ARBEITSGEMEINSCHAFT
PÄDIATRISCHE IMMUNOLOGIE

KiNDER | UKE

37. Jahrestagung der Arbeitsgemeinschaft für pädiatrische Immunologie (API)

und

11. Jahrestagung des Arbeitskreises Pädiatrische Immunologie (AKPI)
der Deutschen Gesellschaft für Immunologie (DGfI)

Clinical Immunology – An Intersection of Specialties

Thursday 29 and Friday 30 April 2021

Welcome to exciting keynote talks and inspiring presentations on current topics from various fields where clinical immunology meets other specialties of medicine (metabolic disorders, hematooncology, pathology, infectious diseases, rheumatology, neurology/ psychiatry) at this year's annual meeting. Attendance of the meeting is not restricted to API members.

Even though we would have preferred to meet in person at the picturesques place Jesteburg in the South of Hamburg (the original site of the meeting), we are sure we will have a productive online meeting together with all of you !

The meeting will be held as Zoom Webinar. To participate as attendee, please register at the links noted below. No fees occur. If you wish to receive CME credits, please enter your number in the dedicated field on the registration page (Einheitliche Fortbildungsnummer, EFN).

Registration Link, Scientific Session Thursday, 29 April 2021:
https://zoom.us/webinar/register/WN_xXs3gxV3QOKwjlfLZI0LvA

Registration Link, Scientific Session Friday, 30 April 2021:
https://zoom.us/webinar/register/WN_vJBiQSfeRoWsSL7_XGFlow

If you experience problems with the Zoom client when entering the conference, use the option "Join from my Browser" further down on your screen. During the meeting you will be able to follow the presentations. No recordings will be made. If you wish to raise a question or make a comment to a speaker, please type it in the Q&A tool.

Session moderators and presenters will receive a link for test sessions (sound and video check) and the respective scientific session by email.

The program and the login for the general assembly (Mitgliederversammlung) will be provided to members by email, separately. During the assembly all members will be able to share audio and video.

Hoping for a meeting in person at API 2022 !

Kai Lehmberg
Meeting President, Hamburg

Thursday, 29 April 2021

14:00	**Welcome**
14:10	**Immunology and Metabolic Disorders** Session Moderator: Susan Farmand, Hamburg
	New treatment options in congenital neutropenias resulting from defects of glucose metabolism Maria Veiga da Cunha, Brussels, Belgium
	Identification of mutations of the gene *FNIP1* involved in energy homeostasis as responsible for autosomal recessive cardiomyopathy, pre-excitation syndrome and immunodeficiency Niehues T., Krefeld
	Transaldolase 1 is required for Neutrophil Extracellular Trap Formation Jakob Paul Morath, Berlin
	Towards the identification of disease-modifying factors in CTLA4 insufficiency Bodo Grimbacher, Freiburg
15:30	**Break**
15:40	**Immunology and Rheumatology** Session Moderator: Catharina Schütz, Dresden
	IL18-opathies Scott Canna, Pittsburgh, USA
	One gene, many facets: phenotypes and functional impairment in SOCS1 haploinsufficiency Catharina Schuetz, Dresden
	Multisystem Inflammation and Susceptibility to Viral infections in Human ZNFX1 Deficiency Jana Pachlopnik Schmid, Zürich
	High prevalence of autoinflammatory diseases in autosomal dominant NF-κB1 insufficiency caused by pathogenic *NFKB1* mutations Bodo Grimbacher, Freiburg
	Severe recurrent ulcers- is there a genetic cause? M Niemuth, Dresden
17:00	**Break**

17:20	**General Assembly (for API members)** including presentations
	Newborn Screening for Severe Combined Immunodeficiencies (SCID) in Germany: Current State of Play Carsten Speckmann, Freiburg
	Activated Phosphoinositide 3-Kinase δ Syndrome: an update from the ESID APDS-Registry Maria Elena Maccari, Freiburg
	Initial presenting manifestations in 16486 patients with primary immunodeficiencies include infections, immune dysregulation, syndromic features, and cancer. Julian Thalhammer, Freiburg
	Clinical Gene Therapy for p47phox-deficient Chronic Granulomatous Disease Janine Reichenbach, Zürich

Friday, 30 April 2021

14:00	**Welcome**
14:05	**Neurology/Psychiatry** Session Moderator: Carsten Speckmann, Freiburg
	Postinfectious Chronic Fatigue Syndrom Including Chronic Covid Syndrom – an Immunologist's Diagnosis ? Carmen Scheibenbogen, Berlin
	Deficiency of the MAP kinase activating death domain protein (MADD) causes defect of cytotoxicity Kerstin Schütze, Hamburg
	Differential DNA damage response of peripheral blood lymphocyte populations Kerstin Felgentreff, Ulm
	Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Ataxia Telangiectasia and Profound Immunodeficiency Peter Bader, Frankfurt
15:20	**Break**

15:30	**Infectious Diseases I** Session Moderator : Jana Pachlopnik, Zürich
	Insights from the Registry of COVID19 in Patients with Primary Immunodeficiencies (COPID19) Isabelle Meyts, Leuven, Belgium
	Phenotypic analysis of the pediatric immune response to SARS-CoV-2 by flow cytometry Freya Sibbertsen, Hamburg
	Lymphocyte cytotoxicity is needed for efficient control of rubella vaccine virus Miriam Groß, Freiburg
	Large deletion of the complete CYBB gene and flanking regions in x-linked CGD Myriam Lorenz, Ulm
	Pityriasis versicolor (Malassezia spec.) as a manifestation of homozygous CARD9 mutation in a Caucasian male Gregor Dückers, Krefeld
	Vaccine-associated Rotavirus Pneumonia in an Infant with Severe Combined Immunodeficiency (SCID) Christian Klemann, Hannover
16:40	**Break**
17:00	**Infectious Diseases II** Session Moderator: Fabian Hauck, München
	Auto-antibodies against type I IFNs in patients with life-threatening COVID-19 or adverse events to yellow fever live-attenuated vaccine Paul Bastard, Paris / New York
	Mild COVID-19 despite neutralizing autoantibodies against type I IFNs in autoimmune polyendocrine syndrome Type 1 (APS-1) Horst von Bernuth, Berlin
	RhoG deficiency: a novel type of familial HLH deciphers a key role of RhoG in granule exocytosis Artem Kalinichenko, Wien

The immune deficiency and dysregulation activity (IDDA) and other clinical scores for inborn errors of immunity
Markus Seidel, Graz

Radiosensitive Omenn Syndrome caused by a Somatic Reversion of a Novel Inborn *DCLRE1C* (ARTEMIS) Variant
Ulrich Pannicke, Ulm

18:20 Break

18:30 Oncology/Pathology
Session Moderator: Sujal Ghosh, Düsseldorf

Lymphomas and Lymphoproliferations in Primary Immunodeficiencies – the Pathologist's Point of View
Ilske Oschlies, Kiel

CARD11 GOF results in heterogeneous phenotypes that are associated with autoreactive/oncogenic BCR repertoires
Fabian Hauck, München

Gain-of-function variants in SYK cause immune dysregulation, systemic inflammation and lymphoma in humans
Daniel Mayr, Wien

Variable immunodeficiency with dermatitis: *CARD11*LOF over three generations
Julia Körholz, Dresden

Allogeneic HSCT for adolescents and adults with inborn errors of immunity: A retrospective study of the Inborn Errors Working Party (IEWP)
Michael H. Albert, München

Allogeneic HSCT for adolescents and adults with inborn errors of immunity: A retrospective single center analysis
Ansgar Schulz, Ulm

19:50 Closing Remarks

Identification of mutations of the gene *FNIP1* involved in energy homeostasis as responsible for autosomal recessive cardiomyopathy, pre-excitation syndrome and immunodeficiency

Niehues T.[1], Turul Özgür T.[1], Bickes MS.[2], Waldmann R.[3], Schöning J.[2], Bräsen JH.[4], Hagel C.[5], Ballmaier M.[6], Klusmann JH.[7,8], Niedermayer A.[3], Pannicke U.[3], Enders A.[9,10], Dueckers G.[1], Siepermann K.[1], Hempel J.[2], Schwarz K.[3,11+], Viemann D.[2,+]

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We report three patients (P1-3 from 2 families) presenting with congenital hypertrophic myocardium, preexcitation syndrome, myopathy of variable severity and motoric developmental delay. Immunological findings comprised low percentages and absolute numbers of B cells, peripheral blood T-cell lymphocytosis, intermittent neutropenia in all 3 patients as well as significantly reduced percentages and absolute numbers of switched memory B cells (P1, P2), hypogammaglobulinemia (IgG, IgM) (P1, P3) and low to absent response to T-cell dependent and T-cell independent vaccination antigens (P2). Flow cytometry analysis of bone marrow (P3, at 6 months of age) showed an enrichment of $CD34^-$ $CD10^+CD21^{low}$ B-cell precursors consistent with a B-cell maturation defect at the pre-B-cell stage. Sequencing revealed homozygous Folliculin-Interacting Protein 1 (FNIP1) mutations in all three patients with unaffected heterozygous family members. FNIP1 and FNIP2 interact with adenosine monophosphate-activated protein kinase (AMPK) which senses nutrient deprivation in cells. In conclusion, we describe cases of human FNIP1 deficiency, which is characterized by severe cardiac disease and complicated by a combined immunodeficiency. Three more patients from unrelated families (including an adult) have recently been described presenting with agammaglobulinemia, recurrent infections, and hypertrophic cardiomyopathy (HCM), two of them with intermittent or severe chronic neutropenia (Blood 2021;137:493-499). Thus, molecular alterations of cellular energy homeostasis (such as an increased AMPK activity) have an unexpectedly significant impact on the immune system apart from the expected effects on high-energy consuming tissues like muscle and brain. This should stimulate a thorough immunological evaluation in patients having inborn errors of energy metabolism.

Transaldolase 1 is required for Neutrophil Extracellular Trap Formation

Jakob Paul Morath[1], Pascale de Lonlay[2], René Santer[3], Konstantinos Tsiakas[3], Robin Kobbe[4], Arturo Zychlinsky[1*]

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Transaldolase 1-deficiency (TALDO-D) is a rare genetic metabolic disease. Transaldolase 1 is an enzymatic part of the non-oxidative pentose phosphate pathway (PPP). TALDO-D results in variable multisystemic clinical symptoms including severe liver failure, potentially lethal in early childhood. Some patients suffer from recurrent infections including the respiratory tract, suggesting primary immunodeficiency.

Neutrophils are the most abundant leukocytes and are essential for the innate immune defense against microbes. The PPP generates reduced NADPH, a cofactor of the NADPH oxidase NOX2 which generates superoxide. NOX2 is required for neutrophil extracellular trap (NET) formation. NETs are expelled through a neutrophil-specific cell death, called NETosis and consist of chromatin decorated with granular proteins. Here we report neutrophilic inability to form NETs in three TALDO-D patients. Deletion of Transaldolase 1 and its partner enzyme, Transketolase, in the neutrophil-like PLB-985 cell line reduced ROS generation and cell death, confirming its essential role in NET formation.

To the best of our knowledge, we present the first genetic evidence that the non-oxidative PPP is required for ROS generation and NET formation. These results provide further insight into the physiology of NET formation and might explain some TALDO-D related symptoms.

Towards the identification of disease-modifying factors in CTLA4 insufficiency

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Introduction**:** Heterozygous mutations in *CTLA4* lead to an inborn error of immunity characterized by immunodeficiency and multi-organ autoimmunity. Interestingly, the penetrance of these deleterious mutations is only about 70%, and their expressivity is highly variable.

Objectives**:** To identify modifying factors explaining the reduced penetrance and the variable expressivity.

Methods**:** We compared two groups of patients with CTLA4 mutations: 1) affected mutation carriers; 2) unaffected mutation carriers. We analyzed the exome sequence, the HLA type, the microbial stool content, the infection history, and epigenetic signatures. In addition, we looked for somatic mutations in *CTLA4* and pathway-related genes.

Results**:** When analyzing the WES data of mutation carriers, we did not identify additional genes which were only mutated in affected but not in the unaffected mutation carriers, or *vice versa*. Moreover, during our studies, we became aware of a monozygotic twin pair with a discordant phenotype, additionally arguing for somatic or environmental modifying factors. In >50 patients tested, we did not identify any additional somatic mutation in candidate genes. The HLA type, however, may add an additional susceptibility factor, as we observed that the haplotype HLA-DRB1 *15:01 and -DQB1 *06:02 in CTLA-4-insufficient patients might be associated with a severe immune dysregulation phenotype. Interestingly, the microbial composition in stool was different in affected CTLA4 mutation carriers when compared to unaffected mutation carriers. Details will be reported at the meeting. The infection history was not revealing, as for all infectious agents investigated, there were infected and not-infected mutation carriers in both groups. The analysis of the epigenetic signatures is pending and will be presented at the meeting.

Conclusion**:** According to our current assessment, the HLA-type and the microbiome are the most promising determinants of disease activity in patients with CTLA4-insufficiency.

Multisystem Inflammation and Susceptibility to Viral infections in Human ZNFX1 Deficiency

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Background: Recognition of viral DNA or RNA is one of the primary triggers for a type-1-interferon-mediated antiviral immune response. Inborn errors of type 1 interferon immunity are associated with either increased susceptibility to viral infections (as a result of impaired interferon production) or autoinflammatory diseases (as a result of enhanced type 1 interferon signaling). ZNFX1 is an interferon-stimulated dsRNA sensor that was recently found to restrict the replication of RNA viruses in mice. ZNFX1's role in the human immune system was not previously known.

Methods: We studied 15 patients with a novel Mendelian immunodeficiency characterized by early-onset seizures, inflammatory episodes with hepatitis and a disease resembling hemophagocytic lymphohistiocytosis, and renal and lung disorders. The disease appeared to be associated with single-stranded RNA or double-stranded DNA viral triggers. With a view to identifying a possible genetic cause, whole exome sequencing was performed in 13 patients. After the stimulation of cells from patients and controls with synthetic RNA and DNA, we investigated viral clearance as well as the transcription and mRNA stability of interferon-stimulated genes.

Results: Homozygous and compound heterozygous mutations were identified in *ZNFX1* in all sequenced patients, resulting in the loss of ZNFX1 protein expression in patient cells. Patient monocytes were less efficient in clearing viral infections after pre-stimulation with synthetic nucleic acids. Stimulation of patients' fibroblasts with synthetic nucleic acids was associated with a deregulated pattern of expression of ISGs linked to alterations in their mRNA half-life.

Conclusions: ZNFX1 has two different roles in type 1 interferon responses; it acts as a "rheostat" for the innate response to viruses. Consistently, ZNFX1-deficient patients suffer concomitantly from (i) increased susceptibility to viral infections, and (ii) life-threatening, virally triggered, hyperinflammatory episodes.

One gene, many facets: phenotypes and functional impairment in SOCS1 haploinsufficiency

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Background: Suppressor of cytokine signaling 1 (SOCS1) has important functions in immune regulation acting on multiple intracellular pathways and it is a key negative regulator of cytokine signaling. Animal models predict an autoimmunity dominated phenotype and early death in complete knockout of SOCS1. Recently *SOCS1* haploinsufficiency has been associated with a novel inborn error of immunity (IEI) in humans. Of patients described to date, it is apparent that *SOCS1* haploinsufficiency has a pleiotropic effect in humans.

Methods: We assessed impacts of reduced *SOCS1* expression across multiple immune cell pathways utilizing patient cells and CRISPR/Cas9 edited primary human T cells.

Results: *SOCS1* haploinsufficiency phenotypes straddle across the International Union of Immunological Societies classification of IEI with a predominance of autoimmunity as opposed to infection. We found that reduced SOCS1 expression led to dysregulation of multiple intracellular pathways in immune cells. STAT1 phosphorylation is enhanced, comparable to STAT1 gain-of-function mutations, and STAT3 phosphorylation is reduced with concurrent reduction of Th17 cells. These findings might explain clinical overlaps between *SOCS1* haploinsufficiency and IEIs like *STAT1*GOF and *STAT3*LOF. Furthermore, reduced E3 ligase functions of SOCS1 led to increased FAK in immune cells resulting in increased AKT and p70 ribosomal protein S6 kinase phosphorylation. We also find Toll-like receptor responses increased in SOCS1 haploinsufficient patients.

Conclusions: *SOCS1* haploinsufficiency is a pleiotropic monogenic IEI. Dysregulation of multiple immune cell pathways may explain the variable clinical phenotype associated with this new condition. Knowledge of these additional dysregulated immune pathways is important when considering specific treatment options for *SOCS1* haploinsufficient individuals.

High prevalence of autoinflammatory diseases in autosomal dominant NF-κB1 insufficiency caused by pathogenic *NFKB1* mutations

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Introduction**:** NF-κB1 (haplo)insufficiency due to heterozygous damaging variants in *NFKB1* has been recognized as an inborn error of immunity with immune dysregulation. Multi-organ autoinflammatory diseases (29.6%), including Behçet's disease (5.6%), are among most frequent clinical complications in affected mutation carriers besides hypogammaglobulinemia (88.9%), respiratory infections (83%) and reduced switched memory B cells (60.3%). The clinical spectrum further comprises autoimmunity (57.4%), lymphoproliferation (52.4%), non-infectious enteropathy (23.1%), opportunistic (15.7%) and gastrointestinal (28.6%) infections, and malignancy (16.8%). Autoinflammatory symptoms in NFKB1 insufficiency include oral and genital aphthous ulcerations (18.5%), non-infectious episodes of fever and systemic inflammation (12%) as well as vasculitis (4.6%).

Objectives**:** Due to the highly heterogeneous immunological phenotypes, including incomplete penetrance (70%) and age-dependent disease severity, attempts to establish clear genotype-phenotype correlations have been unsuccessful so far. Therefore, functional evaluation of each identified *NFKB1* sequence variation is required to demonstrate causality. *NFKB1* encodes the transcription factor precursor p105, which undergoes processing to produce the mature p50.

Methods**:** We use a standard cell culture model, based on transient overexpression of mutant NF-κB1 proteins in HEK293T cells, to test the expression, subcellular localization, precursor processing, DNA binding and promoter activation. In addition, we test protein interactions, cytoplasmic retention/nuclear translocation and DNA-binding competition, following experimentally simulated pathway activation.

Results**:** The disease-causing mechanism presumably originates from imbalanced ratios of NF-κB signaling components, and altered NF-κB signaling dynamics.

Conclusion**:** Due to the high frequency of autoinflammation associated with NF-κB1 insufficiency, mutational and functional analyses of *NFKB1* mutations should be considered, particularly when autosomal-dominant autoinflammatory diseases is suspected.

Severe recurrent ulcers- is there a genetic cause?

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Background: In 2016 an early-onset autoinflammatory disorder with a clinical phenotype resembling Behcet's disease was described by Zhou et al. (*Nat Genetics* 2016) caused by heterozygous loss-of-function mutations in the *TNFAIP3* gene encoding A20. Haploinsufficiency of A20 (HA20) decreases the NF-kB regulatory protein A20 and thereby amplifies action of the transcription factor NF-kB, a central mediator within inflammatory and innate immune signaling pathways.

Patients/Method: A 10y/o girl repeatedly presented with severe oral ulcers, gingivitis, lymphadenopathy and occasionally fever. Serum levels of CrP and ESR were mildly increased, IgE levels considerably elevated. No ulcers were found by endoscopy in stomach and colon, but few intramucous lymphfollicals were seen.
Therapy with steroids and later colchicine temporarily ameliorated her symptoms. Her mother also suffers from recurrent oral ulcers in addition to lung emphysema, autoimmune hepatits of unknown origin, and scleroderma.

Results: Whole exome sequencing revealed a new heterozygous mutation in the *TNFAIP3* gene (NM_006290.4:c.176_177delAG, p.Gln59fs). This variant leads to a frameshift and a premature STOP codon at the beginning of exon 2 and thereby most probably to nonsense mediated decay. Mutations in this gene can vary in location and usually lead to STOP codons or frame shift mutations.
Due to the decreased A20 levels and hence reduced inhibition of NF-kB, patients with HA20 suffer from episodes of fever, recurrent oral and genital ulcers, skin rashes, and polyarthritis, as well as from gastrointestinal and neurological symptoms. Ocular manifestations are far less frequent than in Behcet’s disease and age at manifestation is usually earlier.

Conclusion: In patients with severe “familial” apthosis and unexplained fever, genetic testing may guide clinical treatment decisions as exemplified in this family with unexpected monogenic disease.

Initial presenting manifestations in 16486 patients with primary immune-deficiencies include infections, immune dysregulation, syndromic features, and cancer

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Background: Primary immunodeficiencies (PID) are rare diseases, which makes diagnosis a challenge. Better awareness of the initial presenting manifestations should improve awareness and avoid diagnostic delay. Whilst increased infection susceptibility is a well-known initial PID manifestation, less is known about the frequency of other presenting manifestations.

Methods: We analyzed data on age-related initial presenting manifestations of PID on 16486 patients of the European Society for Immunodeficiencies (ESID) registry. Patients with autoinflammatory diseases were excluded due to the limited number registered.

Results: Overall, 68% of patients initially presented with infections only, 9% with immune dysregulation only and 9% with a combination of both. Syndromic features were the presenting feature in 12%, 4% had laboratory abnormalities only, 1.5% were diagnosed due to family

history only and 0.8% presented with malignancy. Two thirds of PID patients presented before the age of 6 years, but a quarter of patients only developed initial symptoms as adults. Immune dysregulation was most frequently recognized as an initial PID manifestation between 6 and 25 years of age with male predominance until age 10, shifting to female predominance after age 40. Infections were most prevalent as a first manifestation in patients presenting after age 30.

Conclusion: An exclusive focus on infection-centered warning signs would have missed around 25% of PID patients that initially present with other manifestations. We provide a data-based rationale to add immune dysregulation and syndromic features to the PID warning signs, which may significantly improve early PID diagnosis.

Activated Phosphoinositide 3-Kinase δ Syndrome: an update from the ESID APDS-Registry

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Background: Activated phosphoinositide 3-kinase (PI3K) δ Syndrome (APDS) is an autoimmune-lymphoproliferative primary immunodeficiency (AL-PID) caused by autosomal dominant mutations in *PIK3CD* (APDS1) or *PIK3R1* (APDS2). Here we use an updated dataset of the European Society for Immunodeficiencies (ESID)-APDS registry (currently supported by Pharming and previously by Novartis, UCB and GSK) to compare APDS to published datasets from the other autosomal-dominant AL-PID CTLA4 deficiency (n= 173) (Schwab, 2018), NFkB1 deficiency (n= 157)(Lorenzini, 2020) and STAT3 GOF (n= 83) (Faletti, 2021) associated disease.

Patients: By March 20, 2021, 155 patients have been registered and 119 initial datasets have been completed (85 APDS1, 34 APDS2). Age at registration was 0-10y in 38, 11-20y in 43, and above 20y in 38 patients.

Results: The genetic heterogeneity is low compared to other autosomal-dominant AL-PID diseases with 76/85 APDS1 patients carrying the p.E1021K mutation and all APDS2 patients with splice defects leading to loss of exon 11. Disease penetrance appears to be higher than

in other AL-PID diseases with only 2 asymptomatic individuals in the registry. The updated dataset confirms the high overlap of key clinical manifestations between AL-PID disorders with a particularly high incidence of bronchopulmonary infections and benign lymphoproliferation in APDS, while the incidence of autoimmune cytopenia, enteropathy and chronic lung disease is similar across the groups. Similar to CTLA4 or NFkB1 deficiency, 83/119 patients are receiving immunoglobulin replacement treatment.

Emerging features of immune dysregulation in APDS include arthropathies and nephropathies. Growth impairment is as frequent as in STAT3 GOF disease and represents a therapeutic challenge. Similar to CTLA4 and NFkB1 disorders, we confirm a 10% incidence of lymphoma in APDS patients, with malignancy a contributing cause in 4/10 deaths. Although the percentage of transplanted patients (21/119) increases, many APDS patients receive immunosuppressants, in particular rapamycin. Moreover, among the registered patients, 13 have been treated with PI3K□ inhibitors and it will be important to study their long-term disease evolution in this context.

Conclusion: APDS differs from other AL-PID diseases by its limited genetic heterogeneity and strong penetrance. Careful prospective observation of these disease cohorts is required to better delineate APDS-specific health risks and optimal treatment approaches.

Newborn Screening for Severe Combined Immunodeficiencies (SCID) in Germany: Current State of Play

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Background: Severe Combined Immunodeficiencies (SCID) are rare and life-threatening inborn errors of T-cell immunity. Circulating T cells are missing, significantly reduced and/or non-functional. Most patients are asymptomatic at birth, but develop severe (opportunistic) infections and immune dysregulation within the first months of life. Timely diagnosis and curative therapies have improved outcome, otherwise most SCID patients die before the age of 2 years. Treatment consists of hematopoietic stem cell transplantation (HSCT) and, in selected genetic forms of SCID, gene therapy. The outcome of these procedures is significantly better in patients, in whom early diagnosis and initiation of prophylactic treatment prevents critical infections and end-organ damage. A polymerase chain reaction (PCR) analysis for T-cell receptor excision circles (TREC) from dried blood spots allows identification of SCID, and other causes of congenital T-cell lymphopenia, shortly after birth. This test is performed from dried blood spots and can therefore be easily incorporated into the general neonatal screening. Since August 2019 TREC screening is routinely performed within the German screening program, which investigates around 750.000 newborns/year. Children with positive screening results (reduced or absent TREC) are referred to a recently founded API network of Combined Immunodeficieny (CID) Clinics or Centers for confirmatory testing and initiation of treatment.

Methods: Since August 2019 the API screening group has sent out questionnaires to the German CID Clinics and Centers in 6-monthly intervals. The number of referred patients with positive screening, type of SCID or other CID variant and type of initial treatment approach was recorded. Prospectively these data will be cross-analyzed (capture/re-capture analysis) with data the German/European immunodeficiency registry (PID-NET/ESID), neonatal screening database (DGNS report) and treatment registry (GPOH-SCID registry).

Results: One year after initiation of TREC screening the CID-clinics and Centers reported a total of 38 patients with severe congenital T-cell lymphopenia (10 SCID, 7 leaky SCID and 21 syndromal CID). The underlying genetic condition was identified in all but six patients (84%). Eleven (leaky) SCID patients underwent HSCT before four months of age, one patient was planned for HSCT within the first 4 months and one patient with radiosensitive SCID was scheduled to be transplanted at later time point. Two patients presented with in-utero onset of Omenn syndrome and died despite early diagnosis and timely initiation of immunosuppressive treatment. Among 21 patients with syndromal CID, three underwent transplantation of allogenic thymic tissue, one patient received HSCT and 16 were followed under prophylactic treatment. As expected the numbers of new SCID and CID diagnoses was fluctuating between the 6-monthly queries and data from our recent third call (1.5 years after screening initiation) will be evaluated for our presentation. Based on the 1-year follow-up data, the estimated incidence of SCID in Germany is 1:42.000 births (1:19.000 for congenital T-cell lymphopenia including CID?).

Conclusion: TREC screening for SCID and other severe congenital T-cell lymphopenias was successfully incorporated into the prospective German newborn screening program. The incidence of SCID seems significantly higher than in a recent retrospective analysis. The tight interaction of the newly founded API network of CID-Clinics and Centers fosters the tracking of identified patients. Yet, further follow-up and cross-analyses within the existing registries will remain essential to critically evaluate the management and outcome of positively screened neonates.

Clinical Gene Therapy for p47phox-deficient Chronic Granulomatous Disease

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Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by impaired oxidative burst by the phagocyte NADPH oxidase. Patients suffer from recurrent life threatening infections with bacteria and fungi as well as hyperinflammation, often requiring hematopoietic stem cell (HSC) transplantation. In case no matched HSC donor is available, gene therapy of autologous HSC may be performed within clinical trials.

Patients/Methods: The University of Zurich is preparing an international clinical gene therapy trial to treat 10 children and adults with autosomal-recessive CGD, caused by mutations in the NCF1-gene, that result in defects of the p47phox subunit of the NADPH oxidase. The trial using a third generation lentiviral self-inactivated (LV-SIN) vector is planned to start in 2022.

Results: Linked to the use of first-generation gamma-retroviral vectors, oncogenesis was observed in some of the treated patients of early gene therapy trials for CGD and other immunodeficiencies. In addition, in X-CGD patients, silencing of the transgene and of the therapeutic activity were observed over time. Therefore, a novel LV-SIN vector was developed to treat patients with p47phox-deficient CGD that confers specifically myelospecific expression of the therapeutic transgene. This vector results in high levels of p47phox expression and reconstituted NADPH oxidase activity in committed myeloid cells and granulocytes from transduced human p47phox-CGD CD34+ cells and shows resistance to silencing.

Conclusion: Based on these results, the vector was selected for large scale GMP-production in a joint effort between labs in Zürich and Spain, aiming at a multicenter clinical gene therapy trial, comprising study centers in Switzerland and Germany. First patients (pediatric and adult) are planned to be treated by 2022 in this trial.

Deficiency of the MAP kinase activating death domain protein (MADD) causes defect of cytotoxicity

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Background: Deficiency of the MAP kinase activating death domain protein (MADD) results in a protean syndrome with endocrinological and neurological involvement. The immunological function of the MADD protein is poorly understood. It interacts with the death domain of the TNF-alpha receptor 1, which leads to activation of the mitogen-activated protein kinase (MAPK) pathway and propagates an apoptotic signal. Beside the involvement in apoptosis and cell survival, MADD acts as a guanine-nucleotide exchange factor for small GTPases including Rab27a, playing a crucial role in vesicle transport. Hereditary defects of cytotoxicity - e.g. Griscelli Syndrome Type 2 (Rab 27a deficiency) - predispose to hemophagocytic lymphohistiocytosis (HLH).

Patient: A 2-month old female infant born to consanguineous parents presented with a complex clinical picture including enteropathy, marked dystrophy, endocrine and exocrine dysfunctions and developmental delay. Pigmentation was normal. During the course of disease, episodes with certain features of HLH were detected (fever, elevated ferritin, triglycerides, and sCD25), however not the full hyperinflammatory picture.

Results: Immunological workup detected a degranulation defect of resting and Il2-stimulated natural killer (NK) and cytotoxic T cells (CTL). On exome sequencing, the homozygous mutation c.963+1G>A in intron 4 of MADD was identified, which leads to aberrant mRNA splicing. Testing of NK cells and patient derived T cells revealed impaired cytotoxicity comparable to that of Griscelli syndrome 2 patients. To prove causality of MADD deficiency, we introduced a CRISPR/Cas9 based knock-out in the NK cell line NK-92mi. MADD deficient NK cells showed a degranulation defect and impaired killing capacity toward K562 cells similar to that of the patient.

Conclusion: These findings indicate that MADD influences vesicle release of cytotoxic cells, probably through its interaction with Rab27a. MADD deficiency causes a substantial degranulation defect with a consecutive defect of cytotoxicity and may contribute to a partial HLH phenotype.

Differential DNA damage response of peripheral blood lymphocyte populations

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Background: DNA damage is a constant event in every cell triggered by endogenous processes of replication and metabolism, or external influences such as ionizing radiation and intercalating chemicals. Furthermore, DNA double strand breaks are physiologically induced in the process of V(D)J recombination for the generation of diversified T and B cell receptors in lymphocyte development. Large sets of proteins are involved in sensing, stabilizing and repairing this damage including control of cell cycle and proliferation. Some of these factors are phosphorylated upon activation and can be used as biomarkers of DNA damage response (DDR) by flow and mass cytometry. Differential survival rates of lymphocyte subsets in response to DNA damage are well established, characterizing NK cells as most resistant and B cells as most sensitive to DNA damage.

Methods: We investigated DDR to low dose gamma radiation (2Gy) in peripheral blood lymphocytes of 22 healthy donors and 3 patients with ataxia telangiectasia (AT) using mass cytometry. γH2AX, p-CHK2, p-ATM and p53 were analyzed as specific DDR biomarkers for functional readouts of DNA repair efficiency in addition to cell cycle and lymphocyte subsets characterized by 20 surface markers.

Results: We identified significant differences in DDR among lymphocyte populations in both healthy and DNA repair deficient individuals. Whereas $CD56^{+}CD16^{+}$ NK cells showed the strongest γH2AX response to low dose ionizing radiation, the lowest response rate could be observed in $CD19^{+}CD20^{+}$ B cells. Interestingly, γH2AX induction level correlated inversely with ATM-dependent p-CHK2 and p53 response. Differential DDR could be further noticed in naïve compared to memory T and B cell subsets, characterized by reduced γH2AX, but increased p53 levels in naïve T cells. In contrast, DDR was abrogated in all lymphocyte populations of AT patients.

Conclusions: Our results demonstrate differential DDR capacities in lymphocyte subsets that depend on maturation and correlate inversely with DNA damage-related survival. Importantly, DDR analysis of peripheral blood cells for diagnostic purposes should be stratified to lymphocyte subsets.

Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Ataxia Telangiectasia and Profound Immunodeficiency

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Background: Ataxia telangiectasia (A-T) is a primary immunodeficiency with syndromic features caused by biallelic mutations in the *ATM* gene. A slowly progressive neurodegenerative disease, immunodeficiency and increased susceptibility to malignancies result in high premature mortality. One third of all patients will develop severe and fatal pulmonary disease where as one third will develop and succumb due to malignant diseases by the third decade. Pre-emptive HSCT is the only treatment option to correct the underlying immunodeficiency and protect from the development of hematopoietic malignancies. The correction of the patients' immunodeficiency may improve long-term pulmonary function due to lesser respiratory infections. This might help to improve the general conditions and development of these patients. As neurodegeneration probably could not be influenced by allogeneic SCT, and because of potential treatment toxicity this approach is matter of debate for patients with A-T.

Methods: We present four A-T patients with clinical signs of profound immunodeficiency/SCID phenotype and severe complications, leading us to choose HSCT with reduced intensity conditioning (RIC) as an individual treatment strategy intending to restore cellular immunity for proper infection control, treating their granulomatous skin and joint disease, and hematopoietic malignancy prevention.

Results: RIC consisted of fludarabine, cyclophosphamide and ATG-Fresenius (P1,2,4) and busulfan and alemtuzumab (P3), respectively. Treatment was well tolerated in P1-2, whereas P3 developed intermittent acute renal failure, CMV reactivation and thrombotic microangiopathy (TMA). P4 presenting with a clinical phenotype of SCID being infected by a series of viruses pre HSCT, developed an episode of immune hepatitis in the setting of viral clearance during immune reconstitution. This patient developed an autoimmune hemolytic anemia (AIHA) and mild skin autoimmunity (vitiligo) about one year post HSCT. AIHA needed additional treatment with rituximab.

Early and stable hematopoietic engraftment occurred in all patients, as well as adequate T- and B-cell expansion. Initially a mixed donor chimerism in PBMCs was observed, nevertheless T-cell ($CD3^+$) chimerism reached levels above 80% donor origin over time. At last follow-up

(median=3,6 yrs; 1,5-8,5 yrs) all patients are without signs of GVHD or organ toxicity and off immunosuppression. P4 is receiving IVIG after B cell aplasia was induced by rituximab to treat AIHA. Skin granulomas resolved (P1-3).

Conclusion: RIC- HSCT is feasible in a selected group of AT patients with profound/severe combined immunodeficiency phenotype but without short term secondary malignancy. Initial low donor chimerism should be tolerated, as it can evolve to full donor origin over time. Transplantation might be a treatment option for some AT patients as it can correct combined immunodeficiency in AT patients with a high risk of death due to infectious complications, granulomatous joint and skin disease and/or hematological malignancies.

Phenotypic analysis of the pediatric immune response to SARS-CoV-2 by flow cytometry

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Background: Pediatric SARS-CoV-2 infection is often mild or asymptomatic and the immune responses of children are understudied compared to adults. Here, we present an approach to rapidly analyze immune cells using a two-panel (16- and 17-parameter) flow cytometry-based analysis optimized for immune phenotyping of children with an acute and convalescent SARS-CoV-2 infection. The panels have been tailored to specifically target relevant adaptive and innate immune cells, including innate lymphoid cells, and to functionally characterize T and B cell subpopulations using maturation, activation and coinhibitory markers.

Patients/Methods: Samples from a 2-year-old girl with COVID-19, as well as an age- and gender-matched healthy control were analyzed by flow cytometry. Manual gating was used to identify defined subpopulations of T, B and innate immune cells. Furthermore, Uniform Manifold Approximation and Projection (UMAP) was used for unbiased clustering analysis, as well as to distinguish between individual expression patterns of the children.

Results: Both panels performed well in manual gating and in the unbiased UMAP analysis. Manual gating identified both developmental (Tcm, Tem, Ttm, Trm) and functional (Treg, Tfh, Th1, Th17) subsets in both children, and even small subpopulations of B and innate cells, such as plasmablasts and ILC subclasses 1-3. Preliminary unbiased analysis of the panels comparing both children revealed a less pronounced cell density in the CD4+ naïve population, a larger CD8+ effector and memory population accompanied by an increased expression of the co-inhibitory receptors TIGIT and HLA-DR. In addition, we observed a shift from naïve towards mature B-cells and a more pronounced monocyte and NK CD56dim population in the infected child compared to the healthy control.

Conclusion: The approach is suitable to analyze immunologic phenotypes in pediatric cryopreserved samples and could be adapted and standardized for large multi-center studies of pediatric SARS-CoV-2 infection. The rarity of symptomatic SARS-CoV-2 infection of children, the rare occurrence of Multisystem Inflammatory Syndrome in Children (MIS-C) and the appearance of new genetic virus variants necessitate an international, multi-center approach when designing such studies.

Lymphocyte cytotoxicity is needed for efficient control of rubella vaccine virus

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Background: Perforin-mediated cytotoxicity is considered important for antiviral defense. However, for many viruses, it remains unclear whether cytotoxicity is required for their elimination. Rubella virus-induced granulomas have been described in patients with T cell deficiencies, but the molecular mechanism of virus control remains elusive.

Objective: To understand the functional basis of rubella vaccine virus persistence in granulomas.

Methods: Starting from an index case with Griscelli syndrome and rubella skin granulomas, we combined an international survey with a literature search to identify patients with cytotoxicity defects and granuloma. We performed rubella virus immunohistochemistry and PCR and T cell migration assays.

Results: We identified 21 patients with various genetically confirmed cytotoxicity defects, who presented with skin and visceral granulomas. Rubella virus was demonstrated in all 12 accessible biopsies. Granuloma onset was typically before age 2 and lesions persisted from months to years. Granulomas were particularly frequent in MUNC13-4 and RAB27A deficiency, where 50% of patients at risk were affected. Although these genes have also been implicated in lymphocyte migration, 3D migration assays revealed no evidence of impaired migration of patient T cells. Notably, patients showed no evidence of reduced control of concomitantly given measles, mumps or varicella live vaccine or severe infections with other viruses.

Conclusions: We identify lymphocyte cytotoxicity as crucial for control of rubella vaccine virus, without evidence for its need in control of live measles, mumps or varicella vaccines. Cytotoxicity may be less broadly needed for control of viral infections than generally assumed.

Large deletion of the complete CYBB gene and flanking regions in x-linked CGD

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Background: CGD is an x-chromosomal or autosomal-recessive inherited disease characterized by a defect of phagocytes in killing ingested pathogens. Patients affected by CGD suffer from serious bacterial and fungal infections, abscesses, and granulomatous lesions. Detection of the underlying genetic defect is performed by molecular genetic analyses. Large deletions are common among the x-chromosomally inherited *CYBB* gene, which is the most frequently affected gene in CGD. Identification of exact breaking points of large deletions cannot be resolved by standard routine analysis. We established a genetic approach to detect exact breaking points of a large deletion in the CYBB gene enabling rapid genetic testing. The genetic approach presented here delivers significant improvement in genetic testing of CGD patients with large genomic deletions which benefit from prompt diagnosis and early treatment.

Patients/Methods: Samples from a cohort of 96 families with suspicion of CGD were referred to our center between 2010 and 2021 for genetic analysis. We established primers for exon amplification of the *CYBB* gene. For analysis of the 5` and 3` flanking region of the CYBB gene we performed primer walking followed by numerous single PCR steps and as final diagnostic proof a breakpoint-specific PCR product. Purified PCR products were sequenced by a Big Dye Terminator v.1.1 Cycle Sequencing Kit on an Applied Biosystems Prism 3100 Genetic Analyzer.

Results: In our CGD cohort most genetic defects were detected in the *CYBB* gene. In three patients of our CGD cohort we were not able to receive a PCR product concerning exon amplification of the *CYBB* gene. By improving the genetic analysis for one of these patients we were able to unravel a 126kb deletion encompassing the complete *CYBB* and *DYNLT3* genes as well as large parts of the *XK* gene. The patient not only suffered from CGD but also from a McLeod phenotype due to the deletion of the *XK* gene.

Conclusion: In some cases standard genetic testing is insufficient and may lead to failure of genetic confirmation. We present a genetic approach enabling the identification of exact breaking points of large deletions providing faster diagnosis and treatment.

Pityriasis versicolor (Malassezia spec.) as a manifestation of homozygous CARD9 mutation in a Caucasian male

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Background: Autosomal recessive CARD9 deficiency is associated with an increased susceptibility to fungal diseases. Less than half of the patients with autosomal recessive CARD9 deficiency display chronic mucocutaneous candidiasis, they appear more vulnerable to invasive fungal diseases. Despite phenotypical and clinical variability, almost all reported patients suffered from fungal disease caused by phylum Ascomycota. This narrow spectrum of pathogens suggests that *CARD9* appears to be a highly redundant gene.

Results: We present a Caucasian male with a homozygous mutation private variant affecting an essential splice site (c.1434+1G>C) of *CARD9.* He shows normal blood cell count with a normal differentiation of leucocytes, no abnormalities in $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD20^+$, $IgD^-/CD27^+$, $CD14^+$, $CD16^+/56^+/CD3^-$ proportions, normal immunoglobulins (IgG, IgA, IgM, IgE) levels, and normal phagocytic activity (dihydroxyrhodamin expression assay). Functional T-cell studies revealed a comparable IFN-γ but a reduced IL-17 production by patient' $CD4^+$ T cells, as compared to controls. STAT1 phosphorylation in monocytes was slightly elevated in comparison to healthy control' cells. The patient has a chronic (> 6 years) history of pityriasis versicolor of skin, in addition to chronic mucocutaneous *Candida* infection since early childhood. Pityriasis versicolor (*Malassezia species*) belongs to basidiomycete fungi.

Conclusion: To our knowledge, this is the first case with AR CARD9 deficiency and Pityriasis versicolor. The functional characterization of the variant is ongoing. Infection with fungi of basidiomycetes might also occur in patients with CARD9 deficiency, broadening the clinical impact of CARD9 deficiency.

Vaccine-associated Rotavirus Pneumonia in an Infant with Severe Combined Immunodeficiency (SCID)

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Background: Initially considered to be a localized infection confined to the GI tract, rotavirus (RV) disease is now recognized to cause systemic infection also affecting the respiratory tract in immunocompetent children. In infants with SCID, all live vaccines including RV are contraindicated due to life-threatening vaccine-related diseases.

Case report: Born to non-consanguineous German parents, a 2.5 months old girl developed cough and tachypnea two days following RV vaccination. Chest X-ray was consistent with pneumonia, but body temperature, CBC, and CrP proved normal, and screenings for common respiratory viral or bacterial infections were negative. Despite multiple lines of antibiotic treatments, the patient required non-invasive ventilation. Further immunological diagnostics were performed at the age of 4 months, showing a slight reduction of IgG, absent IgA, normal IgM, and, strikingly, a complete lack of T-cells with elevated B- and NK-cells explaining the normal absolute lymphocyte count and pointing to the diagnosis SCID. The child was assigned to our center and we identified a pathogenic homozygous c.202C>T p.(Arg68*) variant in the *CD3D* gene encoding for a T-cell receptor subunit CD3delta. The SCID-screening test performed retrospectively with the original newborn screening sample was highly positive with 0/µl TREC. Further infectiological work-up was initiated and the RV vaccine strain was detected in high concentrations in stool, serum, and in the respiratory tract. The patient underwent HSCT successfully from an HLA-matched unrelated donor. RV ceased to be detectable at day 42 post-HSCT.

Review: Literature research showed 8 patients with SCID due to CD3delta deficiency, but none with RV complications. However, a total of 23 cases of systemic RV infections following vaccinations in other forms of SCID had been reported, but none of them suffered from pulmonary RV complications.

Conclusions:

1. In infants with SCID, live vaccines including RV vaccine can cause life-threatening complications.
2. Pneumonia is among the systemic manifestations of RV vaccine-acquired diseases in immunocompromised patients.
3. The recently introduced SCID screening would have prevented the administration of RV live-vaccine in this patient.
4. As SCID screening does not exclude all forms of SCID, vigorous immunological investigations should be performed in infants with unusual pneumonia.

Mild COVID-19 despite neutralizing autoantibodies against type I IFNs in autoimmune polyendocrine syndrome Type 1 (APS-1)

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Patients with autoimmune polyendocrine syndrome Type 1 (APS-1) caused by biallelic mutations in the gene coding for the autoimmune regulator (*AIRE*) show autoimmunity against endocrine and non-endocrine tissues as well as cytokines.

High titers of autoantibodies against Interferon-α (IFN-α) and IFN-ω are common in almost every patient with APS-1. Autoantibodies to IFN-α and IFN-ω are regarded as causative for severe coronavirus disease-19 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) (Bastard, Rosen et al, Science 2020).

We here describe six patients with APS-1 in whom we isolated high titers of neutralizing autoantibodies to IFN-α and IFN-ω. Four out of these six patients were infected with SARS-CoV-2, yet developed only mild COVID19. None of the patient developed dyspnoe or high temperature.

All patients with APS-1 and neutralizing autoantibodies against type I Interferons who developed only mild COVID19 were females and younger than 26 years of age.

So, autoantibodies to IFN-α and IFN-ω may particularly predispose to severe COVID19 in males older than 65 years of age, but do not show complete penetrance for severe COVID19 in young patients with APS-1.

RhoG deficiency: a novel type of familial HLH deciphers a key role of RhoG in granule exocytosis

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by immune dysregulation with massive, aberrant hyperactivation of cytotoxic T cells and macrophages. The monogenic or primary form of HLH is caused by mutations selectively disrupting perforin-mediated cytotoxicity in human lymphocytes. The genetic etiology and molecular pathology underlying the disease are not always defined.

Patient/Methods: We studied a patient with early-onset severe HLH who fulfilled all diagnostic criteria, including the almost absent killing activity of NK cells. Targeted NGS-based panel sequencing did not reveal any germline mutations in established HLH-associated genes. Using exome sequencing and genome-wide SNP array, we identified biallelic germline mutations in *RHOG*.

Results: Genetic ablation of the *RHOG* gene in a model cell line and primary cytotoxic T lymphocytes (CTLs) from healthy individuals confirmed its crucial role in lymphocyte cytotoxicity. Despite the severe defect in exocytosis, RhoG-deficient lymphocytes showed normal activation, proliferation, and cytokine production.

To investigate the molecular pathomechanism of the RhoG deficiency, we performed interaction proteomics analysis and defined the molecular partners of RhoG in human lymphocytes. This analysis revealed a direct link of RhoG with the exocytosis machinery. Hence, we discovered that RhoG interacts with exocytosis regulator Munc13-4 and mediates docking of Munc13-4-positive cytotoxic granules (CG) to the plasma membrane. This step is required for subsequent fusion of the membranes to release cytolytic cargo toward target cells. Furthermore, we showed that RhoG is essential for the function of hematopoietic Munc13-4, assisting it in binding to the membrane phospholipids. We confirmed that this requirement for the RhoG assistance is unique for Munc13-4, which lacks the C1 membrane-binding domain present in other Munc13 isoforms.

Conclusions: Collectively, our work i) discovers a novel Mendelian disease affecting human immune function and homeostasis, potentially representing familial HLH type 6; ii) defines a molecular pathomechanism of the discovered disorder; iii) identifies a novel layer of exocytosis regulation unique for cytotoxic lymphocytes.

The immune deficiency and dysregulation activity (IDDA) and other clinical scores for inborn errors of immunity

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Background*:* Standardized scores aim to facilitate making a diagnosis, to classify a disorder, and to objectify the disease activity or severity of inborn errors of immunity (IEI). Systematic detection of a set of clinical and laboratory parameters may assist the estimation of the severity of an IEI at its first presentation and during follow-up (intra-individual longitudinal monitoring). Furthermore, it may allow inter-individual (cross-sectional) comparisons, quantification of the effect of treatment, and potentially, even be taken into account for evaluations for transplantation.

Methods*:* Literature search, descriptive comparison of retrieved scores in IEI, and statistical validation of the IDDA score.

Results*:* Most scores or measures applied in IEI are strictly disease-specific. We identified ten different scores, which may be divided into at least three "categories", with variable overlap: 1) *diagnostic* (Hyper-IgE syndrome [HIES] score; H score [hemophagocytosis]), 2) *severity/classification* (Wiskott Aldrich syndrome [WAS] score; common variable immune deficiency [CVID] score), 3) *morbidity and disease activity* (profound combined immune deficiency [P-CID] measure; CTLA-4 haploinsufficiency with autoimmune infiltration [CHAI] morbidity measure; immune deficiency and dysregulation activity [IDDA] score; immune dysregulation, polyendocrinopathy, enteropathy, X-linked [IPEX] organ impairment score; ataxia teleangiectasia [A.T.] score; and the autoinflammatory disease activity index [AIDAI]). Main characteristics of these scores are presented and compared.

Conclusions and discussion*:* Current clinical scores used in IEI are rather heterogenous, some being mere diagnostic assistive tools, others were established for retrospective cohort evaluations or for prospective studies. There are many disease-inherent and user-dependent factors that complicate a broader, standardized use of scores in IEI. For instance, a variable penetrance and inconsistent genotype-phenotype correlations challenge disease-classifying or diagnostic scores. The depth of preclinical and clinical statistical validations varies among the presented tools. Nevertheless, to improve longitudinal documentation of the burden of patients with IEI, their treatment effects, and to allow cross-sectional comparisons between patients and cohorts, a comparable quantification of disease activity should be striven for. For diseases with immune dysregulation, we propose and offer the new version (2.1) of the IDDA "kaleidoscope" score (22 parameters, on a 2-5-step scale) within the ESID registry as optional, simple, but detailed tool for prospective monitoring.

Radiosensitive Omenn Syndrome caused by a Somatic Reversion of a Novel Inborn *DCLRE1C* (ARTEMIS) Variant

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Background: We analysed a patient with typical clinical signs of Omenn syndrome with erythrodermia, diarrhoea, alopecia, lymphadenopathy, hepatosplenomegaly, eosinophilia, elevated levels of IgE and the oligoclonal expansion of autoreactive T cells. We concentrated on the V(D)J-recombination pathway as Omenn Syndrome has been described on the genetic basis of hypomorphic genetic variants in e.g. RAG1/2, LIG4 or ARTEMIS.

Methods: To determine disease causing genetic variants whole exome sequencing was performed using DNA of patient's fibroblasts. Identified variants were confirmed by Sanger sequencing. The functions of the encoded variant proteins were tested in a V(D)J-recombination assay. The origin of the patient's peripheral T cells was specified by short tandem repeat analysis and their clonality was validated by high-throuput next generation sequencing.

Results: The patient's autologous T cells were exclusively of alpha/beta type and showed a restricted T-cell receptor (TCR) repertoire. Genetic screening of the patient's fibroblasts identified two novel compound heterozygous variants in the *DCLRE1C* gene. Both variants did not exhibit residual V(D)J-recombination activity. Sanger sequencing of the patient's T cells revealed a somatic reversion of one variant to the wild-type sequence.

Conclusions: Here we describe a patient with radiosensitive Omenn Syndrome presenting with typical symptoms and an extraordinary high T-cell count. He carries two novel compound heterozygous variants in the *DCLRE1C* gene; one of them is reverted to wild type in the patient's T cells.

CARD11 GOF results in heterogeneous phenotypes that are associated with autoreactive/oncogenic BCR repertoires

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Background: Caspase recruitment domain family member 11 (CARD11) gain-of-function (GOF) variants cause B-cell expansion with NF-κB and T-cell anergy (BENTA) disease.

Objective: We sought to delineate the genetic, pathophysiologic, immune phenotypic and clinical properties of a CARD11 GOF patient cohort.

Results: We report 13 patients from 6 families with 3 CARD11 GOF variants (p.F97Y, p.G126D, p.L251P). All patients had recurrent upper and lower respiratory tract infections, dysgammaglobulinemia and polyclonal B-cell proliferation associated with disturbed peripheral B-cell differentiation. Three patients developed hemophagocytic lymphohistiocytosis (HLH) requiring allogeneic hematopoietic cell transplantation, 1 patient developed acute autoimmune liver failure (AALF) requiring liver transplantation, and 1 patient developed follicular B-cell non-Hodgkin lymphoma requiring chemotherapy. All CARD11 GOF variants led to constitutive activity of the CARD11:BCL10:MALT1-complex and canonical NF-κB signaling in B-cells. B-cell antigen receptor (BCR) deep sequencing identified autoreactive BCR repertoires with predominant IGHV4-34 and IGLV2-18 usage that recognize the N-acetyl-lactosamine epitopes present on both various self-antigens (I/i blood group antigen, B-cell isoform of CD45) and microbial pathogens (Epstein-Barr virus, cytomegalovirus, Mycoplasma pneumoniae). These particular BCRs are also found in diffuse large B-cell lymphoma (DBCL) in conjunction with somatic activating variants of NF-κB signaling molecules and named oncogenic BCRs.

Conclusions: CARD11 GOF variants activate canonical NF-κB signaling predominantly in B-cells and lead to defective B-cell differentiation favoring the selection of autoreactive/oncogenic BCR such as IGHV4-34. The presumable second hits that lead to immune dysregulation such as HLH, AALF and lymphoma/leukemia are object of ongoing investigation.

Gain-of-function variants in SYK cause immune dysregulation, systemic inflammation and lymphoma in humans

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Background**:** Predisposition to malignancy and inflammatory disease can be caused by monogenic inborn defects of immunity. Understanding rare genetic defects that present with cancer susceptibility informs precision medicine diagnostic, therapeutic and screening approaches.

Methods: Whole-Exome Sequencing (WES) was carried out in six patients who presented with intestinal, skin, joint inflammation and lymphoma. Additional functional in vivo approaches in humans and mice and in vitro experiments were used to characterize the immunopathology.

Results**:** We were referred a 31-year-old female patient with hypogammaglobulinemia, recurrent infections and steroid refractory spinal inflammation. WES of the patient revealed a rare, *de novo* monoallelic p. P342T variant in the Spleen Tyrosine Kinase (SYK) gene. Subsequently, together with our consortium partners we identified additional 4 novel monoallelic mutations in the SYK gene (p.A353T, p.M450I, p.S550F, p.S550Y) all of which resulted in gain-of function (GOF) with increased phosphorylation of SYK. Main clinical manifestations of patients were intestinal (6/6), skin (5/6), joint (2/6) and CNS (2/6) inflammation, recurrent infections (4/6) and hypogammaglobinemia, while comparatively early onset diffuse large B-cell lymphoma (49yr, 51yr) was present in 2/4 adult SYK GOF patients. The germline p.A353T variant identified in one lymphoma affected patient is the most common coding somatic SYK variant in the Catalogue Of Somatic Mutations In Cancer database and observed in multiple large intestine, liver and prostate cancer samples. A knock-in mouse model of the human p.S550Y homologue was generated and recapitulated aspects of patient's immune dysregulation including spontaneous joint inflammation and hypogammaglobulinemia.

Conclusion: Our study suggests that SYK GOF variants result in a novel autosomal dominant syndrome of immune dysregulation and lymphoma predisposition and points towards the critical role of SYK in immunopathology.

Variable immunodeficiency with dermatitis: *CARD11*LOF over three generations

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Background: Atopic dermatitis (AD) affects up to 25% of children and 10% of adults in Western countries[1]. When severe or recurrent infections and highly elevated serum IgE levels occur in AD patients, an inborn error of immunity (IEI) may be suspected. The IUIS classification lists mutations in nine different genes for the so-called Hyper IgE syndromes[2,3]. Diagnosing the underlying IEI may influence treatment strategies.

Methods: We report on an 18-year old patient with a long-standing history of infections due to hypogammaglobulinaemia, intermittent agranulocytosis, atopy with elevated IgE, eosinophilia and colitis. The working diagnosis of common variable immunodeficiency was revised when a heterozygous hypomorphic *CARD11* mutation [c.223C>T; p.(Arg75Trp)] was identified. Investigation of the family led to discovery of five other family members affected by severe atopy associated with the above mutation.

Results: Apart from the index patient, recurrent infections or hypogammaglobulinaemia are absent from all affected family members. However, they were found to have eosinophilia and elevated IgE levels and an increased CD4/8-ratio. T-cell proliferation is decreased to mitogens and specific antigens. Interestingly, solid tumors occurred in two generations. A cousin is under treatment with dupilumab, which much improved his severe eczema.

Conclusion: Heterozygous *CARD11*-deficiency was first described by Ma et al. in 2017[4]. Partial T-cell deficiency as well as diminished IFN-γ cytokine production were identified as disease causing mechanisms. The phenotypic spectrum of this PID is broad[5]. However, solid tumors have never been reported. Treatment with dupilumab, a monoclonal IL-4- and IL-13-antibody may be of benefit in some patients, an approach successful in classical AD Hyper-IgE-syndrome (*HIES*) due to *STAT3*LOF mutations[6].

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Allogeneic HSCT for adolescents and adults with inborn errors of immunity: A retrospective single center analysis

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Background: We observe an increased age of CID patients undergoing HSCT (figure). The needs of these adolescents and young adults (AYAs) with inborn errors of immunity differ from young children's needs, medically and psychosocially. In adult BMT these needs are not met adequately whereas in pediatric teams the setting is also not ideal. Due to their prolonged course of illness AYAs with IEI have experienced more complications prior to transplant.

Methods: We present a retrospective single center analysis of adolescents and adults >16 years of age who underwent HSCT for an underlying IEI from 1.1.2000 to 15.10.2020.

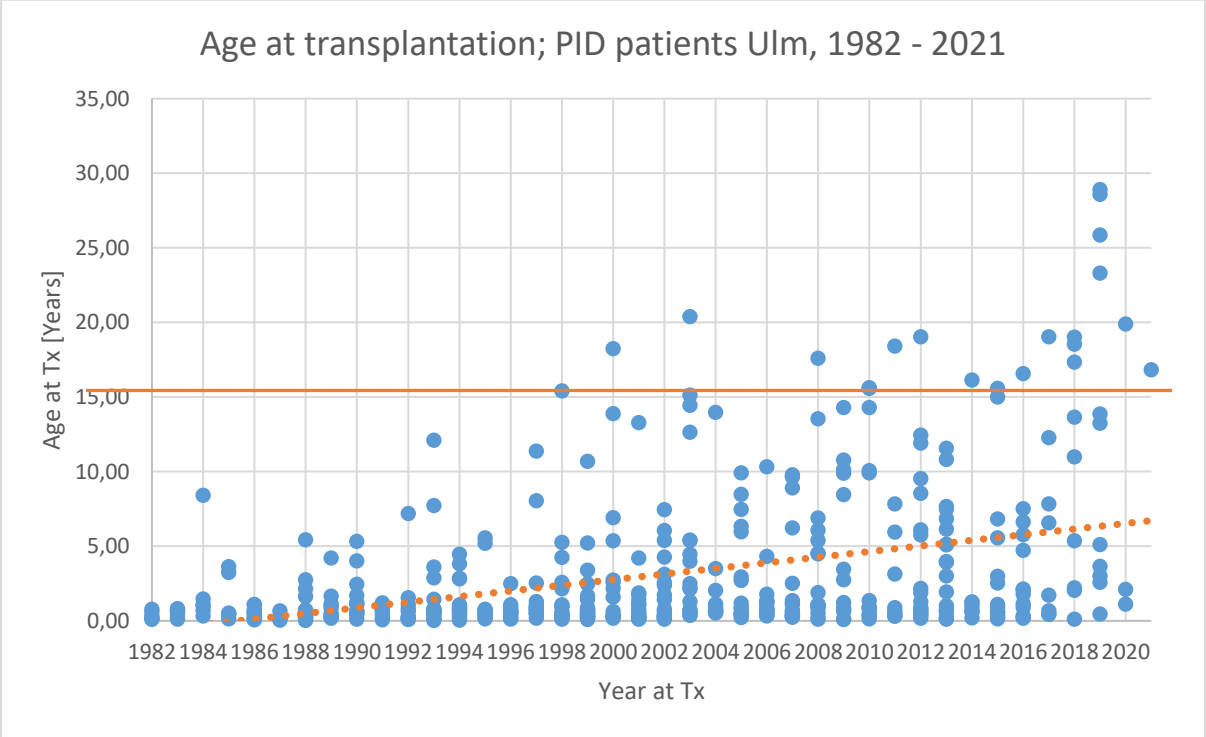
Results: 18 patients were included in this analysis. In this cohort 4 patients with SCID who had undergone HSCT as infants received a second transplantation as adolescents due to poor T cell function. All the other patients received their first transplantation above the age of 16 years. The underlying conditions were CGD (n=6), AR-HIES (n=3), APDS (n=2), IL10-receptor deficiency (n=1), ADA2 deficiency (n=1) and genetically undefined CVID (n=1). The median age at transplant was 19.5 years (range: 17.3-28.9) with a median follow-up of 17.3 months (range: 3.4-110.4). The main source for stem cells was unmanipulated bone marrow (n=12, 67%) whereas PBSC was used in only 33%. In the group of patients that received PBSC two had CD34+ selected stem cells. Donors were matched-unrelated (n=9), matched family donors (n=6) or haploidentical donors (n=3). Conditioning regimens were mainly with reduced intensity based on treosulfan±fludarabine±thiotepa (n=8) or targeted-busulfan/fludarabine (n=5). Two patients received busulfan-cyclophosphamide, one patient had RIT, one patient had post-transplant cyclophosphamide and one patient received serotherapy only. All patients except two with MSD received serotherapy with ATG or alemtuzumab.

The overall survival was excellent with 89% and only two patients died of transplant-related mortality. Complications were mainly related to toxicity, e.g. renal impairment, infections with viral reactivations or bacterial colonization or due to the underlying condition, e.g. stomata and fistulas.

Conclusions: The overall survival of AYAs with inborn errors of immunity is similar compared to young children. The risk of infection is higher because of viral reactivation and bacterial colonisation. There is more toxicity compared to younger children probably due to previous

therapies. Long-term toxicity such as fertility and the psychosocial impact need to be considered as well.

Figure:



Allogeneic HSCT for adolescents and adults with inborn errors of immunity: A retrospective study of the Inborn Errors Working Party (IEWP)

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Background: Recent single center publications from specialist centers have demonstrated that HSCT can be safe and effective for adolescents and adults with inborn errors of immunity (IEI) [1,2]. There remains an urgent need, however, to identify which adult patients are most likely to benefit from HSCT and the optimal timing of transplant.

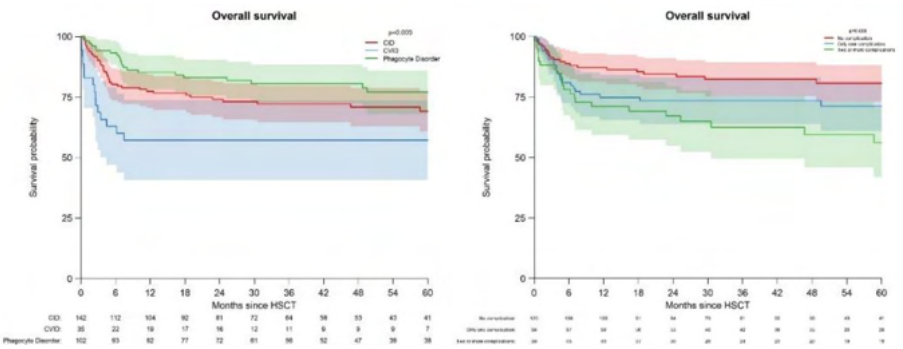
Methods: We present an EBMT IEWP retrospective multicenter study of adolescents and adults ≥15 years of age who underwent HSCT for an underlying IEI from 1.1.2000 to 31.12.2018. The Kaplan-Meier method and the log rank test were applied to assess probabilities of overall survival (OS) and the differences between risk groups. IEI diagnoses were classified as per 2019 IUIS classification[3].

Results: 283 patients were included in this analysis. The most common forms of IEI were combined immune deficiency (CID) including disorders of immune dysregulation (n=142), phagocyte disorders (n=102) and common variable immunodeficiency (CVID; n=35). The median age at transplant was 18 years (range: 15-62) with a median follow-up of 50.8 months (95%CI: 44.3-59.6). Stem cell sources were peripheral blood (n=147) or bone marrow (n=135)

with one cord blood. Donors were unrelated (10/10 MUD, n=153; 9/10 MMUD, n=43; <9/10 MMUD, n=9; UCB, n=1; total unrelated n=206), matched family donors (n=63), haploidentical donors (n=9), or unknown (n=5). Conditioning intensity was predominantly reduced intensity (n=183 reduced toxicity and n=21 non-myeloablative), but 70 patients received myeloablative conditioning, and 9 had unknown conditioning intensity. We examined various IEI-related complications present at the time of transplant for impact on OS, including bronchiectasis, granulomatous lymphocytic inflammatory lung disease (GLILD), colitis, protracted diarrhea, body mass index (BMI), prior splenectomy, and total number of IEI-associated complications. The 2-year OS for the whole cohort was 75% (95%CI: 70-80%). Superior OS at 2 years was achieved in patients with phagocyte disorders (83%, 76-90%) and CID (74%, 67-81%) compared to those with CVID (57%, 41-74%), respectively (log-rank test p=0.003; figure). Neither age, donor type, conditioning intensity, BMI, nor presence of colitis, protracted diarrhea, GLILD or bronchiectasis at HSCT had a significant impact on OS, but the cumulative number of IEI-related complications at the time of HSCT did. OS was 84% (78-91%), 73% (64-83%), and 67% (55-79%), respectively for patients with no complications, only one complication, or $\geq$ 2 complications (p=0.006; figure). Prior splenectomy (60% vs 78%, p=0.003) also adversely affected OS.

Conclusion: This is by far the largest study performed to date examining the outcome following HSCT for adolescents and adults with IEI. OS of this cohort was independent of donor source and conditioning intensity, and very good outcomes were observed for patients with CID and phagocyte disorders. We demonstrate that prior splenectomy and the number of IEI-associated complications at HSCT, but not patient age, adversely affect OS. Data from this study is expected to provide essential guidance for the use of HSCT in adolescent and adult IEI patients.

Figure:



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