ScientistSpotlight

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# Unraveling the complexities of the cancer genome to guide patient-tailored treatment strategies

## Introduction

There is an emerging recognition that an improved ability to assess the cancer genome will help clinicians better stratify patients for diagnosis, prognosis, and therapy selection. This includes an evolving appreciation for the wealth of emerging clinically actionable variants that should be validated and included alongside accepted diagnostic markers to improve cancer diagnosis and disease staging. In this article, Dr. Peter Ambros from the Children's Cancer Research Institute (CCRI) in Vienna, Austria discusses the way in which arrays are helping to unravel the complexities of the cancer genome and guiding patient-tailored treatment strategies.

The work at the CCRI is helping to advance diagnosis, prognosis, and treatment strategies for children and adolescents suffering from cancer. To achieve this aim, the work of Dr. Peter Ambros's group is primarily devoted to understanding the genomic and cell biological aspects of neuroblastomas and other pediatric solid tumors such as nephroblastomas, rhabdomyosarcomas, and rhabdoid tumors. Genomic features of pediatric tumors can frequently help in the diagnostic workup and, importantly, can offer additional prognostic information. They aim to apply the most innovative and robust genomic diagnostic tools and provide this information to the treating oncologist to choose the most suitable treatment options available to young patients.

- **What clinical challenge are you trying to address?**

The biological and clinical behavior of neuroblastomas, the most frequent solid tumors of infancy and early childhood, is unique and does not necessarily correspond to tumor dissemination but is largely dependent on tumor genomics. Over the last two decades a number of research teams, including ours, have provided evidence that genomic features help to differentiate between prognostically different subgroups. This worldwide-accepted concept is now being applied and developed further in European clinical studies

**Peter and Inge Ambros, whose team conducts the neuroblastoma array work at the Children's Cancer Research Institute (CCRI) in Vienna.**

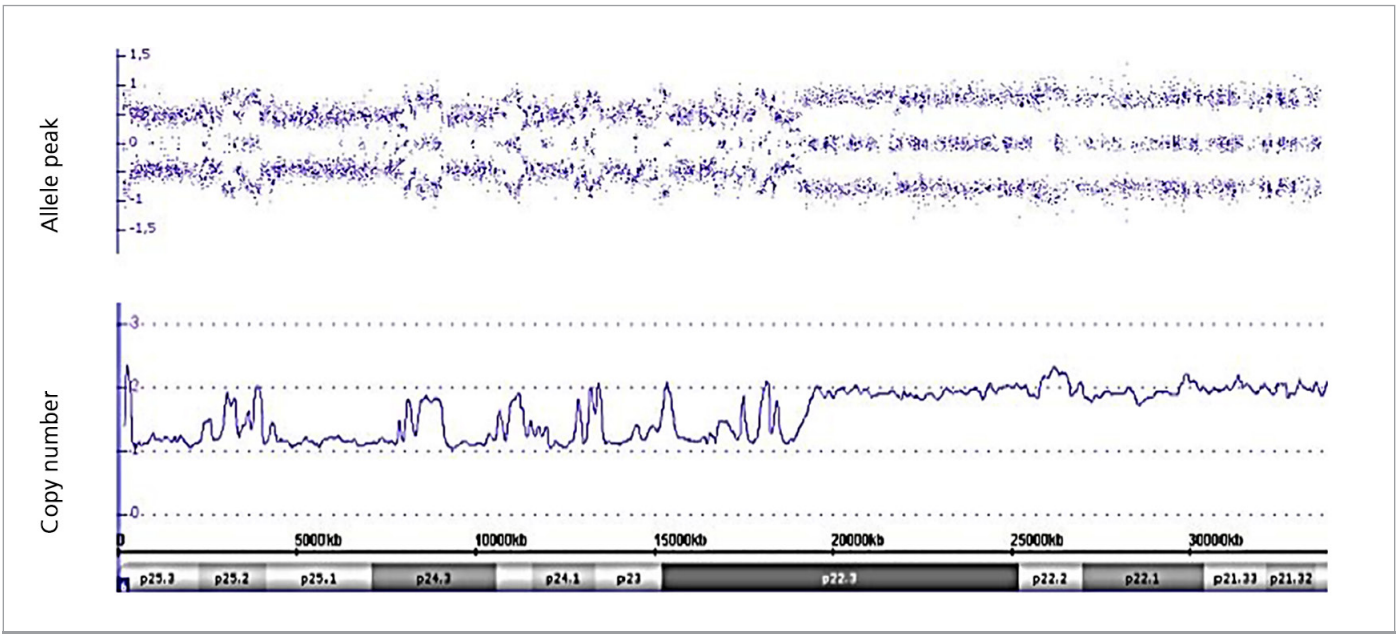
aimed at reducing or intensifying cytotoxic treatment in genetically defined patient groups. Since these current clinical studies range from a "wait-and-see" strategy after complete/incomplete resection, or even no resection at all, to the application of high-dose chemotherapy and stem cell transplantation, the tumor genome-based therapy stratification has to be as secure, reliable, sensitive, and specific as possible. Therefore, we chose CytoScan® HD Array; it meets all the requirements of a comprehensive genomic analysis.

"Tumor genome-based therapy stratification has to be as secure, reliable, sensitive, and specific as possible."

- **Prior to using arrays, what techniques were you using? What prompted your transition to start using a high-density SNP array?**

Before applying CytoScan HD Array routinely in our laboratory, we used a PCR-based technique identifying 100 genomic loci simultaneously. This technique, referred to as MLPA (multiplex ligation-dependent probe amplification), essentially fulfills all the requirements of a cost-effective diagnostic test that analyzes all genomic loci frequently affected in neuroblastomas (so-called "typical" genomic aberrations). The MLPA NB kit is tailored to detect those segmental chromosomal aberrations, typically found in neuroblastoma, which are generally accepted as being of major prognostic impact. However, this technique does not allow visualizing all genomic aberrations present in the tumor genome, as the number of PCR targets that identify copy number changes is limited.

Recently, a number of new genomic aberrations have been described in neuroblastomas, which are assumed to also have prognostic impact. The most spectacular genomic aberration to be found in a large number of tumors, including neuroblastomas, is referred to as chromothripsis. This state of genomic "catastrophe" is assumed to be an indicator of unfavorable prognosis. In addition, also minor aberrations such as deletions of single exons like in the ATRX (alpha thalassemia/mental retardation syndrome X-linked) gene identify a patient subgroup with a defined clinical behavior. However, the prognostic impact of these aberrations has to be validated in a larger patient cohort. As our laboratory is not only doing routine tests but is also




**An example of the recently described "chromothripsis" in a tumor cell. The chromothripsis with >30 breakpoints concerns the short arm of chromosome 6. The y axis depicts DNA copy number (lower) and allele peak (upper). Allele peak panels normally show three distinct "bands," representing all homozygous (top and bottom bands) and heterozygous (middle band) allele calls. The multiple oscillations between the two DNA copy number states are indicative of chromothripsis.**

"Classical CGH techniques cannot identify aberrations only present in a sub-population of cells. The copy number information together with the SNP data provided by CytoScan HD Array frequently overcome this limitation."

searching for new genomic markers to be used in the clinical practice, we have been looking for the best current strategy to achieve both goals. The technology offered by CytoScan® HD Array fulfilled all the requirements concerning coverage of copy number and SNP probes, allowing detection of copy number changes with high resolution (including aberrations spanning only a few exons), exact breakpoint localizations, gene amplifications, uniparental disomies/trisomies, low-level mosaicisms, and chromothripsis.

- **Could you describe your experimental design and sampling strategy?**

Nowadays, in pediatric tumor diagnosis, we are frequently faced with very small amounts of tumor material as more and more true cut biopsies are taken. Therefore, we have adopted methods that use very tiny biopsies or even bone marrow samples to obtain sufficient DNA, routinely ~200 ng, to perform the analysis with CytoScan HD Array. To enable a short turnaround time of 4–5 days and to provide the treating clinician with quick results, we decided to have all wet lab activities located in the CCRI. This has the advantage of fine tuning the experiments and designing the experimental settings in such a way as to address all issues of sample size, sample pretreatment, and quality of the tumor probe with the utmost care.

- **What have you found to be the clinical implications of loss of heterozygosity (LOH) with or without a copy number change?**

In neuroblastoma especially, we are frequently confronted with copy number changes of either whole chromosomes or parts thereof, the latter being usually referred to as segmental chromosomal aberrations. The segmental chromosomal changes can either result in gains or losses of parts of chromosomes. In most instances the copy number losses correlate with LOH. Copy neutral losses of heterozygosity have, so far, not been taken into consideration in many tumor entities. However, preliminary data gained with CytoScan HD Array indicate that this type of LOH occurs more frequently in certain tumor types than expected. This is one research question we are currently following up in more detail.

- **How critical is it to adopt a whole-genome approach to cancer analysis?**

Over recent years we have come to realize that tumors can be extremely heterogeneous, with varying numbers of subclones. Of course, interphase FISH (I-FISH) is still the method of first choice for detecting subpopulations within a tumor. However, usually only one aberration can be traced within the tumor in one FISH experiment. Thus, one has to know which region or which gene to search for—which is often impossible. Whole-genome approaches like conventional CGH techniques provide the full picture of all loci involved, albeit dependent on the number of probes on the array. However, classical CGH techniques cannot identify aberrations only present in a subpopulation of cells. The copy number information together with the SNP data provided by CytoScan HD Array frequently overcome this limitation and can also identify subpopulations that are present in a minority of tumor cells. This is of crucial interest to us because an aberration initially only present in a minority of cells may lead to a relapse. All of these questions are currently under investigation and will probably lead to a more complete picture of the impact of all genomic aberrations present in the tumor.

- **What impact do you think the usage of SNP arrays will be on patient care?**

As already mentioned, the current neuroblastoma clinical trials implement the presence of segmental chromosomal aberrations ( i.e., gains or losses of chromosomal fragments) in the treatment decision process. To my knowledge, the "Low and Intermediate Risk Protocol" of the European Neuroblastoma Study Group (SIOPEN) is the first European clinical study implementing genomic information in addition to amplification events into a clinical decision making process. CytoScan HD Array represents a most reliable technique to unequivocally identify these aberrations, thus providing a sound basis for treatment decisions. Since neuroblastoma is frequently considered a model tumor for the use of genomic information in the treatment stratification process (information on *MYCN* amplification has been used worldwide for decades in a large number of clinical studies), it is expected that the presence of genomic aberrations (other than gene amplifications) in other tumor entities may also become a helpful indicator to guide patient-tailored treatment strategies.

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