Leukaemia diagnosis

Realising the potential of whole transcriptome sequencing

Acute lymphoblastic leukaemia (ALL) is a cancer affecting the white blood cells. Many different subtypes of ALL have been described, which makes accurate diagnosis a challenge that requires a plethora of diagnostic methods. Applying comprehensive high-throughput sequencing tools, Dr Wencke Walter and the team at Munich Leukemia Laboratory show rapid and accurate genetic characterisation of specific types of leukaemia. This identification of patients' genetic fingerprint is key to a precise diagnosis and the design of personalised cancer treatments.

Leukaemia is a cancer of the white blood cells, the cells produced in the bone marrow that help us to fight infection. Normally, white blood cells repair and reproduce themselves in a very controlled way. However, in leukaemia, this process gets out of control: the cells continue to reproduce but do not mature. As these cells are not fully developed, they do not work properly.

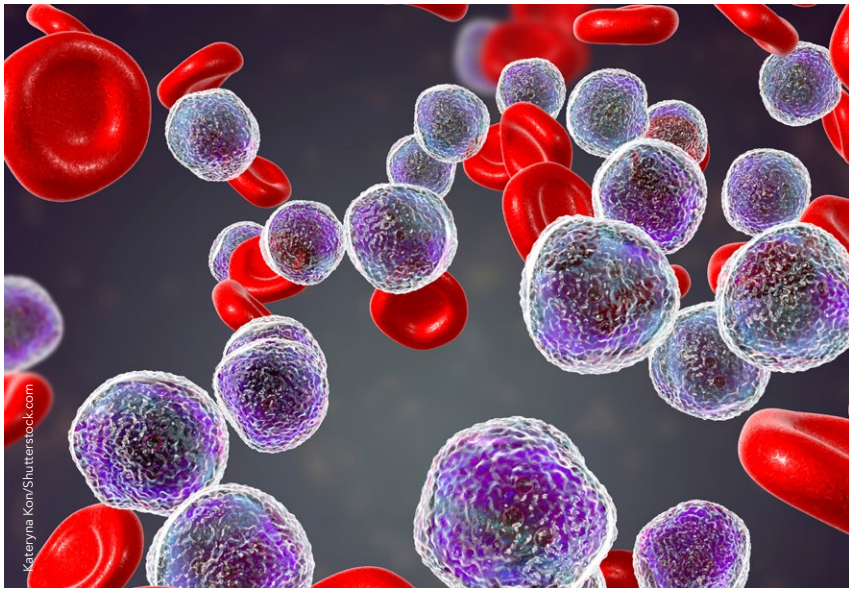
One type of leukaemia called acute lymphoblastic leukaemia (ALL) is caused by overproduction of a specific kind of white blood cell: the lymphoblast. In ALL, immature lymphoblasts fill up the bone marrow, preventing it from making healthy blood cells. ALL makes up three-quarters of leukaemia cases in children but it can also be diagnosed in teenagers and adults.

ALL: A DIVERSE DISEASE

A diverse blood cancer, many different types of ALL exist, and are identified according to the type of lymphoblast cell affected and the stage during the maturation process that the cells become leukaemic.

For example, within the most common type of ALL, B-cell precursor ALL (BCP-ALL), the World Health Organization (WHO) recognises nine different sub-groups. Because of its very diverse nature, ALL diagnosis is very challenging. Currently, several different tests must be carried out to provide an accurate and precise diagnosis, and still it might be difficult to identify the exact subtype.

Looking to change this are Dr Wencke Walter and the team at Munich Leukemia Laboratory. By integrating several cutting-edge technologies, the researchers are opening up new avenues that will enable rapid and accurate diagnosis of every subtype of ALL. As Walter explains, 'the current standard diagnostics require different technologies to deliver a rapid, accurate, and clinically relevant diagnosis for each patient. Even with the plethora of available methods, it is still challenging to comprehensively cover all subtypes.'



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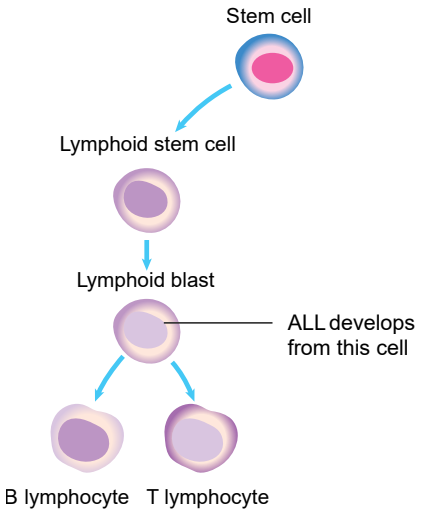

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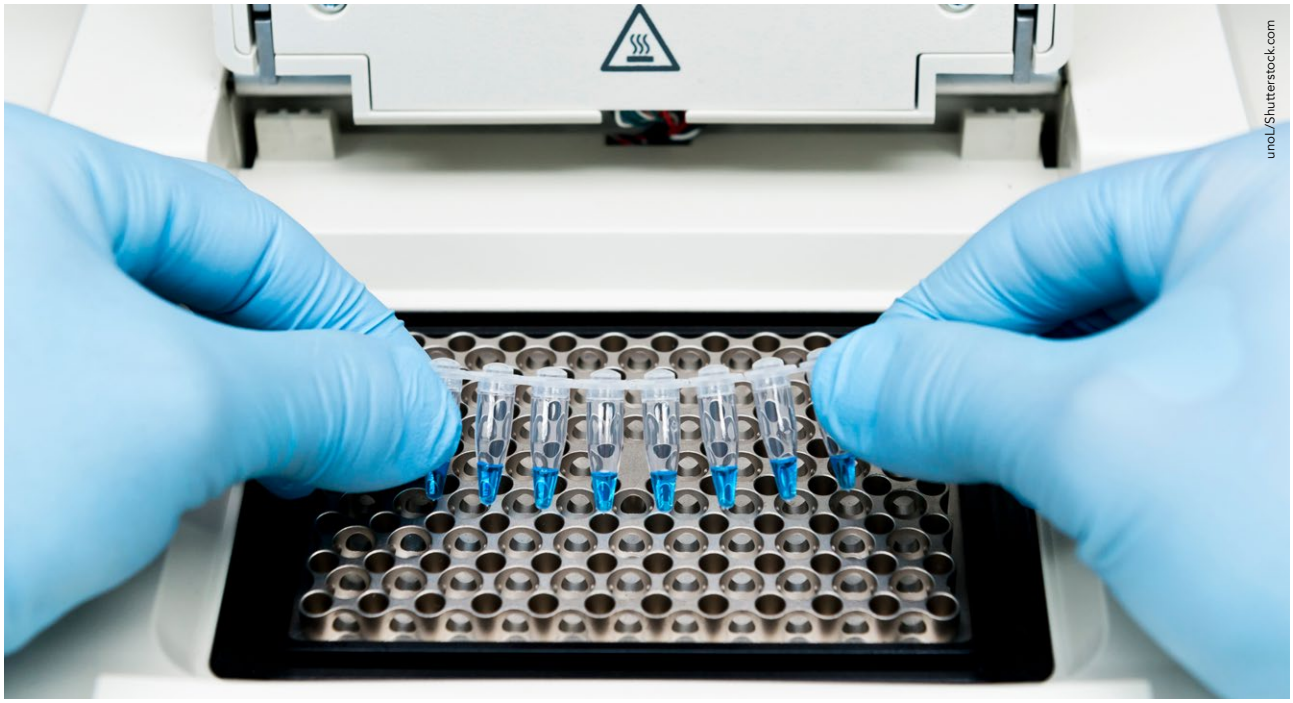
Acute lymphoblastic leukaemia (ALL) is caused by the overproduction of lymphoblasts.



Integrating cutting-edge technologies, Dr Wencke Walter and the team at Munich Leukemia Laboratory are opening up new avenues that will enable rapid and accurate diagnosis of ALL subtypes.

WHOLE GENOME SEQUENCING

In recent years, several ALL subtypes have been identified that are characterised by specific molecular genetic markers. These are best detected by new technologies – called next-generation sequencing – that allow genetic information to be determined. One method is whole genome sequencing (WGS), which determines the complete DNA sequence of a person's genetic information (genome) at a single time. Walter describes, 'because each human cell contains the same DNA regardless of its function, DNA represents the most fundamental building block of the cell. WGS reads a person's complete genetic information and simultaneously detects mutations, copy number alterations, as well as structural variants – all of which play an important role in cancer diagnostics.'

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WHOLE TRANSCRIPTOME SEQUENCING

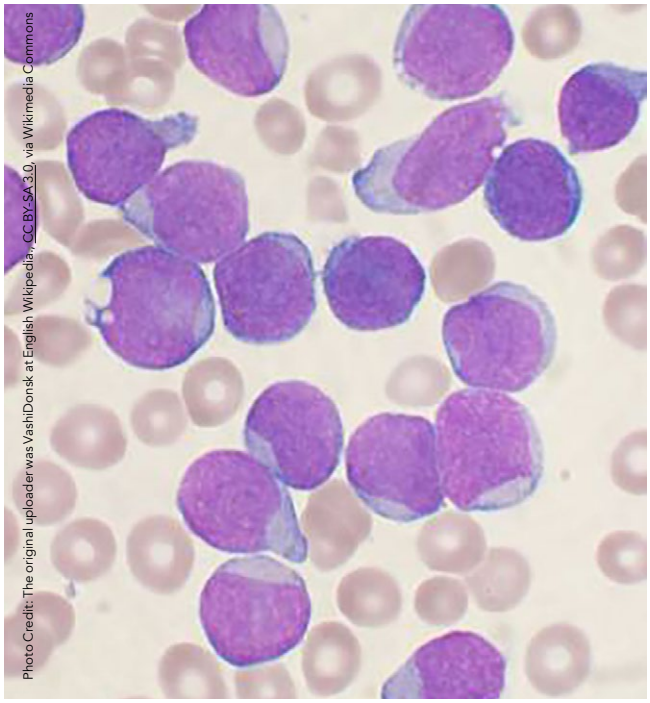
Another method, whole transcriptome sequencing (WTS), gives a snap-shot measurement of the parts of DNA that are 'transcribed' in a cell. That is, the parts which are actively being used to make new molecules. In contrast to a cell's genome, its transcriptome differs. Especially in the case of an illness such as cancer, abnormal gene transcription implements significant changes in the transcriptome, affecting the proportion of genes that are active. This change can be better detected by using the WTS method. Importantly, WTS also enables the unbiased detection of known and novel fusion transcripts which are crucial for subtype classification. A fusion transcript is the result of chromosome rearrangements or deletions, which cause different fragment transcripts to join or fuse together.

The Munich Leukemia Laboratory researchers have been striving to solidify an ALL-subtype classification approach based on WGS and WTS data analysis. Their aim is to reliably subclassify ALL patients with a single assay that can substitute current conventional diagnostic methods.

GENETIC CHARACTERISATION OF ALL

In a recent study, detailed WTS analysis of 279 patients with newly-diagnosed ALL was performed to explore its diagnostic potential to genetically characterise ALL and assess its relevance in everyday practice. The cohort comprised 115 female (41%) and 164 male (59%) patients, with a median age of 49 years at diagnosis and included 211 patients diagnosed with BCP-ALL and 68 patients with T-ALL according to the guidelines from the WHO.

Genetic material was extracted from bone marrow cells (n=196) or peripheral blood cells (n=83) for genome and transcriptome analysis. Samples were assigned to either the T or B lineage based on the expression levels of 14 marker genes. Crucially, compared to the standard methods, 97% of subgroup defining rearrangements were identified as fusion transcripts by WTS. In addition, the WTS data was used to infer copy number variations to identify subtype-specific abnormalities. The



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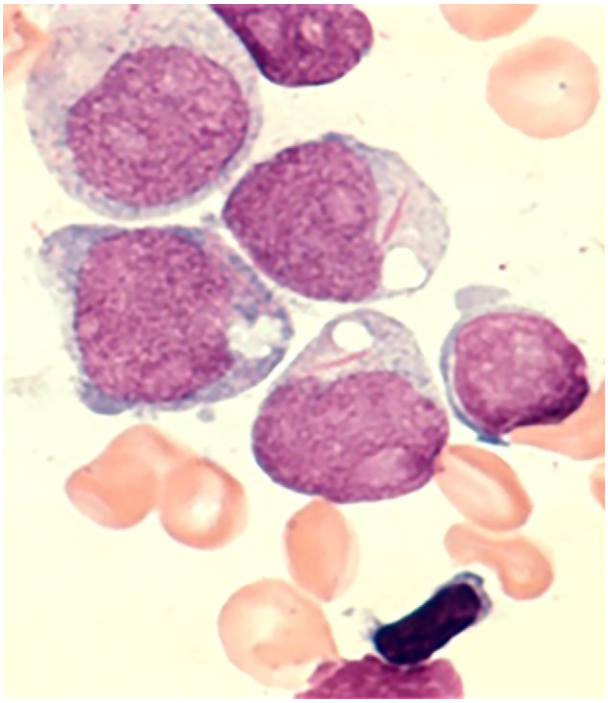
Bone marrow from a person with precursor B-cell ALL. The large purple cells are lymphoblasts.



Bone marrow showing acute myeloid leukaemia.

value of the WTS data was particularly evident in cases which lack typical subtype defining genetic abnormalities.

THE APPLICATION OF WGS AND WTS

A further study was conducted on patients with ALL and acute myeloid leukaemia (AML), a leukaemia that affects the myeloid white blood cells. The study included 738 AML patients and 417 ALL patients. The diagnosis for these patients was established following WHO guidelines.

Notably, the results demonstrated that WGS and WTS provide all necessary genetic information to accurately determine the diagnostic and prognostic subgroup according to WHO guidelines, both for AML and ALL. Moreover, these tools provide a broader and higher resolution genome characterisation than the current gold standard methods, which helps in understanding the molecular connection of a patients' genetic background, offering the basis for more precise diagnosis, prognosis, and targeted treatment selection.

Despite the huge potential of WGS and WTS, the current lack of standardised quality parameters, sample preparation and data analysis workflows must be addressed to integrate these promising methods into routine diagnostic processes.

With the growing number of analysed genomic regions, the quantity and complexity of potentially actionable genomic alterations available for each patient increases, but manual classification, annotation, and validation of these abnormalities lags behind. Walter explains, 'among the existing limitations for a widespread implementation of WGS and WTS in routine diagnostics, is the lack of knowledge about uncommon and not well-described chromosomal abnormalities and gene mutations.'

WTS and WGS are at the brink of turning into valuable tools for the diagnosis of many types of cancer.

A GLIMPSE INTO THE FUTURE: BUILDING DECISION-MAKING TOOLS

Building on their research, the Munich Leukemia Laboratory researchers now aim to use WGS and WTS to prospectively profile patients with a suspected diagnosis of ALL and AML. Their exciting new study will further increase our knowledge on the incidence of chromosome aberrations, somatic mutations, and altered gene expression profiles. This acquisition of large quantities of high-quality, standardised data will also contribute to successful downstream artificial intelligence (AI)-based approaches to produce reliable and trustworthy AI models to advance precision medicine. Excitingly, this could help transform large, multi-modal data into predictive models and decision-making tools.

Walter concludes, 'WTS and WGS are at the brink of turning into valuable tools for the diagnosis many types of cancer, including blood cancer.' The team's promising results are leading the way for a new age in cancer treatment by advancing precision medicine. A more comprehensive genetic profile of a patient not only allows for a more accurate diagnosis but might also inform tailored cancer treatments.

Behind the Research

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Research Objectives

Dr Walter integrates multiple data sets to obtain a comprehensive picture of the underlying molecular regulatory networks of haematological malignancies. Her particular interest is the analysis of transcriptome data.

Detail

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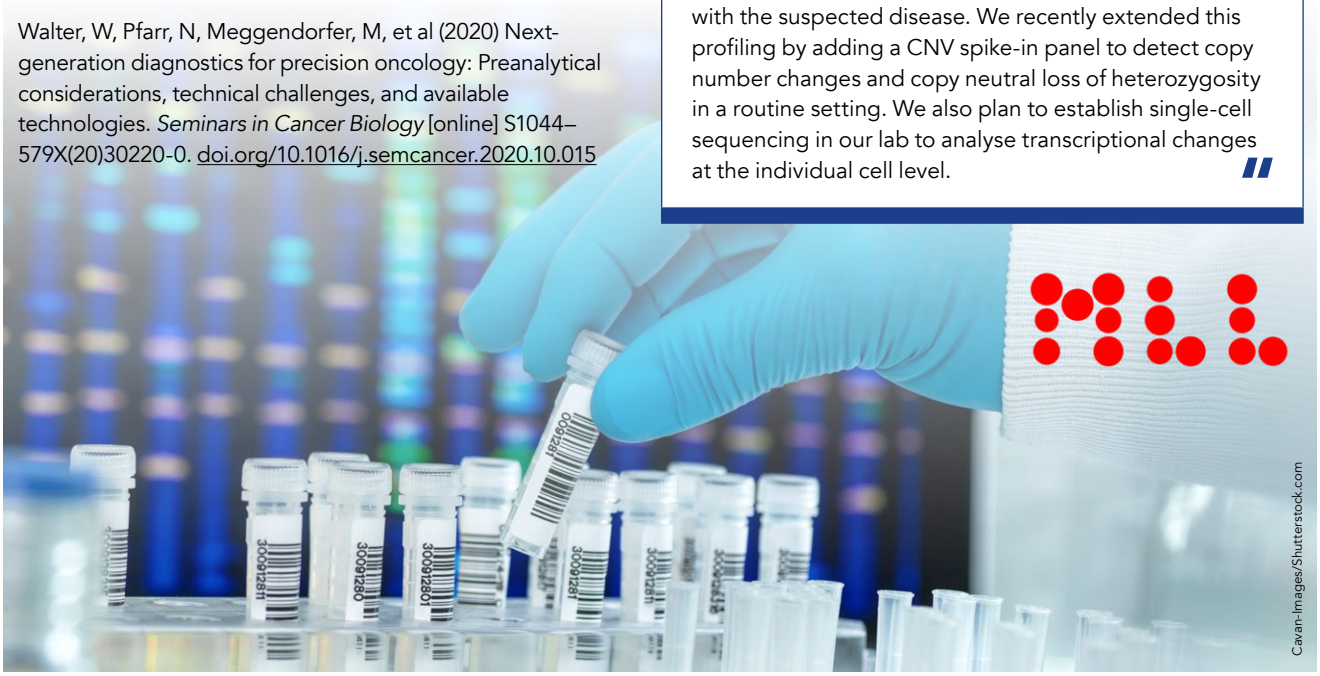
Personal Response

Besides performing WTS and WGS on a larger number of patients in 2022, what other new methods or technologies could your team use to make patient stratification more clinically relevant?

"In addition to WGS and WTS we are also constantly optimising our targeted gene panel workflow. Each patient sample that arrives at our molecular genetics department is analysed for a selected set of genes or gene regions that have been found to be associated with the suspected disease. We recently extended this profiling by adding a CNV spike-in panel to detect copy number changes and copy neutral loss of heterozygosity in a routine setting. We also plan to establish single-cell sequencing in our lab to analyse transcriptional changes at the individual cell level."



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