional molecular information about alternative potential therapy options.

Although immunohistochemistry can be used in specific instances, we are generally able to answer these questions via sequencing or a combination of immunohistochemistry and sequencing. An example that comes to mind now was a case of an inflammatory myofibroblastic tumor, which had recurred with metastatic disease and showed immunoreactivity for our protein and sequencing subsequently identified the RANBP2-ALK fusion, allowing further refinement of the diagnosis to an epitheloid inflammatory myofibroblastic sarcoma. And the oncologist could consider inclusion of crizotinib, an ALK inhibitor to the treatment regimen. So somewhat of a long winded answer there, but I can definitively say that it has been my experience that molecular diagnostics have become an essential tool in our sarcoma diagnoses.

**Venus Brady:**

So seeing that this seems like an innovative approach, Dr. Hiemenez, could you tell us about some relevant guidelines that cover molecular testing against sarcomas?

**Dr. Matthew Hiemenez:**

There's really two big ones I'd like to mention. So the first is for pathologists and the second one is more for oncologists. So the first is the WHO guide, so the so-called Blue Books. The one that covers sarcoma is the WHO Classification of Soft Tissue and Bone Tumors. The fifth edition was released in 2020. And there's a few really interesting things to highlight about the most recent WHO for sarcomas. One is that there's a section on diagnostic molecular pathology for the different sarcomas in the Blue Book. And then another thing that's interesting is that they separate essential and desirable diagnostic criteria for the different sarcomas that are covered. And right now, for the vast majority of the tumors that are in the Blue Book, molecular testing is falling under the desirable category rather than the essential category.

And just as a random example, if we look at CIC-rearrange sarcomas. So these are sarcomas that are defined by a rearrangement of the CIC gene in almost all instances, it's a specific fusion CIC-DUX4. In those particular tumors, actually demonstrating the defining CIC gene rearrangement is called desirable rather than essential. And I think in thinking about this and how the guidelines can help us to think about molecular testing, I think it's important to recognize that the WHO guidelines are international guidelines and molecular testing isn't uniformly available across the world. But I do think that as the testing becomes more widespread and hopefully cheaper, that more of the molecular testing will move into the essential category.

Another interesting thing to highlight about the WHO Blue Books is that in the latest edition, there are a good variety of new tumors that are defined by their central molecular features. So for example, there's NTRK-rearranged spindle cell neoplasms, EWSR1-SMAD3 fibroblastic tumor, and then several new molecularly defined Rhabdomyosarcoma subtypes. So the importance of molecular diagnostics in both defining and diagnosing these tumors is becoming more and more, I think, recognized by the WHO guidelines.

And then the second set of guidelines I'd like to mention are more for oncologists, but they're the National Comprehensive Cancer Network guidelines or NCCN. NCCN has two guidelines that are relevant here. They have guidelines for soft tissue sarcomas and for bone cancers. In the soft tissue sarcoma guidelines, they actually have three pages where they list out all these different molecular alterations. So clearly it's very much on the radar of the practicing oncologists, but they have a broad statement that they feel molecular testing is very useful, but it's just very important that it's performed by pathologists that have experience in soft issue sarcomas, because interpreting these results aren't always completely straightforward. And then the Bone Cancer Guidelines recommend molecular testing in general, to identify targeted therapies for the sarcomas that are covered in those guidelines.

**Venus Brady:**

And you touched on this a bit, but are there any other promising molecular testing methods to be applied in the future for greater diagnostic precision?

**Dr. Matthew Hiemenez:**

Yes. I want to echo what Dr. Olson said previously. I think methylation analysis is very exciting. I'm not an expert in this area, I don't practice in it, but I am trying to follow along and learn as the field evolves. And the idea behind methylation analysis is that different tumor types have different methylation patterns in their genome. And so what you can do is take 1000 plus samples and create a classifier for methylation patterns, use that classifier to help classify a tumor into different methylation groups. And this is a plug for CAP, and this is not solicited, but cap had a really excellent webinar on this just a few weeks ago, called Methylation Analysis: A New Approach to Sarcoma Classification. It was led by two pathologists in Heidelberg, Germany, Dr. Andreas von Deimling and Dr. Christian Koelsche. And they walk through this in a very, I think, interesting and engaging way.

One sort of takeaway for me in watching that webinar and reading some of their papers they publish is that a big application of this for sarcomas is likely going to be the sarcomas that don't have a defining gene fusion. So the three quarters or so that have complex karyotypes, and aren't defined by specific gene fusion. Those could potentially benefit from methylation analysis classifiers as the testing becomes more mainstream over time. And it's a very exciting area. And I think we're all just interested to see how it evolves and how it ends up being integrated into diagnostic practice.

**Venus Brady:**

Sounds very exciting, indeed. And I want to thank you guys again for joining us for this CAPcast. To read Dr. Hiemenez's article on the same topic, search precision medicine on cap.org or MyCAP app. And thank you for listening to this CAPcast. To find other podcasts brought to you by the College of American Pathologists you can find CAPcast on the MyCAP app for CAP members and on your favorite podcasting platform.