A New View of Cancer Pathways in Pediatric Leukemia

Researchers are using the TruSight® RNA Pan-Cancer Panel and the MiSeq® System to understand the role of fusion genes in pediatric leukemia.

Introduction

According to the World Health Organization, leukemia, or cancer of the blood cells, is the most common cancer in children and adolescents.[1] While childhood leukemia remains a relatively rare disease, the treatment options for this cancer are intensive and debilitating. Most patients will receive some type of chemotherapy, and various targeted drugs, radiation, or stem cell therapies might also be used. Patient response to these treatments varies significantly. Determining which therapies will be most effective depends on understanding the genetic differences underlying each individual's disease.

Gianni Cazzaniga, Ph.D., a medical geneticist at the University of Milan-Bicocca's Tettamanti Research Center, and his colleagues study the molecular basis of pediatric leukemia. Using next-generation sequencing (NGS), they are identifying genes that might contribute to the onset of disease and gaining insight into the heterogeneity of treatment response in patients. With this information, they hope to someday help physicians select the safest, most effective treatment for each patient.

Dr. Cazzaniga and his team use the MiSeq System and were involved in beta testing the TruSight RNA Pan-Cancer panel, a targeted panel designed to detect fusion genes and variant expression in over 1300 cancer-associated genes. Dr. Cazzaniga spoke with iCommunity about what his team is learning about the genetic drivers underlying childhood leukemia, and our current understanding of the role of fusion genes in the progression and relapse of the disease.

Q: What sparked your interest in medical genetics, particularly for childhood leukemia?
Gianni Cazzaniga (GC): I'm a biologist by training, and chose to specialize in medical genetics because it can help us better diagnose and treat different diseases. My lab is part of the pediatric department at the University of Milan-Bicocca. Our goal is to improve the cure rate for childhood leukemia. Currently, the cure rate is around 80%, which, for cancer, is quite high.[2] However, the treatments are very aggressive. We'd like to improve the cure rate to 100% and improve disease treatments so that they are less toxic.

I have an emotional link with the pediatric field. I like that my work will help children and I'm enthusiastic about looking for ways to improve the cure rate for leukemia. I also find leukemia to be an interesting disease. We are focused on defining new prognostic subgroups, in particular targetable lesions for treatment. We want to identify gene rearrangements that could be targets for new, specific drugs. Ultimately, we want to make major improvements in how children with leukemia are treated for the disease and reduce their risk of relapse.

Q: What is the focus of the research conducted at the Tettamanti Research Center?
GC: Tettamanti was founded 25 years ago to conduct diagnostic research focusing on pediatric leukemia. It's supported by parent associations and peer-reviewed grants. I've been working here since 1994, more than 20 years now. Originally, we were performing research in a single room. Now, we have a new research center that is 1300 square meters.

Q: Do we know what triggers the onset of childhood leukemia?
GC: Unfortunately, no one knows for certain what triggers pediatric leukemia. This is one of the questions we are trying to answer. There are some epidemiological studies that focus mainly on exposure to toxic situations, such as chemicals and radioactivity. There are also events that occur *in utero*, but because of the long latency, we don't believe that they are sufficient to cause leukemia.

In addition to understanding the causes of pediatric leukemia, we're interested in studying the preleukemia state, or the state of the body before the clinical manifestation of the disease. Our lab contributed to the demonstration of prenatal origin.[3-4] Recently, there has been significant interest in studying predisposition. There are a few studies that show polymorphisms or mutations in genes that predispose certain people to leukemia.[5] This is another area where NGS could be important in helping us understand what is occurring at the genetic level.

Dr. Gianni Cazzaniga is a medical geneticist at the University of Milan-Bicocca's Tettamanti Research Center.

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Q: How are you using NGS in your research and what has it enabled you to discover?
GC: NGS can help us define the genetic lesions that are associated with the risk of relapse. We evaluated different possibilities to identify molecular rearrangement, including fluorescent *in situ* hybridization (FISH) and multiplex PCR. Due to the increasing number of lesions that we were seeing, we decided that NGS, whether whole-genome or targeted, would be the best tool to identify their presence.

We also are interested in minimal residual disease (MRD) detection by NGS. This is research we've been doing for 20 years and we were among the 4 labs that developed a tool that is now used as a main diagnostic factor in all clinical protocols.[6-10] We're hoping that NGS can be used to define clonal targets that will enable us to assess MRD.

Q: How has NGS impacted pediatric leukemia research?
GC: NGS is a very useful tool for clonality assessment and MRD monitoring, as well as for detecting gene aberrations. We aim to develop a targeted panel to define the different aberrations that could be diagnostic and prognostic in pediatric cancer. We're working to define fusion genes (from translocations), gene expression profiling, and mutational patterns with NGS to identify the genetic drivers of the disease. NGS offers faster turnaround and improves the detection of these drivers.

> "NGS is a very useful tool for clonality assessment and MRD monitoring, as well as for detecting gene aberrations."

Q: What NGS workflow did you adopt in your lab?
GC: We started a European consortium, called EuroNGS, 3 years ago to consider the different possibilities for NGS in clonality assessment and minimal residual disease (MRD) monitoring to predict relapse. We started by developing the bioinformatics part of the analysis.

We then created a process where we divided the tasks for the different genes across different labs. Each lab was in charge of developing a specific application for 1 gene. Then we shared what we found and moved on to a validation phase where we plan to validate the applications developed by the other labs. By the end of 2016, we hope to have a method for target detection. In the next year, we aim to use NGS for clonality and MRD risk assessment studies.

As a single lab, we are also working on the definition of molecular aberrations, the rearrangements we see in the genome. We worked at a DNA level and then began thinking about new approaches, such as targeted RNA capture. We have compared various methods and we're preparing to use these methods to develop research protocols.

Q: What are fusion genes and how are they created?
GC: Fusion genes are the consequence of chromosomal translocation or internal deletions.[11] Translocations are the most common molecular rearrangement events that we see in leukemia. With leukemia, there are numerous aberrancies. There can be more or less copies of a chromosome than expected, with the patient being hyperdiploid or hypodiploid as a result of the major gene rearrangement caused by translocations. It's different from lymphoma where a translocation causes the overexpression of a certain gene. In leukemia, the fusion is the rearrangement between half of 1 gene and half of another gene. The result can be restricted to 2 main consequences. One is the creation of aberrant transcription factors and the other is the activation of kinases. Those are the main mechanisms we are trying to understand by using NGS. We want to discover what those genes are and how they work.

Q: When do you use DNA versus RNA sequencing?
GC: We are interested in chromosomal translocations, as these are what cause fusion genes to occur. When we started, the targeted RNA approach was not available. We began with DNA and worked to define the breakpoint of the chromosomal translocations, which occur in the interim region. We applied this system and had 2 major problems. One was the sequencing length. The coverage in introns was not as good as we needed it to be. Several affected sequences did not allow us to design appropriate solutions, and those regions were exactly the ones where the breakpoint occurred. The second problem was that even if we had a sequence with the breakpoint of interest, alignment to the genome was difficult because those are repetitive sequences. They map to different regions of the genome and it's difficult to know which region is the right one. Also, if you have a DNA sequence that could be used for monitoring the disease, aneuploidy might cause problems in correlating the number of DNA copies with the number of cells. So you cannot say how many residual cells are there during treatments by measuring the DNA in those cells, and vice versa.

RNA Sequencing (RNA-Seq) is much better, because it allows us to define the fusion gene directly, as well as the consequence of the chromosomal translocation. Targeting genes that are more relevant in this process enables us to identify the partners in the translocation, which is a major advantage. With RNA-Seq, we can also measure gene expression in the amount of RNA.

> "We cannot discover anything using these old techniques (cytogenetics, PCR, or FISH), we can only use them to monitor what we already know."

Q: Before NGS, what tools were used to study fusion genes?
GC: In the past, we studied fusions using cytogenetics, PCR, or FISH. To use PCR, we need to know the partner gene in the fusion. When we use FISH, it's a logistical problem, in particular for multicentric studies. What we can monitor depends on whether we can collect the cells in the right way. If this is the case, we can detect the broken gene. However, we still have no way of identifying the partner gene using a molecular approach. We cannot discover anything using these old techniques, we can only use them to monitor what we already know.

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Q: Why did you choose an RNA panel to study fusion genes in pediatric leukemia?
GC: We started with DNA but it wasn't successful because of repetitive sequences and the low coverage of the probes. We moved to an RNA panel because we know that the fusion genes need to be expressed to be relevant, so we are quite sure that there will be an RNA molecule with the fusion. We also know that there are several genes that are more frequently involved in fusions. If those genes are in the panel, then we can see what they are doing.

> "We moved to the MiSeq System after comparing it with other technologies. It has a low error rate and provides high reproducibility."

Q: How does the MiSeq System enable you to study fusion genes?
GC: We are a relatively small group, so we do not have access to large sequencing systems in terms of cost or bioinformatics analysis. The MiSeq System is the right compromise, enabling us to discover what we need to discover. It enables us to develop our methods by ourselves and also perform the correct bioinformatics analysis.

We moved to the MiSeq System after comparing it with other technologies. It has a low error rate and provides high reproducibility. Compared to past technologies, we can perform more discovery work on the MiSeq System. We can put what we do, in different ways, with different techniques, onto a single platform. Illumina is also dedicated to sequencers. That made it the right choice for us to invest in this technology.

Q: How does the TruSight RNA Pan-Cancer Panel enable you to make the most of your samples?
GC: Particularly in pediatric research, we're working with small, often degraded, or poor-quality samples and that limits what we can do. The RNA Pan-Cancer Panel sample requirement is small (10–100 ng total RNA or 20 ng FFPE RNA), enabling us to perform the analysis easily no matter the size or quality of the sample. The MiSeq System also has a relatively low throughput, which is good for us because we want to use RNA-Seq in a prospective way. The turnaround is fast, so we're seeing results and making discoveries quickly to move our research forward.

Q: What are the advantages of using BaseSpace® RNA Core Apps to analyze your data?
GC: We are analyzing our data in 2 different ways. We have one person on our team, who is a biologist, not a bioinformatician, who is using BaseSpace RNA Core Apps to analyze our RNA data. She has been able to understand the minimal types of information we could analyze that would be sufficient to detect fusion genes. We are also collaborating with a bioinformatics group to develop our own system of analysis; one young bioinformatician from our group is training with our collaborators.

Q: What interesting discoveries have you found so far using RNA-Seq?
GC: We've discovered new fusion genes including 1 gene of interest, the PAX5 gene.[12-13] We are also using the MiSeq System to define the aberrations in a new subgroup of subjects with poor prognosis that have a gene expression signature in common with cases of Philadelphia chromosome positive acute lymphoblastic leukemia. This group (named Ph-like ALL) has a similar phenotype to the t(9;22) translocation, but does not have the actual translocation. We are defining molecular events in this specific subgroup to help us better understand what is happening.

> "The RNA Pan-Cancer Panel sample requirement is small, enabling us to perform the analysis easily no matter the size or quality of the sample."

Q: What are the next steps in your research studies?
GC: We plan to use NGS to help us to perform MRD assessment. We want to improve, as much as possible, the sensitivity of our methods to identify 1 specific molecule, that is 1 specific leukemia cell, in the context of 100,000 normal cells. We want to push the limit of detection forward. We also will continue to study all the potential molecular aberrations we see with leukemia. We want to move our discovery even further so we can understand how we could apply whole-transcriptome RNA-Seq to benefit patients.

Q: Where do you hope that your work with NGS will take you?
GC: We're hoping our discoveries will support personalized medicine. In the future, we'd like to develop an NGS test that can assess molecular aberrations and be used to identify subgroups of patients so that clinicians can make sure that treatments for each patient are targeted, personalized, and effective.[14]

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