ST. ANNA CHILDREN'S CANCER RESEARCH INSTITUTE

SCIENCE REPORT 2021

St. Anna Kinderkrebsforschung

CHILDREN'S CANCER RESEARCH INSTITUTE

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INTRODUCTION

CONQUERING CHILDHOOD CANCER? NOTHING WILL STOP US.

This science report provides me with the great opportunity to share that throughout the previous year we at St. Anna Children's Cancer Research Institute (CCRI) have worked passionately towards a single objective: rendering childhood cancer a curable disease.

A father whose little daughter was diagnosed with leukemia brought this objective up 35 years ago, when prospects for children with leukemia were bleak. This father, Erwin Senoner, and Helmut Gadner, Medical Director of the St. Anna Children's Hospital at that time, set themselves the ultimate goal of establishing a dedicated childhood cancer research institute. Of course, he and all the other parents who were supporting this pioneering work hoped that their kids might benefit from all these efforts. Striving to fulfill these hopes and expectations is still a tough task, despite the fact that many achievements and milestones in childhood cancer research have been accomplished since then. Conquering childhood cancer is a great challenge.

However, we are determined, we have a clear goal and we are sticking to it based on the model of our founders. By doing so, the year 2021 became a highly successful one that shows how we are getting closer to our goal. To give you just a brief glimpse, let me start with a short list of my personal highlights that made last year so special for our institute.

At the very beginning of 2021, Davide Seruggia joined St. Anna CCRI to establish and lead the "Pediatric Leukemia Biology" research group as Principal Investigator (PI). He previously worked at Harvard Medical School and Boston Children's Hospital in the world-class laboratory of Stuart Orkin that has an impressive track record in investigating mechanisms of hematopoiesis and hematologic disease. To investigate non-coding regions in the DNA in hematopoiesis and leukemia, Davide Seruggia received one of the prestigious Starting Grants of the European Research Council (ERC).

Still during the first quarter of 2021, a team of researchers around Heinrich Kovar received the highly endowed Crazy 8 Initiative award from Alex's Lemonade Stand Foundation (ALSF), a leading funder of pediatric cancer research. In this ALSF call, St. Anna CCRI was the only institution selected for funding outside the United States. It represents a great honor for our institute, but also an unprecedented opportunity to push forward bone sarcoma research in a competitive team on a global scale. I am also delighted that a prestigious Life Science Grant for Precision Medicine of the Vienna Science and Technology Fund (WWTF) was awarded to Eleni Tomazou and her international research collaborators. With her innovative approach, she provides a minimally invasive alternative to conventional tumor biopsies, allowing for a fast detection and regular monitoring of childhood cancers followed by tailored treatment decisions for every patient.

As coordinator of the European Reference Network for Paediatric Oncology (ERN-PaedCan), Ruth Ladenstein at St. Anna CCRI successfully established the ground for the network to expand to 81 full members from 21 countries and eleven affiliated partners from seven countries. This is a remarkable achievement to continue improving outcomes of childhood cancer by reducing the current inequalities in different member states.

Throughout 2021, we published more papers than ever in some of the most recognized and prestigious scientific journals, among others in the *Blood Journal, Nature Genetics, Science Immunology, the Journal of Clinical Oncology* and *Nature Communications*. In these studies, we have discovered new genetic causes of diseases, refined guidelines for treatment decisions, and identified potential new targets for future therapies.

I am proud to notice that our institute has collected numerous prizes and awards in the last year – Michael Dworzak was named "Worldwide Top Leukemia Expert", Benjamin Salzer and Charlotte Zajc from the Christian Doppler Lab for Next Generation CAR-T cells won the Life Science Research Awards Austria, Davide Seruggia received an ISTT Young Investigator Award, Ruth Ladenstein obtained the Clemens von Pirquet Prize, and I myself obtained the first international Isil Berat Barlan Award for Primary Immunodeficiency Diseases. Collectively, these pieces of recognition show that our scientific work has been acknowledged far beyond the walls of our institute. This goes to show how determination, dedication, and persistence pay off.

You may ask yourself why I am listing such grants, prizes and publications here. Ultimately, we want to challenge ourselves by doing internationally competitive research for the benefit of children with cancer. Despite all our efforts, childhood cancer still poses a deadly threat to a part of affected patients and there is a dire need for new and better therapies. To tackle this, we are systematically promoting precision oncology approaches at our institute by bringing together the competencies of different research groups for this larger-scale effort. It is our vision that a molecular understanding of childhood cancer may support treatment decisions allowing us to offer tailored therapies to those children who cannot be cured with standard therapies. Combining molecular profiling, functional chemosensitivity testing and molecular disease monitoring, this program will bridge St. Anna CCRI's long-standing translational and clinical research activities and our diagnostic expertise. There are only few places which provide an adequate framework to bring such a project forward – and we consider our institute as one of them, because of our continuous quest to develop further and to partner with nearby and far-away partner institutions. We are determined to engage with some of the most brilliant minds out there. On this transformative journey, we need to become even more visionary than before, and we need your support to help those children who do not have sufficient chances of survival.

Finally yet importantly, it is my great pleasure to welcome our new head of the institute, Leo Kager. He succeeds Wolfgang Holter in this position, whom I would like to thank for his many years of commitment to the institute. As senior oncologist at St. Anna Children's Hospital, Leo Kager brings in his long-term clinical experience and perspective and he will work towards further strengthening the partnership between St. Anna CCRI and St. Anna Children's Hospital.

Thanks to our dedicated staff, our generous donors, our reliable partners, and everyone who played a role in our scientific endeavors, 2021 is a perfect example of our strength: we have achieved even more than we had anticipated. We hope you enjoy reading this research report! From my perspective, we have every reason to approach the future with confidence and hope.

Kaan Boztug
Scientific Director & Managing Director

SPECIAL EFFORTS FOR A SPECIAL MISSION: DEFEATING CHILDHOOD CANCER TOGETHER

Excellence is never an accident. *-Aristotle*

I am pleased to inform you that the scientific achievements of our researchers in 2021 were outstanding. We have made significant progress in all relevant performance indicators.

This development is inevitably accompanied by growth. We have not only established a new research group, but have also been able to generate considerable competitive third-party funding. At the same time, these growth factors have also revealed the limits of our current spatial resources. Opportunities for optimization were exhausted in 2021 through the creation of new research and work spaces.

In addition, I would like to report on a structural change that we were able to finalize last year. The incorporation of the research institute into St. Anna Kinderkrebsforschung GmbH was also completed from a financial point of view within the framework of the preparation of the annual financial statement and was audited and certified with the appropriate care and accuracy.

Important structural projects were also completed and implemented in the IT area in order to create the resources for further development steps after a consolidation phase.

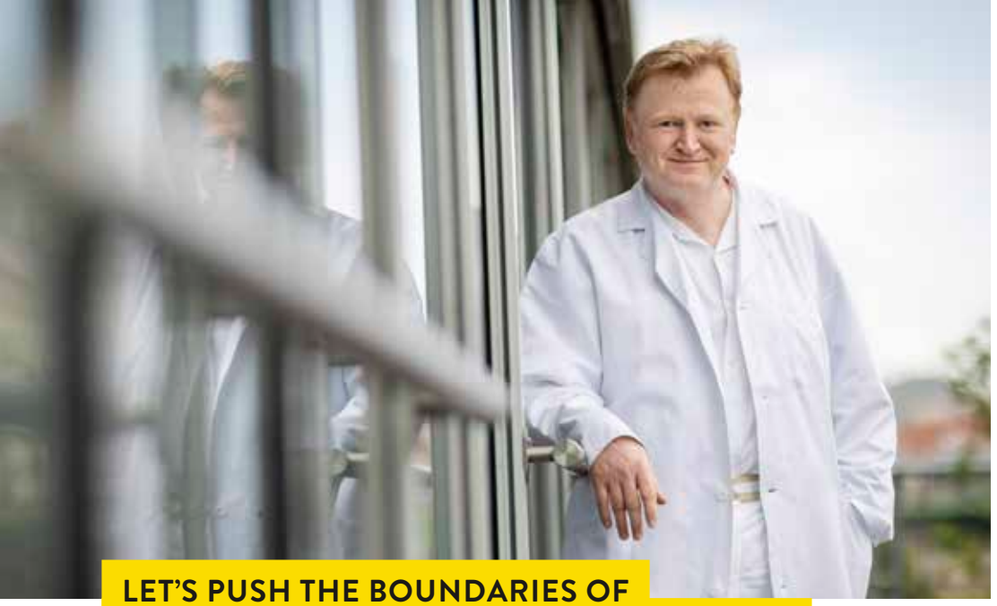
The various activities in the HR area in recent years were rewarded with a special award in 2021. Our "Employee Spotlights – Employee Short Videos", which were developed with a lot of passion but a low budget, were awarded the Silberne Feder of the Public Relations Verband Austria, with first place in the category Image-Video-Sound. I am particularly proud that we were able to prevail against international competition from large corporations thanks to the dedicated efforts of our employees.

It is with great pleasure that I welcome our new Head of Institute, Prof. Dr. Leo Kager, who, as an internationally respected "physician-scientist" of many years' standing, achieved his first research successes in the founding phase of our institute. During the initial and so far excellent collaboration of our newly formed institute management, Prof. Kager has contributed new impulses, ideas and visions, in order to attribute the necessary importance and strategic future value to the connecting "bridge" between the research institute and the hospital.

We would like to thank Prof. Holter, who, in addition to his demanding position as Medical Director of St. Anna Children's Hospital, also held the position of Institute Director for many years. We would like to express our sincere thanks to our Board of Directors for their commitment, especially duringthe past year; in addition to their generous investment of time, also for many hours of dedicated, passionate cooperation, for support in various projects and for the courage to break new ground. Many heartfelt thanks also to you, our supporters, for your loyalty, your trust in our work and your willingness to enable us to live our mission together through your donations.

The first months of 2022 show us that we live in a volatile, uncertain, complex and ambiguous world (VUCA), which will continue to challenge us in the fulfilment of our important goals. In these times, it is all the more important to believe in our mission and to find the necessary support in it to willingly continue working towards achieving something special on a large and small scale and thus also to increase individual satisfaction.

Jörg Bürger
Managing Director & CFO



LET'S PUSH THE BOUNDARIES OF CHILDHOOD CANCER RESEARCH FURTHER

Sixty years ago, almost all children with cancer or severe hematological and immunological diseases had a rapid fatal course of disease. That was about to change, when in 1962 Danny Thomas, a famous US actor and comedian, who founded St. Jude Children's Research Hospital (SJCRH) in Memphis, Tennessee, brought together physicians and researchers to develop, provide and study treatments for these young patients by the promise that 'no child should die in the dawn of life'.

Subsequent collaborative work by scientists and clinicians who believed that children with such devastating diseases deserve a hope for the future resulted in continuous improvements, and currently up to 80% of young cancer patients become long-term survivors in the industrialized world. Among the pioneers in childhood cancer research were also a couple of physicians and scientists, as well as physician-scientists who initiated St. Anna Children's Cancer Research Institute (St. Anna CCRI) together with parents of young cancer patients almost 35 years ago in Vienna.

Similar to the SJCRH in Memphis, St. Anna CCRI and St. Anna Children's Hospital provide a unique infrastructure to perform cutting-edge research and medical care for children with life threatening diseases.

However, every year about 45 children and adolescents still die of cancer in Austria. Moreover, a significant number of adult pediatric cancer survivors suffer from significant long-term morbidities due to the cancer itself and/or the given therapies. Therefore, there is ample room for further improvements in order to increase survival and to reduce toxicity of therapies. As long as only a single child has to die from cancer and its consequences, we must dedicate all our scientific efforts to improve diagnostics and treatment opportunities.

In research in general and especially in pediatric cancer research, it is essential to be open for new ideas and to 'think outside the box'. Every disease is an interplay of multiple factors. Therefore, a holistic approach is necessary to understand the mechanisms that contribute to a disease phenotype. In pediatric cancer, it is important to know how cancer develops, progresses, and becomes resistant to therapies. In this context, for example, expanding insights into defects in the genome that pose an individual to a higher susceptibility to cancer development, are necessary.

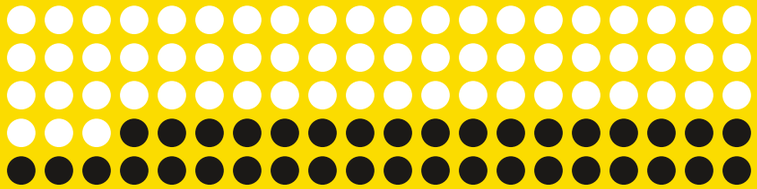
Knowledge on such cancer predisposition syndromes (e.g., Li-Fraumeni syndrome, inherited bone marrow failure syndromes, etc.) is essential for physicians to council patients and parents, to plan and perform screening investigations, to tailor therapies (e.g., avoid strong genotoxic medications and radiotherapy if possible), and to guide long-term follow-up care. In addition, certain inborn immunodeficiency disorders can pose the affected individual to a higher risk for cancer. Cancer predisposition and cancer immunology are important topics in St. Anna CCRI and St. Anna Children's Hospital. Thus, one can be glad to see that the scientific subjects of our institute are continuously expanding to address these complex issues. To give another example, research to refine cancer cell detection in order to better personalize therapies as well as monitoring after cancer therapy is also essential to improve the outcomes for childhood cancer patients. In this, close collaboration between the clinic and the research institute is crucial for success.

In my new position as head of the institute and as an oncologist at St. Anna Children's Hospital, I see it as my duty to link St. Anna CCRI and St. Anna Children's Hospital even more tightly. Close collaboration between clinicians and scientists – with some even taking on a dual role – means that the individual needs of our young patients can be addressed more precisely.

Since my start at St. Anna CCRI (at that time 'under the roof' of the hospital) and St. Anna Children's Hospital in 1993, I have seen too many children deceasing from cancer and other catastrophic pediatric diseases. Therefore, I consider it a privilege to contribute to the task of finding a cure for all young patients suffering from such diseases in the extraordinary environment of 'St. Anna' with its outstanding resources provided from the numerous generous supporters and a long tradition to care for children.

Leo Kager
Head of Institute

STAFF COMPOSITION



63% Female

37% Male

NATIONS

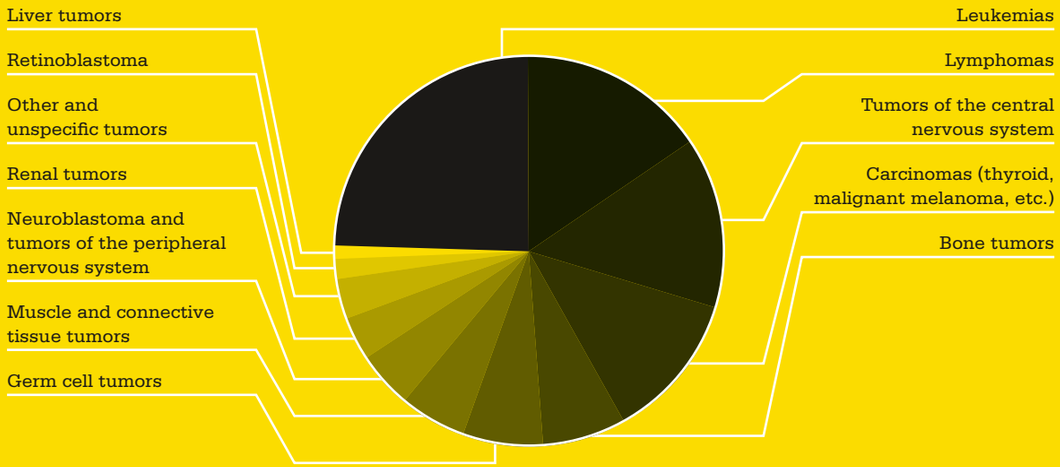
RESEARCH NETWORK

ALBANIA
ARGENTINA
AUSTRALIA
AUSTRIA
BELARUS
BELGIUM
BOLIVIA
BOSNIA AND HERZEGOVINA
BRAZIL
BULGARIA
CANADA
CHILE
CHINA
CROATIA
CZECH REPUBLIC
DENMARK
FINLAND
FRANCE
GERMANY
GREECE
HONG KONG
HUNGARY
ICELAND
INDIA
IRELAND
ISRAEL
ITALY
JAPAN
LITHUANIA
NETHERLANDS
NEW ZEALAND
NORWAY
POLAND
PORTUGAL
ROMANIA
RUSSIA
SERBIA
SINGAPORE
SLOVAKIA
SLOVENIA
SOUTH KOREA
SPAIN
SWEDEN
SWITZERLAND
TANZANIA
TURKEY
UKRAINE
URUGUAY
UNITED KINGDOM
U.S.A.

CHILDHOOD CANCER IN AUSTRIA

- Rare (<1% of all cancer cases)
- New occurrences per year:
  Children up to 14: ~200
  Adolescents: ~100
- Most common cancers: leukemia, lymphoma, brain tumors

St. Anna CCRI strives to improve diagnosis and treatment through innovative research and international cooperation



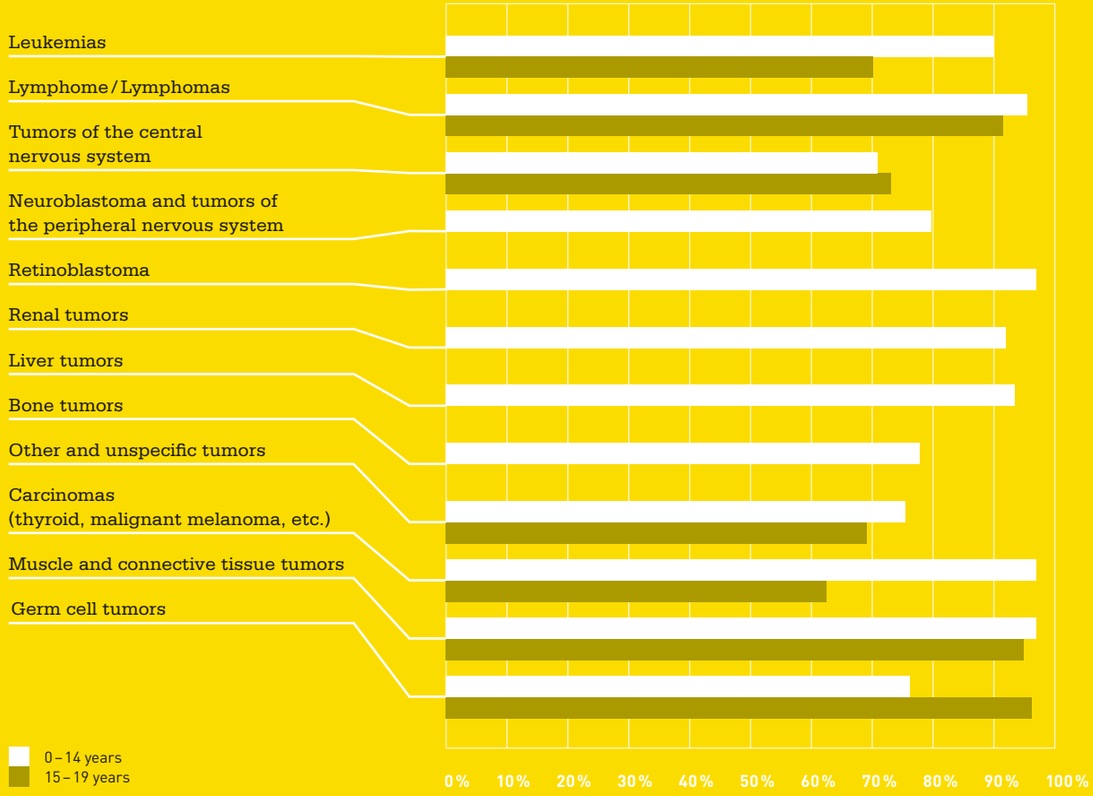
Cancer incidence (new occurrences) in children and adolescents, Austria 2009–2018

Statistik Austria, 2021

SURVIVAL AFTER CHILDHOOD CANCER IN AUSTRIA

- Total 5-year-survival: 85%
- ~30 children and 15 adolescents in Austria die annually from cancer.

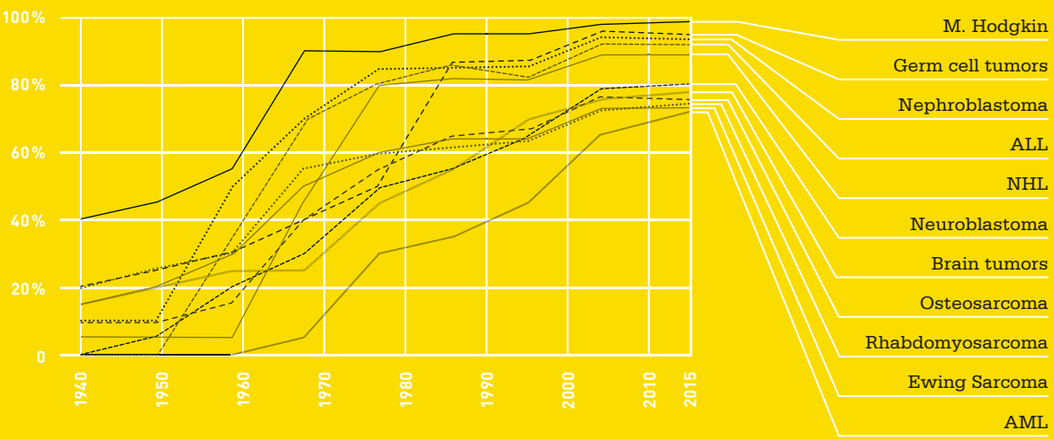
By advancing its pediatric precision oncology program ever further, St. Anna CCRI aims to increase the cure rate and the quality of survivorship for children and adolescents suffering from cancer.



CHILDHOOD CANCER SURVIVAL IN THE PAST AND TODAY

CHILDHOOD CANCER SURVIVAL RATES IN GERMANY, 1940 TO 2015.

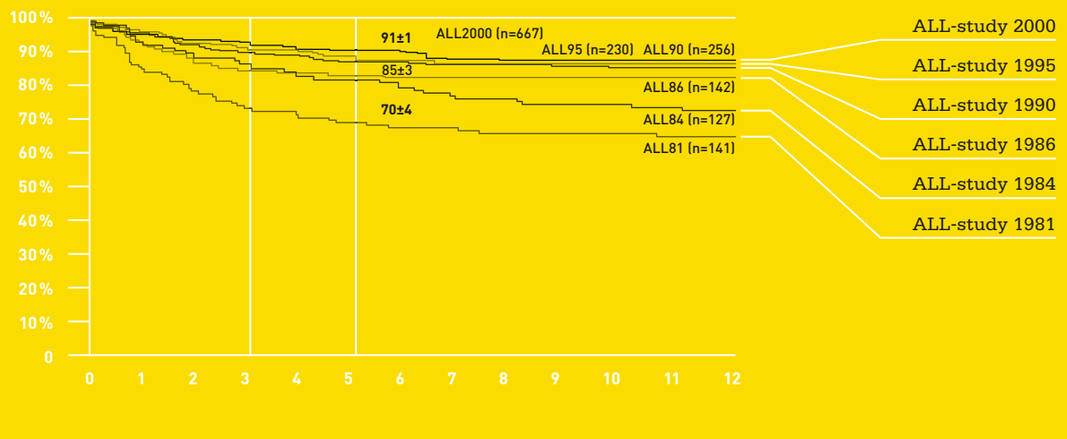
2-year survival until 1980,
5-year survival from 1980.



Burdach et al., Mol Cell Pediatr 2018

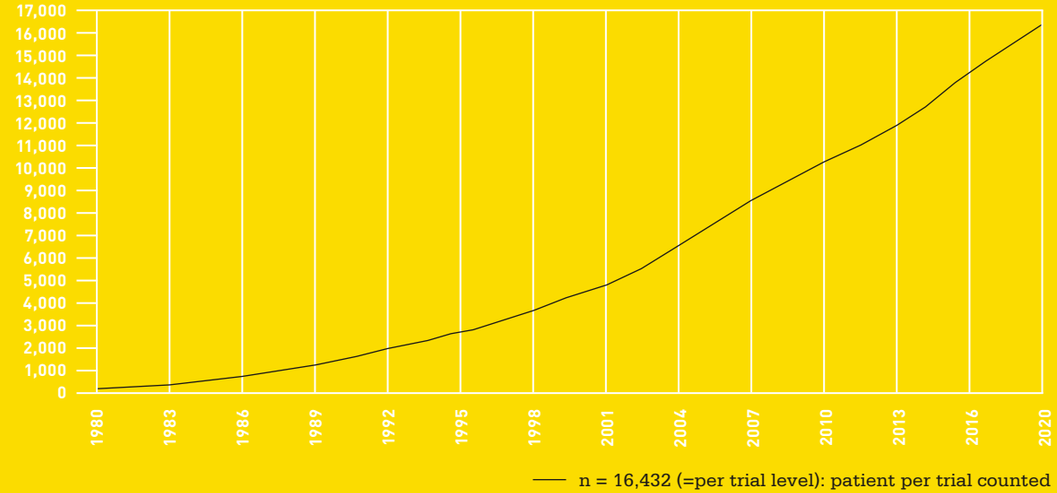
ALL: INCREASE IN LONG-TERM SURVIVAL IN AUSTRIA FROM 70 TO 91%

1,563 children with acute lymphoblastic leukemia (ALL) treated in studies from 1981 until today (AUSTRIAN ALL-BMF 1981–2009).



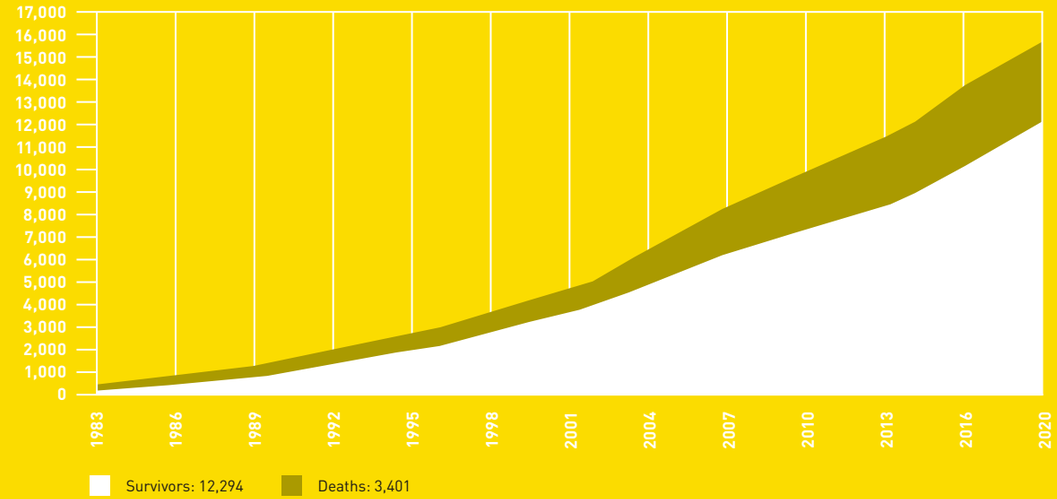
Own data

ANNUAL TOTAL PATIENT ACCRUAL WITHIN S²IRP



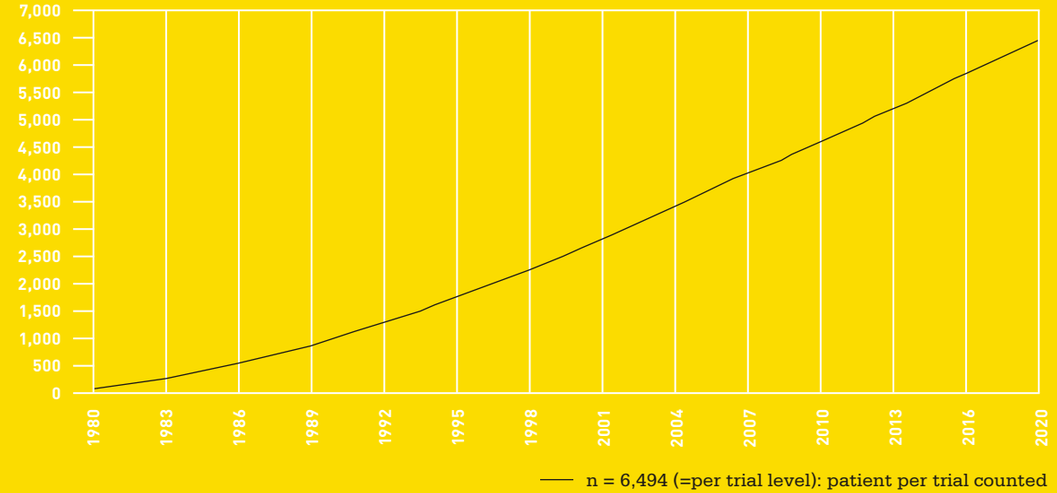
n = 16,432 (=per trial level): patient per trial counted

DEATHS AND SURVIVORS



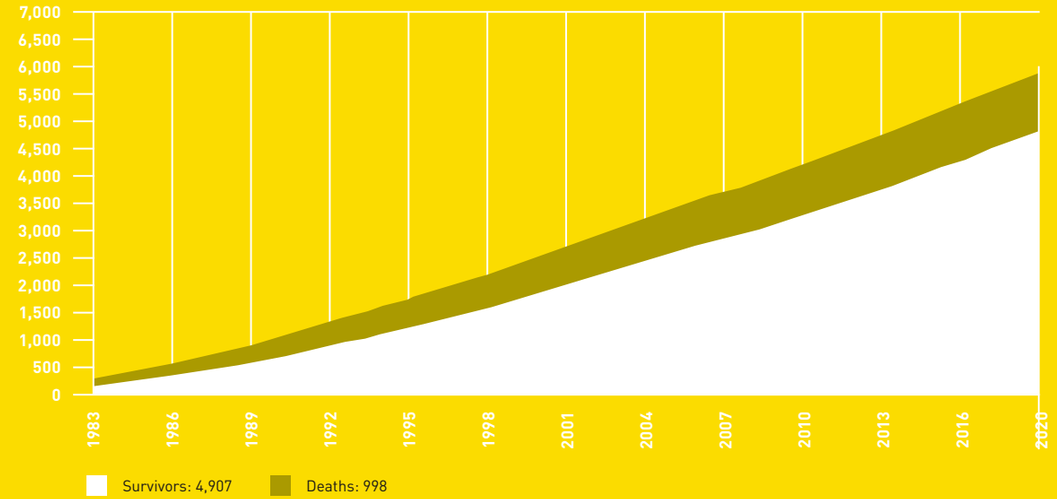
Data: Ulrike Pötschger, Statistical Team Lead, S²IRP

ANNUAL AUSTRIAN PATIENT ACCRUAL



n = 6,494 (=per trial level): patient per trial counted

DEATHS AND SURVIVORS



NEWS TICKER

Ruth Ladenstein advocates for the Survivorship Passport facilitating follow-up care of childhood cancer survivors.

Credit: Gilbert Novy

SURVIVORSHIP PASSPORT: FOR A BETTER LIFE AFTER CHILDHOOD CANCER

(Vienna, 12.7.2021) **EU funds research project to improve long-term follow-up care for childhood cancer survivors with four million euros.**

In Europe, 35,000 children and adolescents are diagnosed with cancer every year, about 300 of them in Austria. Thanks to better cancer treatments, in recent decades the survival rate has increased from 20 to 80 percent in European countries rich in resources. However, 60 to 70 percent of all survivors worldwide struggle with late effects of the disease and its treatment. A particular challenge for long-term follow-up is the transition from pediatric to general adult medical care. Relevant information to identify individual support needs and to respond accordingly is often lacking.

ALL INFORMATION IN ONE PASSPORT

The so-called Survivorship Passport aims to close this supply gap across Europe and to improve long-term follow-up care. To ensure the best possible implementation of the European digital Survivorship Passport, the EU is funding the "PanCareSurPass" research project with four million euros as part of the Horizon 2020 research and innovation program. The passport provides survivors with a detailed and complete overview of their diagnosis, treatment and disease course. In addition, it includes evidence-based, personalized recommendations for long-term follow-up care. This enables survivors' health to be monitored closely and more intensively than before – always based on their individual disease history. This could also make it easier for survivors to enter professional life and maintain a job.

Ruth Ladenstein, head of the Implementation Strategy Development in the PanCareSurPass project and head of the Department of Studies and Statistics S^2IRP at St. Anna Children's Cancer Research Institute in Vienna, comments, "The implementation of the Survivorship Passport at EU level is in many ways a success and a step towards overcoming the treatment inequalities of survivors in society. Based on this tool, adequate screening can be performed, and if necessary, appropriate therapies can be identified by means of targeted diagnostic strategies."

INVESTIGATION AND BROAD IMPLEMENTATION

So far, the Survivorship Passport is not yet available throughout the EU. However, thanks to Ruth Ladenstein's intensive efforts and the support of Austria's "Onkologiebeirat", the concept of the Survivorship Passport has already been anchored as an important strategic goal in the Austrian Cancer Plan. In 2021, Ruth Ladenstein's participation in the European Cancer Mission Board made it possible to anchor the concept for all age groups in Europe's Beating Cancer Plan as well. Together with Survivors Austria, considerations have also been made regarding networking with the ELGA electronic medical record at the federal level.

The new SurPass version will be launched and tested in a multi-national study in Austria, Belgium, Germany, Italy, Lithuania and Spain at selected hospitals/cancer registries. The vision for the future is to anchor the Survivorship Passport in the electronic health records in Europe (ELGA in Austria). The scientific teams involved are analyzing the Survivorship Passport as well as relevant electronic interfaces together with patient organizations and healthcare professionals. The question of how to use available health data from different sources accurately and effectively is one aspect of the project. Furthermore, health economic aspects of the implementation are being analyzed.

In Austria, the association Survivors Austria – Children's Cancer Survivors Initiative will support the implementation of the PanCareSurPass project in terms of content and health policy. "As part of our cooperative framework with St. Anna Children's Cancer Research Institute and CCI Europe, the European umbrella organization for childhood cancer aid organizations, we will actively shape the project with our experience gained from 18 years of work with survivors," says Hannah Gsell, chairwoman of the Survivors Austria association and project manager at Childhood Cancer International (CCI) Europe.

SURVIVORS: "NOW WE NEED THE SUPPORT OF POLITICIANS."

Asked for her point of view as a patient representative, Carina Schneider, a psychologist and board member of the Survivors Austria association and committee member of CCI Europe, says, "Early information about the possible late effects of cancer in childhood, adolescence and young adulthood is essential for one's own health management and for preventive health care. If you do not know what you may face or are likely to face, you cannot know what to pay particular attention to in terms of prevention and early detection. In Europe, it makes little difference whether you live in a rich or a poor country: there are de facto hardly any adequate care structures for the long-term follow-up of adult survivors of pediatric oncological diseases. We are impatient! For so many years, we have been working together with childhood cancer aid organizations and experts throughout Austria and Europe to develop the Survivorship Passport. Now we need the support of our politicians and our health care system to implement it, so that survivors get the long-term care they need."

From left to right:
Carina Schneider, Hanna San Nocoló, Wolfgang Holter, Eleni Tomazou, Stella Kyriakides, Suzanne Rödler, Kaan Boztug, Sabine Taschner-Mandl, Wolfgang Mückstein, Jörg Bürger, Ruth Ladenstein, Davide Seruggia, Erwin Senoner, Katherina Reich.

Credit: St. Anna Kinderspital/Kellner

EU COMMISSIONER VISITS ST. ANNA: "EUROPE'S BEATING CANCER PLAN PUTS THE SPOT LIGHT ON CHILDHOOD CANCER"

(Vienna, 08.09.2021) **Childhood cancer is different from cancer in adults. Thus, it needs research specifically tailored to pediatric tumors, which requires dedicated funding. The need for medicines suitable for children, as well as improvements in long-term follow-up care for cancer survivors have been discussed with EU Health Commissioner Stella Kyriakides and Austrian Health Minister Wolfgang Mückstein at St. Anna Children's Cancer Research Institute and St. Anna Children's Hospital.**

Due to intensive research throughout the last decades, survival rates for childhood cancer have improved considerably. "However, this comes at a high price," explains Prof. Ruth Ladenstein, head of the Clinical Trials Unit S^2IRP at St. Anna Children's Cancer Research Institute. "We owe a great many successes to long-used drugs that are not specifically approved for application in children (i.e., off-label use). Of course, that carries risks."

PUTTING CHILDREN AS THE FOCUS OF RESEARCH

60 to 70 percent of all childhood cancer survivors struggle with late effects of the disease and its treatment. "Therefore, we urgently need new drugs that are specifically tailored to children and are not only more effective, but also better tolerated," says Ruth Ladenstein, who also works as an oncologist at St. Anna Children's Hospital. In this regard, pediatric cancer research needs to focus on both, early drug development and subsequent clinical trials across Europe.
Kaan Boztug, Scientific Director of St. Anna CCRI: "At our Comprehensive Cancer Center, jointly comprising St. Anna CCRI and St. Anna Children's Hospital, we cover the entire spectrum from bench to bedside, i.e., from basic research to new applications in clinical trials."

The selection of suitable drugs for cancer in children and adolescents needs to be based mainly on a molecular understanding of the diseases in order to initiate a revolution in pediatric cancer therapy with higher chances of better long-term survival and minimization of late effects. "Even those tumors that do not respond to established therapies at the moment should be treated efficiently and successfully in the future. To this end, all modern technologies in the research field must be exploited and the understanding of carcinogenesis in childhood cancer entities must be expanded," continues Kaan Boztug, who is also a physician at St. Anna Children's Hospital. In addition, there is a need to clarify which of the results from adult oncology can be translated into childhood cancer. For example, St. Anna CCRI recently showed that so-called *ALK* alterations in childhood nerve tumors reduce the chance of survival. Accordingly, *ALK* inhibitors used in adult cancers could also be helpful in children with nerve tumors.

EU IS AN ESSENTIAL FUNDING SOURCE

St. Anna CCRI closely collaborates with other centers of expertise, both nationally and internationally. "We have built up a network of strong partners and together we can achieve a lot. However, we often still lack adequate funding because drug development in the field of rare diseases – and pediatric cancer is one of them – is not driven economically, compared with common indications in the adult field," explains Ruth Ladenstein. "This means that children need special attention and also special funding opportunities, so that the many open questions are taken into account in future funding programs," says Ruth Ladenstein.

That this hope comes true seems to be a realistic scenario. The EU Commissioner Stella Kyriakides, responsible for Health and Food Safety, points out: "Cancer is the leading cause of death in children over 12 months of age in Europe. The recent pandemic has confronted us with additional challenges. Regular checkups and sometimes even therapies have been postponed. We all know what that means – not only in terms of clinical outcomes, but also of psychological stress for the families. That is why we put a special focus on childhood cancer in Europe's Beating Cancer Plan, which is budgeted at 4 billion euros. We have now launched the first calls in the EU4Health program, which includes setting up a network for young adult survivors of childhood cancer." According to the Austrian Health Minister Wolfgang Mückstein, Austria will fully support not only Europe's Beating Cancer Plan, but also the Mission Cancer. The EU Mission Cancer initiative, co-developed by Ruth Ladenstein as a member of the EU Mission Cancer Board, aims to ensure that the problems associated specifically with pediatric cancers are actively addressed.

EQUAL TREATMENT ACROSS BORDERS

Another topic of discussion has been inequalities of childhood cancer survival across Europe. "We must ensure that children in Europe have access to state-of-the-art therapies," emphasizes Stella Kyriakides. Wolfgang Holter, Medical Director of St. Anna Children's Hospital and Head of St. Anna CCRI, points out: "Our Children's Cancer Center has a long tradition in cross-border healthcare. It is our strong desire that the best clinical expertise as well as the latest research findings benefit all children in Europe, regardless of where they live."

One tool to achieve equal chances of survival for all children with cancer in Europe is the European Reference Network for Pediatric Oncology, ERN PaedCan – coordinated by Ruth Ladenstein." This network connects comprehensive pediatric cancer centers, accredited by the European Commission to create a clear governance structure for knowledge sharing and care coordination," says Ruth Ladenstein. Wolfgang Mückstein comments, "For the first time, such networks provide a structural collaboration in healthcare across Europe. This will continue to be very important in the future."

ERN PaedCan comprises numerous strategies to make specialized know-how and life-saving pediatric oncology treatments accessible throughout the EU. One of them is the so-called twinning: Centers with high potential in "widening countries" become affiliated partners. Through twinning programs, these centers receive support from an established center to achieve higher standards of education, research and care. "To overcome existing inequalities in Europe, we need more twinning projects, which must be funded through non-competitive grants," Ruth Ladenstein urges. Stella Kyriakides agrees: "Dedicated training programs to support the pediatric cancer community in building strong multidisciplinary workflows are essential. With Europe's Beating Cancer Plan, we have set ourselves an ambitious program. What matters now is the implementation, and here I ask all relevant stakeholders, media professionals and patients to work together. Thank you once again for the commitment and research you are doing here. Together, we can support children much better."

MEETING THE NEEDS OF CHILDHOOD CANCER SURVIVORS

A key goal of Europe's Beating Cancer Plan is to improve the quality of life for cancer patients and survivors. Late effects of childhood and adolescent cancer can remain undetected, or follow-up care is insufficient or rather it comes too late. After the age of 18, pediatric care is no longer responsible for the necessary long-term follow-up care. However, most adult medical facilities do not meet the special needs of this patient group. This is due to a lack of complete quality-assured information transfer in a condensed form for follow-up care with targeted medical check-ups as well as treatment plans for follow-up care for the diagnosis and therapy of late effects after successful cancer therapy.

One tool designed to counteract this lack of information and to facilitate individual follow-up care is the Survivorship Passport (SUPA), which was developed in several EU-funded projects and has since been awaiting implementation throughout Europe. As a first step, the Austrian Cancer Plan included the implementation of SUPA as an operational goal back in 2014. According to this, every person suffering from childhood cancer in Austria should receive a SUPA, which summarizes oncological diagnoses and therapies and contains individual follow-up recommendations.

"Survivors Austria", a patient organization that has been a strong supporter of the project from the beginning, is once again calling for SUPA to be implemented in the electronic health record ELGA. Board member Carina Schneider, who has become involved as a patient representative since her own cancer diagnosis in her adolescence, calls on politicians to wait no longer: "We established the Survivorship Passport model in Austria so many years ago, all of Europe has invested a lot in its development. Now its integration into ELGA is the necessary next step, so that SUPA can finally support survivors. When will our healthcare system make it possible for us to make use of SUPA to better deal with late effects?"

"You are right. The Survivorship Passport has been postponed because of the pandemic. Now we care about moving this important issue forward," announces Wolfgang Mückstein.

Katharina Reich, Head of Department VII for Public Health and the Health Systems, adds, "It is of great concern to us that survivors of childhood cancer can have their detailed medical history available like a common thread, by means of using state-of-the-art structures. Intensive work is being done on the project."

In her new role, Sabine Taschner-Mandl would like to introduce significantly more targeted therapies, not only in early phase I or II studies, but also in larger trials.

Credit: H. Eisenberger

SABINE TASCHNER-MANDL ELECTED TO THE EXECUTIVE BOARD OF EUROPE'S NEUROBLASTOMA NETWORK SIOPEN

(Vienna, 20.10.2021) **Developing new therapies for childhood nerve tumors and identifying more molecular markers for better diagnosis – this is what Sabine Taschner-Mandl strives for in her new role as Executive Committee Board Member of the European Neuroblastoma Research Group SIOPEN.**

Sabine Taschner-Mandl, head of the Tumor Biology Research Group at St. Anna Children's Cancer Research Institute, has recently been elected to the Executive Committee Board of the International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN). For the first time a molecular biologist is joining the Board, which until now has been composed mainly of clinicians. Sabine Taschner-Mandl's election was confirmed at this year's SIOPEN Annual General Meeting in Barcelona at the beginning of October.

DISCOVERING TUMOR TARGETS

SIOPEN's declared aim is to improve the survival of children with neuroblastoma – a tumor that arises from immature nerve cells. When asked how she plans to drive this goal forward, Sabine Taschner-Mandl explains, "I want to promote translational research and interdisciplinary teamwork within the study group. Two aspects are particularly important to me. First, we would like to introduce significantly more targeted therapies, not only in early phase I or II studies, but also in larger trials. To identify the most effective drugs, detailed knowledge of the tumor biology is essential. This is why molecular tumor profiles need to be incorporated into translational studies."

Secondly, Sabine Taschner-Mandl emphasizes the prognostic role of biomarkers in predicting which patients will benefit from treatment, and indicating if treatment may need to be adapted. "We need more molecular markers to tell us how efficient a therapy is. Such markers allow us to early identify those children who do not respond or do not respond sufficiently to standard therapies. To improve treatment for the latter patient groups in the future, it is essential to implement translational research findings into clinical trial designs as quickly as possible in order to guide treatment decisions."

DREAM OF BORDERLESS RESEARCH

The Executive Committee Board meets once a month – at the moment virtually – to decide on clinical trials and projects and to promote networking. "In addition, we also aim at solving problems in currently running translational studies," Sabine Taschner-Mandl points out. She refers, for instance, to difficulties in cross-border research activities. To give an example, sometimes it is not possible to ship samples from one country to another because national interpretations of European directives can differ substantially.

"We hope to raise awareness of such problems with the aim of supporting joint SIOPEN projects with the necessary infrastructure, for example by creating a central database, namely the BIOPORTAL."

Austria is one of the smaller countries with comparatively few study patients within SIOPEN. "As Country Representative, I am particularly excited to represent Austria and St. Anna Children's Cancer Research Institute internationally," Sabine Taschner-Mandl adds.

ABOUT SIOPEN

The International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN) Association has its registered office in Vienna and was founded during the presidency period of Ruth Ladenstein for SIOPEN in Vienna in 2009. The SIOPEN Association fosters neuroblastoma research and enables long-term sustainability of the group. Currently, the SIOPEN Association has 432 active members in 34 countries and 29 countries are involved in SIOPEN studies.
www.siopen.net

KAAN BOZTUG TAKES OVER PROFESSORSHIP

In his research, Kaan Boztug aims at expanding activities in the field of rare diseases and fostering the synergies between interdisciplinary experts.

Credit: Ian Ehm

(Vienna, 7.12.2021) **Kaan Boztug, consultant in pediatrics and adolescent medicine and internationally renowned expert in rare hematopoietic and immunological diseases, has accepted a professorship (§ 99 Para. 4 UG) in the subject field Pediatrics and Inflammation Research at the Medical University of Vienna's Department of Pediatrics and Adolescent Medicine with effect from December 1st 2021.**

During the course of his medical and scientific work in the field of congenital immunodeficiencies and hematopoietic disorders, Kaan Boztug has played a leading role in the description and molecular characterization of more than 15 previously unknown diseases. These research projects have explained essential mechanisms of hematopoiesis and bone marrow failure, T- and B-cell regulation and neutrophil function, thereby paving the way for future personalized therapeutic approaches.

He has received numerous prizes and international awards, including the FWF START Prize, one ERC Starting Grant and one ERC Consolidator Grant, for this outstanding work, which has been published in renowned high-impact journals. He has also received the Merit Award of the American Society of Hematology, the Kind-Philipp Prize for Leukemia Research, the Clemens von Pirquet Prize of the Austrian Society for Pediatrics and Adolescent Medicine, the City of Vienna Research Prize for medical sciences, and the Johann Wilhelm Ritter von Mannagetta Prize for Medicine awarded by the Austrian Academy of Sciences.

As a physician-scientist, Kaan Boztug has always combined research with the care and treatment of patients with rare and undiagnosed hematopoietic and immunological diseases. In his clinical and experimental research, his aim is to expand activities in the field of rare diseases and foster the synergies that exist between interdisciplinary experts and encourage collaborative working. He is particularly committed to imparting this translational approach in the teaching and training of talented young researchers and clinicians.

"Excellent collaboration between clinicians and experts from different disciplines and institutions is essential for medical progress and success, especially in the field of rare diseases. This is my greatest driver, as is my need for a deep molecular understanding of the causes of immunological and hematological diseases, so that we can use this as a basis for developing precision medicine approaches!" states Boztug.

Martin Distel, Florian Halbritter, and Heinrich Kovar receive the prestigious ALSF Crazy 8 Initiative Award for their project "Tracking the origin of Ewing sarcoma (...)".

Credit: St. Anna CCRI

ALEX'S LEMONADE STAND FOUNDATION CRAZY 8 INITIATIVE AWARD GOES TO VIENNA: TRACKING THE TUMOR ORIGIN TO CURE CHILDHOOD CANCER

(Vienna, 16.3.2021) **The project "Tracking the origin of Ewing sarcoma (...)", coordinated by St. Anna Children's Cancer Research Institute, has been selected for the prestigious Crazy 8 Initiative Award. With this funding, Viennese scientists aim to elucidate the still unsolved mystery of the origin and development process of pediatric bone sarcomas. This knowledge is fundamental to lay a path for new and more effective therapies to cure childhood cancer.**

A team of researchers led by Heinrich Kovar has received the highly endowed Crazy 8 Initiative Award from Alex's Lemonade Stand Foundation (ALSF), a leading funder of pediatric cancer research, for their research project "Tracking Ewing sarcoma origin by developmental and trans-species genomics".

Through a rigorous review process, ALSF received over 100 letters of intent from researchers that resulted in 83 full grant applications which were ultimately narrowed down to four projects. One of them is now starting in Vienna.

Together with researchers from St. Anna Children's Cancer Research Institute, the Medical University of Vienna and the University of Natural Resources and Applied Life Sciences, Vienna, Heinrich Kovar strives to answer a tricky question that has occupied scientists for generations: What is the origin of bone sarcomas and how do they form?

Ewing sarcoma is a very aggressive bone tumor in children and adolescents, and associated with poor long-term survival in about one third of the patients. The need for novel treatment options to improve the outcome is high, but the clinical development of innovative new medicines is severely hampered by low incidence rates and lack of pre-clinical models.

CHALLENGING LIKE CRAZY, BUT CRITICALLY NEEDED

Despite intensive research throughout the last decades, the tissue origin of Ewing sarcoma as well as the timing of its genetic onset remains unknown. This fact prevents the development of suitable disease models to test drug mechanisms and efficacy, resulting in a lack of innovative therapies. "Now, we are breaking new ground to decipher the secret of this disease," says Heinrich Kovar. "The overall goal of this project is to develop an appropriate pre-clinical tumor model recapitulating the human disease. This would allow for high-throughput pre-clinical drug screenings."

"To decipher the tissue and differentiation state of origin for Ewing sarcoma, we will follow two complementary approaches," announces Heinrich Kovar.

EXPLORING HUMAN EPIGENETIC MEMORY IN FISH

Led by St. Anna CCRI's Martin Distel, the first approach takes advantage of the cell's epigenetic memory, "remembering" its tissue of origin, to find the cells in zebrafish larvae analogous to the cells of bone sarcoma origin in humans. Heinrich Kovar explains, "Gene expression is widely regulated by epigenetics. From the cell's epigenetic memory, we utilize several gene-regulating elements, which are typically active in human Ewing sarcoma and which are most likely activated in the cell of cancer origin. We use these elements to regulate fluorescent reporter genes in zebrafish larvae, coding for fluorescent proteins." As soon as the sarcoma-specific regulating elements are activated in specific cells of the zebrafish larvae, these cells light up.

"We closely watch this process throughout the whole fish development and ask the following questions: When do cells start to light up? In which tissue? And for how long?" explains Martin Distel.

Subsequently, the researchers isolate the fluorescent cells to determine their identity. They then express the disease-driving oncogene in the identified cell type, which is supposed to result in tumor formation in the fish.

Until now, it has not been possible to grow human-like Ewing sarcoma in an animal model. If the researchers succeed, this would be a huge step forward in the investigation of bone sarcoma in children.

CREATING A GENE EXPRESSION ATLAS TO MAP CANCER ON HEALTHY TISSUE

The second approach, co-led by Florian Halbritter, focuses on mesenchymal and neural crest stem cells, which were proposed as candidate cell types of Ewing sarcoma origin. "By using single-cell transcriptome and epigenome analyses, we will create an exact reference map of human stem cell development. We will differentiate these stem cells into cartilage, bone, fat or nerve cells. At different time points of stem cell differentiation, we will then activate a cancer-inducing oncogene and dynamically monitor sarcoma-like transformation."

"We want to know: how do these cells differentiate with and without the oncogene?" says Florian Halbritter. This knowledge will be summarized in a differentiation atlas of healthy versus cancer development. The hereby created map will be compared with data from human tumors, to see which cell types and differentiation states best match with the tumors.

"In these independent but complementary approaches, the novel and crucial aspect of our strategy is to not only localize the cell of tumor origin but also to investigate at which developmental stage tumor formation is induced. If successful, we expect our approach to converge on few candidate cell types and differentiation states, which will be used to model the disease," explains Heinrich Kovar.

WHEN TAKING A HIGH RISK, THE RIGHT TEAM IS CRUCIAL FOR SUCCESS

This multidisciplinary research project is only possible in close collaboration of St. Anna CCRI's research groups of Ewing sarcoma expert Heinrich Kovar, molecular biotechnologist Martin Distel, and computational biologist Florian Halbritter (all St. Anna CCRI) with developmental biologist Igor Adameyko and genomics expert Matthias Farlik (both Medical University of Vienna) and cell engineering expert Cornelia Kasper (University of Natural Resources and Life Sciences, Vienna).

The Crazy 8 Initiative, provided by Alex's Lemonade Stand Foundation, strives to fund "impactful research that is critically needed for children with cancer types that have been historically difficult to cure." It refers to eight unsolved, burning research questions in childhood cancer, which are most difficult to tackle. "Therefore, we need to take a high risk and break new ground in research," says Heinrich Kovar. "There is a risk of failure, but a chance to change the game in the fight against childhood cancer.

ST. ANNA CCRI EARNS WWTF GRANT TO PROMOTE PRECISION MEDICINE IN CHILDHOOD CANCER

Award winner Eleni Tomazou: "We are very excited. Our liquid biopsy assay could be implemented for personalized adaptations of childhood cancer therapy."

Credit: Harald Eisenberger

(Vienna, 8.6.2021) **St. Anna Children's Cancer Research Institute is receiving a prestigious Life Science Grant for Precision Medicine, provided by the Vienna Science and Technology Fund (WWTF). Award winner Eleni Tomazou and her colleagues will clinically validate a promising new diagnostic approach, expected to enable precision medicine in childhood tumors based on blood samples.**

Eleni Tomazou, a Prinicipal Investigator at St. Anna CCRI, is being awarded one of the highly endowed Life Science Grants for Precision Medicine, which are issued by the Vienna Science and Technology Fund (WWTF). The selected project focuses on the clinical validation of a minimally invasive diagnostic toolkit for pediatric sarcomas. Out of 82 short applications, 24 projects were invited for a full proposal and seven projects were selected for funding with an overall amount of € 6.07 million. Eleni Tomazou, together with Christoph Bock from the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Markus Metzler from the University Hospital Erlangen, and Uta Dirksen from the University Hospital Essen, will be able to devote a funding amount of almost € 900,000 to their research project on childhood tumors.

The project is based on the concept of liquid biopsies, where blood samples are collected and analyzed for short DNA fragments that a tumor has leaked into the blood stream. This approach provides a minimally invasive alternative to conventional tumor biopsies, holding great promise for precision medicine. However, clinical uptake in pediatric oncology has been slow compared to adult cancers, in part because low mutation rates in childhood cancer hamper the use of genetic markers for identifying tumor-derived DNA fragments.

"We have developed a liquid biopsy assay that is independent of genetic defects. It combines whole genome sequencing of cell-free DNA with machine learning algorithms to detect epigenetic and gene-regulatory patterns characteristic of tumor cells. Epigenetic patterns help to determine under which circumstances a gene is switched on and when it becomes mute," explains Eleni Tomazou, who leads the Epigenome-based Precision Medicine research group at St. Anna CCRI. "In Ewing sarcoma, a bone cancer in children and young adults, we found widespread epigenetic aberrations. We exploit these alterations to improve diagnosis and monitoring of this disease, simply by analyzing fragmentation patterns of tumor-derived DNA circulating in the blood."

NEW DIAGNOSTIC TOOL KIT FOR PEDIATRIC BONE CANCER

A promising route towards better outcomes for patients with Ewing sarcoma is to personalize chemotherapy, such that each patient receives as much treatment as needed to maximize the chances of curing the cancer, while minimizing the long-lasting side effects that come with high-dose chemotherapy. "As the next step in our research, this funding allows us to clinically validate our liquid biopsy assay and thereby take a decisive step towards a more personalized treatment strategy for Ewing sarcoma," says Eleni Tomazou.

The research team will pursue three applications of their minimally invasive diagnostic kit:

- Discrimination between patients with localized Ewing sarcoma with high versus low risk and detection of occult metastases at diagnosis
- Real-time monitoring of the initial response to chemotherapy
- Disease monitoring for early detection of relapse during continued chemotherapy and follow-up

PAVING THE WAY TOWARDS ADAPTIVE CLINICAL TRIAL DESIGN

"We are very excited. Our project will establish the foundations for innovative clinical trial design in pediatric cancers. Our liquid biopsy assay could be implemented for personalized adaptations of therapy, and for monitoring of patients to detect and interfere with relapses. Our approach complements other initiatives that focus on the genome or transcriptome. This has limitations in Ewing sarcoma and other childhood cancers, which we will address by looking at the characteristic epigenomes of these tumors," says Eleni Tomazou.

Kaan Boztug, Scientific Director of St. Anna CCRI comments, "This is a very promising project with high clinical relevance. The active participation of the German project partners as well as the close ties with the iEuroEwing consortium strengthens our international standing."

Successful completion of the funded project will qualify epigenome-based liquid biopsy as molecular biomarkers for inclusion in prospective clinical trials.

“1000 IDEAS” TO BOOST CHILDHOOD CANCER RESEARCH: ST. ANNA CCRI EARNS TWO GRANTS IN COMPETITIVE FWF PROGRAM

(Vienna, 29.6.2021) **Two projects from St. Anna Children’s Cancer Research Institute are receiving funding from the “1000 Ideas Program” of the Austrian Science Fund (FWF). The aim of this program is to support daring and original research ideas that lie outside the current scientific understanding. In the second call for funding in this program, Eleni Tomazou and Florian Halbritter, together with Martin Distel from St. Anna CCRI were among 22 awardees from a total of 270 applicants.**

Recently, the Austrian Research Fund FWF identified an area that has been largely neglected to date, namely high-risk ideas that may be too premature to have good chances of obtaining funding via existing grant programs due to unconventional design or lack of validating data. The FWF’s “1000 Ideas Program” funds exactly such projects, characterized by a high risk of failure but also comprising great innovative potential. Among the recent funding grants, two projects from St. Anna CCRI were selected, one from Eleni Tomazou and one from Florian Halbritter and Martin Distel. Since childhood cancers are rare diseases, projects such as from our grant winners are an effective and useful complement to classical, conventional research projects, and may open new paths on the way to improving childhood cancer diagnostics and therapy:

Florian Halbritter, Eleni Tomazou and Martin Distel (from left) receive funding from the FWF’s “1000 Ideas Programme”.

Credit: St. Anna CCRI

CRACKING THE RIBOSOME CODE OF CANCER DRUG RESISTANCE

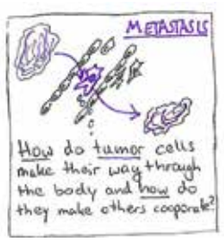
Drug resistance is the biggest challenge in cancer therapy. It exists across all types of cancer and all modes of treatment. Understanding the underlying mechanisms by which cancer cells escape cell death is important for the design of effective and sustainable therapeutic interventions. This project, led by Eleni Tomazou, seeks to establish selective protein translation as a novel mechanism of chemotherapeutic drug resistance. “It is based on the hypothesis that heterogeneity in the composition of ribosomes (the “ribosome code”) affects translation in a cell-specific manner and enables some cancer cells to adapt swiftly to chemotherapy by acquiring a metastable phenotype of drug tolerance,” informs Eleni Tomazou. If confirmed, this hypothesis will establish a new resistance mechanism and open up novel therapeutic strategies to prevent therapy resistance by specific targeting of drug tolerance-conferring ribosomes.

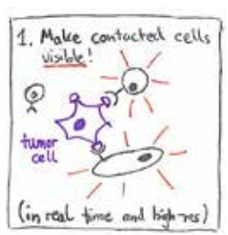
CELL CONTACT TRACING TO CATCH METASTASIS IN THE ACT

Cells in humans do not exist in isolation. Interactions between cells critically shape their development and behavior. Yet, the molecular effects of cell contacts remain impossible to track at scale. Florian Halbritter, Martin Distel, and their teams want to elucidate when, where and how tumors manipulate cells to pave their way to forming metastases. To track cell contacts and their effects on the interactors simultaneously, they will develop a system for molecular recording of these contacts by fusing live imaging with deep genomic analysis. The goal of this project is to establish a prototype system for comprehensive cell contact tracing with broad applicability, or a refined blueprint for how such a system could be implemented.

“Our project will provide the means to answer fundamental questions in biomedical research. It is expected to lay the grounds for future, better-informed bioengineering and robust study design. We are happy to make the first step with this ambitious pilot,” says Florian Halbritter.

Florian Halbritter and Martin Distel aim to dissect tumor-normal cell interactions during metastasis by making cell contacts “visible” – in parallel, under the microscope and for genomics (or other *-omics) assays. This will enable the first comprehensive, multi-scale tracing of cell contacts and the effects of those contacts.








Thomas Lion, Eva König, Eleni Tomazou and Kaan Boztug being awarded an FWF grant.

Credit: Harald Eisenberger (3), Ian Ehm (1)

FOUR FWF STAND-ALONE PROJECTS RECENTLY APPROVED AT ST. ANNA CCRI

(Vienna, 12.8.2021) **The Austrian Science Fund (FWF) is awarding four scientists from St. Anna Children's Cancer Research Institute with Stand-Alone Grants, each of which is worth almost € 400,000. Congratulations to the grantees Kaan Boztug, Eva König, Thomas Lion, and Eleni Tomazou!**

Getting a Stand-Alone Project approved from the Austrian Science Fund (FWF) is what every basic scientist in Austria strives for at least once. This program successfully fulfills the FWF's mission of providing a solid foundation for basic research at the highest level in Austria. Thus, Stand-Alone Projects are an important cornerstone of the scientific work at St. Anna CCRI, an organization solely financed by donations and grants from national and international funding bodies. We are therefore proud to present four FWF projects from St. Anna CCRI scientists.

UNRAVELING THE KILLING FUNCTION OF IMMUNE CELLS

In their recently approved Stand-Alone Project, Kaan Boztug, Scientific Director of St. Anna CCRI, and co-authors Artem Kalinichenko, Senior Postdoc, and Alexis Lomakin, Program Leader & Deputy Lab Head in Kaan Boztug's group, aim to investigate the role of a small GTPase in coordinating the killing function (i.e., cytotoxicity) of immune cells. They have recently discovered an essential role for this protein for the cytotoxic function of human lymphocytes mediated through its capacity to control both the actin cytoskeleton and the exocytosis machinery. Exocytosis is used by specific immune cells to release cytotoxic molecules to attack and kill infected or tumor cells. "Discovering the specific mechanisms through which the identified protein controls and coordinates the actin cytoskeleton and exocytosis in space and time is the main goal of our project," explains Kaan Boztug. He is convinced that the outcome of this project will directly contribute to the understanding of the molecular mechanisms of inborn errors of immunity. Furthermore, it might predict potential therapeutic avenues for such disorders.

POTENTIATING NATURAL KILLER CELLS TO FIGHT CANCER

Another FWF Stand-Alone grantee, Eva König, Principal Investigator at St. Anna CCRI, is investigating mechanisms that inhibit the function of natural killer cells (NK) in order to find novel ways to improve their cytotoxic capacity. Ultimately, this project aims to find immuno-therapeutic agents to fight against cancer more efficiently.

In recent years, new anti-cancer therapies, such as immunotherapy with natural killer (NK) cells, have reached the clinics and shown promising results. However, oncologists are still facing challenges caused by resistance to therapy and secondary metastasis. In order to find more potent and long-lasting therapeutics against cancer it is essential to determine the signaling events that are induced in NK cells upon their recognition of tumor or infected cells and that are required for effective target cell elimination. The transcription factor STAT1 plays a key role in NK cell function, regulating fundamental aspects such as cell

maturation and cytotoxicity. Eva König and colleagues previously suggested a transcription-independent function and so far unrecognized involvement of STAT1 in the immunological synapse of NK cells – a discovery, which will be investigated further in this project.

BACTERIA AND FUNGI: TWO PARTNERS IN CRIME

In their Stand-Alone Project, Thomas Lion, Medical Director of Labdia Labordiagnostik and Principal Investigator at St. Anna CCRI, and colleagues investigate polymicrobial infections involving bacteria and fungi. They aim at identifying biomarkers for diagnostics to detect these infections early. To date, interactions between bacteria and fungi in the context of invasive infections have not been widely addressed. Neither are current diagnostic approaches designed to routinely assess infections by more than one pathogen. The presence of bacterial-fungal co-infections may therefore be missed. However, polymicrobial infections are of paramount importance because of the potential severity of clinical manifestations, often associated with increased resistance to antimicrobial treatment.

INTERPLAY OF ONCOGENIC FUSIONS AND CELLULAR CONTEXT IN SARCOMA

The FWF Stand-Alone Project of Eleni Tomazou, Principal Investigator at St. Anna CCRI, tackles a fundamental question in cancer biology: Why and how do certain oncogenic driver genes promote cancer in one cellular context but not in another. It focuses on fusion oncogenes relevant to sarcoma. Despite intensive research, the mechanism how fusion-driven sarcomas are induced and the cells of their origin remain unresolved until today. Together with her team, Eleni Tomazou will combine pluripotent stem cell differentiation with forced expression of sarcoma-linked fusion oncogenes, single-cell analysis, and functional perturbation experiments, to systematically probe the cellular contexts and molecular mechanisms of fusion-driven sarcomagenesis in human cells. This project will not only lead to new molecular insights into fusion-driven carcinogenesis but could also help to identify cell context-specific therapeutic vulnerabilities.

Branka Radic-Sarikas and Heinrich Kovar are developing innovative in vitro models recapitulating the full complexity of lung metastases to test novel treatments.

Credit: St. Anna CCRI

ALTERNATIVE METHOD TO ANIMAL TESTING FOR DEVELOPING THERAPIES AGAINST CHILDHOOD CANCER

(Vienna, 22.12.2021) **Congratulations to St. Anna CCRI scientists Heinrich Kovar and Branka Radic-Sarikas for winning a grant from the Austrian Science Fund (FWF) under the program "Alternative methods to animal testing".**

By using state-of-the-art technologies like single cell and spatial transcriptomics, organoids and 3D bioprinting, the research project MetLung aims at creating and validating patient-specific 3D models of lung metastatic solid tumors, ultimately guiding personalized drug selection. Metastasis is a major cause of death for children and adolescents with cancer, and the most common location of pediatric solid tumor metastases is the lung.

"Our failure to cure patients with metastasis is partly due to a lack of understanding of tumor and niche cell interactions, and the associated mechanisms of treatment resistance. Knowledge about mediators of metastasis common to different tumor types may unravel novel therapeutic targets," explains Branka Radic-Sarikas.

"In this project we are developing innovative in vitro models recapitulating the full complexity of lung metastases to test novel treatments. This approach will allow us to prioritize the most promising compounds for clinical development thus significantly reducing the need for pre-clinical testing in animals," concludes Heinrich Kovar.

Irfete Fetahu intends to determine if epigenetic defects are associated with the development of neuroblastoma, the most common cancer in infants.

Credit: St. Anna CCRI

NEW FWF STAND-ALONE PROJECT AIMS AT DECODING THE EPIGENOME IN NEUROBLASTOMA

(Vienna, 23.11.2021) **Congrats to Irfete Fetahu, postdoc at St. Anna CCRI on receiving a Stand-Alone Grant from the Austrian Science Fund FWF.**

Together with her Principal Investigator Sabine Taschner-Mandl from the Tumor Biology group and colleagues, Irfete Fetahu has the ambitious goal to decode the epigenome and its regulation in neuroblastoma, a tumor that forms in certain types of nerve tissue and is the most common cancer in infants.

Whereas approximately 30% of neuroblastoma cases can be attributed to genetic mutations, the rest of the cases are still an enigma that captivates scientists and physicians.

To bring more light into the mechanisms involved in neuroblastoma development, Irfete Fetahu intends to explore the genome-wide epigenetic landscapes of neuroblastoma, to determine if epigenetic defects are associated with the development of this malignant disease, and, finally, to study the molecular mechanisms governing its epigenetic regulation.

FWF's Lise Meitner Fellowship aims to integrate fellows like Michael Kraakman into Austrian research institutions and thus generate "brain gain".

Credit: Gilbert Novy

MICHAEL KRAAKMAN STARTS RENOWNED LISE MEITNER FELLOWSHIP

(Vienna, 7.1.2021) **Congratulations to Michael Kraakman, who is now embarking on his FWF Lise Meitner Fellowship at St. Anna CCRI to investigate severe immunodeficiencies.**

The Austrian Science Fund's (FWF) Lise Meitner Program is dedicated to promoting highly qualified researchers from abroad who could contribute to the scientific development of an Austrian research institution. "In this project, we are investigating four patients with a severe immunodeficiency disease caused by mutations in a gene that is not well characterized," explains Michael Kraakman from the group of Kaan Boztug.
"These mutations render the patient's cells unable to produce a particular protein whose function was not previously known to be important in the human immune system. Based on the limited information about this protein, we hypothesize that it is critical for the control of proteostasis." Representing an essential asset for all human cells, proteostasis is a collective term for processes that ensure the production of all proteins required for cells to function and respond to their environment. "To explore the role of this protein in proteostasis, we will closely examine the immune cells of our patients. We aim to understand how the impairment of this protein causes immune deficiency, and thus draw conclusions about its function in healthy individuals," states Michael Kraakman.

PARTICULARLY AT RISK OF PERTURBED PROTEOSTASIS: IMMUNE CELLS

Results of Michael Kraakman and colleagues to date have shown that B cells – the immune cells responsible for antibody production – are particularly sensitive to disrupted proteostasis, significantly reducing their cellular survival. In addition, loss of proteostasis control in another important immune cell subtype, namely T cells, caused these cells to become "exhausted," which was associated with decreased metabolic capacity, and impaired functionality.

"Our goal is to deepen our understanding of proteostasis control in the human immune system by using the latest technologies to study defective proteostasis and to map altered metabolic pathways and associated signals that promote cell death. State-of-the-art molecular techniques will allow us to identify additional molecular interactors that may be relevant and contribute to our patients' disease and hopefully identify avenues for therapeutic intervention." Finally, after combining these emerging data with currently available information, the research team will apply computational models to predict additional proteins whose dysfunctions may also cause similarly severe immunodeficiencies.

ABOUT FWF'S LISE MEITNER PROGRAM

The Austrian Science Fund's (FWF) Lise Meitner training and career development program aims to attract highly qualified researchers of all disciplines from abroad to research institutions and research programs in Austria and take targeted measures that provide them with a maximum of support in their research work and career development during the postdoc stage. Mentoring by a co-applicant is an especially important part of this program.

Furthermore, the program intends to create added value through the research cooperation between co-applicants and the Lise Meitner Fellows by opening up new fields of research, by establishing new research approaches, methods, processes, and techniques, and by sustainably enhancing the quality of research at the host institutions.

KAAN BOZTUG RECEIVES THE “IŞIL BERAT BARLAN AWARD FOR PRIMARY IMMUNODEFICIENCY DISEASES”

(Vienna, 3.9.2021) **Congratulations! Kaan Boztug, Scientific Director of St. Anna Children’s Cancer Research Institute and Director of Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, has been awarded the Işil Berat Barlan Award for his outstanding research in the field of primary immunodeficiencies, which will be presented for the first time this year at the European Congress of Immunology (ECI).**

The scientist is receiving this award for his pioneering research on immunodeficiencies. Disturbances in the balance of the immune system can be the starting point for a number of diseases such as increased susceptibility to infections, autoimmune diseases and cancer. The study of congenital defects of the immune system makes it possible to uncover the molecular mechanisms underlying such severe diseases. This forms the basis for new effective treatment approaches.

Awardee Kaan Boztug about his research: “Uncovering the molecular mechanisms underlying congenital immune defects forms the basis for new effective treatment approaches.”

Credit: Ian Ehm

“I have been very fortunate to meet Prof. Işil Berat Barlan personally and to have the opportunity to work with her. Therefore, this award has a very special meaning for me. I see it in memory of Prof. Barlan as a wonderful way to honor her life and her many achievements in pediatrics and immunology – so I am particularly pleased to receive this award,” says Kaan Boztug.

ABOUT THE AWARD

The Turkish Society of Immunology (TSI), together with Professor Talal Chatila of Harvard Medical School, has presented three awards in honor of the late colleague Prof. Işil Berat Barlan, a distinguished physician-scientist and an educator who made invaluable contributions in the fields of allergy and immunology in Turkey and internationally, every year since 2016.

The award winners Benjamin Salzer and Charlotte Zajc designed new tools to switch immune cells on and off and direct them more specifically against tumor cells.

Credit: ÖGMBT/Philipp Schuster

BENJAMIN SALZER AND CHARLOTTE ZAJC WIN LIFE SCIENCE RESEARCH AWARDS

(Vienna, 20.09.2021) **Congratulations to Benjamin Salzer and Charlotte Zajc from our Christian Doppler Laboratory for Next Generation CAR T Cells! With their publication resulting from their doctoral thesis, both have won first place at the Life Science Research Awards Austria 2021 – Benjamin Salzer (St. Anna CCRI) in the category Applied Research, and Charlotte Zajc (University of Natural Resources and Life Sciences, Vienna (BOKU) / St. Anna CCRI alumna) in the category Excellence and Societal Impact.**

In the award-winning publications, Charlotte Zajc, Benjamin Salzer, and colleagues designed novel molecular switches and Chimeric Antigen Receptor (CAR) prototypes. With these new tools, immune cells can be reliably switched on and off and directed more specifically against tumor cells. This reduces the risk of CAR T cells attacking healthy tissue.

The official award ceremony took place during the 13th Annual Meeting of the ÖGMBT – Austrian Association of Molecular Life Sciences and Biotechnology on Monday, September 20. The award is supported by the Austrian Federal Ministry for Digital and Economic Affairs.

ABOUT THE AWARD

The promotion of young scientists is one of the main goals of the Austrian Association of Molecular Life Sciences and Biotechnology (Österreichische Gesellschaft für Molekulare Biowissenschaften und Biotechnologie, ÖGMBT). Each year, the ÖGMBT initiates a unique nationwide search in order to find the brightest young researchers in the Life Sciences field across all of Austria. There are different award categories available: Life Science Research Awards Austria (Basic Science, Applied Research, Excellence & Societal Impact) and Life Science PhD Awards Austria (Basic Science, Applied Science). The award ceremony takes place at the ÖGMBT Annual Meeting.

PUBLICATIONS

Salzer et al., Engineering AvidCARs for combinatorial antigen recognition and reversible control of CAR function. Nature Communications 2020.

Zajc et al., A conformation-specific ON-switch for controlling CAR T cells with an orally available drug. PNAS 2020.

Thomas Lion,
Ruth Ladenstein and
Daniela Karall

Credit: Tobias Zimmermann

ST. ANNA SCIENTIST HONORED TWICE

(Vienna, 2.10.2021) **The Austrian Society for Pediatrics and Adolescent Medicine (ÖGKJ) is honoring and presenting an award to Ruth Ladenstein: The pediatric cancer researcher and physician is receiving not only the Clemens von Pirquet Award but also a prize for the best oncological publication.**

Every year, the Austrian Society for Pediatrics and Adolescent Medicine (ÖGKJ) honors its members for outstanding scientific work with scientific awards and prizes.

CLEMENS VON PIRQUET PRIZE FOR THE BEST IMPACT FACTOR

On the occasion of the 59th ÖGKJ Annual Meeting, Ladenstein, Head of the Clinical Trials Unit S^2IRP at St. Anna Children's Cancer Research Institute, was awarded the prestigious Clemens von Pirquet Award, since her publications (papers as first, last and/or corresponding author) within the last three years have received the most citations in total.

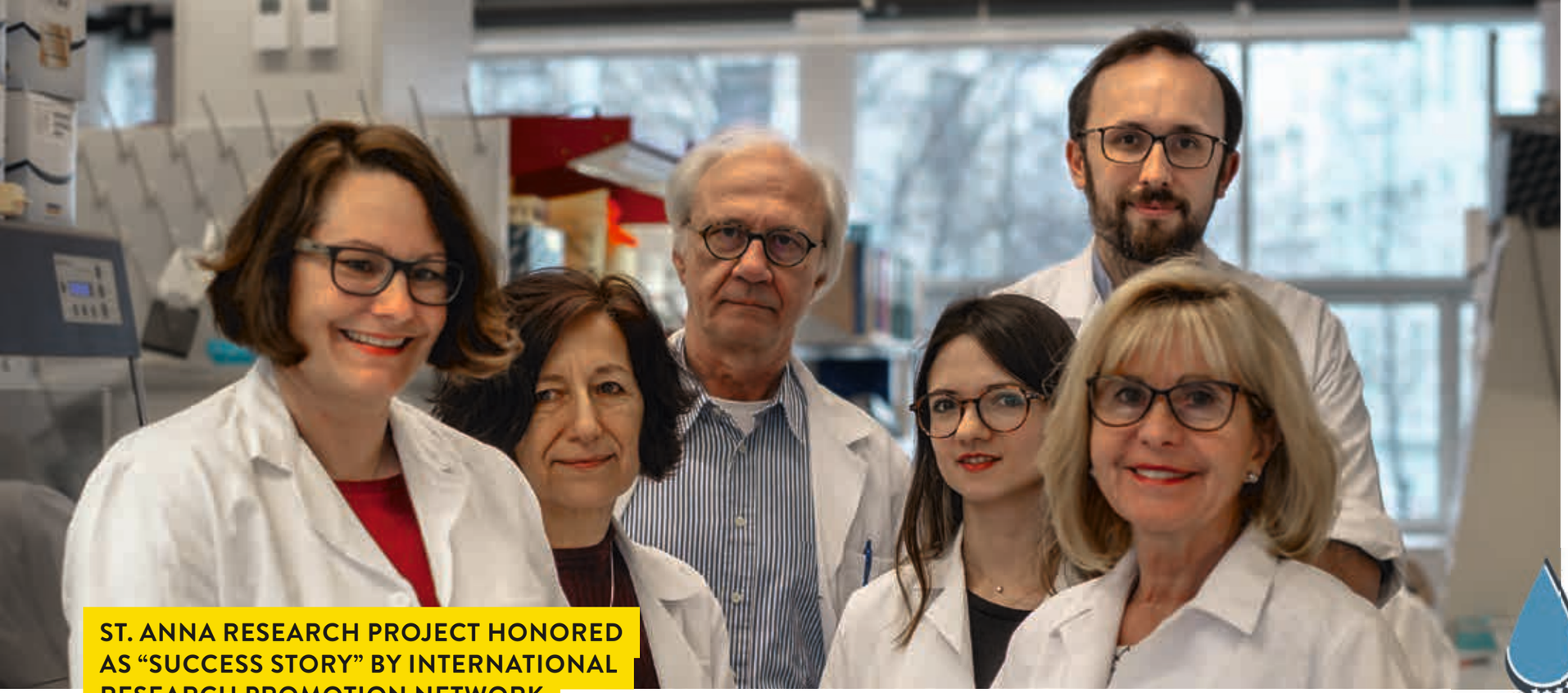
PRIZE FOR THE BEST ONCOLOGICAL PAPER

Furthermore, Ruth Ladenstein is receiving the prize for the best oncological paper. The study shows that the complete surgical removal of certain nerve tumors, so-called high-risk neuroblastomas, in a multimodal treatment approach, improves patient survival. This was the result of a study conducted by Ruth Ladenstein in collaboration with colleagues worldwide.

The work was published in the Journal of Clinical Oncology *(Holmes K et al. Influence of Surgical Excision on the Survival of Patients With Stage 4 High-Risk Neuroblastoma: A Report From the HR-NBL1/SIOPEN Study. J Clin Oncol 2020).*

ABOUT THE AWARDS

The Clemens von Pirquet Prize is a scientific prize awarded by the Austrian Society for Pediatrics and Adolescent Medicine (ÖGKJ) in memory of Clemens von Pirquet. The prize is awarded to members of the Society for their "scientific achievements in the field of pediatrics and its border areas". In addition, the ÖGKJ grants an annual Pediatric Hematology-Oncology Science Award for the best hemato-oncology paper of the previous year.



The Austrian ONTHETRRAC research team (f.l.t.r.): Sabine Taschner-Mandl, Inge Ambros, Peter Ambros, Polyxeni Bozatzi, Fikret Rifatbegovic and Ruth Ladenstein.

Credit: St. Anna CCRI

ST. ANNA RESEARCH PROJECT HONORED AS "SUCCESS STORY" BY INTERNATIONAL RESEARCH PROMOTION NETWORK

(Vienna, 16.12.2021) **The ONTHETRRAC research team discovered new aspects of tumor heterogeneity and developed tools that are important for accurate diagnosis, prognosis and for overcoming treatment resistance of the nerve tumor neuroblastoma in children. In the December 2021 newsletter, after evaluation of the final report, the project was therefore honored as a "success story" by the research promotion network European Research Area (ERA-NET TRANSCAN-2).**

Neuroblastoma is one of the most common malignant cancers in young children and still poses a deadly threat to high-risk patients. What makes neuroblastoma such a complex disease to tackle are the tumor's heterogeneity, its resistance to treatment and recurrence. Overcoming these issues is at the heart of ONTHETRRAC's efforts – a European research collective under the direction of Sabine Taschner-Mandl, Principal Investigator of the Tumor Biology Group at St. Anna Children's Cancer Research Institute (St. Anna CCRI), and former CCRI scientists Peter Ambros and Inge Ambros.

The ONTHETRRAC team has invested in cutting-edge technologies to gain new insight into the tumor heterogeneity of childhood neuroblastoma and relevance for the patient's prognosis. Based on these novel insights the scientists developed new clinical diagnostic tools, such as minimally invasive liquid biopsy tests, as well as recommendations that have been implemented into the European high-risk neuroblastoma trial protocol.

Investigations revealed that liquid biopsy analyses can help detect more aggressive and treatment-resistant subclones of a tumor. This information is instrumental in discovering a potential relapse after treatment early on. The project funded by ERA-NET (program TRANSCAN-2), the Austrian Science Fund (FWF), and other national funding agencies was a cooperation of experts in the fields of neuroblastoma, molecular genetics, and clinical research at St. Anna CCRI, Charité Berlin, Institut Curie Paris, the University of Ghent, and the German Cancer Research Institute in Heidelberg (see right).

"The earlier we detect recurrent leukemia cells, the faster we can intervene therapeutically and the higher the chances of survival are," explains award winner Michael Dworzak.

Credit: Harald Eisenberger

TOP LEUKEMIA EXPERT WORLDWIDE: MICHAEL DWORZAK HONORED BY INDEPENDENT RANKING PLATFORM

(Vienna, 7.12.2021) **Developing new diagnostic methods for early detection and effective therapy of children and adolescents with leukemia – Michael Dworzak's research pursues goals that are vital for pediatric cancer patients. For his tireless efforts, he has now been honored by the international evaluation platform "Expertscape" as a global top expert in the field of leukemia.**

As head of the research group for Immunological Diagnostics at St. Anna CCRI, deputy medical director and senior consultant of the Department of Oncology and Hematology at St. Anna Children's Hospital, Michael Dworzak is aware of the dire need for innovative diagnostic and treatment options for leukemia in children and adolescents in the clinics and he knows first-hand where research efforts are necessary to cover these needs.

FROM RESEARCH INTO PRACTICE

Since its foundation in 1993, Michael Dworzak's research group has been dedicated to the characterization of leukemias and lymphomas in children using immunophenotyping. By means of multi-color analysis in flow cytometry, this method examines blood and bone marrow samples. Cell suspensions are provided with various defined antibodies that bind specifically to different cells, which are then illuminated in a flow chamber using laser light. Depending on the cell type, a characteristic light reflection pattern is created, which enables precise detection and diagnosis (minimal residual disease, MRD) in malignant blood cancer.

"Our goal is to develop new and better flow cytometric methods that can be used clinically for refined diagnosis as well as for risk assessment and therapy planning," explains Michael Dworzak, who is currently focusing on the second most common and very aggressive form of blood cancer in childhood and adolescence, acute myeloid leukemia (AML). If this malignant disease relapses, the chances of survival are significantly worse than with the more common form of leukemia, acute lymphoblastic leukemia (ALL). "The earlier we detect recurrent cancer cells in AML, the faster we can intervene therapeutically and the higher the chances of survival are for the affected children," says Michael Dworzak.

INTERNATIONAL TOP EXPERT

Expertscape – an impartial ranking platform in the field of biomedicine – now lists Michael Dworzak in the top 0.2% of over 200,000 experts in leukemia worldwide, number 1 in Austria for children and adolescents with leukemia. This assessment is based on Michael Dworzak's contribution to 75 publications in high-ranking international scientific journals between 2011 and the present day.

"I am very pleased about this award because it shows how successful a close link between research and clinic can be and which improvements can be achieved for patients with leukemia in treatment. Cross-border cooperation in international networks and across institutes is essential because such success can only be achieved in an international scientific and clinical joint effort," explains Michael Dworzak.

David Seruggia wins an award, honoring young scientists with outstanding achievements in transgenic technologies.

Credit: St. Anna Children's Cancer Research Institute

CONGRATS TO DAVIDE SERUGGIA FOR RECEIVING THE ISTT YOUNG INVESTIGATOR AWARD!

(Vienna, 20.12.2021) **Davide Seruggia received an award for his successful complex projects requiring advanced CRISPR technology: He pioneered CRISPR genome-editing techniques and used these CRISPR methods for the functional investigation of regulatory elements found at the non-coding area of mammalian genomes (Seruggia et al. 2015, 2020).**

ABOUT THE AWARD
The ISTT (International Society for Transgenic Technologies) Young Investigator Award identifies and recognizes young scientists who advance the science and technologies in the generation and analysis of transgenic animal models for bio-medical research and biotechnology applications.

"Silberne Feder" Award 2021: f.l.: Lukas Lach, Martina Teufner, Lisa Huto.

Credit: PRVA

"AND THE SILVER FEATHER GOES TO ...!"

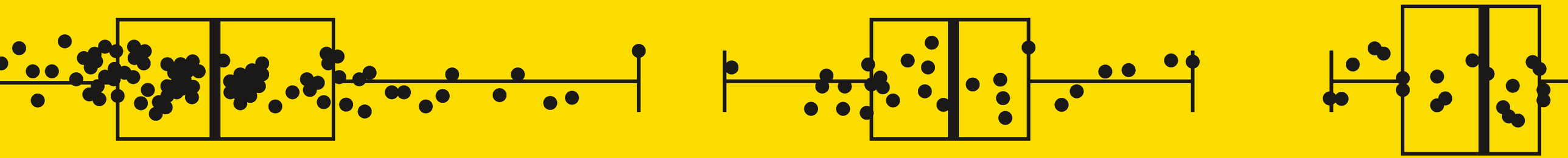
(Vienna, 23.09.2021) **Congratulations! The Public Relations Association Austria (PRVA) has awarded the "Silberne Feder" Awards of the year 2021. Among them, first place in the category "Image – Video – Sound" went to Lukas Lach from St. Anna CCRI for the video series #EMPLOYEESPOTLIGHT.**

The video series, developed together with Martina Teufner (Head of HR), aims to address potential applicants via LinkedIn and YouTube, but it also functions as an echo platform for its employees.

ABOUT THE AWARD
The aim of the award for employee media "Die Silberne Feder" is to help raise the quality of internal communications in German-speaking countries.

The award is presented in four categories: Digital, Print, Image Video Sound, Employee Story

SOLID TUMORS



“To properly tackle a disease, we must understand how it develops.”

CAROLINE HUTTER

Group: Langerhans Cell Histiocytosis Biology (LCH)

Caroline Hutter earned an MD from the Medical University of Vienna before obtaining a PhD at the Imperial Cancer Research Fund in London, where she focused on investigating the role of integrins in stem cells in Fiona Watt’s lab. She then joined the lab of Meinrad Busslinger at the Research Institute of Molecular Pathology (IMP) in Vienna and trained in pediatrics at the Medical University of Vienna as well as St. Anna Children’s Hospital. Her special interest lies in precision oncology, hard-to-treat cancers and histiocytosis. She is leader of the LCH Biology group at St. Anna CCRI where her laboratory research focuses on a better understanding of the biology of Langerhans Cell Histiocytosis (LCH), a rare histiocytic disorder whose most severe clinical course predominantly affects young children. The goal is to gain insight into the pathogenesis that will subsequently allow new treatment approaches for this disease. Caroline Hutter is also an attending physician in pediatric oncology at St. Anna Children’s Hospital where she leads the clinical precision medicine team.

CAROLINE HUTTER GROUP

Langerhans Cell Histiocytosis Biology (LCH)

PRINCIPAL INVESTIGATOR
Caroline Hutter

POSTDOCTORAL FELLOWS
Raphaela Schwentner
Giulio Abagnale

BIOINFORMATICIAN, CLINICAL RESEARCHER
Sebastian Eder

PHD STUDENT
Wouter Van Midden

UNDERGRADUATE STUDENT
Philipp Ben Soussia

TECHNICIAN
Eva Krivec (until 2021)

HEINRICH KOVAR

Group: Molecular Biology of Solid Tumors

Univ.-Prof. Heinrich Kovar studied biology and chemistry at the University of Vienna, where he then also obtained a PhD in molecular biology. He joined CCRI in 1988 as the head of the Molecular Biology of Solid Tumors group. His research focuses on Ewing sarcoma, in particular on the influence of tissue origin, cellular and molecular context, and driver gene biology. The goal is to translate clinical observations into molecular patterns, and molecular patterns into diagnostic/prognostic tools and novel treatment options. Heinrich Kovar served as scientific director of CCRI from 2001 to 2017 and has received numerous awards for his research and a number of highly endowed research grants. He is a scientific advisor to renowned international research institutions and programs and member of the ITCC Solid Tumor Steering Board. His current research focus lies on the generation and characterization of Ewing sarcoma models, and on mechanisms of Ewing sarcoma plasticity as the basis for disease susceptibility and progression.

“Cancer is a problem of metastasis. Killing metastases is saving lives. We therefore need to target the complex tumor interactions in the metastatic niche.”

HEINRICH KOVAR GROUP

Molecular Biology of Solid Tumors

PRINCIPAL INVESTIGATOR
Heinrich Kovar

STAFF SCIENTISTS
Dave Aryee
Jozef Ban

POSTDOCTORAL FELLOWS
Branka Radic Sarikas
Lisa Bierbaumer (until 2021)
Utkarsh Kapoor
Valerie Fock

PHD STUDENTS
Rahil Noorizadeh
Veveeyan Suresh

MASTER STUDENT
Mathias Eduard Ilg (until 2021)

TECHNICIAN
Karin Mühlbacher

SABINE TASCHNER-MANDL

Group: Tumor Biology

Sabine Taschner-Mandl, PhD, has been head of the Tumor Biology group at St. Anna CCRI, since 2018, where she has been working as a scientist since 2008. The aim of her research group is to tackle unresolved questions of neuroblastoma pathogenesis and develop new diagnostic and therapeutic approaches to facilitate precision medicine for children with malignant tumors. In addition, Sabine Taschner-Mandl is a lecturer at the Medical University of Vienna and Vienna University of Technology. She completed her studies of biology at the University of Vienna with a diploma thesis in vaccine development. This was followed by a dissertation and a postdoc position at the Institute of Immunology at the Medical University of Vienna. Besides her research work at CCRI, the researcher was a visiting scientist at Significo and the University of Helsinki (EC-FP7 Marie Curie Program). She is leading and participating in international (e.g. ERA-NET/ TRANSCAN LIQUIDHOPE, EC H2020 PRIMAGE) and national initiatives. As a member of the Executive Board and co-chair of the Biology Committee of the European Society of Pediatric Oncology Neuroblastoma (SIOPEN) and in other international working groups (INRG, ITCC), she is fostering innovative research for the benefit of pediatric patients with cancer.

SABINE TASCHNER-MANDL GROUP

Group: Tumor Biology

TUMOR BIOLOGY

PRINCIPAL INVESTIGATOR
Sabine Taschner-Mandl

SENIOR POSTDOCTORAL FELLOW
Irfete Fetahu

POSTDOCTORAL FELLOWS
Eva Bozsaky
Polyxeni Bozatzi

PHD STUDENT
Daria Lazic

MASTER STUDENTS
Donya Esmaeiligoudarzi (until 2021)
Sylvia Ramirez
Virág Gehl (until 2021)

BACHELOR STUDENT
Sophie Neswadba

TECHNICIAN
Teresa Gerber (until 2021)

TUMOR BIOLOGY DIAGNOSTICS

SENIOR STAFF SCIENTIST & TEAM LEADER TUMOR BIOLOGY DIAGNOSTICS
Marie Bernkopf

SENIOR STAFF SCIENTIST
Fikret Rifatbegovic

BACHELOR STUDENT
Christiane Paukner (until 2021)

ELENI TOMAZOU

Group: Epigenome-based Precision Medicine

Eleni Tomazou has been a Principal Investigator at the St. Anna CCRI since January 2018. She has established a research program focusing on epigenome-based precision medicine at St. Anna CCRI. By studying how fusion oncoproteins rewire healthy cells for malignancy, she aims to exploit this knowledge towards precision medicine against pediatric sarcomas. Eleni Tomazou has a strong background in epigenomics research and two years' experience in clinical diagnostics and management of a high-throughput diagnostics lab. Prior to joining St. Anna CCRI, she did her PhD at the Wellcome Sanger Institute (Cambridge, UK) and postdoctoral training at the Broad Institute and the Harvard Department for Stem Cell and Regenerative Biology (Cambridge, USA). She is a 2016 recipient of the Elise Richter Fellowship, a career development grant for female scientists offered by the Austrian Science Foundation (FWF).

"Pediatric sarcomas are hard-to-treat cancers with unique molecular characteristics. To develop precise molecular therapies, we need to understand when, why and how they arise."

ELENI TOMAZOU GROUP

Epigenome-based Precision Medicine

PRINCIPAL INVESTIGATOR
Eleni Tomazou

POSTDOCTORAL FELLOW
Stefan Terlecki-Zaniewicz

PHD STUDENTS
Marcus Tötzl
Peter Peneder
Lisa Daniel (until 2021)

MASTER STUDENT
Nikolaus Mandlburger

TECHNICIANS
Adrian Stütz (until 2021)
Abdelgawad Abdelrahman (until 2021)
Daria Pajak

PUBLICATIONS

UNDERNEATH THE TIP OF THE ICEBERG

Langerhans cell histiocytosis (LCH) is a rare disease characterized by the accumulation of CD1A+CD207+ histiocytic cells. It is most commonly driven by a somatic, activating mutation in the BRAF serine-threonine kinase (BRAFV600E). While the focus of most studies lies on the tip of the iceberg – CD1a+CD207+ LCH cells – Caroline Hutter and her team pointed their interest in what lies beneath and might be the source of morbidity in patients with severe LCH. They propose that BRAF-mutated hematopoietic cells generate systemic inflammation and contribute to high-risk disease. Single-cell transcriptome analyses of peripheral blood and bone marrow cells, as well as cytokine profiling at the time of diagnosis and during therapy with a BRAF-specific inhibitor, show that inhibiting BRAF causes inflammatory cytokines to be downregulated, leading to a better clinical outcome.

Treating LCH patients for years, Caroline Hutter observed that patients with LCH are often not mainly suffering from their lesions but rather from inflammation and organ failure caused by mutated cells. In most cases, no or few infiltrating CD1A+CD207+ cells are found in risk organs as liver, spleen or the hematopoietic system, suggesting that these pathogenic phenotypes might be caused by other cells. To uncover what lies beneath, she assembled a team of experimental and computational researchers from St. Anna CCRI and medical doctors from St. Anna Children's Hospital.

CLINICALLY BETTER – MOLECULARLY WORSE

One intriguing and unexpected observation was that while patients with BRAFV600E-positive LCH treated with the BRAF inhibitor vemurafenib immediately respond with clinical improvement and even remission, BRAFV600E levels in the peripheral blood rise. On the other hand, as soon as the inhibitor was discontinued, these patients, including the patient in this study, relapsed. Accordingly, vemurafenib discontinuation immediately led to fever and other signs of inflammation. However, on resumption the fever subsided within hours. This pointed towards an inflammation-inhibitory effect of vemurafenib.

ANTI-INFLAMMATORY EFFECTS OF VEMURAFENIB LEAD TO CLINICAL REMISSION…

Using state-of-the-art single cell RNA sequencing technology, changes in gene expression in peripheral blood and bone marrow cells from one patient carrying the BRAFV600E mutation were studied. To follow changes over time and the impact of BRAF inhibition, cells were analyzed at time of diagnosis and during treatment with the combination of vemurafenib and salvage chemotherapy. Pathway enrichment analysis recapitulated the clinical picture as pathways associated with inflammation were enriched, illustrating broad anti-inflammatory effects of vemurafenib on immune cells. In line, serum levels of inflammatory cytokines exactly mirror vemurafenib administration.

…BUT DOES NOT ERADICATE THE DISEASE

As mentioned above, discontinuation of vemurafenib immediately resulted in clinical deterioration. However, the patient in this study was treated with 2-chlorodeoxyadenosine (2- CdA, cladribine) and cytarabine (ARA-C), which also led to the eradication of BRAFV600E mutated immune cells. After the third cycle of chemotherapy the dose of vemurafenib was reduced and subsequently discontinued and the patient achieved remission.

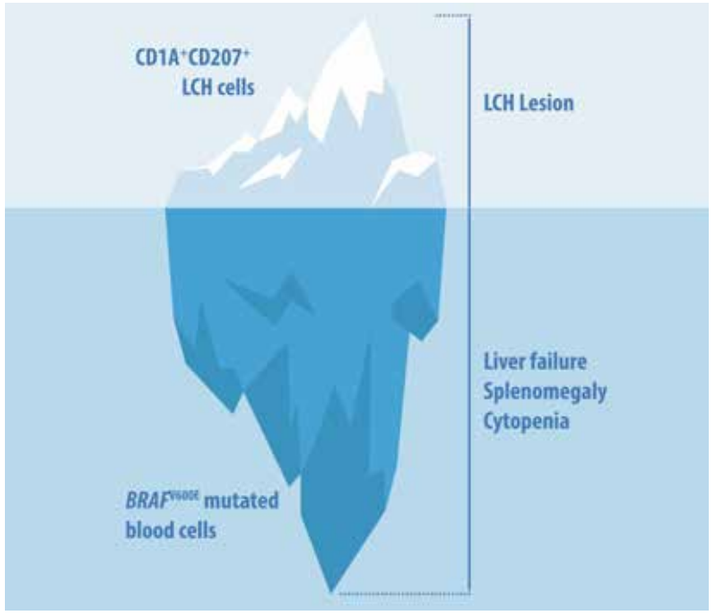
FUTURE THERAPY

Although the CD1A+CD207+ histiocytes are the hallmark of LCH, other BRAF-mutated cells contribute significantly to morbidity in patients with multisystem LCH by causing systemic inflammation and hence risk organ involvement. Therefore, it is essential to understand better how these cells lead and enhance LCH and eradicate them from the patient's hematologic system. Vemurafenib can lead to an immediate response and clinical stabilization but to achieve sustained remission, combination with chemotherapy as presented in this study might be necessary.

PUBLICATION

Eder, S. K.*, Schwentner, R.*, Ben Soussia, P., Abagnale, G., Attarbaschi, A., Minkov, M., Halbritter, F., & Hutter, C. (2021). Vemurafenib acts as molecular on-off switch governing systemic inflammation in Langerhans cell histiocytosis. Blood Adv. https://doi.org/10.1182/bloodadvances.2021005442

*Shared first authorship



Model of LCH pathogenesis which states that while the CD1A+CD207+ histiocytes are the hallmark of LCH, other BRAF-mutated cell populations may contribute significantly to morbidity in patients with multisystem LCH.

Eder, Schwentner, et al., Blood Adv 2022

YAP/TAZ TARGETING REDUCES EWING SARCOMA METASTASIS

This publication reveals that inhibition of the YAP/TAZ pathway may prevent Ewing sarcoma cell dissemination and metastasis, thus presenting a promising new therapeutic target for a disease with a long-standing stagnant poor clinical outcome.

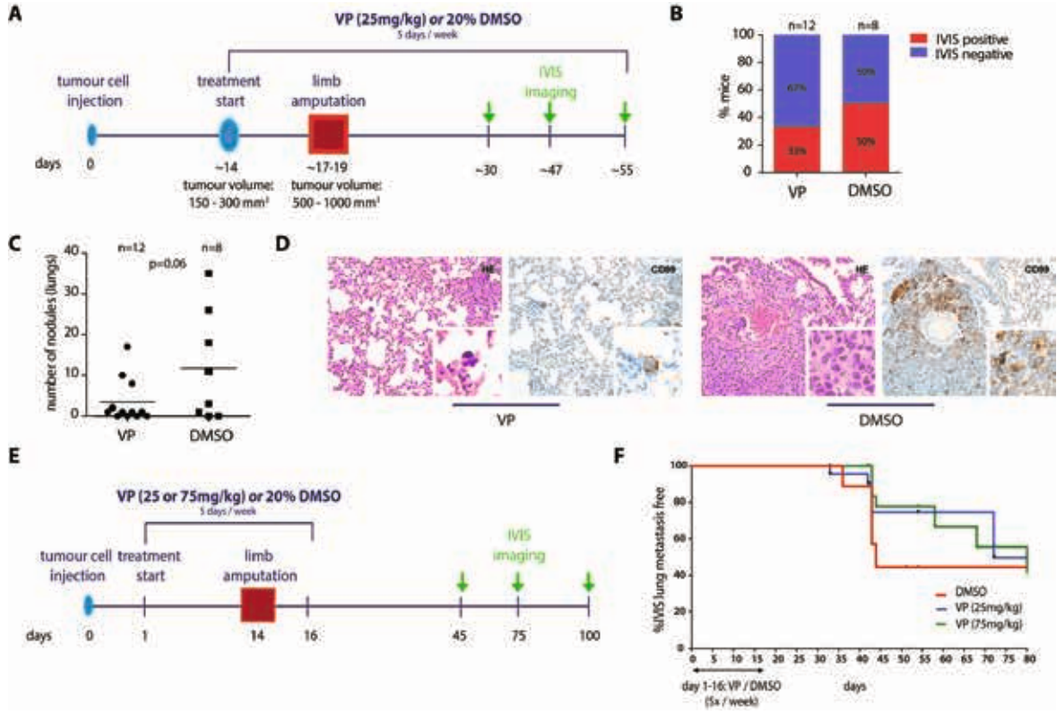
Verteporfin, a potent YAP/TAZ/TEAD complex inhibitor, reduced Ewing sarcoma cell migration and metastasis in a recent study of the Molecular Biology of Solid Tumors group at St Anna CCRI. Therefore, YAP/TAZ inhibitors may be considered for combination therapy with cytotoxic standard-of-care chemotherapy to prevent onset of the metastatic cascade. The study provides a proof of concept for the clinical promise of future drugs targeting YAP/TAZ with even higher specificity and improved safety features.

WHAT MAKES THE YAP/TAZ/TEAD AXIS A POTENT TARGET

Ewing sarcoma is a pediatric bone cancer with high and early dissemination potential and an overall survival rate of less than 30 percent for metastatic disease. The tumor is most commonly driven by the EWS-FLI1 fusion gene, inducing cell transformation and oncogenesis.

Recent studies suggest that EWS-FLI1 may oscillate between high and low expression states, thereby orchestrating distinct phenotypic programs: Whereas cells with high EWS-FLI1 expression proliferate, cells that are low on EWS-FLI1 are migratory and invasive.

A prerequisite for the initiation of metastasis is the cell's ability to transform from a highly organized into a loose, migratory phenotype. In the case of EWS-FLI1-low tumor cells, factors from the Hippo pathway are activated, a signaling cascade that controls organ size through the regulation of cell proliferation and apoptosis. In particular, TAZ levels are increased in the migratory EWS-FLI1-low state and are associated with adverse prognosis in Ewing sarcoma patients. This makes the YAP/TAZ/TEAD axis a potent metastasis target in this type of cancer.



Verteporfin treatment reduces Ewing sarcoma (EwS) lung metastasis in a mouse xenograft model.
(a) Experimental setting 1: set-up and VP treatment scheme. Luciferase-expressing TC71 cells were injected into the tibial crest of mice. Intra-peritoneal injections of VP (25 mg/kg) or solvent control (20% DMSO) started once tumours reached a specific size. When tumours reached a volume of 150–300mm3, tumour-bearing limbs were amputated and VP and control treatments (5 days/week) were continued for a maximum of 35 days. (b) Proportions of mice with IVIS-detectable pulmonary metastases per treatment group. (c) Number of histopathologically detectable tumour nodules in lung sections of control- and VP-treated mice based on evaluation of H&E and CD99 stainings. The mean number ±s.e.m. of tumour nodules per condition is shown. P value was calculated by two-tailed Student's t-test. (d) Exemplary H&E and CD99 stainings showing a reduced size of EwS lung metastatic nodules (200x magnification, inserts: 600x magnification). (e) Experimental setting 2: set-up and VP treatment scheme. As for setting 1 in (A), luciferase-expressing TC71 cells were injected, but VP (25 mg/kg or 75 mg/kg) and control treatments were started one day after tumour cell injection and stopped two days after limb amputation. (f) Lung metastasis free survival of control- and VP-treated mice from experimental setting 2. Although data are not statistically significant, VP-treated mice show a delay in metastatic on-set.

PUBLICATION

Bierbaumer, L., Katschnig, A. M., Radic-Sarikas, B., Kauer, M. O., Petro, J. A., Hogler, S., Gurnhofer, E., Pedot, G., Schafer, B. W., Schwentner, R., Muhlbacher, K., Kromp, F., Aryee, D. N. T., Kenner, L., Uren, A., & Kovar, H. (2021). YAP/TAZ inhibition reduces metastatic potential of Ewing sarcoma cells. Oncogenesis,10(1), 2. https://doi.org/10.1038/s41389-020-00294-8

AI TO DETECT CANCER CELLS IN MICROSCOPY IMAGES

This publication on artificial intelligence presents novel insights on automated image analyses of cancer cells. Based on their results, the researchers of the Tumor Biology Lab led by Sabine Taschner-Mandl recommend the creation of artificial images to train deep neural networks. Furthermore, they propose methods to effectively segment complex microscopy images, such as tumor tissue.

Biomedical image analysis is used to characterize cells in tissue sections or bone marrow aspirates and play an important role in diagnostics as well as research. The characterization of cells enables us, for example, to understand pathophysiological features of tumors which are required for risk assessment and therapy choice in pediatric cancer. Complex algorithms, such as deep learning architectures, allow the detection of even subtle biological changes, while benefitting from the statistical power of analyzing thousands of cells. This study for the first time systematically benchmarks multiple deep learning architectures against classical approaches for the accurate detection and separation of cell nuclei in complex fluorescence microscopy images, as frequently found in tumor tissue.

CHALLENGE OF COMPLEX IMAGES

Deep neural networks can detect cells and cell nuclei accurately only if expert-annotated datasets can be used for training. Unfortunately, available datasets are limited in size and complexity. Complexity of images is high if e.g. nuclei are tightly aggregated and cannot be separated by the segmentation algorithm. This can lead to inaccurate image segmentation and wrong biological conclusions (Figure 1). Separating and labeling each 'nuclear instance' (=termed instance-aware segmentation) is the key challenge in nuclear image segmentation.

To evaluate state-of-the-art segmentation methods on complex images, the scientists from the St. Anna CCRI Tumor Biology Group use their recently published dataset of expert-annotated images (Kromp et al., Scientific Data, 2020, https://doi.org/10.1038/s41597-020-00608-w).

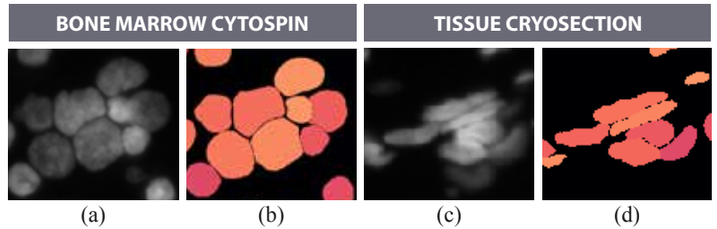


FIGURE 1

Examples of nuclear morphologies in various cell and tissue preparations. (a) Neuroblastoma bone marrow cytospin presenting varying nuclear intensity and size. (b) Annotated mask of (a). (c) Ganglioneuroma tissue cryosection presenting overlapping/aggregated nuclei with varying morphology and intensity. (d) Annotated mask of (c).

Kromp et al., IEEE Transactions on Medical Imaging 2021

LET MACHINES LEARN BETTER

To increase the training material for deep learning architectures, the scientists propose to create artificial images focusing on overlapping nuclei, thereby simulating complex nuclear images. These artificially generated images can now be used to train instance-aware segmentation algorithms, such as Mask R-CNN, which are more accurate as compared to classical machine learning-based approaches.

Based on their results, the scientists further recommend “silver-standard” image annotation (Figure 2) to train deep learning algorithms. Silver-standard training sets contain partially inaccurate annotation masks. However, they have the advantage that they can be generated by trained undergraduate students, whereas gold-standard training sets need time-consuming curation by biology and pathology experts. The researchers could prove that in combination with artificial images and simple annotations, deep learning architectures deliver an excellent detection and separation of each cell in a complex tissue. This is crucial for the exact measurements of biological changes in the tumor.

WIDE RANGE OF APPLICATION

By using automated imaging, the scientists aimed to characterize tumor cells more precisely, which will help to stratify patients for more personalized cancer treatment in the long run. The study has the potential to improve accuracy and enable broad applications of microscopy-based image analysis workflows on complex images of various cells and tissues.

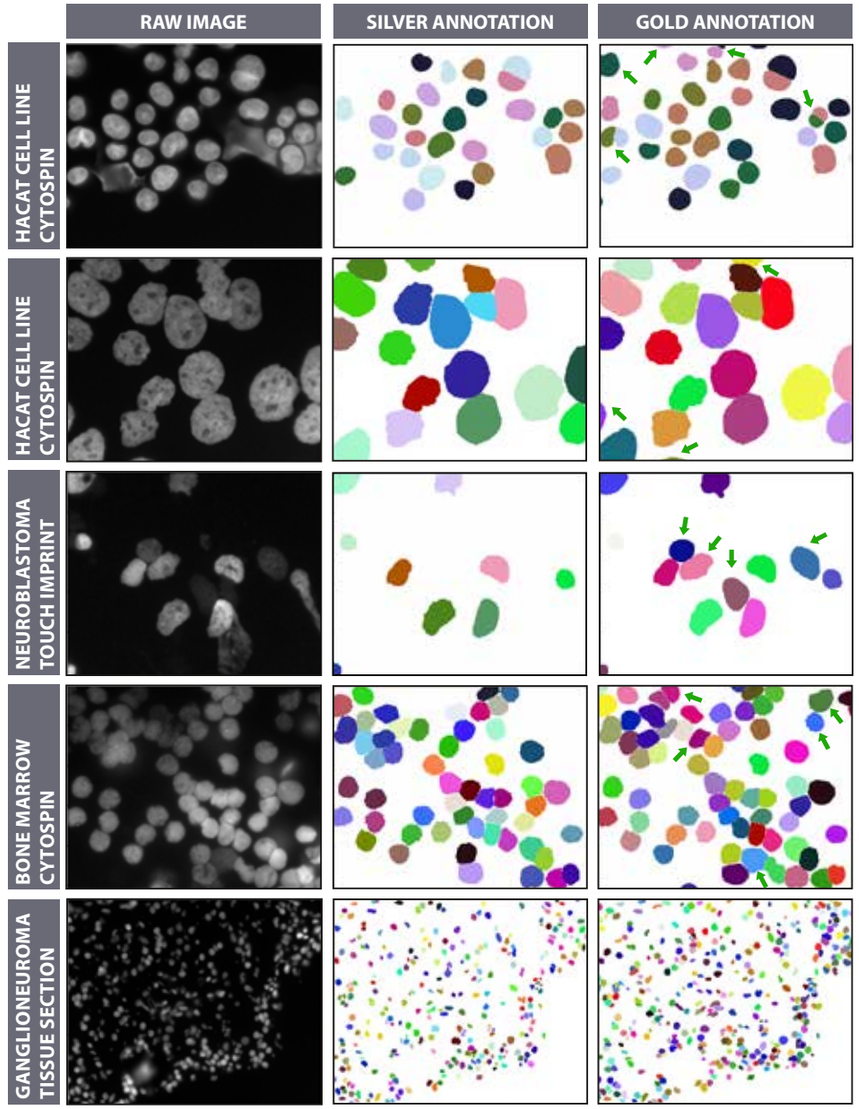


FIGURE 2

Examples of all types of preparations/specimen including a comparison between annotations from undergraduates (silver-standard) and from experts (gold-standard). Green arrows indicate differences between silver-standard and gold-standard annotations.

Kromp et al., IEEE Transactions on Medical Imaging 2021

PUBLICATION

Kromp, F., Fischer, L., Bozsaky, E., Ambros, I. M., Dorr, W., Beiske, K., Ambros, P. F., Hanbury, A.#, & Taschner-Mandl, S.# (2021). Evaluation of Deep Learning architectures for complex immunofluorescence nuclear image segmentation. IEEE Trans Med Imaging,PP. https://doi.org/10.1109/TMI.2021.3069558

Shared senior authorship

BONE MARROW METASTASES FOR THE FIRST TIME ANALYZED CELL BY CELL

When childhood nerve tumors, so-called neuroblastomas, form metastases, they preferentially do so in the bone marrow. This study shows for the first time the exact cell composition of such metastases. This knowledge forms the basis for the development of therapy concepts that target all individual tumor cell types in metastatic cancers.

In this study, the Tumor Biology research team led by Sabine Taschner-Mandl describes the exact composition of the individual cells of bone marrow metastases from a solid tumor at the single-cell level using highly multiplex imaging. The study showed that cells that have spread into the bone marrow from childhood nerve tumors, so-called neuroblastomas, are heterogeneous. The researchers therefore conclude that in order to therapeutically attack all these different tumor cell types, combinations of therapeutic approaches are required. Also cells in the microenvironment of a bone marrow metastasis are likely to play a crucial role. In this context, the scientists identified a cell type that apparently prevents immune cells from fighting the tumor.

NEW TARGET, BETTER TAILORED THERAPY?

By applying single cell multiplex microscopy analysis, the team could describe which cell types are present in a bone marrow metastasis compared to healthy bone marrow. Using this method, the research team could distinguish not only whether the cell is a neuroblastoma cell or a specific immune or stem cell, but also which molecules or markers they carry on their surface. These markers, for example GD2 or B7H3, are also used as therapeutic targets. The study shows that some tumor cells in the bone marrow have a lot of these already known markers, while others have few or none.

In addition, the researchers have discovered a completely new marker protein, Fas Apoptotic Inhibitory Molecule 2 (FAIM2). It allows the detection of neuroblastoma cells that were not found with previous markers. Thus, a broader range of different tumor cell variants can be detected. FAIM2 could also serve as a target for future therapies. Immunotherapies have already been developed against GD2 and B7H3. However, since these markers are not present on all neuroblastoma cells, the scientists assume that only appropriate combination therapies can attack all individual tumor cells. FAIM2 is a possible target for the development of new immunotherapies.

SCANNING "SABOTEURS" IN THE MICROENVIRONMENT

The Tumor Biology group is currently evaluating FAIM2 to be included in routine diagnostic assessment of bone marrow minimal residual disease in addition to the currently used markers in order to more reliably detect tumor cells in bone marrow. Moreover, it could be relevant to detect also other cells in the metastatic bone marrow microenvironment, so-called "granulocytic myeloid-derived suppressor cells" (G-MDSCs), in routine diagnostics. These cells are likely to downregulate the immune response against tumor cells, while, according to the data of Taschner-Mandl and colleagues, at the same time an inflammatory response is detected in the metastasized bone marrow samples. These results provide the first evidence that metastatic tumor cells remodel their bone marrow microenvironment. How this is accomplished on a mechanistic level has to be investigated in a follow-up study.

HIGH-END TECHNOLOGY ENABLES SINGLE CELL ANALYSIS

The researchers investigated 20 cell-specific markers, which were analyzed from eight patient samples. In cooperation with Dr. Christian Ostalecki, head of the MELC Facility at the University Hospital Erlangen, the team used the multiplex imaging method multi-epitope ligand cartography (MELC), which enables automated antibody staining followed by immunofluorescence microscopy (Figure 1). To screen each cell for its markers, the authors developed a software for image processing and analysis (DeepFLEX) that integrates our novel deep learning-based strategy for the detection of cell nuclei (Kromp et al, Scientific Data, 2020; Kromp et al, IEEE TMI, 2021). The researchers constructed a single-cell atlas of more than 35,000 cells metastasized to the bone marrow, as well as the immune and bone marrow cells in their microenvironment (Figure 2). To support their findings, they additionally sequenced the genes expressed as messenger RNA from samples of 38 patients with and without bone marrow metastases.

The presented findings indicate that metastatic tumor cells shape the bone marrow micro-environment, warranting deeper investigations of spatio-temporal dynamics at the single-cell level and their clinical relevance.

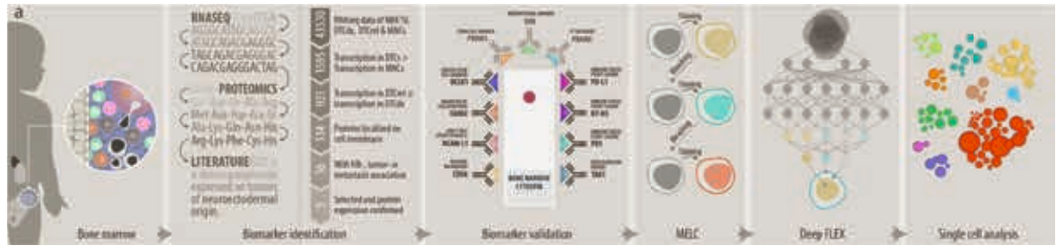


FIGURE 1

Data mining and establishment of a 20-plex imaging panel for multi-epitope ligand cartography (MELC). Eight bone marrow metastases (aspirates) were analyzed using MELC by repeated staining, imaging and bleaching cycles. Images were processed by DeepFLEX followed by single-cell analysis of tumor cells and cells of the bone marrow metastatic niche

Lazic et al., Cancers (Basel) 2021

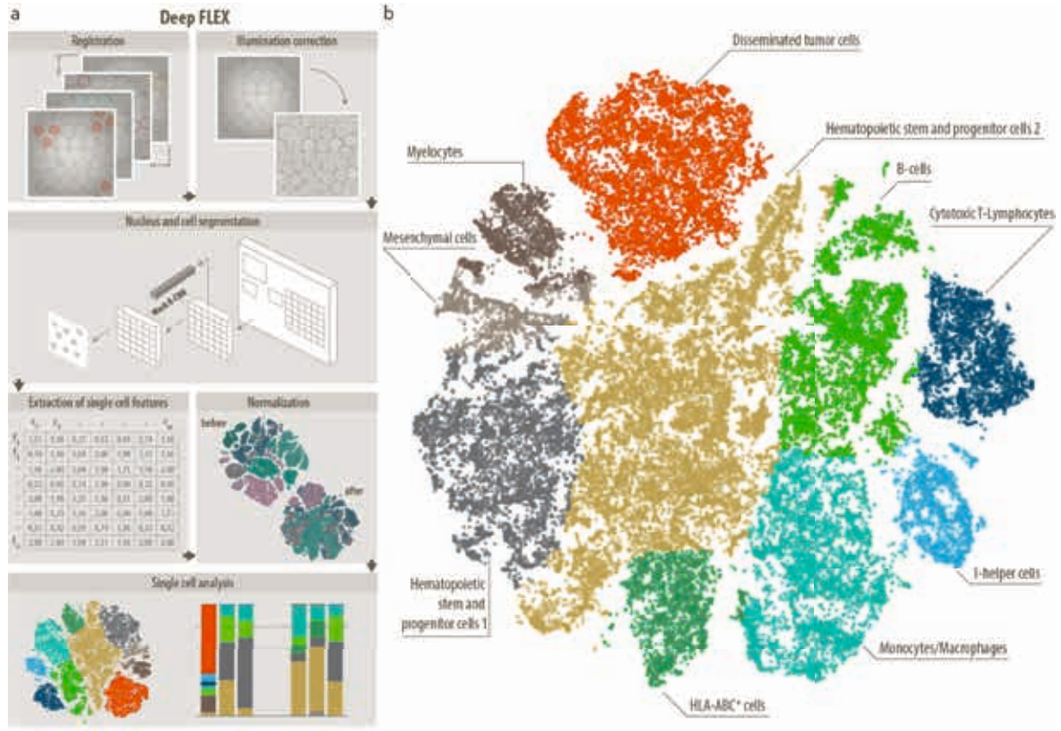


FIGURE 2

DeepFLEX and single-cell map of 8 bone marrow metastases from 4 patients with metastatic neuroblastoma. a, Schematic overview of the deep learning-based image processing, segmentation, normalization and single-cell analysis pipeline DeepFLEX. b, t-SNE (t-distributed Stochastic Neighbor Embedding) plot of 35,700 single cells colored by cell type.

Lazic et al., Cancers (Basel) 2021

PUBLICATION:

Lazic, D., Kromp, F., Rifatbegovic, F., Repiscak, P., Kirr, M., Mivalt, F., Halbritter, F., Bernkopf, M., Bileck, A., Ussowicz, M., Ambros, I. M., Ambros, P. F., Gerner, C., Ladenstein, R., Ostalecki, C., & Taschner-Mandl, S. (2021). Landscape of Bone Marrow Metastasis in Human Neuroblastoma Unraveled by Transcriptomics and Deep Multiplex Imaging. Cancers (Basel),13(17). https://doi.org/10.3390/cancers13174311

NATURAL "BRAKE" AGAINST MALIGNANT NEUROBLASTOMA

A factor that turns malignant tumors into benign ones? That is exactly what was discovered by this study. Scientists of the Tumor Biology Lab led by Sabine Taschner-Mandl together with colleagues studied tumors of the peripheral nervous system in children, namely neuroblastomas. The team discovered that the uncontrolled growth of benign neuroblastomas is stopped by a signal molecule produced by Schwann cells that are present within these tumors. This natural "brake" also works on malignant neuroblastoma cultures. The study describes for the first time the function of this signal molecule – not only in tumors, but also in injured nerve fibers.

What sounds contradictory at first glance, namely firing a tumor with a growth factor, makes sense in neuroblastoma. Neuroblastoma is a tumor of the peripheral nervous system and the most common solid cancer in early childhood. In contrast to malignant neuroblastomas, benign neuroblastomas contain, next to tumor cells, many "Schwann cells". These cells normally protect and repair nerve cells. The results of the now published study indicate that Schwann cells in neuroblastoma stimulate tumor cells to mature, thereby halting their unchecked growth.

A CELL THAT STOPS TUMOR GROWTH…

To accomplish this, Schwann cells produce, among other factors, a signaling molecule called epidermal growth factor-like 8 (EGFL8). The research team demonstrates that EGFL8 stimulates the differentiation, or maturation, of neuroblastoma cells. Until recently, it was only known that this protein existed, but its function was not clear. This study shows for the first time where EGFL8 is produced and how it acts. Furthermore, the study results show that high levels of EGFL8 were associated with better survival rates in neuroblastoma patients.

It could be demonstrated in cell culture that Schwann cells as well as their secreted signaling molecules exert anti-tumor effects, even in aggressive neuroblastoma cells (Figure 1). Thus, the researchers were able to exploit a process that occurs naturally in benign neuroblastomas to stop the malignant ones. In addition to EGFL8, other, yet uncharacterized Schwann cell molecules could also provide targets for cancer therapies in the future.

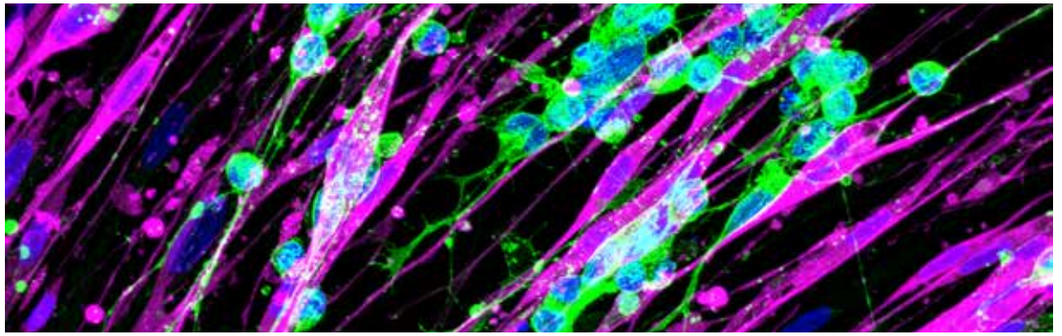
However, the effects of Schwann cells are presumably much more extensive: the research team is currently investigating how Schwann cells manipulate immune cells in their environment.

…AND PROMOTES THE HEALING OF INJURED NERVE FIBERS

The present study provides another significant finding: Schwann cells in benign neuroblastomas have a similar cellular status to those Schwann cells that support the healing of injured peripheral nerves. Direct comparison revealed that Schwann cells in the tumor express certain repair-associated genes and show specific repair functions, showing that this signaling molecule plays a role in both tumor development of benign neuroblastomas and the regeneration of injured nerves. Since EGFL8 stimulates the formation of nerve cell extensions, it could be of great importance for the treatment of injured nerve fibers.

PROSPECTIVE APPLICATION IN AGGRESSIVE TUMORS

EGFL8 and other factors produced by Schwann cells could be applied in the treatment of nerve damage as well as aggressive neuroblastoma. Using phosphoproteomics, the signaling pathways that are activated by EGFL8 in neuroblastoma cells were deciphered. Major differences compared to cells that have not been stimulated with EGFL8 were identified and illustrate a rewiring of cellular signaling in tumor cells by EGFL8. In addition to the EGFL8 protein itself, these downstream signaling pathways also represent potential targets for future treatments. The scientists conclude that while there is still a long way to go before these findings ultimately reach the patient, this publication has laid the foundation for taking the next steps.



Immunofluorescence image of co-cultures of Schwann cells and neuroblastoma cells. Schwann cells express S100B (magenta), while neuroblastoma cells show high abundance of the tumor marker GD2 (green). Cell nuclei are stained with DAPI (blue). Weiss and Taschner-Mandl et al., Nature Communications, 2021.

Weiss et al., Nat Commun 2021

PUBLICATION:

Weiss, T.*, Taschner-Mandl, S.*, Janker, L., Bileck, A., Rifatbegovic, F., Kromp, F., Sorger, H., Kauer, M. O., Frech, C., Windhager, R., Gerner, C., Ambros,P. F., & Ambros, I. M. (2021). Schwann cell plasticity regulates neuroblastic tumor cell differentiation via epidermal growth factor-like protein 8. Nat Commun,12(1), 1624. https://doi.org/10.1038/s41467-021-21859-0

* Shared first authorship

BLOOD TEST DETECTS CHILDHOOD TUMORS BASED ON THEIR EPIGENETIC PROFILES

This study exploits the characteristic epigenetic signatures of childhood tumors to detect, classify and monitor the disease. The scientists analyzed short fragments of tumor DNA that are circulating in the blood. Such "liquid biopsy" analyses exploit the unique epigenetic landscape of bone tumors and do not depend on any genetic alterations, which are rare in childhood cancers. This approach promises to improve the personalized diagnostics and, possibly, future therapies of childhood tumors such as Ewing sarcoma.

The study was led by scientists from the Epigenome-based Precision Medicine group at St. Anna CCRI in collaboration with CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences as well as with scientists from Germany, France, and Norway and it was performed in the context of the EURO EWING Consortium. It provides an innovative method for "liquid biopsy" analysis of childhood tumors. This method exploits the fragmentation patterns of the small DNA fragments that tumors leak into the blood stream, which reflect the unique epigenetic signature of many childhood cancers. Focusing on Ewing sarcoma, a bone tumor of children and young adults with unmet clinical need, the team demonstrated the method's utility for tumor classification and monitoring, which permits close surveillance of cancer therapy without highly invasive tumor biopsies.

In tumors, cancer cells constantly divide, with some of the cancer cells dying in the process. These cells often release their DNA into the blood stream, where it circulates and can be analyzed using genomic methods such as high-throughput DNA sequencing.

Such "so-termed liquid biopsy" analyses provide a minimally invasive alternative to conventional tumor biopsies that often require surgery, holding great promise for personalized therapies. For example, it becomes possible to check frequently for molecular changes in the tumor. However, the use of liquid biopsy for childhood cancers has so far been hampered by the fact that many childhood tumors have few genetic alterations that are detectable in DNA isolated from blood plasma.

EXPLOITING TUMOR-SPECIFIC EPIGENETIC PROFILES

Cell-free DNA from dying tumor cells circulates in the blood in the form of small fragments. Their size is neither random nor determined solely by the DNA sequence. Rather, it reflects how the DNA is packaged inside the cancer cells, and it is influenced by the chromatin (i.e., complex of DNA, protein and RNA) structure and epigenetic profiles of these cells. This is very relevant because epigenetic patterns – sometimes referred to as the "second code" of the genome – are characteristically different for different cell types in the human body. Epigenetic mechanisms lead to changes in gene function that are not based on changes in the DNA sequence but are passed on to daughter cells. The analysis of cell-free DNA fragmentation patterns provides a unique opportunity to learn about the epigenetic regulation inside the tumor without having to surgically remove tumor cells or even know whether and where in the body a tumor exists.

The researchers previously identified unique epigenetic signatures of Ewing sarcoma. They reasoned that these characteristic epigenetic signatures should be preserved in the fragmentation patterns of tumor-derived DNA circulating in the blood. This would provide a much-needed marker for early diagnosis and tumor classification using the liquid biopsy concept.

MACHINE LEARNING INCREASES SENSITIVITY

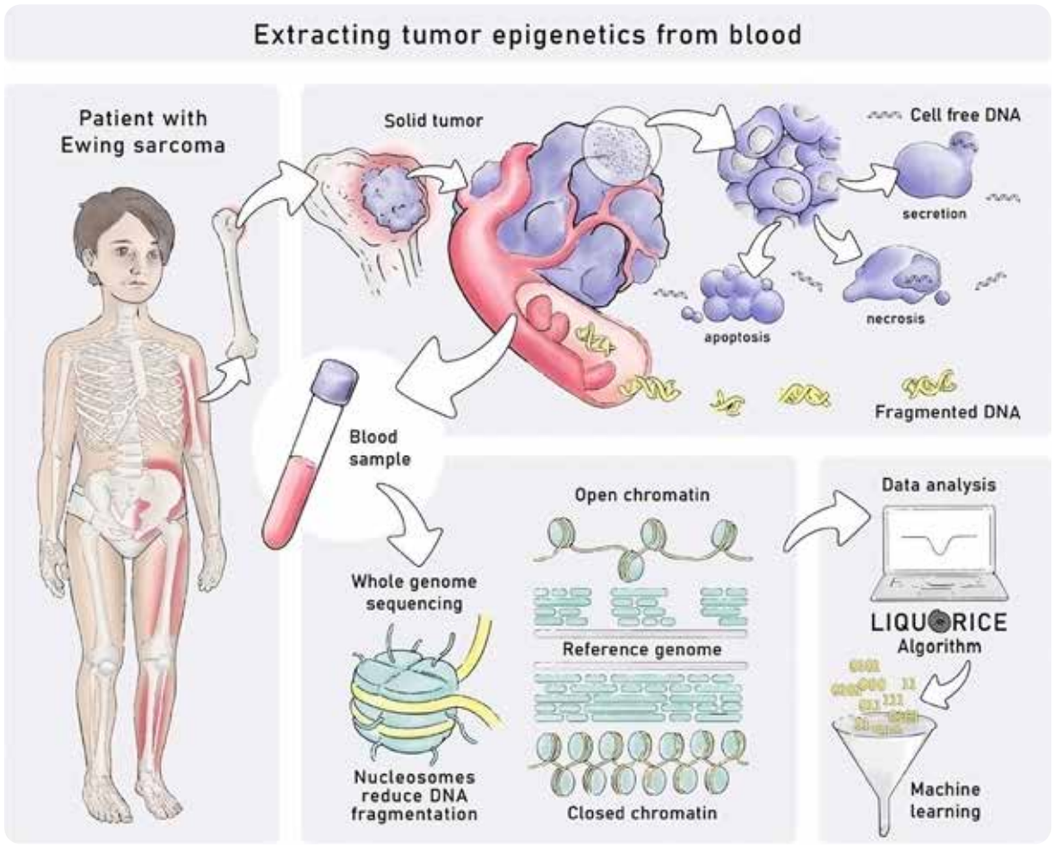
The new study benchmarks various metrics for analyzing cell-free DNA fragmentation, and it introduces the LIQUORICE algorithm for detecting circulating tumor DNA based on cancer-specific chromatin signatures. The scientists used machine-learning classifiers to distinguish between patients with cancer and healthy individuals, and between different types of pediatric sarcomas. By feeding machine learning algorithms with the extensive whole genome sequencing data of tumor-derived DNA in the blood stream, the analysis becomes highly sensitive and in many instances outperforms conventional genetic analyses.

The assay works well, however, further validation will be needed before it can become part of routine clinical diagnostics. According to the scientists, their approach could be used for minimally invasive diagnosis, but also as a prognostic marker to monitor which patient responds to therapy. Additionally, it might serve as a predictive marker during neoadjuvant therapy (i.e., chemotherapy before surgery), potentially enabling dose adjustments according to treatment response. Right now, most patients receive very high doses of chemotherapy, while some patients may be cured already with a less severe therapy, which would reduce their risk of getting other cancers later in life. There is a real medical need for adaptive clinical trials and personalized treatment of bone tumors in children.

PUBLICATION

Peneder, P.*, Stutz, A. M.*, Surdez, D., Krumbholz, M., Semper, S., Chicard, M., Sheffield, N. C., Pierron, G., Lapouble, E., Totzl, M., Erguner, B., Barreca, D., Rendeiro, A. F., Agaimy, A., Boztug, H., Engstler, G., Dworzak, M., Bernkopf, M., Taschner-Mandl, S., Ambros, I. M., Myklebost, O., Marec-Bérard, P., Burchill, S. A., Brennan, B., Strauss, S. J., Whelan, J., Schleiermacher, G., Schaefer, C., Dirksen, U., Hutter, C., Boye, K., Ambros, P .F., Delattre, O., Metzler, M., Bock, C.#, Tomazou, E. M.# (2021). Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden. Nat Commun, 12(1), 3230. https://doi.org/10.1038/s41467-021-23445-w

* Shared first authorship
\# Shared senior authorship



Extracting tumor epigenetics from blood

NEW APPROACH EXPLAINS HOW PROTEIN COMPLEXES REGULATE CANCER GENES

While NUP98 fusion proteins have been shown to cause leukemia, the molecular mechanisms have so far been unknown. In this study led by Vetmeduni Vienna, with the participation of St. Anna CCRI and others, researchers have now been able to decipher an important part of these mechanisms. According to the study, a possible driver for the activation of leukemia genes is the very dynamic complex formation of NUP98 fusion proteins with other proteins in a process known as biomolecular condensation. This finding could contribute to the development of more effective and targeted cancer therapies that would be of particular benefit to children suffering from acute myeloid leukemia (AML).

Cancer-associated chromosomal rearrangements often result in the expression of pathogenic fusion proteins. Leukemia features a particularly high frequency of fusion oncogenes, and the functional investigation of leukemia-associated fusion proteins has provided invaluable insights into the molecular mechanisms of cancer development. The protein complexes around fusion proteins play a defining role in shaping oncogenic gene expression patterns, and the investigation of protein interactions is therefore crucial to identifying new targets on the basis of which more effective cancer therapies can be developed.

EXTREMELY POOR PROGNOSIS FOR CHILDREN WITH AML

While the most common leukemia fusion proteins have been extensively characterized, functional understanding of rare fusions that affect a significant number of patients and have limited treatment options is still lacking. In acute myeloid leukemia (AML), the N-terminal part of the Nucleoporin 98 (NUP98) gene (N-NUP98) is fused to over 30 different C-terminal partner loci. While NUP98 rearrangements are rare (accounting for about 2% of all AML), they are over-represented in pediatric AML, where they are associated with a particularly poor prognosis.

HOW NUP98 FUSION PROTEINS DRIVE AML

All NUP98 fusion proteins share an intrinsically disordered region (IDR) in the NUP98 N-terminus featuring repeats of phenylalanine-glycine (FG), whereas C-terminal fusion partners often function in gene control. Under the supervision of Florian Grebien, Head of the Institute for Medical Biochemistry at the University of Veterinary Medicine Vienna, the researchers investigated whether the protein interactomes of NUP98 fusion proteins provided any insights into the mechanisms of oncogenic transformation. Analyses of affinity purification coupled to mass spectrometry and confocal imaging of five different NUP98 fusion proteins revealed that shared interactors were enriched for proteins involved in biomolecular condensation. These factors co-localized with NUP98 fusion proteins in characteristic condensates in the cell nucleus. An artificial FG fusion protein phenocopied the nuclear localization patterns of NUP98 fusion proteins and their capability to drive oncogenic gene expression programs.

NEW STARTING POINT FOR IMPROVED CANCER THERAPIES

First author Stefan Terlecki-Zaniewicz and colleagues used a newly developed method called biotinylated isoxazole-mediated condensome mass spectrometry (biCon-MS) to show that NUP98 fusion proteins alter the global composition of biomolecular condensates to drive oncogenic gene expression programs. On the basis of the data gathered in this study, they propose that IDR-containing fusion proteins combine biomolecular condensation with transcriptional control to induce cancer. The alteration of biomolecular condensation could therefore be a fundamental principle of cancer development driven by oncogenic fusion proteins. The researchers believe this could be a new approach to developing more effective and targeted cancer therapies.

PUBLICATION
Terlecki-Zaniewicz, S., Humer, T., Eder, T., Schmoellerl, J., Heyes, E., Manhart, G., Kuchynka, N., Parapatics, K., Liberante, F. G., Muller, A. C., Tomazou, E. M., & Grebien, F. (2021). Biomolecular condensation of NUP98 fusion proteins drives leukemogenic gene expression. Nat Struct Mol Biol,28(2), 190-201. https://doi.org/10.1038/s41594-020-00550-w

Biomolecular condensates (purple) in the nucleus (green) of leukemia cells might contribute to leukemogenesis.

CLINICAL STUDIES

RUTH LADENSTEIN

Group: Studies & Statistics for Integrated Research and Projects (S²IRP)

Ruth Ladenstein obtained a medical degree from the University of Vienna and an MBA from the University of Salzburg. She is Senior Consultant in Pediatric Oncology at the St. Anna Children's Hospital and Professor of Pediatrics at the Medical University of Vienna. She became head of the Clinical Trials Unit 'Studies and Statistics for Integrated Research and Projects' (S2IRP) at the CCRI in 1996 where together with her team she conducts Therapy Optimization Studies (TOS) to guarantee better chances of survival of children and adolescents with cancer. In addition, Ruth Ladenstein coordinates the European Reference Network for Pediatric Oncology ERN PaedCan and the Austrian Research Network for Pediatric Medicine OKIDS. She co-founded the association SIOPEN (Society of Paediatric Oncology European Neuroblastoma Network) in 2009. Ruth Ladenstein is chair of the Austrian Working Group for Pediatric Hematology-Oncology, a member of the Oncology Advisory Board of the Austrian Ministry of Health and was a member of the EU Mission Board for Cancer (Horizon Europe) from 2019 – 2021 (new candidacy February 2022) and since 2021 a member of the Mission Action Group Austria.

"Our research goes beyond borders. It is our strong desire that the latest research findings benefit all children in Europe, regardless of where they live."

RUTH LADENSTEIN GROUP

Group: Studies & Statistics for Integrated Research and Projects (S²IRP)

GROUP LEADER
Ruth Ladenstein

SCIENTIFIC ASSISTANT
Claudia Zeiner-Koglin

SENIOR OPERATION MANAGERS
Anke Emberger
Sonja Schneider-Schwarz (until 2021)

STATISTICIANS TEAM LEADER
Ulrike Pötschger

MONITORING
Alexandra Rüben (until 2021)
Barbara Tiefenböck (until 2021)
Heatherheart Ablaza

PHARMACOVIGILANCE
Anna Schlaffer (until 2021)
Merete Tschokert

ADMINISTRATIVE SUPPORT
Corinne Grafl

CLINICAL RESEARCH ASSOCIATES
Adnan Becirovic (until 2021)
Barbara Kristufek (until 2021)
Birgit Hochgatterer
Ekaterina Werderits (maternity leave)
Elfriede Thiem
Eva Sorz
Iris Bachtrog
Jenny Mader (until 2021)
Ljubica Mandic
Lena Brandt
Marek Nykiel
Markus Mair (until 2021)
Merete Tschokert
Naomi Linton-Romir
Nora Mühlegger
Susanne Karlhuber
Theresa Brügmann
Tijana Frank

STATISTICIANS
Evgenia Glogova
Gülsah Sabirli-Goek (until 2021)
Helga Björk Arnardóttir
Paulina Kurzmann

PROJECT MANAGER
Andrea Mikolasek (OKIDS)

PUBLICATIONS

SCIENCE REPORTS

PEDIATRIC HIGH-RISK TUMORS: GENE DEFECT SHORTENS SURVIVAL AND NEEDS TARGETED TREATMENT

Neuroblastomas, the most common solid tumors outside the brain in children, are associated with poorer survival if they have genetic alterations in the *ALK* gene and belong to the high-risk group. If such alterations are detected, it is conceivable that treatment with ALK inhibitors could be used as upfront therapy in future trials

Genetic alterations in the so-called *ALK* gene and the associated protein can fuel the growth of malignant nerve tumors in children. These tumors, namely neuroblastomas, are neoplasms outside the brain that arise from nervous tissue during embryonic development. In a very aggressive form of neuroblastoma (high-risk neuroblastoma), certain genetic alterations in *ALK* have now been identified for the first time as independent predictive markers of decreased survival. Affected children should therefore receive targeted treatment with an ALK inhibitor in future studies. This was the conclusion of a team from St. Anna CCRI in collaboration with St. Anna Children's Hospital and research groups throughout Europe and Israel.

BETTER CHANCES FOR SURVIVAL THANKS TO TARGETED ALK INHIBITION?

ALK stands for "anaplastic lymphoma kinase", a protein that promotes tumor growth when activated. "Our results convincingly argue for the use of an ALK inhibitor together with chemo-therapy and immunotherapy as initial treatment for high-risk neuroblastoma harboring an *ALK* mutation or amplification (i.e., massive replication of the affected DNA sequence). The presence of an *ALK* mutation or amplification worsens survival outcome of affected patients. These patients should therefore receive an ALK inhibitor as tailored therapy in future clinical studies. *ALK* alterations are also a risk factor for survival in a later phase of treatment, when patients receive immunotherapy to maintain previous treatment success. This result strongly suggests that integration of ALK inhibitors throughout all phases of modern era high-risk neuroblastoma therapy is warranted.

ALK ALTERATIONS ARE A RELEVANT RISK FACTOR

The international, randomized Phase III High-Risk Neuroblastoma trial (HR-NBL1), conducted by SIOPEN, enrolled a total of 3,334 patients between 2002 and 2019. Of these, 1,092 patients were included in the *ALK* analysis group, which did not differ in overall survival from the general study population. 132 institutions/hospitals in 19 different countries participated in the trial. Inclusion criteria included stage 2 to stage 4S neuroblastoma according to the International Neuroblastoma Staging System and *MYCN* amplification or stage 4 without *MYCN* amplification in patients aged over 12 months at diagnosis up to 20 years. Within the study, multiple randomized treatment arms consisting of chemotherapy, radiation, and immunotherapy were defined over different time periods.

A large proportion of children were older than 18 months at diagnosis (81%) and had reached an advanced stage of disease (88%, stage 4). *MYCN* amplification, a significant independent risk factor, was present in 47%. 762 study participants were screened for an *ALK* mutation and 901 for an ALK amplification. ALK mutations were detected in 14 percent (106/762) and *ALK* amplifications in 4.5 percent (41/901) of these patients.

It could be shown that *ALK* alterations were a significant predictor for poorer survival in high-risk neuroblastoma (5-year overall survival: 48 vs. 67% with *ALK* alteration vs. no *ALK* alteration, p=0.03). This was also evident in the subgroup already treated with current standard high-dose chemo-therapy (busulfan/melphalan) including anti-GD2 immunotherapy.

Furthermore, evaluation of *ALK* amplification alone was also associated with poorer long-term survival (5-year overall survival: 28 vs. 51% with *ALK* amplification vs. no *ALK* amplification, p<0.001), particularly in cases with metastatic *MYCN* amplified disease.

A subset of *ALK* mutations, namely those with a high "mutation dose" (i.e., clonal mutations; mutant allele fraction >20%) also proved to be a risk factor for poorer long-term survival (5-year overall survival: 34 vs. 59 vs. 49% for clonal *ALK* mutation vs. subclonal vs. no *ALK* mutation, p=0.018). *ALK* mutations with high "mutation dose" comprise approximately 10% of all high-risk neuroblastomas.

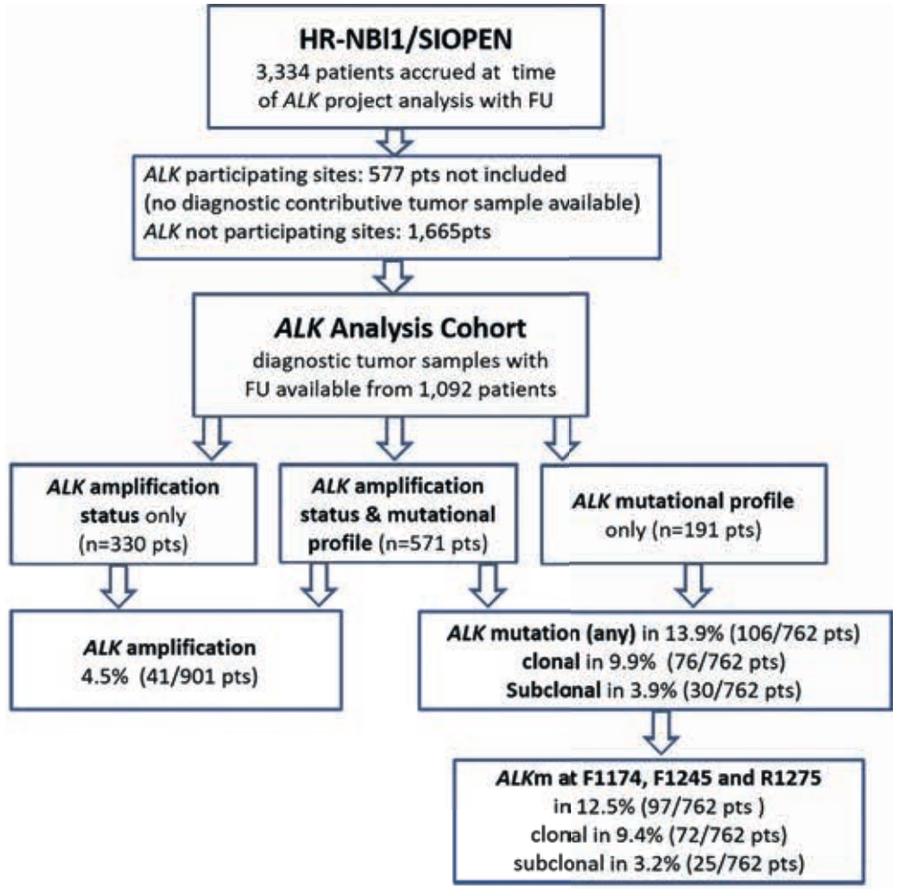


FIGURE 1

***ALK* analysis cohort within the HR-NBL1/SIOPEN trial: Patient inclusion**

Bellini, Pötschger et al., J Clin Oncol 2021

	Overall Survival (OS)					Event-free Survival (EFS)				
parameter in the *ALK* analysis cohort (n=1.092)	Patients	Events	5-Year OS (%; 95% CI)	HR (95% CI)	p	Patients	Events	5-Year EFS (%; 95% CI)	HR (95% CI)	p
***ALK*-amplification (ALKa)**										
no	860	418	51 (47 -54)	ref.	<0.001	860	492	40 (36-43)	ref.	<0.001
yes	41	29	28 (15-42)	2.3 (1.6-3.4)		41	31	24 (13-38)	2 (1.4-2.9)	
***ALK*-mutation (ALKm)**										
nonmutated	656	347	49 (45-53)	ref.	0.18	656	395	38 (35-42)	ref.	0.081
ALK m clonal*	76	48	34 (23-45)	1.4 (1.1-2)		76	51	31 (21-42)	1.3 (1-1.7)	
ALK m subclonal**	30	13	59 (39-74)	0.7 (0.4-1.2)		30	16	49 (30-65)	0.8 (0.5-1.3)	
known *ALK*-alteration status										
nonmutated	465	241	51 (46-55)	ref.	0.001	465	280	38 (33-43)	ref.	0.057
ALK a	19	14	26 (10-47)	2.2 (1.3-3.8)		19	14	26 (10-47)	1.7 (1-2.9)	
ALK m clonal	65	42	33 (21-44)	1.7 (1.2-2.3)		65	43	33 (22-44)	1.4 (1-2.9)	
ALK m subclonal	22	12	48 (26-67)	1 (0.5-1.8)		22	14	39 (19-59)	1 (0.6-1.8)	

* clonal level: >20% mutated allele fraction (MAF)
** subclonal level : MAF 0.1-20%

FIGURE 2

EFS and OS according to *ALK* Alterations.

Bellini , Pötschger et al., J Clin Oncol 2021

PUBLICATION:

Bellini, A.*, Potschger, U.*, Bernard, V., Lapouble, E., Baulande, S., Ambros, P. F., Auger, N., Beiske, K., Bernkopf, M., Betts, D. R., Bhalshankar, J., Bown, N., de Preter, K., Clement, N., Combaret, V., Font de Mora, J., George, S. L., Jimenez, I., Jeison, M., Marques, B., Martinsson, T., Mazzocco, K., Morini, M., Mühlethaler-Mottet, A., Noguera, R., Pierron, G., Rossing, M., Taschner-Mandl, S., Van Roy, N., Vicha, A., Chesler, L., Balwierz, W., Castel, V., Elliott, M., Kogner, P., Laureys, G., Luksch, R., Malis, J., Popovic-Beck, M., Ash, S., Delattre, O., Valteau-Couanet, D., Tweddle, D.A.#, Ladenstein, R.#, Schleiermacher, G.# (2021). Frequency and Prognostic Impact of ALK Amplifications and Mutations in the European Neuroblastoma Study Group (SIOPEN) High-Risk Neuroblastoma Trial (HR-NBL1). J Clin Oncol, JCO2100086. https://doi.org/10.1200/JCO.21.00086

* Shared first authorship

LONG-TERM OUTCOME AND ROLE OF BIOLOGY WITHIN RISK-ADAPTED NEUROBLASTOMA TREATMENT STRATEGIES

This study evaluated long-term outcome and genomic profiles in the Austrian Neuroblastoma Trial A-NB94 which applied a risk-adapted strategy of treatment using stage, age and *MYCN* amplification status for stratification. The risk-adapted approach resulted in an excellent long-term survival for the majority of patients with acceptable long-term morbidity. An age- and stage-dependent frequency of segmental chromosomal aberrations was confirmed and should be considered in future treatment decision-making processes.

FROM SPONTANEOUS REGRESSION TO HIGH MALIGNANCY

Neuroblastoma is the most common extracranial malignancy of childhood and originates from the sympathetic nervous system. It shows a highly heterogeneous behavior ranging from spontaneous regression or maturation into a benign ganglio-neuroma to an aggressive and intractable disease. Risk classification systems are using clinical and biological characteristics to predict survival and adapt treatment intensity.

At this study's initiation, recognized risk driving factors included stage defined by the International Neuroblastoma Staging System (INSS), age at diagnosis, and *MYCN* oncogene amplification (MNA) status. MNA, a strong biologic marker associated with rapid tumor growth, transforms otherwise favorable risk profiles of infants and children with localized resectable or unresectable disease into high-risk.

Metastatic disease in children older than 18 months constitutes per se an unfavorable risk group regardless of *MYCN* status. Intratumoral heterogeneous MNA refers to the coexistence of clustered or scattered single MNA cells and non-*MYCN*-amplified tumor cells, a phenomenon that was largely unexplored at the initiation of the trial. A recent study highlights the importance of viewing it separately from the MNA profile and its unfavorable risk implication, however, prognostication and therapy allocation are still unsolved issues.

RISK-ADAPTED TREATMENT STRATEGY IS OF UTMOST IMPORTANCE

The A-NB94 trial was the first in Austria to stratify therapy intensity according to tumor staging, patient's age, and *MYCN* amplification status, the latter being a biologic marker turning otherwise low-risk tumors into high-risk disease.

Risk-adapted strategy of treatment ranged from surgery only to intensity-adjusted chemotherapy, single or multiple courses of high-dose chemotherapy followed by autologous stem cell rescue depending on response to induction chemotherapy, and irradiation to the primary tumor site. Segmental chromosomal alterations were investigated retrospectively using multi- and pan-genomic techniques. The A-NB94 trial enrolled 163 patients. Patients with localized disease had an excellent ten-year (10y) event-free survival (EFS) and overall survival (OS) of 99 ± 1% and 93 ± 2% whilst it was 80 ± 13% and 90 ± 9% for infants with stage 4S and for infants with stage 4 non-MNA disease both 83 ± 15%. Stage 4 patients either >12 months or ≤12 months but with MNA had a 10y-EFS and OS of 45 ± 8% and 47 ± 8%, respectively.

Segmental chromosomal alterations were present in increasing frequencies according to stage and age: in 29% of localized tumors but in 92% of stage 4 tumors ($p < 0.001$), and in 39% of patients ≤ 12 months but in 63% of patients > 12 months ($p < 0.001$). By evaluating long-term outcome and genomic profiles in the Austrian Neuroblastoma Trial A-NB94, it was shown that a risk-adapted strategy of treatment successfully reduced chemotherapy exposure in low- and intermediate-risk patients with excellent long-term results while the outcome of high-risk disease met contemporary trials.

PUBLICATION:

Fiedler, S., Ambros, I. M., Glogova, E., Benesch, M., Urban, C., Mayer, M., Ebetsberger-Dachs, G., Bardi, E., Jones, N., Gamper, A., Meister, B., Crazzolara, R., Amann, G., Dieckmann, K., Horcher, E., Kerbl, R., Brunner-Herglotz, B., Ziegler, A., Ambros, P. F., & Ladenstein, R. (2021). Long-Term Outcome and Role of Biology within Risk-Adapted Treatment Strategies: The Austrian Neuroblastoma Trial A-NB94. Cancers (Basel), 13(3). https://doi.org/10.3390/cancers13030572

NERVE TUMOR IN CHILDREN: STUDY IDENTIFIES BETTER TOLERABLE CHEMOTHERAPY WITHOUT LOSS OF EFFICACY

The initial chemotherapy of aggressive childhood nerve tumors, so-called high-risk neuroblastomas, is crucial for ultimate survival. It has now been shown that the chemotherapy regimen used by the European Neuroblastoma Study Group is equally efficacious but better tolerated than a highly effective regimen from the US.

For particularly aggressive nerve tumors in children, so-called high-risk neuroblastomas, various combination chemotherapies are used with the intention to shrink the tumor before surgery (i.e., induction chemotherapy). The efficacy of such an induction therapy significantly impacts the chances of survival. The European Neuroblastoma Study Group of the International Society of Pediatric Oncology (SIOPEN) has now compared two of the most effective combination therapies in an international study, coordinated by St. Anna CCRI. The result: equal efficacy, but significantly lower rates of side effects with the therapy regimen considered the standard of the European Neuroblastoma Group SIOPEN. With the European rCOJEC regimen, high-grade vomiting, nausea, diarrhea, infections and stomatitis were significantly lower than in the US reference group. It will therefore be implemented as a standard of care for high-risk neuroblastoma.

EQUAL EFFICACY, FEWER SIDE EFFECTS

The study team compared the treatment regimen of the renowned Memorial Sloan Kettering Cancer Center, i.e., MSKCC-N5 regimen, which has the best efficacy results to date, with the so-called rCOJEC regimen, the standard SIOPEN treatment in Europe. In the present international phase III study, a total of 630 patients were randomly assigned to one of the two regimens. The intention was to evaluate whether the MSKCC-N5 regimen improves the response of tumor metastases (metastatic complete response) compared with rCOJEC. Furthermore, the clinicians investigated whether MSKCC-N5 therapy reduces the likelihood of relapse within three years (3-year event-free survival), compared with the rCOJEC regimen. Neither was the case. This study showed that the efficacy did not significantly differ between the two treatment regimens. However, the rate of high-grade (grade 3–4), acute adverse events was significantly higher with the MSKCC-N5 regimen.

Further studies should now examine the efficacy of immunotherapies in addition to the rCOJEC regimen, given that only about 60 and 65 percent of children receiving the rCOJEC and -MSKCC-N5 regimens, respectively, are still alive after three years. Therefore, there is an urgent need to further improve the survival outcome. For example, the combination of chemotherapy with anti-GD2 antibodies is very promising – a combination that will be tested in randomized trials by SIOPEN and others.

HIGH NEED FOR RANDOMIZED TRIALS

Thanks to continuously improving therapies, an increasingly larger number of children with high-risk neuroblastoma are already surviving their disease nowadays. Furthermore, those who survive have a long life expectancy. For this reason it is even more important today to also investigate sequelae resulting from intensive chemotherapies. This study is only the fourth randomized trial investigating induction therapies in high-risk neuroblastoma, because randomized clinical trials require a lot of organizational effort at the international level due to small case numbers in each participating country. However, they are essential for providing the best treatment for childhood cancer patients.

ABOUT THE STUDY

The SIOPEN international randomized phase III HR-NBL1.5 trial included a total of 630 subjects. The median age was 3.2 years. The study included children or adolescents between one and 20 years of age with stage 4 neuroblastoma older than 12 months or infants younger than one year of age with stage 4S and MYCN amplification. Study participants were randomly assigned in a 1:1 ratio to the rCOJEC (cisplatin, vincristine, etoposide, cyclophosphamide, and carboplatin) or MSKCC-N5 regimen (cyclophosphamide, doxorubicin, vincristine, and cisplatin). Induction therapy with one of these regimens was followed by tumor surgery, high-dose chemotherapy (busulphan, melphalan), radiotherapy, and isotretionin combined with immunotherapy with dinutuximab beta. Co-primary study endpoints were metastatic complete response and 3-year event-free survival. The metastatic complete response rate was not significantly different between the rCOJEC (32%) and MSKCC-N5 (35%, p=0.368) groups. There were also no significant differences in 3-year event-free survival between the groups (44±3% vs. 47%±3%, p=0.527). High-grade adverse events (grade 3–4) were significantly more frequent in the MSKCC-N5 group. These included non-hematologic side effects (68 vs. 48%, p<0.001), infections (35 vs. 25%, p=0.011), stomatitis (25 vs. 3%, p<0.001), nausea/vomiting (17 vs. 7%, p<0.001) and diarrhea (7 vs. 3%, p=0.011).

PUBLICATION

Garaventa, A., Poetschger, U., Valteau-Couanet, D., Luksch, R., Castel, V., Elliott, M., Ash, S., Chan, G. C. F., Laureys, G., Beck-Popovic, M., Vettenranta, K., Balwierz, W., Schroeder, H., Owens, C., Cesen, M., Papadakis, V., Trahair, T., Schleiermacher, G., Ambros, P., Sorrentino, S., Pearson, A. D. J., Ladenstein, R. L. (2021). Randomized Trial of Two Induction Therapy Regimens for High-Risk Neuroblastoma: HR-NBL1.5 International Society of Pediatric Oncology European Neuroblastoma Group Study. J Clin Oncol, JCO2003144. https://doi.org/10.1200/JCO.20.03144

HOW TO TREAT CHILDREN WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA, IN NEED OF A STEM CELL TRANSPLANTATION

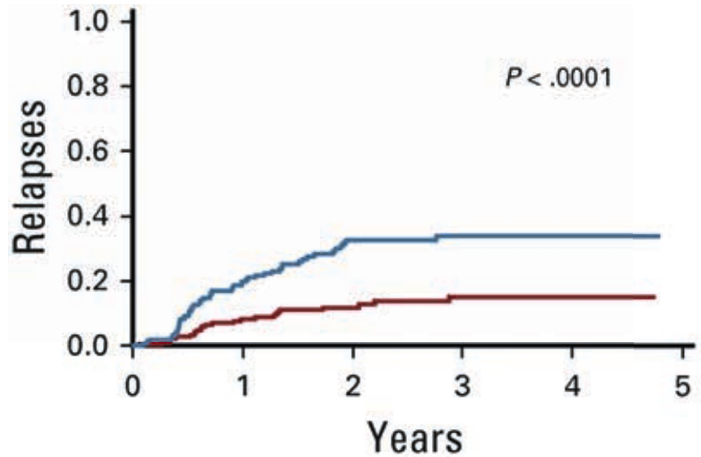
What started as a scientific discussion about whether total body irradiation can be omitted to avoid long-term side effects in patients with acute lymphoblastic leukemia (ALL) before allogeneic hematopoietic stem cell transplantation (HSCT), led to an international randomized study, eventually showing the contrary. Total body irradiation turned out to be the better strategy in avoiding relapse.

AN ALTERNATIVE TO TOTAL BODY IRRADIATION?

Following a study showing that stem cell transplantation works just as well when donors are not related, an international cooperation of scientists and clinicians from 35 countries brought the best expertise in the field of acute lymphoblastic leukemia (ALL) together. ALL is a childhood blood cancer that causes certain abnormal lymphoid cells to build up in the bone marrow. These interfere with the production of normal white blood cells. If standard chemotherapy is not sufficient to cure the disease, stem cell transplantation is an effective but intensive and complex treatment that can cause life-threatening and long-term side effects. Therefore, the experts posed the question whether there is a way to reduce the treatment burden and omit total body irradiation – a pre-conditioning procedure before undergoing allogeneic stemcell transplantation – in children over the age of four. Despite its effectiveness, total body irradiation goes along with multiple long-term side effects, e.g., infertility or a second cancer type, the latter being far less likely to occur when patients receive chemo-conditioning instead.

IRRADIATION DECREASES RELAPSES

In 2012 a randomized controlled, open-label, international, multicenter, phase III, noninferiority study with about 1,000 patients ≤ 18 years at diagnosis, 4–21 years at HSCT, in complete remission pre-HSCT, and with an HLA-compatible related or unrelated donor,was initiated to determine if chemo-conditioning (fludarabine, thiotepa, and either busulfan or treosulfan) in young patients with ALL should be the preferred treatment option before HSCT. After significant results pointing towards irradiation being the treatment to favor, the randomization was closed in 2018. Total body irradiation (myeloablative conditioning with fractionated 12 Gy TBI) in combination with etoposide unmistakably turned out to be the best conditioning treatment for children with high-risk acute lymphoblastic leukemia in need of a stem cell transplantation – also because of fewer severe acute side effects and because relapses turned out to be far less likely after irradiation (see figure). This treatment is therefore recommended for all children with high-risk ALL over 4 years of age, with a sibling or suitable unrelated donor. The vision for the future is that innovative therapies on the rise like CAR T cells render the burden of transplantation and its associated pre-conditioning with irradiation avoidable.



	Patients	Eval.	Relapses	2-year CIR
TBI	212	209	24	0.12 (0.08-0.17)
CHC	201	200	55	0.33 (0.25-0.40)

The most frequent reason for treatment failure was relapse, commonly in bone marrow (56/70 patients). Two-year cumulative incidence of relapse was 0.12 (95% CI, 0.08 to 0.17) following total body irradiation (TBI) and 0.33 (95% CI, 0.25 to 0.40) following chemo-conditioning (CHC, p < 0.0001).

Peters et al., J Clin Oncol 2021

PUBLICATION

Peters, C., Dalle, J. H., Locatelli, F., Poetschger, U., Sedlacek, P., Buechner, J., Shaw, P. J., Staciuk, R., Ifversen, M., Pichler, H., Vettenranta, K., Svec, P., Aleinikova, O., Stein, J., Gungor, T., Toporski, J., Truong, T. H., Diaz-de-Heredia, C., Bierings, M., Ariffin, H., Essa, M., Burkhardt, B., Schultz, K., Meisel, R., Lankester, A., Ansari, M., Schrappe, M., IBFM Study Group; von Stackelberg, A., IntReALL Study Group; Balduzzi, A., I-BFM SCT Study Group; Corbacioglu, S., EBMT Paediatric Diseases Working Party; Bader, P. (2021). Total Body Irradiation or Chemotherapy Conditioning in Childhood ALL: A Multinational, Randomized, Noninferiority Phase III Study. J Clin Oncol,39(4), 295-307. https://doi.org/10.1200/JCO.20.02529

IMMUNOLOGY, HEMATOLOGY
& IMMUNOTHERAPY

KAAN BOZTUG

Group: Immune Deficiency, Cancer Predisposition & Precision Oncology

Univ-.Prof. Kaan Boztug completed his medical studies in Düsseldorf and Freiburg as well as London. He also obtained a doctorate at the Scripps Research Institute in La Jolla, USA, before completing his clinical training and postdoctoral research at Hannover Medical School. In 2011, he started working at the Medical University Vienna's Department of Pediatrics and Adolescent Medicine and became a Principal Investigator at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences. Kaan Boztug became Scientific Director at St. Anna CCRI in 2019. His research group focuses on inborn immune disorders and inherited predisposition to childhood tumors, aiming to understand fundamental mechanisms of immune surveillance relevant to pediatric oncology and immunotherapy approaches. Besides his work at CeMM and St. Anna CCRI, Kaan Boztug is Director of the CeRUD Vienna Center for Rare and Undiagnosed Diseases at Medical University Vienna, Consultant for Pediatric Hematology/ Oncology & Head of Pediatric Immunology at St. Anna Children's Hospital, and Director of the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD).

"Our goal is to understand the mechanistic details of disease: to enable more advanced, personalized treatments in the future."

KAAN BOZTUG GROUP

Group: Immune Deficiency, Cancer Predisposition & Precision Oncology

ST. ANNA CHILDREN'S CANCER RESEARCH INSTITUTE

PRINCIPAL INVESTIGATOR
Kaan Boztug

SCIENTIFIC PROJECT MANAGER, EXECUTIVE ASSISTANT
Caroline Stremnitzer

SENIOR SCIENTIFIC PROJECT MANAGER
Isabel Grießhammer (until 2021)

LAB MANAGER
Wojciech Garncarz

PROGRAM LEADER & DEPUTY LAB HEAD
Alexis Lomakin (until 2021)

SENIOR STAFF SCIENTIST
Irinka Castanon Ortega

SENIOR POSTDOCTORAL FELLOWS
Michael Kraakman
Artem Kalinichenko

POSTDOCTORAL FELLOWS
Juraj Konc
Cheryl van de Wetering

PHD STUDENTS
Jana Block
Sören Strohmenger
Ben Haladik
Jakob Berner (until 2021)
Tala Shahin (until 2021)

MD STUDENT
Daniel Mayr

TECHNICIANS
Sarah Giuliani
Anna Segarra Roca
Alexandra Frohne

LUDWIG BOLTZMANN INSTITUTE FOR RARE AND UNDIAGNOSED DISEASES (LBI-RUD)

ADMINISTRATION
Johannes Pfeifenschneider

POSTDOCTORAL FELLOWS
Vanessa Hertlein
Sevgi Köstel Bal
Samaneh Zoghi

PHD STUDENTS
Bernhard Ransmayr
Jakob Huemer
Yirun Miao

TECHNICIANS
Raul Jimenez Heredia
Christina Rashkova

MANFRED LEHNER

Christian Doppler Laboratory for Next Generation CAR T Cells

Manfred Lehner received his PhD in 2001 at St. Anna CCRI on the topic of “Immune response of dendritic cells after infection and interaction with T cells”. In 2012 he habilitated at the Friedrich Alexander University Erlangen-Nuremberg on cellular therapies. In 2013, back at St. Anna CCRI, he focused on a new concept for the promising CAR T cell therapy. After a stay abroad at the National Cancer Institute in Bethesda (USA) in 2014, he further developed this concept and started to realize it in 2016 in cooperation with Michael Traxlmayr from the University of Natural Resources and Life Sciences, BOKU. This eventually led to the international filing of three patents. Based on these results, he applied for a Christian Doppler Laboratory for Next Generation CAR T Cells together with BOKU and the industrial partner Miltenyi Biotec. The CD Lab was approved in 2019 and has been running successfully since then.

“Three factors are extremely motivating for me: our research addresses key problems in the field, our approaches to solving them have a high therapeutic potential, and the team spirit is great.”

CHRISTIAN DOPPLER LABORATORY

Christian Doppler Laboratory for Next Generation CAR T Cells

ST. ANNA CHILDREN'S CANCER RESEARCH INSTITUTE – CAR BIOLOGY

PROJECT COORDINATOR
Manfred Lehner

MASTER STUDENT
Patrick Gano (until 2021)

PHD STUDENT
Elise Sylvander

POSTDOCTORAL FELLOW
Benjamin Salzer (until 2021)

TECHNICIANS
Dominik Emminger
Katja Nettermann (until 2021)

BOKU – UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES – PROTEIN ENGINEERING

HEAD OF EXTERNAL MODULE
Michael Traxlmayr

MASTER STUDENTS
Alex Alton (until 2021)
Fabian Schubert
Elisabeth Lehner

PHD STUDENT
Magdalena Teufl

POSTDOCTORAL FELLOW
Charlotte Zajc

INTERN
Urban Leitgeb (until 2021)

MILTENYI BIOTEC

PROJECT PARTNER INDUSTRY
Joerg Mittelstaet

EVA KÖNIG

Group: Tumor Immunoediting

Eva König graduated from the University of Vienna with a degree in Chemistry in 2007 before starting a PhD with a focus on tumor immunology in the laboratory of Veronika Sexl at the Medical University of Vienna and University of Veterinary Medicine Vienna. She proceeded as a postdoctoral fellow at the QIMR Berghofer Institute of Medical Research in Brisbane (Australia) and the Austrian Max Perutz Laboratories in Vienna. In 2019, Eva König established the Tumor Immunoediting group at the St. Anna CCRI. The focus of their research is to study the role of innate lymphocytes called natural killer (NK) cells in tumor surveillance. They implement an unbiased screening approach to study the mechanisms of tumor evasion from NK cells. Complementing this, they are interested in specific molecular pathways activated in NK cells upon the recognition of tumor cells. Together with her team of PhD and Master students and technical assistants, she aims to find novel targets to increase the susceptibility of tumor cells towards NK cell-mediated surveillance and/or to enhance NK cell functionality per se.

“We aim to better understand the complex interaction of immune and tumor cells to develop improved cancer therapies.”

EVA KÖNIG GROUP

Group: Tumor Immunoediting

GROUP LEADER/ PRINCIPAL INVESTIGATOR
Eva Maria König

LAB MANAGERS
Hayeon Baik (until 2021)
Faith Hall Herbold

MASTER STUDENT
Susanne Stofner

PHD STUDENTS
Michelle Buri
Faith David

PUBLICATIONS

IMMUNE CELLS OUT OF CONTROL: HOW LETHAL HYPERINFLAMMATION EMERGES FROM A NOVEL GENE DEFECT

CCRI researchers along with their international cooperation partners have discovered a novel subtype of a genetic disease: genetically determined deficiency of the protein RhoG abrogates the normal cytotoxic function of specific immune cells, causing hemophagocytic lymphohistiocytosis (HLH). These new findings may help with the genetic diagnosis for patients with a clinical suspicion of HLH. Published in the high-ranked scientific journal *Blood*, the study provides a basis for both, a deeper understanding of the biology of HLH and the exploration of new therapeutic approaches.

As part of an international collaborative effort, scientists at St. Anna CCRI are shedding light on a new etiology of a disease called familial hemophagocytic lymphohistiocytosis (HLH). Occurring usually in early childhood, familial or genetically-determined HLH is one of the most dramatic hematologic disorders. It is characterized by the inability of specific immune cells, namely T lymphocytes and natural killer (NK) cells, to kill an infected (e.g., virus-infected) target cell. As a consequence, the body may secrete biological messengers (so-called cytokines) that generate massive immune activation and hyperinflammation throughout the entire body. If untreated, the hyperinflammation associated with HLH can be lethal in a short period of time.

Until recently, four subtypes of familial HLH had been known, caused by mutations in genes involved in regulating the immune defense. This study discovered a new type of this disease, caused by inherited mutations in the gene that encodes the protein RhoG. The researchers show how deficiency of RhoG specifically impairs the cytotoxic function of T lymphocytes and NK cells. This results in their uncontrolled activation and ultimately causes HLH. In particular, RhoG deficiency impairs the process of exocytosis in specific immune cells and disables their killing ability. Immune cells like T and NK cells use exocytosis to release cytotoxic molecules to attack and kill infected or tumor cells. When RhoG deficiency abrogates this function in immune cells, they cannot kill their target cells as intended. The paper explores in detail how this potentially affects the propensity to develop cancer.

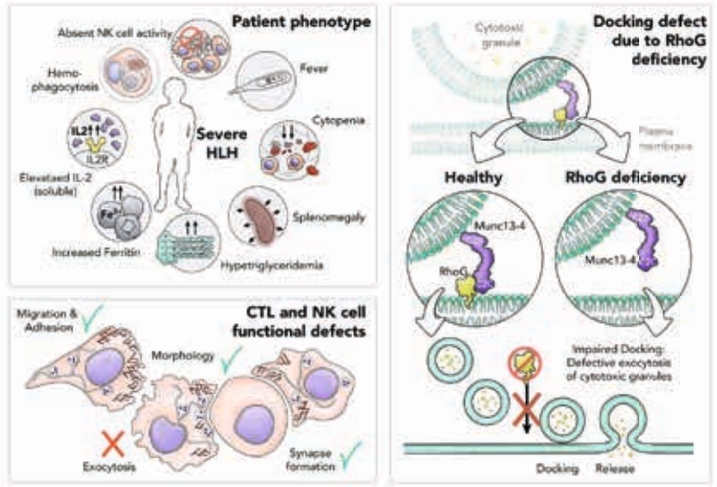
RHOG REGULATES LYMPHOCYTE CYTOTOXICITY

In their study, the scientists investigated an infant who developed severe HLH at the age of four months. While the disease was associated with impaired cytotoxicity of T and NK cells, no mutations were found in known HLH-associated genes. Further genetic analysis revealed deleterious mutations in the gene encoding RhoG. By experimental ablation of RhoG, the scientists confirmed the previously unknown role of RhoG in the cytotoxic function of human lymphocytes. Despite a drastic and specific effect on cytotoxic function, RhoG deficiency does not affect other functions of immune cells that play an important role for the disease development.

The researchers discovered a pivotal role of RhoG interaction with an exocytosis protein called Munc13-4, essential for anchoring of cytotoxic granules to the plasma membrane. This docking is a critical step in exocytosis. It is required for further fusion of the vesicles with the plasma membrane and the release of the cytotoxic granules. Thus, the study illuminates RhoG as a novel essential regulator of human lymphocyte cytotoxicity, and provides the molecular pathomechanism behind this previously unreported genetically determined form of hemophagocytic lymphohistiocytosis.

ACCELERATED DIAGNOSIS ENABLING EFFICIENT TREATMENT OF PATIENTS

Based on the understanding of the underlying molecular mechanism of familial HLH, the researchers are looking forward to an improved prognosis and treatment of the disease in the long-term. As a short-term consequence, the discovered RhoG deficiency can help HLH patients by enabling a genetic diagnosis. Better understanding of the molecular pathomechanisms of HLH may impact disease management and prognosis: The discovery of RhoG deficiency has revealed new insights into the molecular functions of this protein and revealed highly relevant questions. The paper shows that RhoG regulates both the 'cell skeleton' and the exocytosis machinery and triggers new research questions on how RhoG coordinates their activity in space and time.



RhoG deficiency abrogates the cytotoxic function of CTLs and NK cells, causing a life-threatening condition called familial hemophagocytic lymphohistiocytosis (FHL). At a molecular level, we showed that RhoG interacts with Munc13-4 at the surface of cytotoxic granules to promote their anchoring to the plasma membrane and subsequent fusion required for the release of effector molecules.

Kalinichenko et al., Blood 2021
2021 Elsevier
Graphics: Tatjana Hirschmugl

PUBLICATION

Kalinichenko, A., Perinetti Casoni, G., Dupre, L., Trotta, L., Huemer, J., Galgano, D., German, Y., Haladik, B., Pazmandi, J., Thian, M., Yuce Petronczki, O., Chiang, S. C. C., Taskinen, M. H., Hekkala, A., Kauppila, S., Lindgren, O., Tapiainen, T., Kraakman, M. J., Vettenranta, K., Lomakin, A. J., Saarela, J.#, Seppänen, M. R. J.#, Bryceson, J. T.#, Boztug, K.# (2021). RhoG deficiency abrogates cytotoxicity of human lymphocytes and causes hemophagocytic lymphohistiocytosis. Blood. https://doi.org/10.1182/blood.2020008738

Shared senior authorship

DO YOU SPEAK IMMUNOLOGY? A NEW LANGUAGE FOR CONGENITAL IMMUNE DISORDERS

A detailed vocabulary to identify and explore rare, congenital immune disorders around the world? This is exactly what is now available thanks to a new study. Congenital immune disorders often affect only a handful of children around the world. The now published expansion of the nomenclature allows better analysis, identification, and treatment of these diseases.

When a child in Austria falls ill with a rare immunodeficiency, their treating physician will often search for experience with similar cases around the world. Has there ever been a comparable case before? What has been the treatment experience in the other patient? But even if a child in – for example – Japan has been diagnosed with the same disease, the treating physicians will probably never get to know about this fact. Researchers at St. Anna CCRI and the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD) strive to change that. They realized that – to identify and eventually treat children with rare diseases – there needs to be a global exchange between researchers and physicians. This exchange can only be successful if everyone speaks the same language, describes diseases in a uniform way, and if local registries are internationally interconnected.

YOU ONLY FIND WHAT YOU CAN NAME

Boztug's research group at St. Anna CCRI and the LBI-RUD, together with the group of Marielle van Gijn at the University Medical Center Groningen, have therefore brought together relevant partners around the world and leading medical societies in the field of immune diseases including the European Society for Immunodeficiencies (ESID) and the European Reference Network on Rare Primary Immunodeficiency, Autoinflammatory and Autoimmune diseases (ERN-RITA) to further develop and extend the so-called Human Phenotype Ontology (HPO). HPO provides a language-independent standardized vocabulary for phenotypes of human diseases. Matthias Haimel and Julia Pazmandi, shared first authors of the study, initiated a collaboration with Peter Robinson, who originally developed HPO, and together with their cooperation partners systematically expanded the vocabulary to describe immunological diseases. More accurate and detailed descriptions of the symptoms of rare, congenital immune disorders will enable consistent diagnoses worldwide. Until now, different terms have been applied in different countries for the same disease and its symptoms. But: only what you can name can be found. At the same time, an accurate description helps to discover new diseases and gene mutations.

HPO includes 2,120 rare diseases, of which congenital immune disorders form a subgroup. For the latter, HPO has not been specific enough and therefore has hardly been used in the expert community. To change this, leading researchers and clinicians in the field have met regularly both at CCRI and LBI-RUD in Vienna as well as in virtual workshops to systematically revise and expand the terms of four disease groups relevant to congenital immune disorders within HPO. New terms such as "recurrent fever" or "unusual infections" were added. To accomplish this, selected articles were analyzed for technical terms using machine learning, which were then assigned as correct or incorrect by experts in a review process.

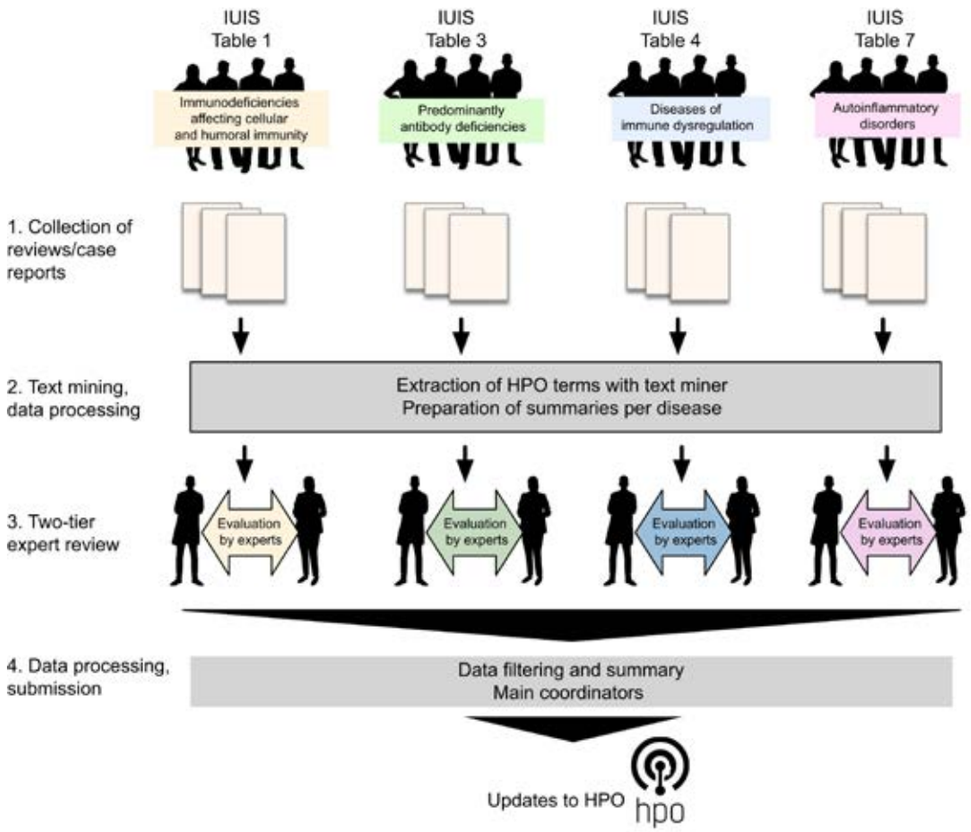
TOP 10 RANKING NARROWS DOWN THE DIAGNOSIS

To showcase the efficiency of their expansion of HPO terms for immune defects, the researchers analyzed 30 patients from a database. HPO terms were assigned to disease entities and based on this, the similarity to all previously revised HPO diseases was calculated. In most cases, the correct diagnosis was found in the top 10 diseases calculated for each symptom. Artificial intelligence now makes it easier to assign certain symptoms to a rare disease.

It is impossible for doctors nowadays to know about every rare disease. Further, some immune disorders are very similar to each other. Patients could thus be spared years of searching for their correct diagnosis. In this way, treatment can be initiated earlier, and the outcome may be improved.This study uses machine learning to narrow down the selection of possible diagnoses for patients and to retrieve the full spectrum of terms from the literature at the touch of a button. The same process could be applied to unstructured clinical notes. Abnormal clinical values in medical records could thus be automatically translated into HPO codes, promoting more accurate diagnosis.

INTERNATIONAL COLLABORATION – THE KEY TO RARE DISEASES

Identifying at least a few individuals worldwide suffering from the same disease is often critical to gain insight into typical manifestations. The now expanded and reannotated HPO terms allow for a better description of symptoms and enable the creation of an electronic disease profile. Efficiently sharing such anonymized disease profiles across institutions and borders is a major challenge. Platforms facilitating this are already being used by the LBI-RUD as part of the Undiagnosed Diseases Network International (UDNI) with the ultimate goal to efficiently identify patients with rare diseases and to enable them to receive the best possible treatment approaches.



Pipeline for standardized reannotation of Inborn Errors of immunity (IEI) diseases. First, scientific publications were collected by experts for each disease within the subgroups. Second, HPO terms were extracted from the provided publications for each disease using machine learning and summarized in Excel documents. Third, a 2-tier expert review evaluated the text-mined terms, suggested additional terms if required, and the responsible working group agreed on the final HPO annotations for each disease. Fourth, data were collated, and the agreed terms were submitted to HPO.

PUBLICATION

Haimel, M.*, Pazmandi, J.*, Heredia, R. J., Dmytrus, J., Bal, S. K., Zoghi, S., van Daele, P., Briggs, T. A., Wouters, C., Bader-Meunier, B., Aeschlimann, F. A., Caorsi, R., Eleftheriou, D., Hoppenreijs, E., Salzer, E., Bakhtiar, S., Derfalvi, B., Saettini, F., Kusters, M. A. A., Elfeky, R., Trück, J., Rivière, J.G., van der Burg, M., Gattorno, M., Seidel, M. G., Burns, S., Warnatz, K., Hauck, F., Brogan, P., Gilmour, K. C., Schuetz, C.,Simon, A., Bock, C., Hambleton, S., de Vries, E., Robinson, P. N., van Gijn, M.#, Boztug, K.# (2021). Curation and Expansion of Human Phenotype Ontology for Defined Groups of Inborn Errors of Immunity. J Allergy Clin Immunol. https://doi.org/10.1016/j.jaci.2021.04.033

* Shared first authorship
\# Shared senior authorship

FROM THE GOD OF THE SUN TO IMMUNODEFICIENCY: IDENTIFICATION OF A NEW RARE DISEASE AFFECTING HEMATOPOIESIS AND IMMUNITY

Together with international collaborators, scientists at the St. Anna CCRI discover a new inborn error of hematopoiesis and immunity, caused by an inherited genetic defect of the transcription factor Helios. In their project, a team of researchers was able to define previously unknown roles of Helios in immune activation and homeostasis.

The immune system is one of the most complex and fascinating networks in the human body. Comprised of cellular and humoral components, it not only protects us against external intruders such as viruses or bacteria, but also plays a fundamental role in detecting aberrant cells developing into cancer cells. The development and function of immune cells is tightly regulated by the temporal and spatial control of gene expression. This is mainly achieved by so-called transcription factors – special proteins that bind to regulatory sequences of genes and turn their expression on or off. In addition, there is another level of control: so-termed epigenetic remodelers control whether certain regions of the DNA are active or inactive, and thereby determine if transcription factors can bind to their target sequences or not.

The Ikaros family of zinc finger transcription factors represents a group of proteins that have been shown to play a central role in hematopoiesis and immune cell development and function. It is comprised of five known members: Ikaros, Helios, Aiolos, Eos and Pegasus. The roles of some of these transcription factors, in particular Ikaros, have been studied in detail with their aberrant function more recently being linked to the development of leukemia. The precise function of Helios, however, has only partially been known.

HELIOS – A KEY PLAYER IN T CELL DEVELOPMENT AND FUNCTION

The research team investigated a patient with an unknown defect of immunity and hematopoiesis, who had been suffering from recurrent respiratory infections and hypogammaglobulinemia since birth. In their study, the scientists identified an inherited biallelic mutation – i.e., a mutation carried on both copies of the gene – in the gene encoding Helios, a transcription factor named after the Greek god of the sun.

Helios is predominantly expressed in developing thymocytes, activated T cells and regulatory T cells – a subset of T cells that play a role in controlling the immune activation response. In regulatory T cells, Helios is known to be important for regulating the transcription of several genes. Although Helios has already been studied in mice, its role in human immune homeostasis and T cell development remains unclear.

BIALLELIC MUTATION OF HELIOS CAUSES EPIGENETIC DEFECT AND IMMUNODEFICIENCY

At a molecular level, the defect resulting from the biallelic mutation in *IKZF2* did not affect DNA binding or dimerization of Helios but had other consequences. The mutation affecting Helios leads to disruptions in the interaction with other proteins, including epigenetic remodelers, thereby compromising the precise control of activation of genes.

The scientists used single-cell transcriptomics and in vitro functional assays to further study the effects of the mutation at a cellular level. They were able to show that the defect in Helios had detrimental effects on immunity:

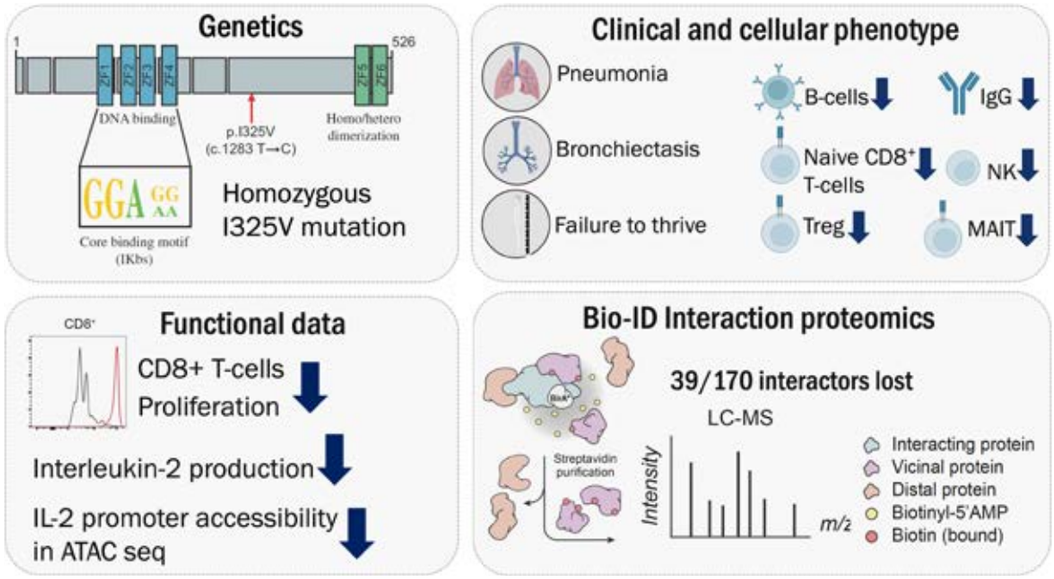
While CD8+ T cells carrying the mutation shifted towards a pro-inflammatory, effector-like status, patient CD4+ cells showed impaired activation. Additionally, a B cell defect was detected: there was a B cell loss over time, and peripheral memory B cells and plasmablasts were reduced, while transitional B cells were increased.

IMPACT ON FUTURE RESEARCH AND TREATMENT

As the proper activation of conventional T cells and the presence of B cells is important for mounting an immune activation response against infections and (pre)cancerous cells, these findings are of great interest. The results do not only characterize a novel inborn error of immunity, but also further define and expand our knowledge of the role of Helios in immune activation and homeostasis. The Helios-dependent epigenetic regulation defect represents a novel molecular mechanism leading up to the severe loss of balance (homeostasis) of immunity as seen in the disease. In summary, this study represents a significant advance in the understanding of the precise role Helios plays in hematopoiesis and immunity and it will help future efforts to potentially target these regulators in both immunodeficiency and malignancy.

PUBLICATION

Shahin, T., Kuehn, H. S., Shoeb, M. R., Gawriyski, L., Giuliani, S., Repiscak, P., Hoeger, B., Yuce Petronczki, O., Bal, S. K., Zoghi, S., Dmytrus, J., Seruggia, D., Castanon, I., Rezaei, N., Varjosalo, M., Halbritter, F., Rosenzweig, S. D., & Boztug, K. (2021). Germline biallelic mutation affecting the transcription factor Helios causes pleiotropic defects of immunity. Sci Immunol, 6(65), eabe3981. https://doi.org/10.1126/sciimmunol.abe3981



(Top left) Whole exome sequencing was performed in a seventeen-year-old male patient and revealed a biallelic germ-line variant in IKZF2 that is ultra rare and has been predicted to be damaging by various prediction algorithms. (Top right) The patient was affected by recurrent bacterial lower respiratory tract infections complicated by bronchiectasis and failure to thrive and responded well to intravenous immunoglobulin substitution. Flowcytometry of patient PBMCs revealed hypogammgaglobulinemia, low B-cell counts and a maturation defect of CD8+ T-cells. Furthermore propensities of Treg, NK- and MAIT cells were reduced in the patient. (On the bottom left) Functional experiments revealed a reduction of CD8+ T-cell proliferation upon stimulation with anti CD3/CD28 dynabeads, which was likely due to significantly impaired IL-2 production of CD8+ T-cells. Patient PBMCs demonstrated reduced accessability of the IL-2 promoter locus in an ATAC sequencing assay, explaining the diminished IL2 production in patient cells. (On the bottom right) Bio-ID proximity labelling studies of HEK293 cells expressing the patient HELIOS variant showed loss of affinity to 38/170 interaction partners.

Shahin et al., Sci Immunol. 2021
Graphics:Tala Shahin, Daniel Mayr

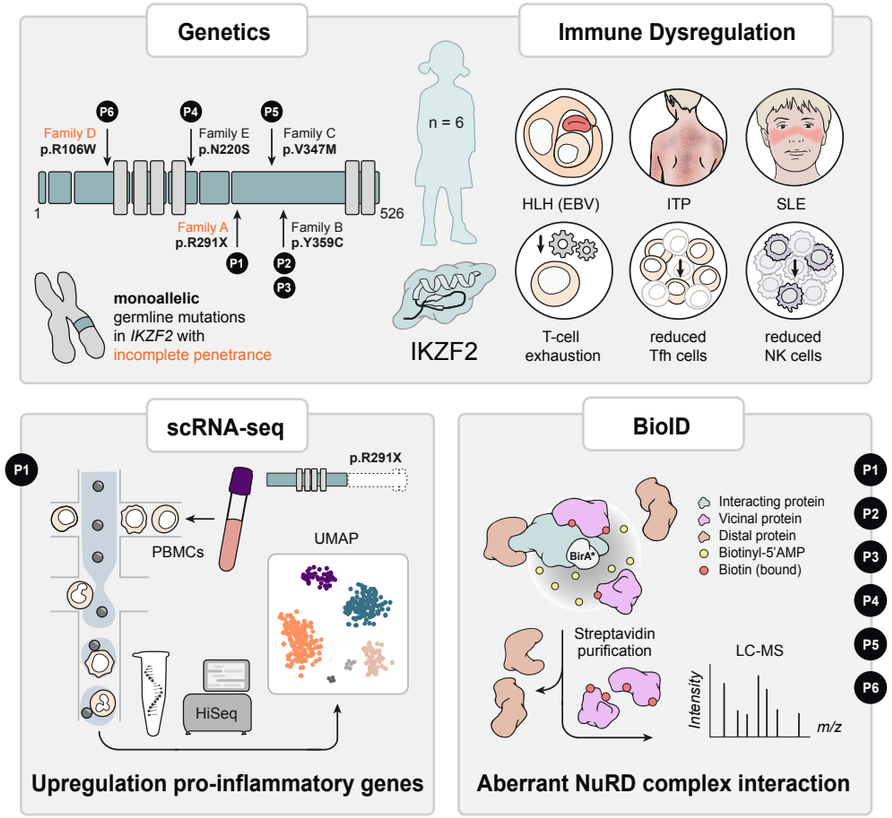
THE GOD OF THE SUN AT THE FOREFRONT OF IMMUNODEFICIENCY RESEARCH

In a parallel project to the discovery of mutations affecting both copies of the *IKZF2* gene (so-termed biallelic mutations), the scientists link heterozygous (monoallelic) mutations in the transcription factor Helios to a novel disease characterized by immunodeficiency and immune dysregulation.

Together with their colleagues, shared first authors Tala Shahin and Daniel Mayr from St. Anna CCRI identified a total of five different monoallelic mutations in six patients with immune dysregulation. All six patients presented with compromised immune systems, suffering from systemic inflammations (hemophagocytic lymphohistiocytosis, HLH), excessive bruising and bleeding (immune thrombocytopenia, ITP) or widespread tissue inflammation (systemic lupus erythematosus, SLE).

Using state-of-the-art biochemical approaches, the researchers uncovered the underlying mechanism by which Helios causes immune dysregulation in these patients. They found that, like other transcription factors, Helios also associates with many other proteins and creates a protein network essential for transcriptional regulation. Among these interacting proteins were members of the NuRD complex (nucleosome remodeling deacetylase complex), which is a group of epigenetic factors that play a critical role in transcriptional regulation. The researchers found that a common feature for all the patients analyzed in this work was the dysregulation in the ability of Helios to interact with the NuRD complex, resulting, as the authors had proposed, in an aberrant transcription of target genes.

These findings are crucial for the understanding of the spectrum of clinical manifestations that are associated with mutations in Helios. Furthermore, future discovery of additional patients with mutations in Helios will enable even more detailed understanding of the roles of Helios in immunity and homeostasis. This, in turn, could lay the groundwork for the development of targeted treatments for affected patients with Helios mutations.



Mutations in Helios were linked to immunodeficiency and immune dysregulation through state-of-the-art biomechanical technologies.

PUBLICATION

Shahin, T.*, Mayr, D.*, Shoeb, M. R., Kuehn, H. S., Hoeger, B., Giuliani, S., Gawriyski, L., Yuce Petronczki, O., Hadjadj, J., Kostel Bal, S., Zoghi, S., Haimel, M., Jimenez-Heredia, R., Boutboul, D., Triebwasser, M. P., Rialland-Battisti, F., Costedoat-Chalumeau, N., Quartier, P., Tangye, S. G., Fleisher, T. A., Rezaei, N., Romberg, N., Latour, S., Varjosalo, M., Halbritter, F., Rieux-Laucat, F., Castanon, I., Rosenzweig, S. D.#, Boztug, K.# (2021). Identification of Germline Monoallelic Mutations in *IKZF2* in Patients with Immune Dysregulation. Blood Adv. https://doi.org/10.1182/bloodadvances.2021006367

* Shared first authorship
# Shared senior authorship

MUTATIONS IN *SYK* CAUSE SEVERE IMMUNE DISORDER WITH CANCER PREDISPOSITION

This publication deciphers the underlying mechanism of a previously unknown immune disorder that causes severe immune dysfunction involving the intestine, skin, and nervous tissues. The international research team could demonstrate that this disorder is caused by mutations affecting the signaling molecule termed spleen tyrosine kinase (SYK). The newly discovered mutations in the corresponding gene lead to a permanent activation of SYK – resulting in aberrant immune responses, severe inflammation and a susceptibility for lymphoma. By elucidating the mechanism of this disease, it is possible that affected patients may be offered targeted ("personalized") treatment options in the future.

Scientists at St. Anna CCRI and the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD), together with an international research team, have succeeded in identifying a previously unknown genetic cause of severe multi-organ inflammation. Genetic analysis revealed mutations in the gene encoding spleen tyrosine kinase (SYK) – a protein instrumental in signal transduction for activation of the immune system. The patients identified in this newly published study showed a similar clinical picture with recurrent inflammation, impaired immune regulation, and a clear predisposition to the development of lymphomas. Lymphomas represent a type of cancer that originates from infection-fighting cells of the immune system, called lymphocytes.

TENDENCY TO INFECTION AND IMMUNE DEFENSE DIRECTED AGAINST THE OWN BODY

This research paper shows for the first time that a specific type of mutation in the *SYK* gene which leads to continuous activation of the corresponding protein (so-termed gain-of-function mutation) is the cause of this severe immune disorder. SYK is a key molecule in immune regulation and thus a potential target for therapies. Its mutation-induced hyperactivity leads to an impaired immune response, and both increased infections and attacks against endogenous structures by the immune system occur. In the individuals described in the study, SYK over-activation resulted in immune defects and inflammatory responses of varying severity in the intestine, skin, joints, liver, and nervous system.

TAILORED PREVENTION AND TREATMENT FOR *SYK* MUTATION?

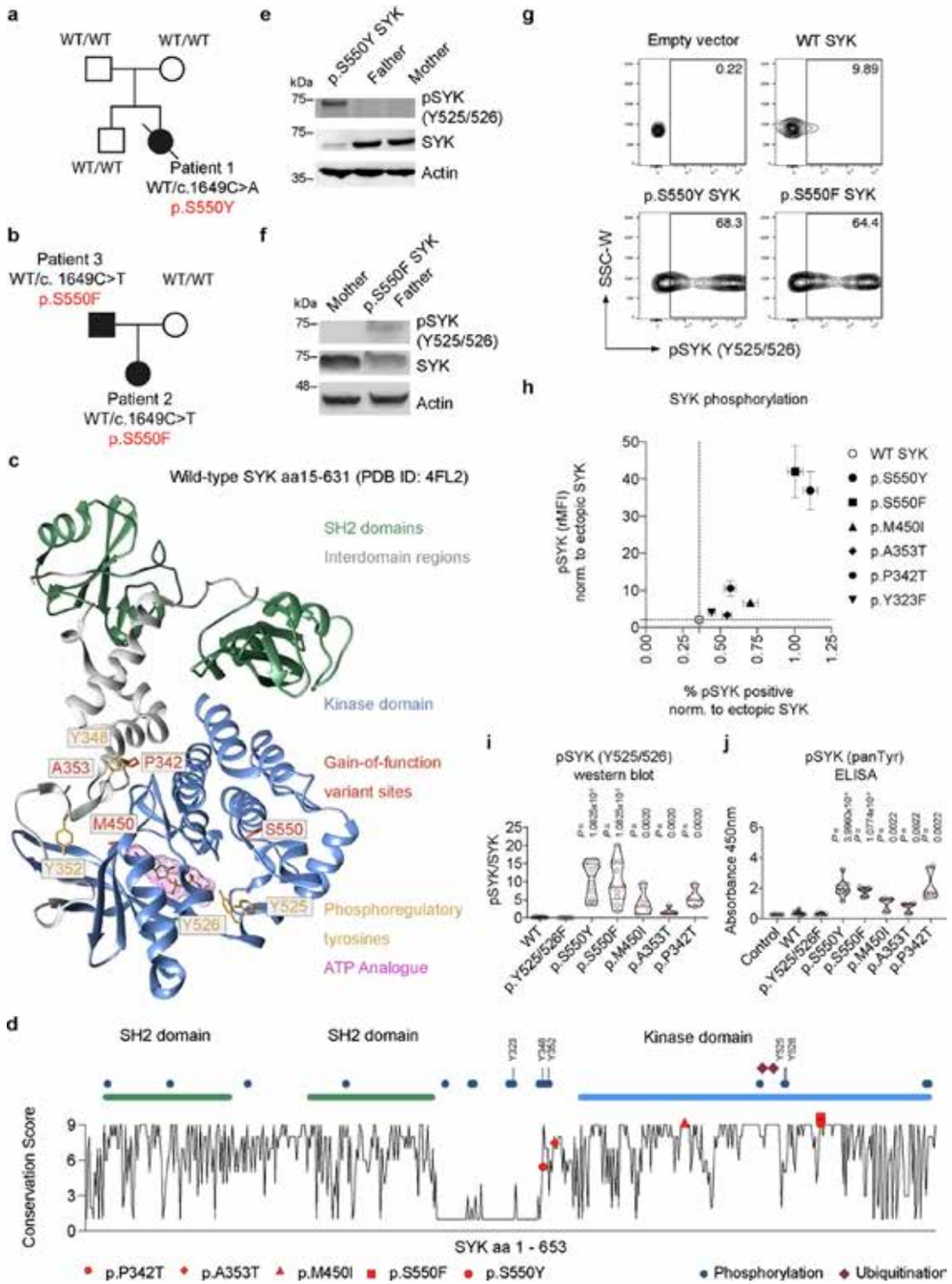
Patients with *SYK* gain-of-function mutations are also at increased risk for blood cancers and therefore require regular check-ups. The six patients analyzed here are located in different regions around the world but had similar symptoms. Therefore, this study provides again an example of how fundamental knowledge can be gained in rare diseases through a global collaboration and the scientists are particularly excited about the very productive partnership between their institutions all around the world. The resulting new insight about the same cause of disease facilitated a productive exchange of experience between the treating centers. Given the cancer predisposition identified in this disease, patients will now be examined regularly as part of an appropriate screening program.

Although so far only six individuals are known to have a molecular disorder in the *SYK* gene, a potential targeted treatment with a SYK inhibitor would already be available to them, as this drug has already been approved for other diseases. Within the study presented here, no individual has yet been treated with a SYK inhibitor. However, future studies will investigate whether such an approach can be considered as a targeted ("personalized") treatment option, particularly for severe forms of the disease that are not well controlled with other treatment options.

PUBLICATION

Wang, L .*, Aschenbrenner, D.*, Zeng, Z.*, Cao, X., Mayr, D., Mehta, M., Capitani, M., Warner, N., Pan, J., Wang, L., Li, Q., Zuo, T., Cohen-Kedar, S., Lu, J., Ardy, R. C., Mulder, D. J., Dissanayake, D., Peng, K., Huang, Z., Li, X., Wang, Y., Wang, X., Li, S., Bullers, S., Gammage, A. N., Warnatz, K., Schiefer, A. I., Krivan, G., Goda, V., Kahr, W. H. A., Lemaire, M.; Genomics England Research Consortium, Lu, C. Y., Siddiqui, I., Surette, M.G., Kotlarz, D., Engelhardt, K. R., Griffin, H. R., Rottapel, R., Decaluwe, H., Laxer, R. M., Proietti, M., Hambleton, S., Elcombe, S., Guo, C. H., Grimbacher, B., Dotan, I., Ng, S. C., Freeman, S. A., Snapper, S. B., Klein, C., Boztug, K.#, Huang, Y.#, Li, D.#, Uhlig, H.H.#, & Muise, A.M.# (2021). Gain-of-function variants in *SYK* cause immune dysregulation and systemic inflammation in humans and mice. Nat Genet, 53(4), 500-510. https://doi.org/10.1038/s41588-021-00803-4

* Shared first authorship
\# Shared senior authorship



Functional characterization of SYK variants.
(a and b) Pedigree and *SYK* variant in Patients 1 to 3. (c) Structural model of wild-type human SYK (PDB ID: 4FL2), highlighting the sites of SYK gain-of-function variants (p.P342T, p.A353T, p.M450I, p.S550F and p.S550Y), the ATP binding pocket (occupied by an ATP analog), and key phosphoregulatory tyrosine residues that determine SYK activity. (d) Graphical illustration of SYK sequence conservation (black line) based on ConSurf conservation score (see Methods). Domain structure, phosphorylation sites (blue dots), ubiquitination sites (dark red squares) and positions of identified patient variants (colored triangles) are indicated. (e and f) Total and phosphorylated SYK expression in PBMC from family 1 and 2 by western blot (representative blots are shown of 3 independent repeats). (g) Contour plot presentation of pSYK (Y525/526) expression in HEK293 cells transfected with an empty vector, wild-type SYK or mutated p.S550Y or p.S550F SYK assessed by intracellular staining and flow cytometry. (h) SYK phosphorylation in wild-type SYK or mutated SYK assessed by intracellular staining and flow cytometry. Data are shown as normalized relative mean fluorescence intensity (rMFI) of pSYK (Y525/526) to ectopically expressed SYK (y-axis) and normalized % of pSYK positive cells (x-axis) to ectopically expressed SYK; (mean±SD; n of independent experiments/n of cell culture replicates: eV(8/30), WT(8/24), p.S550Y(8/28), p.S550F(6/21), p.M450I(8/28), p.A353T(8/30), p.P342T(8/30),p.Y323F(3/12)). (i) Quantification of western blot analysis of total and phosphorylated levels of SYK (Y525/526) in HEK293 cells according to Extended Data Fig. 4g. (j) SYK phosphorylation (panTyr) in HEK293 cells expressing an empty vector (Control), wild-type SYK (WT) or the diverse range of SYK variants identified in patients as measured by ELISA. i and j: quartiles and median; n of independent experiments: WT(10), p.Y525/526F(10) p.S550Y(10), p.S550F(10), p.M450I(4), p.A353T(4), p.P342T(4). Mann-Whitney test.

Wang et al., Nat. Genet. 2021

LEUKEMIAS & LYMPHOMAS, MOLECULAR MICROBIOLOGY

MICHAEL DWORZAK

Group: Immunological Diagnostics

Michael Dworzak obtained his MD from the University of Vienna and joined St. Anna CCRI in 1993 as a clinician scientist, combining research and its adaption to clinical work. He is vice chair of the St. Anna Kinderspital in Vienna, Section Head of pediatric Oncology & Hematology, and head of the Immunological Diagnostics laboratory at Labdia Labordiagnostik GmbH and CCRI, its focus lying on the development of new diagnostic methods for children and adolescents with leukemia and lymphomas using flow cytometry immunophenotyping. Michael Dworzak coordinates several international FLOW study groups and networks (iBFM-FLOW, EuPAL-FLOW). His major achievements include the establishment, clinical validation, and international dissemination of an innovative landmark-technology for response assessment in pediatric leukemias based on flow-cytometric minimal residual detection (FLOW-MRD). This eventually led to the integration of FLOW-MRD into clinical treatment protocols, which are applied by an intercontinental consortium for stratification of pediatric patients into relapse-risk-based treatment strata worldwide.

“My work is dedicated to the goal of curing every child with acute myeloid leukemia – by means of high-end diagnostics that allows for individualized therapy!”

MICHAEL DWORZAK GROUP

Group: Immunological Diagnostics

PRINCIPAL INVESTIGATOR
Michael Dworzak

STAFF SCIENTIST
Margarita Maurer-Granofszky

PROJECT TEAM MEMBER
Michael Reiter

PROJECT TEAM MEMBER (EXTERNAL, TU WIEN)
Matthias Gerold Wödlinger

DIPLOMA STUDENT
Manfred Visagie (until 2021)

TECHNICIANS
Claudia Mitteregger
Angela Schumich
Susanne Suhendra-Chen
Alice Bramböck

THOMAS LION

Group: Molecular Microbiology

Univ.-Prof. Thomas Lion obtained his medical degree from the University of Vienna, a PhD in Genetics from the University of Prague, and a MSc in Social Sciences from the University of Vienna. He is a professor at the Medical University of Vienna and head of the Division for Molecular Microbiology at the St. Anna CCRI. His research focuses on the complexity of viral, fungal and bacterial infections in immunocompromised patients as well as on the subclonal architecture of Ph-positive leukemias. He serves as chairman of the Scientific Board of the Austrian Society of Pediatrics, and, being a certified specialist in pediatric hemato-oncology and medical and chemical laboratory diagnostics, as the Medical Director and Managing Director of Labdia Labor-diagnostik, a center developing new diagnostic tools and offering molecular diagnostics in the fields of microbiology and leukemia. He has published about 200 papers in peer-reviewed journals and a number of book chapters, and served as editor of scientific textbooks. He has been section editor and editorial board member of various journals. He has received 20 national and international research awards and filed different patents on molecular diagnostic techniques.

“I strive to generate new knowledge that will benefit patients with cancer.”

THOMAS LION GROUP

Group: Molecular Microbiology

PRINCIPAL INVESTIGATOR
Thomas Lion

POSTDOCTORAL FELLOWS
Maria Filomena De Almeida Nogueira (until 2021)
Klara Obrova
Petra Pusic

PHD STUDENT
Triin Laos (until 2021)

BACHELOR STUDENT
Paola Fürhacker

INTERN
Asma Bouazizi

TECHNICIANS
Isabella Sponseiler
Michaela Fortschegger
Sandra Preuner

DAVIDE SERUGGIA

Group: Pediatric Leukemia Biology

Davide Seruggia obtained a degree in Biotechnology at the University of Milano-Bicocca (Italy) in 2010, and a PhD in Molecular Biology at the National Centre for Biotechnology (CNB-CSIC) in Madrid (Spain) in 2014. During his PhD under the supervision of Lluis Montoliu, he focused on non-coding DNA regulatory sequences and generated several mouse lines carrying deletions of selected enhancers. In 2015 he joined the laboratory of Stuart H. Orkin at Boston Children's Hospital and Harvard, where he trained in hematology, stem cell biology and genomics. In Boston, Davide used genomics and genome editing to explore the role of epigenetic factors, chromatin modifiers and transcriptional co-activators in the context of mouse embryonic stem cells, and generated a series of mouse models to study how chromatin modifiers control hematopoiesis, erythropoiesis and the expression of globin genes. In 2019, he was promoted to Instructor in Pediatrics at Harvard Medical School and attracted funding from the WES Foundation and Pedals for Pediatrics to investigate non-coding sequence variation in pediatric leukemia. In 2021 Davide joined the St. Anna CCRI as Principal Investigator and CeMM as Adjunct Principal Investigator, supported by an ERC Starting Grant.

"We explore mechanisms of gene regulation to understand how leukemia develops. It is an intricate, fascinating puzzle with enormous potential to improve the lives of young patients."

DAVIDE SERUGGIA GROUP

Group: Pediatric Leukemia Biology

PRINCIPAL INVESTIGATOR
Davide Seruggia

POSTDOCTORAL FELLOWS
Ana Patricia Kutschat
Maciej Piotr Zaczek

PHD STUDENT
Leonie Gabriela Katharina Lehmayer

MASTER STUDENT
Sandra Wittibschlager

LAB MANAGER
Anzhelika Karjalainen

SABINE STREHL

Group: Genetics of Leukemias

Sabine Strehl obtained her PhD in biology from the University of Vienna in 1987, at that time working on the replication domain organization of plant chromosomes. In 1988 she was one of the first researchers who joined the newly founded St. Anna CCRI. She started to focus on the molecular cytogenetic analysis of both neuroblastoma and leukemia and became an expert in *in situ* hybridization. In 1995 she obtained a fellowship from the Max-Kade foundation and spent three years in the Genetics Division, Children's Hospital and Harvard Medical School in Boston (USA) where she received extensive training in molecular biology. Upon her return to the St. Anna CCRI in 1998, she specialized in pediatric leukemia and in July 2006 was appointed as leader of the *Genetics of Leukemia group*. Since then she has dedicated her research to understanding the pathogenesis and progression of leukemia with a focus on the comprehensive characterization of its genetic features and their biological and functional consequences. It is of particular importance to her to link basic and translational research to foster optimal treatment strategies and to achieve this goal she closely collaborates with the pediatric hemato-oncologists of the St. Anna Children's Hospital.

"Research is full of potholes waiting to trip you but my curiosity in the mysteries of biology and the rewarding glimpses of success keep me going."

SABINE STREHL GROUP

Group: Genetics of Leukemias

PRINCIPAL INVESTIGATOR
Sabine Strehl

BIOINFORMATICS DATA ANALYST
Dagmar Schinnerl

STAFF SCIENTIST
Klaus Fortschegger

POSTDOCTORAL FELLOW
Manel Lladó Santaeularia

MASTER STUDENT
Sophie Charlotte Knoll

TECHNICIAN
Marion Riebler

PUBLICATIONS

QUALITY CONTROL OF FLOW CYTOMETRY FOR THERAPY RESPONSE ASSESSMENT IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Therapy response assessment by monitoring of residual leukemic cells during chemotherapy (minimal residual disease, MRD) by flow cytometry (FCM-MRD) is a powerful prognostic tool for predicting outcomes in acute lymphoblastic leukemia (ALL). To apply this methodology in large, collaborative clinical trials, dedicated laboratory staff must be educated to concordantly high levels of expertise and their performance quality should be continuously monitored. In this publication, we demonstrate that the training of laboratories using experienced training/twinning partners, along with continuous educational feedback significantly improves the performance of laboratories in detecting and quantifying MRD in pediatric ALL patients.

MULTI-STEP QUALITY CONTROL OF THERAPY RESPONSE ASSESSMENT USING FCM-MRD

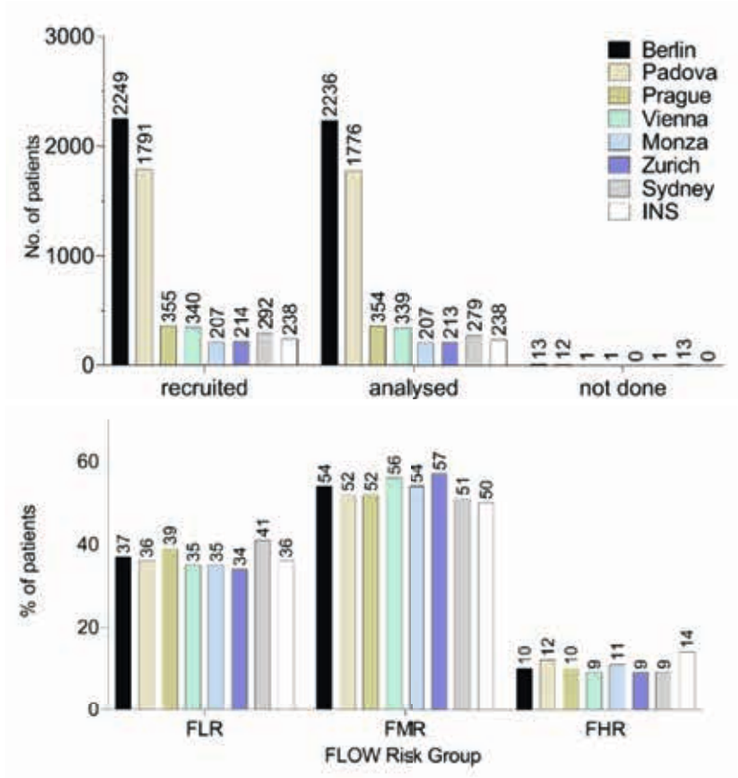
A unique and comprehensive training and quality control (QC) program involving a large number of reference laboratories was installed to complement standardization of the FCM-MRD methodology with an educational component as well as with persistent quality control measures. The QC and quality assurance (QA) program was based on four major cornerstones: (i) a trainee/expert-trainer program, (ii) obligatory participation in external QA programs (sample send around), (iii) regular participation in data file ring trials (data file send arounds), and (iv) surveys of independent data derived from clinical trial results.

TECHNICAL HARMONIZATION AND CONTINUOUS TRAINING OF THE STAFF

While the technical aspects of the methodology can be harmonized well, the human factor, i.e. the interpretation of the electronic files of the acquired sample, is the actual Achilles heel of the methodology and strongly relies on operator skills and expert knowledge. Hence, discrepancies in MRD values between laboratories can be due to wrong interpretation of the data. Therefore, the data file send arounds especially focused on assessment of the analysis performance of each laboratory. They also contained an educational component, where problematic cases were discussed in joint meetings. As part of such meetings, suggestions, and solutions for proper identification of the aberrant cells were provided. External quality control programs, on the other hand also allowed assessment of the performance of the whole methodology including preparation of the sample and acquisition of the data.

IMPROVEMENT OF LABORATORY PERFORMANCE

In summary, the extensive training and quality assurance program contributed significantly to improving and continuously ensuring the performance of individual laboratories. Of note, the structure based on a coordinating center responsible for quality oversight and feed-back proved particularly effective and relevant. Overall, this led to a very high inter-laboratory concordance rate of FCM-MRD assessments and to a high conformity of risk estimates in independent patient cohorts (see figure). This is essential for conducting large ALL treatment trials which use multi-laboratory FCM-MRD assessments for stratification.



High data conformity of well-trained reference laboratories regarding risk stratification based on flow cytometric therapy response assessment (FCM-MRD); FLOW low-risk (FLR), FLOW medium -risk (FMR), FLOW high-risk (FHR).

PUBLICATION

Maurer-Granofszky, M., Schumich, A., Buldini, B., Gaipa, G., Kappelmayer, J., Mejstrikova, E., Karawajew, L., Rossi, J., Suzan, AÇ., Agriello, E., Anastasiou-Grenzelia, T., Barcala, V., Barna, G., Batinić, D., Bourquin, JP., Brüggemann, M., Bukowska-Strakova, K., Burnusuzov, H., Carelli, D., Deniz, G., Dubravčić, K., Feuerstein, T., Gaillard, MI., Galeano, A., Giordano, H., Gonzalez, A., Groeneveld-Krentz, S., Hevessy, Z., Hrusak, O., Iarossi, MB., Jáksó, P., Kloboves Prevodnik, V., Kohlscheen, S., Kreminska, E., Maglia, O., Malusardi, C., Marinov, N., Martin, BM., Möller, C., Nikulshin, S., Palazzi, J., Paterakis, G., Popov, A., Ratei, R., Rodríguez, C., Sajaroff, EO., Sala, S., Samardzija, G., Sartor, M., Scarparo, P., Sędek, Ł., Slavkovic, B., Solari, L., Svec, P., Szczepanski, T., Taparkou, A., Torrebadell, M., Tzanoudaki, M., Varotto, E., Vernitsky, H., Attarbaschi, A., Schrappe, M., Conter, V., Biondi, A., Felice, M., Campbell, M., Kiss, C., Basso, G., Dworzak, MN., On behalf of the I-BFM-Flow-Network. An Extensive Quality Control and Quality Assurance (QC/QA) Program Significantly Improves Inter-Laboratory Concordance Rates of Flow-Cytometric Minimal Residual Disease Assessment in Acute Lymphoblastic Leukemia: An I-BFM-FLOW-Network Report. Cancers (Basel). 2021 Dec 6; 13(23):6148. doi: 10.3390/cancers13236148. PMID: 34885257; PMCID: PMC8656726.

INVASIVE VIRUS INFECTIONS DURING CHEMOTHERAPY: AN UNDERESTIMATED FACTOR

While the importance of virus infections as a leading cause of morbidity and mortality is well documented in patients undergoing allogeneic stem cell transplantation, their impact in cancer patients receiving chemotherapy is less well studied. This publication reveals that invasive viral infections can be clinically relevant in this setting both in pediatric and adult patients. Routine screening for viremia at the onset of neutropenic fever is therefore warranted and could prevent unnecessary use of antibacterial or antifungal agents.

Cancer chemotherapy can induce neutropenia, defined by pronounced reduction of specific white blood cells called "neutrophilic granulocytes". In this weakened state of the immune system, patients are more susceptible to infections and commonly develop fever referred to as febrile neutropenia. Owing to the clear predominance of bacterial and fungal infections in this setting, screening for systemic viral infections is rarely part of current routine diagnostic surveillance. According to this study led by scientists from the St. Anna Children's Cancer Research Institute, invasive viral infections are an underestimated cause of febrile complications in cancer patients receiving chemotherapy. Routine screening for viremia could therefore prevent overtreatment with antibiotic or antifungal agents in some instances.

DORMANT VIRUSES CAN BE RE-ACTIVATED

Highly immunosuppressive treatment approaches favor opportunistic infections, in which viruses can take advantage of the weakened immune system. This includes virus reactivation, a process by which latent viruses persisting from earlier infections switch back into an active replication mode. Persistent viral infections that are commonly reactivated during states of impaired immune function include various members of the herpes virus family (HHV), for example Epstein-Barr virus (EBV) or cytomegalovirus (CMV), and different other viral pathogens, such as human adenoviruses (HAdV).

INVASIVE VIRAL INFECTIONS CAN PLAY A ROLE DURING CANCER CHEMOTHERAPY

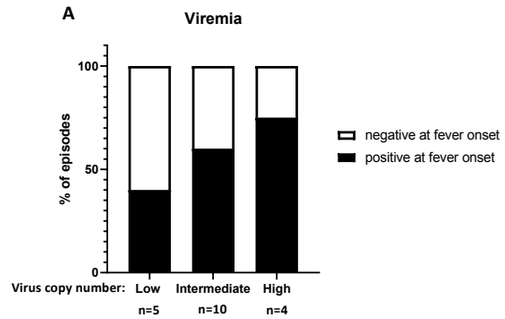
To shed more light on the incidence and potential role of viral infection or reactivation, the authors of this multinational study have prospectively screened 237 febrile neutropenic episodes in pediatric and adult patients undergoing intensive chemotherapy, primarily for treatment of acute leukemia, and tested for the presence and quantity of select clinically relevant viruses.

Viremia was documented in 35 cases, including 27 pediatric (18%), and eight adult (9%) patients, representing 15% of all febrile neutropenic episodes studied. In adult patients, EBV was detected most frequently, while in pediatric patients HHV6 (human herpes virus 6) showed the highest prevalence, followed by EBV and CMV. The detectability of viremia already at first onset of fever correlated with the virus concentration, and fever persisting for more than 24 hours correlated with increasing virus copy numbers (see figure). Most importantly, high virus copy numbers exceeding 10^4 copies/ml blood were generally observed in absence of detectable bacterial or fungal pathogens, indicating that the virus identified was a probable cause of the febrile episode, and the patients affected may therefore not have required antibacterial or antifungal therapy in these instances.

PREVENTION OF OVERTREATMENT WITH INAPPROPRIATE MEDICATION

While low or intermediate levels of viremia were commonly associated with bacterial or fungal co-infection, viremia at higher levels was documented in patients without evidence for other infections. These findings highlight the role of viruses as potentially important and currently underdiagnosed pathogens in patients with malignant neoplasms undergoing chemotherapy. Patients with febrile neutropenia not responding to antibiotic treatment for 72–96 hours commonly receive systemic antimycotic therapy without any evidence for the presence of fungal infection. This empirical treatment strategy can be associated with considerable toxicity and costs. It is therefore desirable to reduce or prevent overtreatment by antifungal agents.

In summary, the study indicates that viral infection or reactivation are probably responsible for a proportion of febrile episodes in the clinical setting studied. Screening for viral infections might therefore be warranted to elucidate their potential role in patients undergoing intensive chemotherapy for malignant disorders. Detection of high-level viremia in the absence of evidence for other microbial infections might serve as a basis for more judicious administration of antimicrobial treatment.



Correlation of virus copy numbers with viremia at onset and during the febrile episode.

Obrova et al., Am J Hematol 2021

PUBLICATION

Obrova, K., Grumaz, S., Remely, M., Czurda, S., Krickl, I., Herndlhofer, S., Gleixner, K. V., Sperr, W. R., Grosslinger, L., Frank, T., Andrade, N., Egger-Matiqi, T., Peters, C., Engstler, G., Dworzak, M., Attarbaschi, A., van Grotel, M., van den Heuvel-Eibrink, M. M., Moiseev, I. S., Rogacheva, Y., Zubarovskaya, L., Zubarovskaya, N., Pichler, H., Lawitschka, A., Koller, E., Keil, F., Valent, P., Sohn, K., Lion, T. (2021). Presence of viremia during febrile neutropenic episodes in patients undergoing chemotherapy for malignant neoplasms. Am J Hematol. https://doi.org/10.1002/ajh.26177

NOVEL BIOINFORMATIC ALGORITHM FOR OPTIMIZED DETECTION OF MUTATIONS IN CANCER GENES

Mutations in critical genes can act as cancer drivers and can alter the efficacy of anticancer drugs targeting these genes. It is essential therefore to identify these point mutations and to monitor the number of cells carrying them for the employment of alternative treatment strategies. Currently available next generation sequencing (NGS) technologies are not economic for small patient numbers or have limitations in the assignment of multiple mutations to the same cell. We have therefore designed a novel bioinformatic approach to NGS data analysis on the Oxford Nanopore platform that facilitates sensitive detection and monitoring of (point) mutations by eliminating the current error rate and that permits inexpensive analyses even in individual patients.

In different types of malignant diseases, mutations in critical genes can act as drivers of the neoplastic process and can alter the efficacy of anticancer drugs targeting these genes. The ABL1 gene, whose uncontrolled activity is a hallmark of chronic myeloid leukemia (CML) and Ph-positive acute lymphoblastic leukemia (Ph+ALL), can be effectively suppressed by specifically designed small molecules referred to as tyrosine kinase inhibitors (TKIs). However, minimal mutations (point mutations) at critical sites of the ABL1 gene can hamper or even eliminate the efficacy of TKIs, thus setting the stage for life-threatening disease progression. It is essential therefore to identify these point mutations to permit the employment of alternative treatment strategies. Moreover, monitoring of the number of cells carrying these mutations is important for assessing the efficacy of the ongoing treatment.

ALTERNATIVE APPROACHES NEEDED TO OVERCOME LIMITATIONS OF CURRENT TECHNOLOGIES

Next generation sequencing (NGS) technologies are commonly used to detect and monitor cells carrying point mutations that mediate resistance to TKIs, but currently available technologies can only be employed at acceptable costs if multiple patient samples can be pooled and analyzed simultaneously. This is an important issue particularly in the pediatric setting, where small patient numbers often preclude economic employment of this diagnostic screening. Moreover, the most commonly used NGS platform (Illumina) has serious limitations in the assignment of multiple mutations to the same cell (same ABL1 molecule), which is important for reliable identification of the most resistant constellations of point mutations. In view of these shortcomings, alternative technical approaches were urgently needed.

NEW BIOINFORMATICS TOOL FOR RELIABLE DETECTION OF POINT MUTATIONS

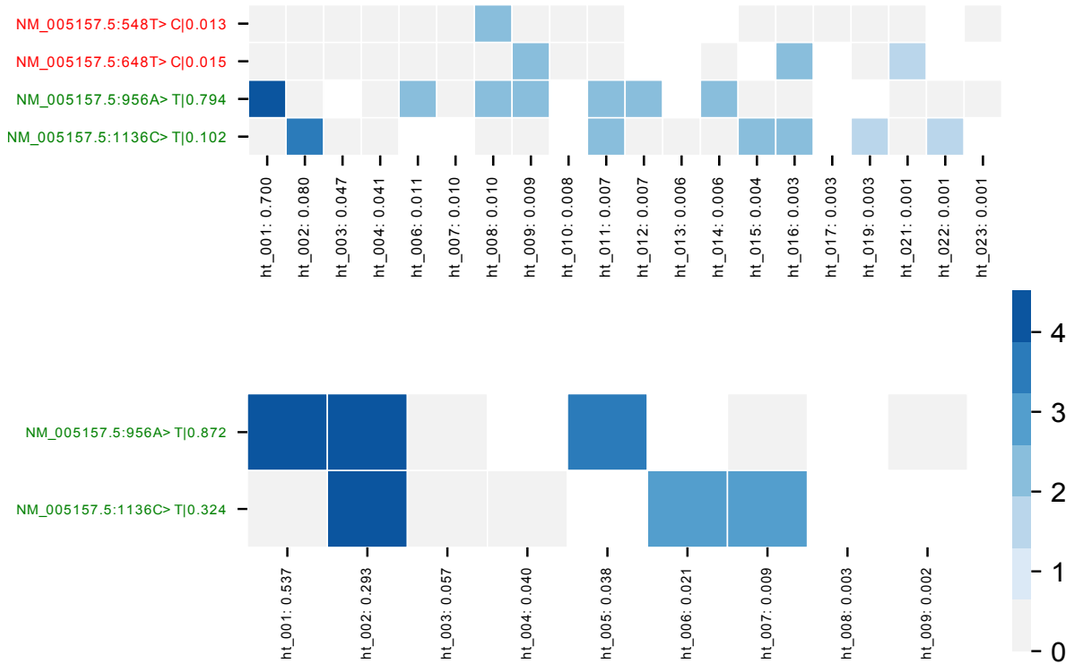
The Oxford Nanopore NGS technology offers a very attractive alternative due to its low costs enabling economic sequence analysis even of individual patient samples and due to very long reads facilitating easy assignment of mutations to the same or to different ABL1 molecules. However, clinical application of this technology was hampered by the intrinsically high error rate, which prevented reliable detection of point mutations at the clinically desirable level of 1–3%. Preliminary insights indicated that the technical problem is mainly attributable to the lack of adequate bioinformatic algorithms for analysis of the sequencing data. Our research team has therefore designed a completely novel bioinformatics approach to NGS data analysis on the Oxford Nanopore platform that eliminates the high error rate and facilitates detection of point mutations at the desired level of sensitivity. More specifically, Nanopanel2 has been developed, a variant caller for Nanopore panel sequencing data (see figure). Nanopanel2 works directly on base-called FAST5 files, uses allele probability distributions and several other filters to robustly separate true from false positive (FP) calls. It effectively calls SNVs and INDELs with variant allele frequencies as low as 1% and 5% respectively and produces only few low-frequency false-positive calls (~1 FP call with VAF¡5% per kb amplicon). Haplotype compositions are then determined by direct phasing. Nanopanel2 is the first somatic variant caller for Nanopore data, enabling accurate, fast (turnaround <48h) and economic (sequencing costs ~100$/sample) diagnostic workflows.

IMPACT FOR PATIENT OUTCOME BY DIAGNOSTIC IMPLEMENTATION OF NANOPANEL2

The researchers are currently in the process of further optimizing this bioinformatics approach exploiting the Oxford Nanopore NGS platform for clinical application, using mutations in the ABL1 gene as a model. They expect to implement this technology in the diagnostic setting in the foreseeable future. Exploitation of this approach in patients with CML and Ph+ALL will greatly improve and economize molecular diagnostics in these malignant disorders and will expectedly provide a basis for personalized treatment strategies leading to better outcome in these patients. Moreover, this diagnostic platform should be readily adaptable to the detection of mutations in other genes and malignant disorders, thus greatly extending its clinical applicability for the benefit of our patients.

PUBLICATION

Popitsch, N., Preuner, S., & Lion, T. (2021). Nanopanel2 calls phased low-frequency variants in Nanopore panel sequencing data. Bioinformatics. https://doi.org/10.1093/bioinformatics/btab526



Exemplary haplotype maps as plotted bynp2 for two benchmark samples containing the same mutations (Y axis, green labels. Red labels denote low-frequency false positive calls; raw VAF as determined by np2 after the pipe symbol). The X axes list found haplotypes sorted by their relative fraction (number after the colon). Blue and grey squares denote present/absent variations, white squares indicate that the respective reads did not span the respective positions or had a deletion at this position. Colour intensity corresponds to log(#reads). In the upper sample, both mutations were introduced using different plasmids. Targeted VAFs were 80% E255V (ht_001), 10% T315I (ht_002) and 10% wt (ht_003+4) respectively. The lower sample contained a mix of 70% single E255V (ht_001), 20% compound E255V+T315I (ht_002) and 10% wt(ht_003+4). The haplotype maps confirm the respective linkage status of the benchmark variants although we find a low-frequency ht in the upper map carrying both mutations (ht_011; freq=0.7%), possibly resulting from sequencing errors.

Popitsch et al., Bioinformatics 2021

MODELING LEUKEMIA DEVELOPMENT IN A DISH

Model systems are invaluable to improve our understanding of cancer development and to ultimately find better treatment options for patients suffering from leukemia. The *Genetics of Leukemia* team at the St. Anna Children's Cancer Research Institute takes advantage of a powerful combination of two novel biological tools to study leukemogenesis.

NEW TOOLS ENABLE IMPROVED DISEASE MODELING

It remains a challenging task to decipher the temporal course of cancer development. Patients diagnosed with cancer already present with a full-blown disease, but researchers aim to go back in time to investigate earlier, still covert stages. In addition, tumorigenesis is frequently studied in mice, but such models do not account for potential differences to humans and on top of that pose ethical issues regarding animal welfare.

The combination of two recent major scientific breakthroughs, both awarded with the Nobel Prize in 2012 and 2020, enables to model cancer development in an innovative and elegant way. First, somatic adult tissue can be reprogrammed to so-called human induced pluripotent stem cells (hiPSCs) resembling an early embryonic stage, as if turning back the developmental clock. Such hiPSCs can again be *in vitro* differentiated into diverse specialized cell types mimicking tissue development. Second, the molecular scissors, CRISPR-Cas9, permit the precise editing of the cellular genetic material facilitating the introduction or even the correction of disease-causing mutations.

The *Genetics of Leukemia* team took advantage of these tools and combined them in an innovative approach, which facilitates the establishment of *in vitro* disease models and the investigation of human cells recapitulating the stepwise processes of normal and cancer development. Specifically, they generated hiPSCs harboring a rare B-cell acute lymphoblastic leukemia associated genetic alteration, namely *RUNX1::JAK2*. Subsequently, they differentiated the genetically modified hiPSCs into blood progenitor cells and during this process expressed the leukemic fusion protein. This approach allowed the team to investigate how the oncogenic chimeric protein influences cell development over time.

PRECISELY GENETICALLY ENGINEERED STEM CELLS

Activation of oncogenes in mammalian cell models is crucial for cancer research and usually achieved by transfection with vectors such as viruses. In this experimental set-up, unfortunately, the oncogene may, on the one hand, randomly integrate at unknown sites into the genome causing unintentional damage to one or the other gene. On the other hand, it may lead to oncogene expression levels, which significantly differ from those in the actual human cancer. Hence, the Genetics of Leukemia team utilized hiPSCs from a healthy donor and targeted the oncogenic mutation precisely to the genomic site where the lesion also occurs in the human disease. Since they aimed to avoid any collateral damage potentially elicited by the CRISPR-Cas9 scissors, an improved genome editing strategy was used which cuts only one but not both strands of the DNA double helix at exactly the targeted site.

Consequently, their newly generated stem cell lines turned out to harbor, as intended, the oncogenic *RUNX1::JAK2* fusion but showed no other undesirable genetic alterations.

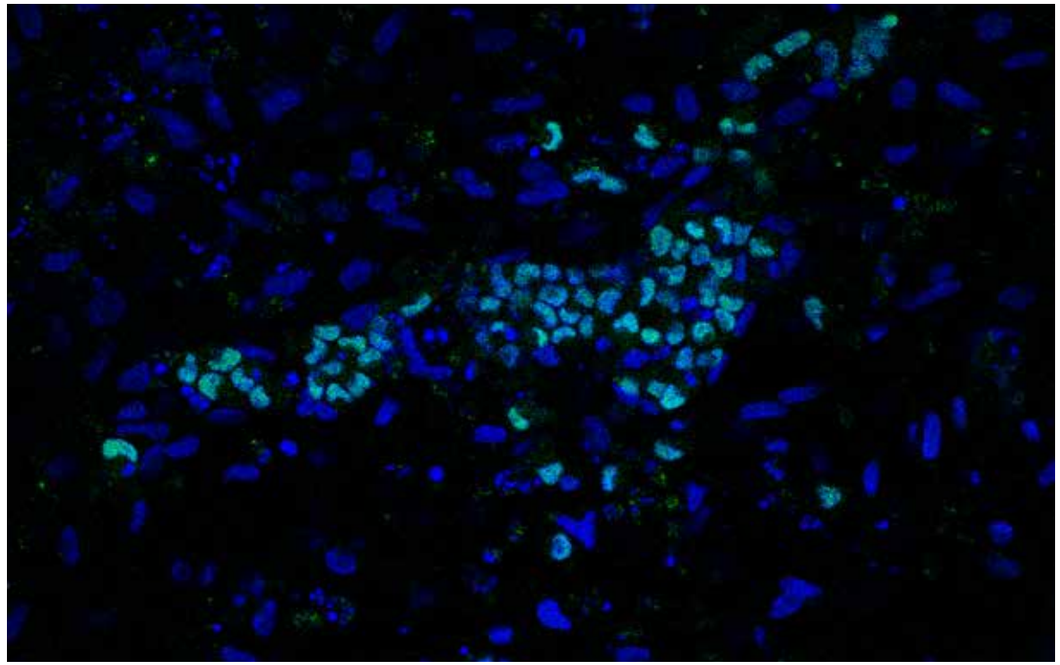
THE ONCOGENE CAUSES SUBTLE CHANGES IN HEMATOPOIESIS

Next, the team *in vitro* differentiated the genetically modified hiPSC lines toward blood progenitor cells and compared them to matched normal cells. Intriguingly, they obtained less hematopoietic cells from the RUNX1::JAK2 expressing hiPSCs, supposedly because the insertion into one *RUNX1* allele reduced the expression level of normalRUNX1, which is known to be essential for blood cell development. However, this constellation accurately accounts for the loss of one copy of wild-type *RUNX1* in the human leukemia.

When they analyzed the gene activity profile by RNA-sequencing they noticed that the JAK/STAT signaling pathway was induced, which has already been described for similar fusion on coproteins. This pathway plays an important role in cell growth, differentiation and survival. Unexpectedly, another cellular mechanism related to cancer was highly active, namely the MYC pathway. Consequently, the scientists hypothesized that cooperation of these two pathways may play a crucial role in RUNX1::JAK2-driven leukemia development.

Rather surprisingly, although RUNX1::JAK2 activated these two prominent cancer-related pathways, the altered cells did not show typical hallmarks of cancer such as increased growth potential or blocked development. This further suggested that onegenetic alteration alone might be insufficient to cause such profound phenotypic changes in the model system being used. However, the research team observed at least a minor bias in the composition of cell types derived from the genetically engineered as compared to normal hiPSCs: a lower number of macrophages and granulocytes (white blood cells of the innate immune system), but an increase in erythrocytes (oxygen-transporting red blood cells). Although the overall changes elicited by the RUNX1::JAK2 oncoprotein were only subtle, the results nevertheless clearly demonstrate that this approach to disease modeling in a dish is feasible.

In the meantime, the *Genetics of Leukemia* team has applied the described approach to other leukemia-driving oncogenes some of which display substantial effects on blood progenitor cell development, consistent with key events taking place during human leukemogenesis.



RUNX1::JAK2 expressing blood progenitor cells derived from genetically engineered human induced pluripotent stem cells. The RUNX1::JAK2 fusion protein (green) localizes to the cell nucleus stained with a blue dye.

Fortschegger et al., Int J Mol Sci 2021

PUBLICATION

Fortschegger, K., Husa, A. M., Schinnerl, D., Nebral, K., & Strehl, S. (2021). Expression of *RUNX1-JAK2* in human induced pluripotent stem cell-derived hematopoietic cells activates the JAK-STAT and MYC pathways. Int J Mol Sci, 22(14), 7576. https://doi.org/10.3390/ijms22147576

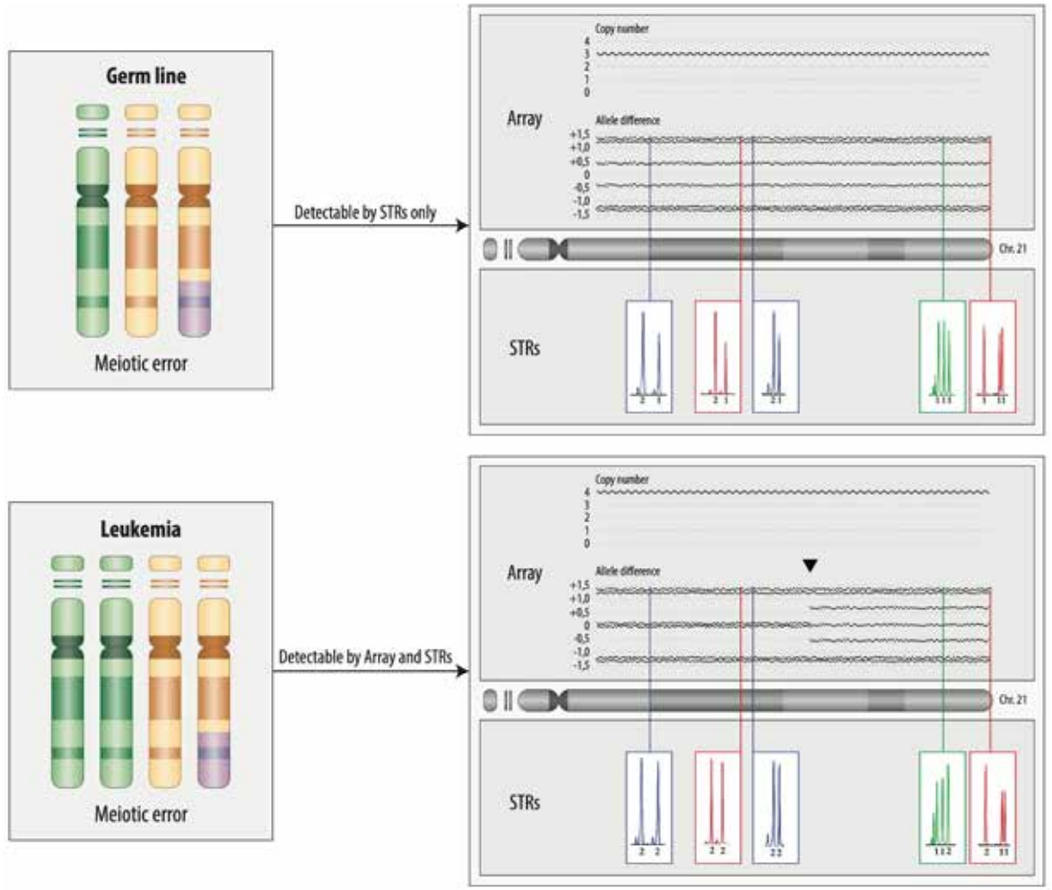
HIGHLIGHTING THE RELEVANCE OF CHROMOSOME 21 FOR CHILDHOOD LYMPHOBLASTIC LEUKEMIA

Chromosome 21 is the most affected chromosome in childhood acute lymphoblastic leukemia. Many of its numerical and structural abnormalities define diagnostically and clinically important subgroups. In this publication, investigators from St. Anna CCRI, St. Anna Children's Hospital and Labdia Labordiagnostik GmbH have provided a comprehensive overview of all these types as well as their respective subtype-specific incidence and distribution.

The results of this study are based on 578 cases that were analyzed using DNA arrays, the currently most advanced and preferred method for the precise simultaneous genome-wide assessment of leukemia-associated copy number alterations with an extraordinary high resolution.

By doing so, they uncovered some unusual and difficult to explain discrepancies between the number of extra chromosome copies and their anticipated parent-of-origin-specific sequence patterns. Such phenomena only become apparent in leukemias with four or even five copies of chromosomes 21 that develop in Down syndrome individuals with an inborn trisomy 21.

A more in-depth analysis of such intriguing cases may eventually provide important clues about the mechanisms that cause the maldistribution of chromosomes and the way in which certain gene regions may contribute to the transformation process and increase the likelihood for developing such types of leukemias.



Array analysis uncovers a meiotic recombination site in the tetrasomic leukemia cells of a patient with constitutional trisomy 21.

Abbasi M. R. *et al.*, Cancers (Basel) 2021

PUBLICATION

Abbasi, M. R. *, Nebral, K. *, Haslinger, S., Inthal, A., Zeitlhofer, P., Konig, M., Schinnerl, D., Kohrer, S., Strehl, S., Panzer-Grumayer, R., Mann, G., Attarbaschi, A., & Haas, O. A. (2021). Copy Number Changes and Allele Distribution Patterns of Chromosome 21 in B Cell Precursor Acute Lymphoblastic Leukemia. Cancers (Basel), 13(18). https://doi.org/10.3390/cancers13184597

* Shared first authorship

COMPUTATIONAL BIOLOGY, MODELS & CORE FACILITIES

MARTIN DISTEL

Group: Innovative Cancer Models

Martin Distel studied Molecular Biotechnology in Munich, Germany and Lund, Sweden before entering a PhD program at the Helmholtz Center in Munich under the supervision of Reinhard Köster. His main focus there was on the engineering of genetic gene expression tools to study the development of the cerebellum in zebrafish. He also worked with Daniel Razansky at the Helmholtz Center to bring non-invasive opto-acoustic imaging to zebrafish. For his postdoctoral work in the field of zebrafish hematopoiesis, he joined the laboratory of David Traver at the University of California in San Diego, USA. In 2014 Martin Distel was recruited to St. Anna CCRI as a group leader, where he established the "Innovative Cancer Models"lab and a new zebrafish facility. His research group leverages the excellent imaging and drug screening possibilities of zebrafish to unravel pediatric cancer etiology and disease-driving mechanisms and to develop novel therapeutic strategies. In 2017 he became the head of the Zebrafish platform Austria for preclinical drug screening (ZANDR) at CCRI, a platform specifically designed to carry out small compound screens on zebrafish disease models.

"Being able to see how cancer cells really behave in an intact organism and how they respond to drugs has tremendous potential for the development of novel treatment strategies."

MARTIN DISTEL GROUP

Group: Innovative Cancer Models

PRINCIPAL INVESTIGATOR
Martin Distel

STAFF SCIENTIST
Stefanie Kirchberger

POSTDOCTORAL FELLOW
Hugo Poplimont

PHD STUDENTS
Sarah Grissenberger
Adam Varady

TECHNICIANS
Caterina Sturtzel
Andrea Wenninger-Weinzierl
Eva Scheuringer
Benjamin Natha

STUDENT ASSISTANTS
Alexander Kaptenja
Daniya Zakai (until 2021)
Ekin Dogan

RENÉ GEYEREGGER

Group: Clinical Cell Biology and FACS Core Unit

René Geyeregger obtained a degree in biology focused on Genetics from the University of Vienna. He then completed a PhD at the University of Vienna and Medical University Vienna, focusing on immunology, polyunsaturated fatty acids, and their effect on immune cells. In the following years, René Geyeregger worked as a Postdoctoral Fellower at the Department of Internal Medicine III and the Institute for Cancer Research at the Medical University Vienna and joined the St. Anna CCRI as a senior scientist in 2008. In 2010, he completed postgraduate studies in Pharmaceutical Quality Management at the University of Vienna. Since 2017, he has been Principal Investigator, head of the Clinical Cell Biology and FACS Core Unit, and the concomitant good manufacturing practice (GMP) laboratory. In 2021, he completed his bachelor's degree in biomedical science at the FH Campus Wien. His research focuses on detecting virus-specific T cells (ongoing projects on SARS-CoV-2), developing innovative adoptive cellular therapies (for viral infections and osteosarcoma), and flow cytometry-based diagnostics after hematopoietic stem cell transplantation and graft-versus-host disease.

"My ambition is to discover something new together with my team and apply it to young cancer patients."

RENÉ GEYEREGGER GROUP

Group: Clinical Cell Biology and FACS Core Unit

PRINCIPAL INVESTIGATOR
René Geyeregger

STAFF SCIENTIST
Wolfgang Paster

TECHNICIANS
Barbara Haigl
Laura Domnanovich
Dieter Printz
Daniela Scharner
Dijana Trbojevic
Elke Zipperer
Julia Stemberger
Christine Hoffmann-Freimüller
Andreas Sparer
Anna-Maria Husa
Svea Pfefferkorn

FLORIAN HALBRITTER

Group: Developmental Cancer Genomics

Florian Halbritter graduated from the University of Osnabrück with a degree in Cognitive Science. In 2008 he entered a PhD program in Stem Cell Bioinformatics under the supervision of Simon Tomlinson and Ian Chambers at the MRC Centre for Regenerative Medicine at the University of Edinburgh. After obtaining his PhD in 2012, he moved to Christoph Bock's lab at CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, and joined St. Anna CCRI as a Principal Investigator in 2018. At St. Anna CCRI, his group now focuses on studying aberrant development in pediatric cancers. The main goal is to achieve a mechanistic understanding of the underlying biology to inspire diagnostics and treatments. Throughout his career Florian Halbritter has been an advocate of open and collaborative science and has participated in numerous international consortia.

"Tumors are parts of our body that have turned against us. We turn terabytes of molecular data into knowledge to find out why and to inspire new treatments."

FLORIAN HALBRITTER GROUP

Group: Developmental Cancer Genomics

PRINCIPAL INVESTIGATOR
Florian Halbritter

POSTDOCTORAL FELLOWS
Luis Fernando Montano Guiterrez
Christoph Hafemeister

PHD STUDENT
Mohamed Refaat Elsahaat Shoeb

PUBLICATIONS

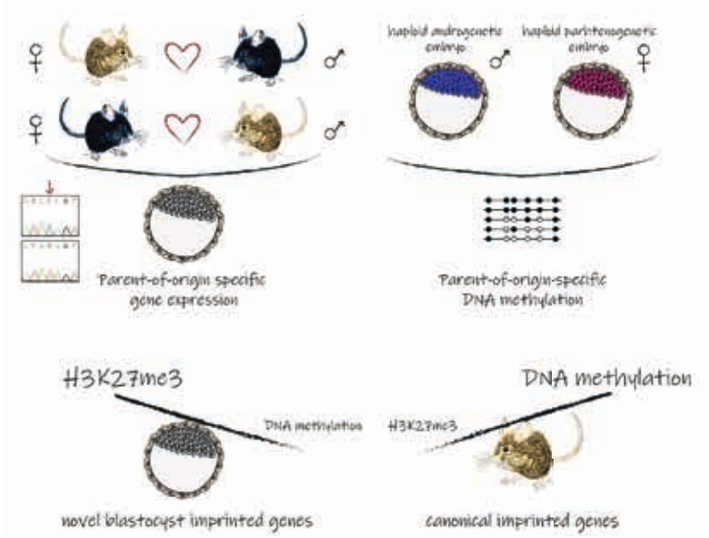
A NEW ROLE FOR HISTONE MODIFICATIONS IN GENOMIC IMPRINTING

Imprinted genes are expressed from either the paternal or maternal allele. A team of scientists led by Martin Leeb have discovered 71 previously unrecognized imprinted genes in preimplantation blastocysts. The study found that imprinting created by differential histone marks plays a more prominent role in the preimplantation blastocyst than canonical DNA methylation mechanisms.

We all inherit traits from our parents, but not only as one set of genes from our father and one from our mother. Epigenetic marks change the way our genes work – without altering the DNA sequence. For most genes, both copies of the gene are active, but a minority of genes is exclusively expressed from either the maternal or paternal allele. The epigenetic mechanism first attributed to this phenomenon, termed genomic imprinting, was DNA methylation. Methylation occurs primarily on cytosine, one of the four bases that together make up our genetic code, and typically leads to the repression of transcription. However, a second mechanism implicated in imprinting relies on histone modifications. Here, it is the methylation of histone proteins that changes the transcriptional activity of genes. Historically, DNA methylation has been the subject of most imprinting studies, but in recent years it has been reported that trimethylation of lysine 27 on histone H3 is an important imprinting mark. Hundreds of imprinted genes have been identified and defects in imprinting are implicated in several developmental disorders.

In cooperation with Florian Halbritter from St. Anna CCRI and Tony Perry from the University of Bath, the scientists have now identified 71 novel imprinted genes and profiled their methylation status. Co-first author Laura Santini and colleagues realized that the majority of the novel genes with uniparental expression in preimplantation blastocyst were not residing in close proximity to methylation marks, implying that DNA methylation was not the major driver of imprinting at this developmental stage. They found out that also some of the genes historically associated with DNA methylation are actually regulated by H3K27 trimethylation specifically in blastocysts. This suggests that histone modifications play a more significant role in early development than previously thought.

The phenomenon of imprinting is still a mystery, and the precise mechanisms involved in the regulation of imprinted genes are not fully understood. H3K27 trimethylation is a reversible modification, and many of the imprinted genes lose their H3K27 marks during development. DNA methylation on the other hand is a more stable mark, but the levels in pre-implantation blastocysts are low. Study leader Martin Leeb concludes that histone modification may serve as a backup system to ensure that imprinting is maintained during early development before DNA methylation takes over as the primary imprinting mechanism.



Mice in Love
Top left: allele-specific RNA-Seq performed on pre-implantation blastocysts from reciprocal mice hybrids to identify parent-of-origin specific gene expression; top right: genome-wide methylation analysis performed on uniparental haploid blastocysts to identify DNA methylation regions that are differentially methylated between the maternal and paternal genomes; bottom: H3K27me3-dependent imprinting has a major impact at the pre-implantation blastocyst stage, especially for the "transient" novel imprinted genes in contrast to the more prominent role for DNA methylation-dependent imprinting at later developmental stages for canonical imprinted genes.

Laura Santini et al., Nat Commun 2021b

PUBLICATION

Santini, L.*, Halbritter, F.*, Titz-Teixeira, F., Suzuki, T., Asami, M., Ma, X., Ramesmayer, J., Lackner, A., Warr, N., Pauler, F., Hippenmeyer, S., Laue, E., Farlik, M., Bock, C., Beyer, A., Perry, A. C. F., & Leeb, M. (2021). Genomic imprinting in mouse blastocysts is predominantly associated with H3K27me3. Nat Commun, 12(1), 3804. https://doi.org/10.1038/s41467-021-23510-4

* Shared first authorship

FINANCIAL REPORT

GUIDELINES FOR THE USE OF DONATIONS

St. Anna Children's Cancer Research Institute is mainly financed by private donations. For the operation of the research institute more than ten million euros are required annually, whereas the association has no basic funding from the public sector. Additional funds are acquired through competitive project grants from recognized national and international agencies.

We are committed to our donors to use the funds entrusted economically and efficiently. The annual financial statement is prepared in accordance with the provisions of §22 of the Federal Act on Associations. As a large association, the financial management as well as the annual financial statements of the association are audited by a public accountant who provides an independent auditor's certificate. Thus, proper and appropriate handling and allocation of the donations in alignment with the statutes can be assured.

SEAL OF APPROVAL FOR DONATIONS AND TAX DEDUCTIBILITY

Since 2002, St. Anna Children's Cancer Research Institute has been one of the first organizations in Austria to receive the seal of approval for donations from the Chamber of Public Accountants and Tax Advisors. For the annual re-awarding, an auditor carries out an additional audit to the one already provided for the annual accounts, scrutinizing for transparency and proper use of funds in accordance with the strict guidelines of the Donation Quality Certificate. On the basis of a notice (Bescheid) issued by the Federal Ministry of Finance, St. Anna Children's Cancer Research Institute is classed as a tax-privileged group of recipients, so donations are tax-deductible from either private or corporate income tax.

QUALITY ASSURANCE OF SCIENTIFIC WORK

The research institute has a Scientific Advisory Board – a committee of external experts – with the task of continuously evaluating the scientific work and advising the Institute's management. In addition, new scientific projects are regularly submitted to renowned national and international research funding bodies and research results are published in internationally recognized scientific journals. In addition, an objective assessment of the scientific performance by recognized external experts in the field takes place at regular intervals.

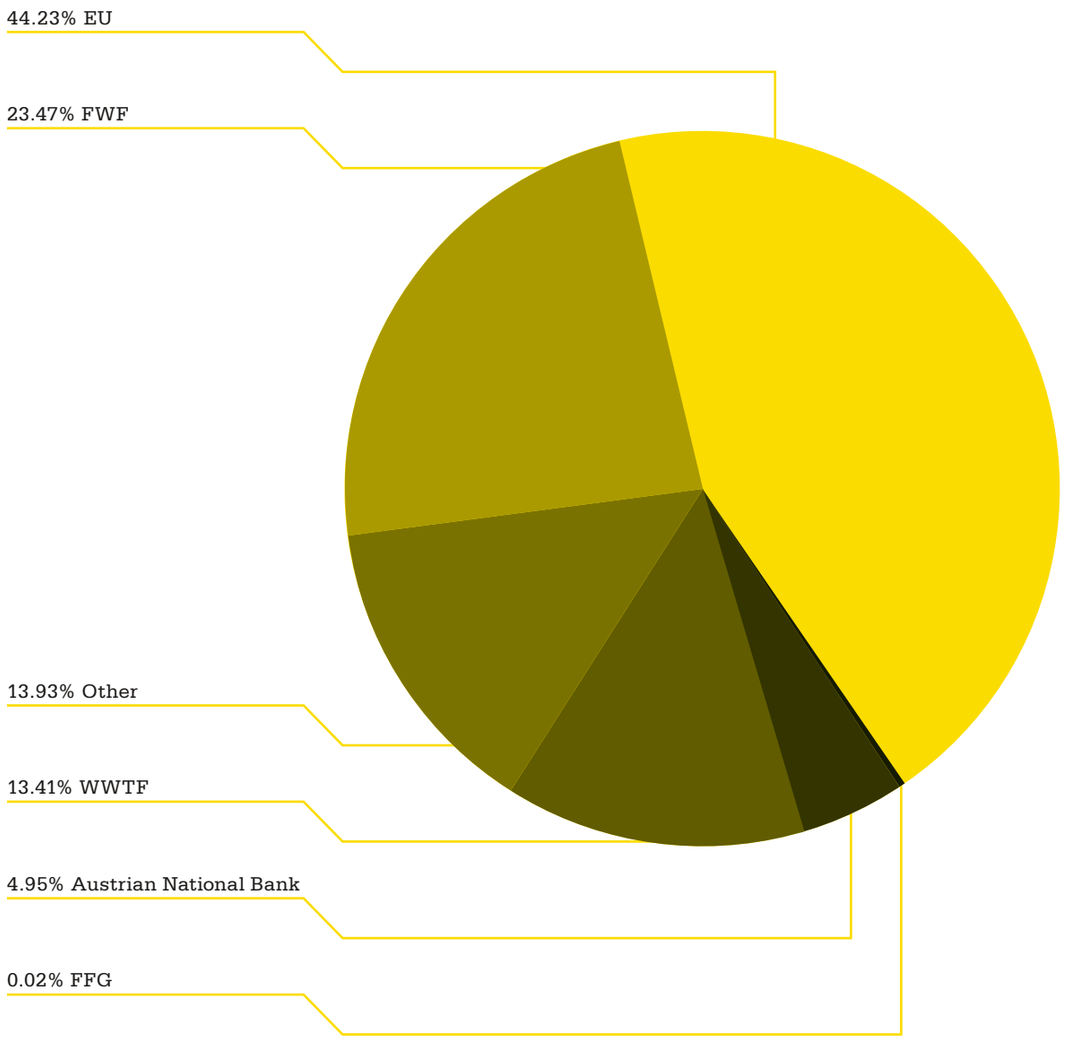
SOURCE OF FUNDS

		2020	2021
I.	Donations		
	a) undedicated donations	€ 0.00	€ 0.00
	b) dedicated donations	€ 24,898,666.38	€ 14,264,254.32
II.	Membership fees	€ 940.00	€ 860.00
III.	Operating income		
	a) operating income from public funds	€ 0.00	€ 0.00
	b) other operating income	€ 1,009,922.50	€ 2,120,478.91
IV.	Public subventions and subsidies	€ 0.00	€ 0.00
V.	Other income		
	a) asset management	€ 36,610.69	€ 42,326.90
	b) other income not included in positions I to IV	€ 0.00	€ 0.00
VI.	Revenue from release of donations and subsidies not yet used for the intended purpose	€ 0.00	€ 0.00
VII.	Release of reserves	€ 0.00	€ 0.00
VIII.	Annual loss	€ 0.00	€ 0.00
TOTAL		**€ 25,946,139.57**	**€ 16,427,920.13**

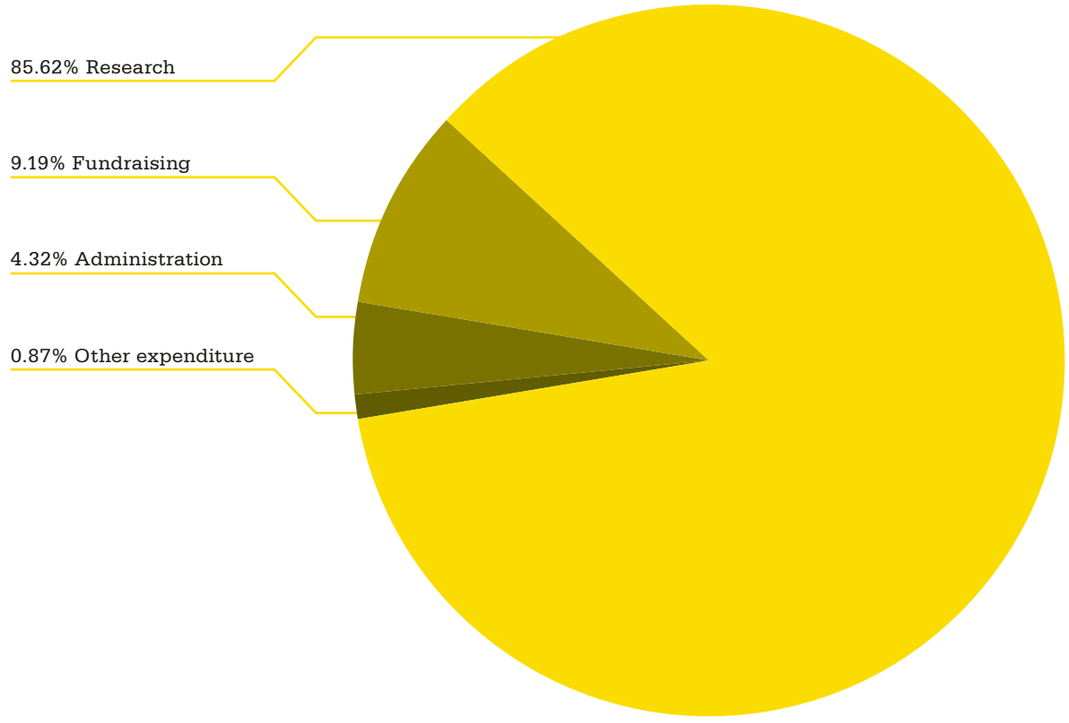
USE OF FUNDS

		2020	2021
I.	Expenditures for statutorily defined purposes	€ 10,481,817.55	€ 10,573,462.96
II.	Fundraising	€ 892,554.21	€ 1,135,445.65
III.	Administration	€ 384,097.91	€ 533,195.24
IV.	Other expenditures not included in posititons I to III	€ 58,253.00	€ 107,366.00
V.	Donations and subsidies not yet used for the intended purpose (allocation to liabilities)	€ 14,129,416.90	€ 4,078,450.28
VI.	Allocation of funds to reserves	€ 0.00	€ 0.00
VII.	Annual profit	€ 0.00	€ 0.00
TOTAL		**€ 25,946,139.57**	**€ 16,427,920.13**

COMPETITIVE RESEARCH GRANTS 2021



ALLOCATION OF FUNDS 2021



ANNEX

ADMINISTRATIVE DEPARTMENTS

MANAGEMENT
Managing Director / CFO: Jörg Bürger
Executive Assistant: Theresa Kröswagn

RESEARCH MANAGEMENT OFFICE
Head of: Nuno-Miguel Andrade Gomes
Ana Pudja (until 2021)
Adriana Planinic
Caterina Barresi (until 2021)
João Manuel Rodrigues Frade
Jellena Vanessa Düster
Martin Schalling
Zoltán Dobai

SCIENCE COMMUNICATION & PR
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Anna Egger
Barbara Brunmair
Lukas Lach
Robina Maria Kuntara

FUNDRAISING
Head of: Lisa Huto
Andrea Prantl
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Vanessa Wally

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David Obradovic
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Mark Rossiwall
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Daniela Hell-Böckl (until 2021)
Sandra Ehrenhofer-Weinzettl

HUMAN RESOURCE
Head of: Martina Teufner
Agata Staniak
Caroline Schmid
Jennifer Horvath

LEGAL COUNSEL
Dragana Gojevic
Nina Zobernig-Krejci
Sabrina Jevremovic (until 2021)

FACILITY MANAGEMENT
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Daniel Dworak
Ferdinand Knierim
Lukas Voglmüller

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Yvonne Schnetzinger (Maternity Leave)
Alexandra Lidy
Anita Polgári
Viktoria Bauer

SECRETARIAT
Marion Zavadil

LEGACIES
Monika Gomez-Beran (Maternity Leave)
Monika Gabrle

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St. Anna CCRI is supported by a board of international childhood oncology and immunology experts who advise us on scientific and strategic questions.

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* work in both groups

INTERNATIONAL AND NATIONAL GRANTS 2020/2021

INTERNATIONAL GRANTS

European rare disease research coordination and support action (ERICA)
CCRI responsible Principal Investigator: Ruth Ladenstein
Coordinator: Alberto Pereira, Leiden University Medical Center, the Netherlands
H2020 Grant Agreement ID –964908
Duration: 01/03/2021 to 28/02/2025

Tracking Ewing sarcoma origin by developmental and trans-species genomics (ORIGIN)
CCRI responsible Principal Investigator and Coordinator: Heinrich Kovar
Additional CCRI Principal Investigators:
Martin Distel, Florian Halbritter
Alex´s Lemonade Stand Foundation (ALSF),
Crazy 8 Initiative Award Program
Duration: 01/03/2021 to 28/02/2025

Functional Interrogation of Non-coding DNA Sequences in leukemia development and drug resistance (FIND-seq)
CCRI responsible Principal Investigator and Awardee: Davide Seruggia
H2020 ERC Starting Grant, Grant Agreement ID - 947803
Duration: 01/03/2021 to 28/02/2026

Implementing the digital survivorship passport to improve person-centered survivorship care (PanCareSurPass)
CCRI responsible Principal Investigator: Ruth Ladenstein
Coordinator: Desiree Grabow, Universitätsmedizin Mainz
H2020 Grant Agreement ID – 899999
Duration: 01/03/2021 to 28/02/2025

Twinning research and education to improve survival in childhood solid tumors in Lithuania (TREL)
CCRI responsible Principal Investigator: Ruth Ladenstein
Coordinator: Jelena Rascon, Vilnius University Hospital Santaros Klinikos, Lithuania
H2020 Grant Agreement ID – 952438
Duration: 01/01/2021 to 31/12/2023

Validation of Actionable Genomic Aberrations in a Paediatric Oncology Network for Doctorate students (VAGABOND)
CCRI responsible Principal Investigator: Heinrich Kovar
Coordinator: Jan Molenaar, Prinses Máxima Centrum, the Netherlands
H2020 Grant Agreement ID - 956285
Duration: 01/12/2020 to 30/11/2024

Integrated and standardized NGS workflows for Personalised therapy (INSTAND-NGS4P)
CCRI responsible Principal Investigators:
Ruth Ladenstein, Kaan Boztug
Coordinator: Kurt Zatloukal, Medical University, Graz
H2020 Grant Agreement ID – 874719
Duration: 01/01/2020 to 31/05/2025

Charting key molecules and mechanisms of human immune Dysregulation (iDysChart)
CCRI responsible Principal Investigator and Awardee: Kaan Boztug
H2020 - ERC Consolidator Grant, Grant Agreement ID - 820074
Duration 01/06/2019 to 31/05/2024

Childhood Leukemia: Overcoming distance between South America and Europe Regions (CLOSER)
CCRI responsible Principal Investigator: Sabine Strehl
Coordinator: Mireia Camos, Hospital Sant Joan de Déu de Barcelona, Spain
H2020 Grant Agreement ID - 825749
Duration: 01/01/2019 to 31/12/2023

European Joint Programme on Rare Diseases (EJP RD)
CCRI responsible Principal Investigator: Ruth Ladenstein
Coordinator: Daria Julkowska, Inserm, France
H2020 Grant Agreement ID - 825575
Duration: 01/01/2019 – 31/12/2023

Comprehensive heatmap for TKI-resistance of mutations in BCR-ABL1 kinase domain
CCRI responsible Principal Investigator: Thomas Lion
Incyte Corporation - Incyte open calls
Duration: 01/01/2019 to 31/07/2021

PRedictive In-silico Multiscale Analytics (PRIMAGE)
CCRI responsible Principal Investigator: Ruth Ladenstein
Coordinator: Luis Martí-Bonmatí, La Fe, Spain
H2020 Grant Agreement ID – 826494
Duration: 01/12/2018 to 30/11/2022

European Reference Network on Paediatric Cancer Connecting Facility-3
CCRI responsible Principal Investigator and Coordinator: Ruth Ladenstein
EU-CEF Grant Agreement ID – INEA/CEF/ICT/A2020/2393583
Duration: 01/09/2021 to 31/08/2023

ERN-PAEDCAN Partner: Paediatric Rare Tumours Network – European Registry (PARTNER)
CCRI responsible Principal Investigator: Ruth Ladenstein
H2020 Grant Agreement ID – 777336
Duration: 01/01/2018 to 31/12/2021

Paediatric Cancer European Reference Network (ERN PaedCan)
CCRI responsible Principal Investigator and Coordinator: Ruth Ladenstein
EU-3HP Grant Agreement ID – 739538
Duration: 01/03/2017 to 28/02/2022

ITCC Pediatric Preclinical POC Platform (ITCCP4)
CCRI responsible Principal Investigator: Heinrich Kovar
Coordinator: Stefan Pfister, Deutsches Krebsforschungszentrum DKFZ, Germany
EU-IMI Grant Agreement ID – 116064
Duration: 01/01/2017 to 31/12/2022

Children's Liver Tumour European Research Network (ChiLTERN)
CCRI partner and Principal Investigator: Heidrun Boztug
Coordinator: Keith Wheatley, University of Birmingham, UK
H2020 Grant Agreement ID - 668596
Duration: 01/01/2016 to 31/12/2021

Modeling Langerhans Cell Histiocytosis with patient derived iPSCs
CCRI responsible researcher: Giulio Abagnale
(supervisor: Caroline Hutter)
Histiocytosis Association
Duration: 01/03/2021 to 28/02/2025

NATIONAL GRANTS 2021

Decoding the epigenome and its regulation in neuroblastoma
CCRI responsible researcher: Irfete Fetahu
(supervisor: Sabine Taschner-Mandl)
Grant from the Austrian Science Fund (FWF), Stand-Alone Project,
ID - P 35072
Duration: 01/12/2021 to 30/11/2024

FemTECH for HR-NBL
CCRI responsible researcher: Sylvia Ramirez
(supervisor Sabine Taschner-Mandl)
Grant from the Austrian Research Promotion Agency (FFG),
FemTech Praktika – Fellowship, ID - 891784
Duration: 17/12/2021 to 14/06/2022

Interplay of fusion genes and cellular context in sarcoma
CCRI responsible Principal Investigator: Eleni Tomazou
Grant from the Austrian Science Fund (FWF), Stand-Alone Project,
ID - P 34958
Duration: 01/10/2021 to 30/09/2025

Comprehensive cell contact tracing (C3T)
CCRI responsible Principal Investigator: Florian Halbritter
Additional CCRI Principal Investigators: Martin Distel
Grant from the Austrian Science Fund (FWF), TAI-1000 Ideas
Program, ID – TAI 454
Duration: 01/09/2021 to 31/12/2022

Validation of a liquid biopsy based molecular diagnostic toolkit for pediatric sarcomas
CCRI responsible Principal Investigator and Coordinator:
Eleni Tomazou
Grant from the Vienna Science and Technology Fund (WWTF),
Life Sciences 2020, ID – LS20-045
Duration: 01/09/2021 to 31/08/2025

Uncovering immune evasion mechanisms of leukemic cells from natural killer cells
CCRI responsible Principal Investigator: Eva König
Grant from the Fellinger Krebsforschung
Duration: 01/08/2021 to 30/06/2022

How do leukemic cells escape natural killer cell-mediated surveillance?
CCRI responsible researcher: Michelle Buri (supervisor: Eva König)
Grant from the Austria Academy of Sciences (ÖAW), DOC fellowship
Duration: 01/08/2021 to 31/08/2024

Establishing light-mediated clonal cancer models to investigate tumor initiation
CCRI responsible Principal Investigator: Adam Varady
(supervisor: Martin Distel)
Grant from the Austria Academy of Sciences (ÖAW),
DOC fellowship, ID – 25931
Duration: 01/08/2021 to 31/01/2024

Characterization of bacterial-fungal interactions: a basis for discovery of microbial biomarkers (BacFun)
CCRI responsible Principal Investigator: Thomas Lion
Grant from the Austrian Science Fund (FWF), Stand-Alone Project,
ID - P 34152
Duration: 01/08/2021 to 31/07/2024

Molecular mechanisms of exocytosis coordination
CCRI responsible Principal Investigator: Kaan Boztug
Grant from the Austrian Science Fund (FWF), Stand-Alone Project,
ID - P 34834
Duration: 01/07/2021 to 30/06/2024

EWS-FLI1 fluctuations in Ewing Sarcoma
CCRI responsible Principal Investigator: Heinrich Kovar
Grant from the Austrian Science Fund (FWF), Stand Alone Project,
ID - P 34341
Duration: 01/04/2021 to 31/03/2024

Cold atmospheric plasma for viral decontamination
CCRI responsible Principal Investigator: Thomas Lion
Grant from the Austrian Science Fund (FWF), CEUS Bilateral Joint
Projects, ID - I 5293
Duration: 01/04/2021 to 31/03/2024

Crossroads of immunometabolism and human deficiency
CCRI responsible researcher: Michael Kraakman
(supervisor: Kaan Boztug)
Grant from the Austrian Science Fund (FWF), Lise Meitner
Program, ID - M 3013
Duration: 01/01/2021 to 28/02/2023

SARS-CoV-2 infections and virus shedding in pediatric patients displaying different risk constellations
CCRI responsible Principal Investigator: Thomas Lion
Grant from the City of Vienna, Bürgermeisterfonds, ID – COVID016
Duration: 01/09/2020 to 31/07/2021

Detailed characterization of the cellular immune response to SARS-CoV-2 infection
CCRI responsible Principal Investigator: René Geyeregger
Grant from the Vienna Science and Technology Fund (WWTF),
WWTF-Covid 19 Rapid Response, ID – COV20-009
Duration: 01/06/2020 to 30/06/2021

Implementation of the first validated diagnostic peptide-MHC multimer test for measuring Sars-CoV-2 T cell memory (Covid-2-tag)
CCRI responsible Principal Investigator: René Geyeregger
Grant from the Austrian Research Promotion Agency (FFG),
KLIPHA-COVID 19, ID - 880938
Duration: 01/05/2020 to 31/07/2021

Art4Science
CCRI responsible Principal Investigator: Eva König
Grant from the Austrian Science Fund (FWF),
Science Communication Program, ID - WKP 132
Duration: 01/05/2020 to 30/04/2022

A novel gene defect affecting actin dynamics reveals unexplored links between immunodeficiency and autoinflammation
CCRI responsible researcher: Jana Block
(supervisor: Kaan Boztug)
Grant from the Austria Academy of Sciences (ÖAW),
DOC fellowship, ID - 25590
Duration: 01/03/2020 to 01/03/2022

Automated minimal residual disease assessment in childhood acute myeloid leukemia (MYEFLOW)
CCRI responsible researcher: Margarita Maurer-Granofszky
(Principal Investigator: Michael Dworzak)
Grant from the Vienna Business Agency, From Science to Products
2019, ID - 2841342
Duration: 15/03/2020 to 14/03/2023

Identifying the Ewing Sarcoma cell-of-origin by cross-species enhancer activity analysis
CCRI responsible researcher: Sarah Grissenberger
(supervisor: Martin Distel)
Grant from the Austria Academy of Sciences (ÖAW),
DOC fellowship, ID - 25607
Duration: 01/03/2020 to 01/09/2022

CD Laboratory for "Next generation CAR-T cells"
Head of CD Laboratory and Coordinator: Manfred Lehner
Christian Doppler Association, Christian Doppler Lab
Duration: 01/11/2019 to 31/10/2026

Detection and prognostic relevance of DUX4 rearrangements in childhood leukemia
CCRI responsible Principal Investigator: Sabine Strehl
Grant from the Austrian National Bank (OeNB), Jubiläumsfonds,
ID - 18281
Duration: 01/08/2019 to 31/01/2022

Advancing Liquid Biopsies for Monitoring and Personalized Treatment of Children with Neuroblastomas (LIQUIDHOPE)
CCRI responsible Principal Investigator: Sabine Taschner-Mandl
Coordinator: Angelika Eggert, Charité, Germany
Grant from the Austrian Science Fund (FWF), TRANSCAN-2 JTC
2020, ID – I 4162
Duration: 01/04/2019 to 31/03/2022

Find tumor immune evasion strategies by cellular barcoding
CCRI responsible Principal Investigator: Eva König
Grant from the Austrian Science Fund (FWF), Stand Alone Project,
ID - P 32001
Duration: 15/03/2019 to 14/09/2023

Ultra-high-risk pediatric cancer - combinatorial drivers and therapeutic targets for precision medicine
CCRI responsible Principal Investigator: Sabine Taschner-Mandl
Grant from the Vienna Science and Technology Fund (WWTF),
Life Sciences 2018, ID – S18-11
Duration: 01/03/2019 to 28/02/2022

Characterizing and targeting the Ewing sarcoma microenvironment to overcome resistance to therapy
CCRI responsible Principal Investigator and Coordinator:
Eleni Tomazou
Grant from the Vienna Science and Technology Fund (WWTF),
Life Sciences 2018, ID – LS18-049
Duration: 01/03/2019 to 28/02/2023

Analysis of temporal and spatial Ewing sarcoma tumor evolution during chemotherapy and validation of its clinical implication
CCRI responsible Principal Investigator: Eleni Tomazou
Grant from the Austrian National Bank (OeNB), Jubiläumsfonds,
ID - 17876
Duration: 01/09/2018 to 31/08/2021

Role of stress granules in Ewing sarcoma susceptibility
CCRI Responsible Principal Investigator: Heinrich Kovar
Grant from the Austrian Science Fund (FWF), Stand Alone Project,
ID - P 29773
Duration: 01/06/2017 to 30/11/2021

ACKNOWLEDGEMENTS

- 3rd Health Program of the European Commission (EU)
- Alex´s Lemonade Stand Foundation for Childhood Cancer
- All employees of St. Anna Children's Hospital
- All institutions, associations and societies related to St. Anna Children's Cancer Research Institute
- Austrian Academy of Sciences (ÖAW)
- Austrian Group for Paediatric Haemato-Oncology (AGPHO)
- Austrian National Bank (OeNB)
- Austrian Research Promotion Agency (FFG)
- Austrian Science Fund (FWF)
- Austrian Society of Hematology and Oncology (ASHO)
- Austrian Society for Pediatric Medicine (ÖGKJ)
- Austrian Stem Cell Registry
- CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences
- Christian Doppler Research Association
- City of Vienna
- District organization Alsergrund
- EBMT European Society for Blood and Marrow Transplantation
- Ethics Commission of the Medical University of Vienna
- European Consumers, Health, Agriculture and Food Executive Agency (CHAFEA) – now European Health and Digital Executive Agency (HaDEA)
- EU4Health Program (EU)
- European Research Council (ERC, EU)
- European Society for Pediatric Oncology (SIOP Europe)
- Federal Ministry Republic of Austria, Education, Science and Research
- Federal Ministry Republic of Austria, Digital and Economic Affairs
- Federal Ministry Republic of Austria, Social Affairs, Health, Care and Consumer Protection
- Fellinger Cancer Research Association
- Fund of the City of Vienna for innovative interdisciplinary cancer research
- German Cancer Registry, University of Mainz
- Gigax Private Trust FL
- Histiocytosis Association
- Horizon 2020 (EU)
- Incyte Biosciences Austria GmbH
- Innovative Medicines Initiative
- International Society of Pediatric Oncology (SIOP)
- Research and Innovation Programs of the European Commission
- Kapsch AG
- Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD)
- Medical University of Vienna
- Medical-Scientific Fund of the Mayor of the capital city of Vienna
- Private sponsors, mentors, association members and donors for the St. Anna Children´s Cancer Research Institute
- Senior City Councilor for Social Affairs, Health and Sport
- Vienna Science and Technology Fund (WWTF)
- Vienna Business Agency

BACHELOR-, MASTER (DIPLOMA)- AND PHD-THESES

GRADUATED IN 2021

ABDELRAHMAN ABDELGAWAD
Dissecting Sensitivity to Trabectedin in Fusion-Oncogene Driven Sarcomas
> Supervised by Eleni Tomazou
MSc thesis

LISA BIERBAUMER
Exploring the potential of YAP/TAZ inhibition as anti-metastatic strategy in Ewing sarcoma
> Supervised by Heinrich Kovar
PhD thesis

MATHIAS ILG
Towards 3D modeling of the metastatic niche:
Establishing and characterizing osteosarcoma tumor spheroids and lung organoids
> Supervised by Heinrich Kovar
MSc thesis

CHRISTIANE PAUKNER
Etablierung der quantitativen Fluoreszenz-in situ-Hybridisierung als diagnostisches Verfahren [Establishment of quantitative fluorescence in situ hybridization as a diagnostic method]
> Supervised by Marie Bernkopf
BSc thesis

MANFRED VISAGIE
Functional testing of pharmacological signal intervention in long-term in vitro and in-vivo models of paediatric AML
> Supervised by Michael N Dworzak
MSc thesis

PUBLICATIONS

2021

1. Abbasi, M. R. *, Nebral, K. *, Haslinger, S., Inthal, A., Zeitlhofer, P., Konig, M., Schinnerl, D., Kohrer, S., Strehl, S., Panzer-Grumayer, R., Mann, G., Attarbaschi, A., & Haas, O. A. (2021). Copy Number Changes and Allele Distribution Patterns of Chromosome 21 in B Cell Precursor Acute Lymphoblastic Leukemia. Cancers (Basel), 13(18). https://doi.org/10.3390/cancers13184597
* Shared first authorship

2. Agerer, B., Koblischke, M., Gudipati, V., Montano-Gutierrez, L. F., Smyth, M., Popa, A., Genger, J. W., Endler, L., Florian, D. M., Muhlgrabner, V., Graninger, M., Aberle, S. W., Husa, A. M., Shaw, L. E., Lercher, A., Gattinger, P., Torralba-Gombau, R., Trapin, D., Penz, T., Barreca, D., Fae, I., Wenda, S., Traungott, M., Walder, G., Pickl, W. F., Thiel, V., Allerberger, F., Stockinger, H., Puchhammer-Stöckl, E., Weninger, W., Fischer, G., Hoepler, W., Pawelka, E., Zoufaly, A., Valenta, R., Bock, C., Paster, W.,Geyeregger, R., Farlik, M., Halbritter, F., Huppa, J. B., Aberle, J. H., Bergthaler, A. (2021). SARS-CoV-2 mutations in MHC-I-restricted epitopes evade CD8(+) T cell responses. Sci Immunol, 6(57).

3. Al-Kershi, S., Golnik, R., Flasinski, M., Waack, K., Rasche, M., Creutzig, U., Dworzak, M., Reinhardt, D., & Klusmann, J. H. (2021). Recommendations for Diagnosis and Treatment of Children with Transient Abnormal Myelopoiesis (TAM) and Myeloid Leukemia in Down Syndrome (ML-DS). Klin Padiatr. https://doi.org/10.1055/a-1532-2016 (Empfehlungen fur die Diagnose und Behandlung von Kindern mit transienter abnormer Myelopoese (TAM) und myeloischer Leukamie bei Down Syndrom (ML-DS).) https://doi.org/10.1055/a-1532-2016

4. Andreana, M. S., C.; Yang, M.; Latham, R.; Leitgeb, R.; Member, IEEE, Drexler, W.; Zimmer, M.; Le, T.; Distel, M.; and Unterhuber, A. (2021). Molecular Multicolor Multiphoton in Vivo Bioimaging Based on a Direct Diode Pumped Ti:sapphire Oscillator. IEEE Journal of Selected Topics in Quantum Electronics, 27. https://doi.org/10.1109/JSTQE.2021.3060289.

5. Andreana, M., Sturtzel, C., Spielvogel, C. P., Papp, L., Leitgeb, R., Drexler, W., Distel, M., & Unterhuber, A. (2021). Toward Quantitative in vivo Label-Free Tracking of Lipid Distribution in a Zebrafish Cancer Model. Front Cell Dev Biol, 9, 675636. https://doi.org/10.3389/fcell.2021.675636

6. Ban, J. *, Fock, V. *, Aryee, D. N. T., & Kovar, H. (2021). Mechanisms, Diagnosis and Treatment of Bone Metastases. Cells, 10(11). https://doi.org/10.3390/cells10112944
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7. Baumgartner, A., Stepien, N., Mayr, L., Madlener, S., Dorfer, C., Schmook, M. T., Traub-Weidinger, T., Lotsch-Gojo, D., Kirchhofer, D., Reisinger, D., Hedrich, C., Arshad, S., Irschik, S., Boztug, H., Engstler, G., Bernkopf, M., Rifatbegovic, F., Holler, C., Slavc, I., Berger, W., Müllauer, L., Haberler, C., Azizi, A. A., Peyrl, A., Gojo, J. (2021). Novel Insights into Diagnosis, Biology and Treatment of Primary Diffuse Leptomeningeal Melanomatosis. J Pers Med, 11(4). https://doi.org/10.3390/jpm11040292

8. Bayram, O., Haskologlu, S., Bayrakoglu, D., Bal, S. K., Islamoglu, C., Cipe, F. E., Kendirli, T., Kursun, N., Guner, S. N., Yildiran, A., Bozdogan, G., Yuksek, M., Reisli, I., Dalva, K., Aytekin, C., Boztug, K., Dogu, F., & Ikinciogullari, A. (2021). Single-Center Study of 72 Patients with Severe Combined Immunodeficiency: Clinical and Laboratory Features and Outcomes. J Clin Immunol. https://doi.org/10.1007/s10875-021-01062-y

9. Bellini, A.*, Potschger, U.*, Bernard, V., Lapouble, E., Baulande, S., Ambros, P. F., Auger, N., Beiske, K., Bernkopf, M., Betts, D. R., Bhalshankar, J., Bown, N., de Preter, K., Clement, N., Combaret, V., Font de Mora, J., George, S. L., Jimenez, I., Jeison, M., Marques, B., Martinsson, T., Mazzocco, K., Morini, M., Mühlethaler-Mottet, A., Noguera, R., Pierron, G., Rossing, M., Taschner-Mandl, S., Van Roy, N., Vicha, A., Chesler, L., Balwierz, W., Castel, V., Elliott, M., Kogner, P., Laureys, G., Luksch, R., Malis, J., Popovic-Beck, M., Ash, S., Delattre, O., Valteau-Couanet, D., Tweddle, D.A.#, Ladenstein, R.#, Schleiermacher, G.# (2021). Frequency and Prognostic Impact of ALK Amplifications and Mutations in the European Neuroblastoma Study Group (SIOPEN) High-Risk Neuroblastoma Trial (HR-NBL1). J Clin Oncol, JCO2100086. https://doi.org/10.1200/JCO.21.00086
* Shared first authorship
# Shared senior authorship

10. Bertrums, E. J. M., Zwaan, C. M., Hasegawa, D., De Haas, V., Reinhardt, D. N., Locatelli, F., De Moerloose, B., Dworzak, M., Buijs, A., Smisek, P., Kolenova, A., Pronk, C. J., Klusmann, J. H., Carbone, A., Ferster, A., Antoniou, E., Meshinchi, S., Raimondi, S. C., Niemeyer, C. M., Hasle, H., van den Heuvel-Eibrink, M. M., Goemans, B. F. (2021). Guideline for management of non-Down syndrome neonates with a myeloproliferative disease on behalf of the I-BFM AML Study Group and EWOG-MDS. Haematologica. https://doi.org/10.3324/haematol.2021.279507

11. Bierbaumer, L., Katschnig, A. M., Radic-Sarikas, B., Kauer, M. O., Petro, J. A., Hogler, S., Gurnhofer, E., Pedot, G., Schafer, B. W., Schwentner, R., Muhlbacher, K., Kromp, F., Aryee, D. N. T., Kenner, L., Uren, A., & Kovar, H. (2021). YAP/TAZ inhibition reduces metastatic potential of Ewing sarcoma cells. Oncogenesis, 10(1), 2. https://doi.org/10.1038/s41389-020-00294-8

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* Shared first authorship
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WE SAY THANK YOU!
Our donor family is big and awesome! We are grateful for the long-standing, loyal support of our numerous sponsors who, despite challenging times, have faithfully stood by our mission of conducting research for the benefit of children with cancer for almost 35 years. The helpfulness of the people and companies with their wonderful ideas and their great commitment to donate is never-ending. St. Anna Children's Cancer Research Institute, which has held the Austrian Donation Certificate since 2002 and belongs to the tax-privileged group of recipients, has been financed mainly by donations from the very beginning. Therefore, we say thank you. Because all of you give children with cancer the chance of a healthy future.

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For a long time, St. Anna Children's Cancer Research Institute has been accompanied by a prominent honorary committee. Our mentors from Austrian politics, business and culture have a special interest in organizing charity projects for the benefit of St. Anna Children's Cancer Research Institute.

Therefore, we would like to express our sincere thanks to the dedicated members of our support committee:
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For 30 years, the cuddly animals of St. Anna Children's Cancer Research Institute have been very popular with young and old; as collector's items, they are also highly sought after! Every year, the mascot family welcomes a new addition.

EVERY DONATION HELPS!
With your personal donation, you enable the continuation of our research in the fight against childhood cancer. For example, more and more companies are donating the amount intended for customer Christmas gifts. Commit yourself at *www.actforstanna.at* and donate on a special occasion! This can be an anniversary, a sportive challenge or any other extraordinary idea. Together we are strong against cancer!

Even renouncing flowers and wreaths at funerals in order to donate instead helps children suffering from cancer. A will or a legacy in favor of St. Anna Children's Cancer Research Institute also helps to provide childhood cancer patients with a healthy future.

LET'S SPREAD THE WORD TOGETHER
Our science communication team works closely together with our researchers to make the scientific work of St. Anna Children's Cancer Research Institute accessible to the public in the most understandable way possible. By internationally disseminating our scientific results, we also aim to reach out to scientists worldwide and attract the brightest minds to join St. Anna Children's Cancer Research Institute. We make it a priority to clearly communicate that every cent that goes into our research is put to the best possible use for the benefit of children with cancer. On our websites and social media channels, we keep you up to date on new study results, awards and grants, as well as on all other important innovations at St. Anna Children's Cancer Research Institute. Thus, you can always get a good overview of our work in pediatric cancer research and its cure. However, new findings are not always major breakthroughs. But each new finding is another piece of the jigsaw that, together with many other pieces, makes real breakthroughs possible. Stay tuned and follow the work of our research teams at *www.ccri.at*.

WE ARE HERE FOR YOU:
The team of St. Anna Children's Cancer Research Institute is very grateful to all of you for the many years of enthusiastic support. Thank you so much!

WOULD YOU LIKE TO GET INFORMATION, DOCUMENTS OR DO YOU HAVE ANY QUESTIONS?
The donation team, the science communication team and I look forward to hearing from you:
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www.kinderkrebsforschung.at

Lisa Huto
Head of Marketing,
Communication & Fundraising

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PUBLISHER AND MEDIA OWNER
St. Anna Kinderkrebsforschung
Zimmermannplatz 10, 1090 Wien
www.ccri.at, www.kinderkrebsforschung.at

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EDITORIAL
Dr. Barbara Brunmair
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Vorstand / Board

RESPONSIBLE FOR DATA PROTECTION
Arnold Redhammer (datenschutz@ccri.at)

CONCEPT & DESIGN
Büro X, www.buerox.at

PHOTO
Harald Eisenberger, www.eisenberger.co.at

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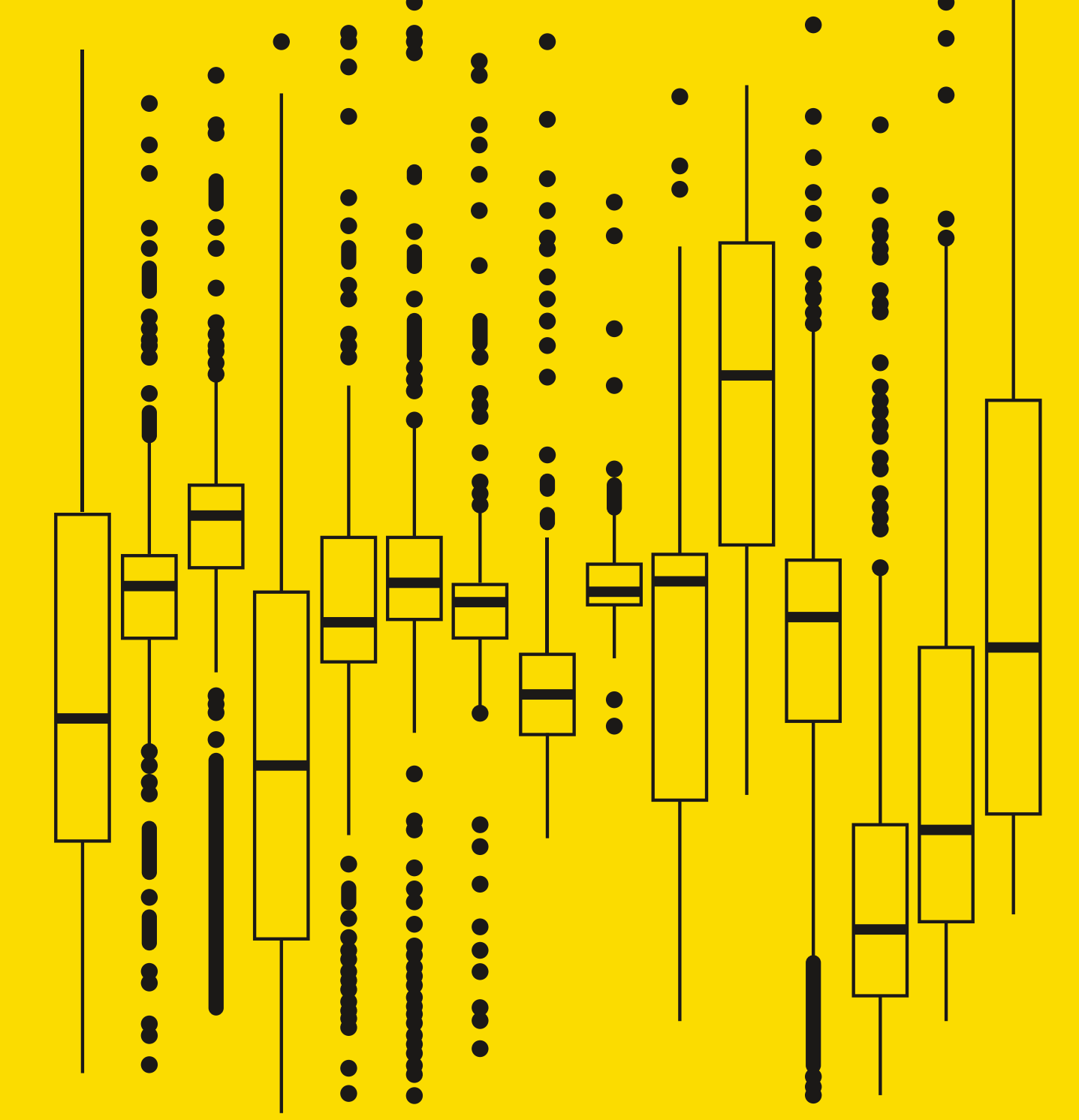

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