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How to recognize inborn errors of immunity in a child presenting with a malignancy: guidelines for the pediatric hemato-oncologist

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ABSTRACT

Inborn errors of immunity (IEI) are a group of disorders caused by genetically determined defects in the immune system, leading to infections, autoimmunity, autoinflammation and an increased risk of malignancy. In some cases, a malignancy might be the first sign of an underlying IEI. As therapeutic strategies might be different in these patients, recognition of the underlying IEI by the pediatric hemato-oncologist is important. This article, written by a group of experts in pediatric immunology, hemato-oncology, pathology and genetics, aims to provide guidelines for pediatric hemato-oncologists on how to recognize a possible underlying IEI and what diagnostic tests can be performed, and gives some consideration to treatment possibilities.

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Introduction

Inborn errors of immunity (IEI), are a group of disorders caused by genetic defects in the immune system, leading to infections, autoimmunity, autoinflammation and an

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increased risk of malignancy.¹⁻¹⁰ A majority of those malignancies are of lymphoid origin, but other malignancies occur as well.¹⁻⁸ Over 400 different IEI were described, a number likely to increase.¹⁰ The incidence of IEI is estimated to be approximately 1 in 1200,¹¹ meaning that pediatric hemato-oncologists (PHO) are very likely to be confronted with malignancies in children with IEI. In many cases, the IEI diagnosis has already been established before tumor onset, but in some children, the malignancy can be the presenting feature of their underlying IEI, as important subgroup of patients is un- or misdiagnosed because of the variable phenotype and lack of awareness among physicians.¹²⁻¹⁴

Malignancy is a leading cause of mortality in some groups of patients with IEI, with the relative risk from disease-related and treatment-related mortality varying between subtypes. Consequently, defining uniform management guidelines remains challenging.^{15,16} Similar to pediatric patients with other cancer predisposition syndromes, such as trisomy 21, some children with an underlying IEI experience increased toxicity and/or decreased efficacy of standard cancer treatment but this remains difficult to predict, even within a single IEI subtype but some children with co-existent IEI and malignancy might benefit from dose modifications of chemotherapy, avoidance of radiotherapy, increased use of antimicrobial prophylaxis, or immunoglobulin replacement therapy. Conversely, for some patient groups, cancer recurrence poses the greatest threat and dose reductions may not be uniformly desirable.¹⁶ The role of early hematopoietic stem cell transplantation (HSCT) should be considered in some patients, both to achieve malignant disease control and treat the underlying IEI. However, before any of these treatment modifications can be implemented, the diagnosis of IEI has to have been considered (Table 1).

Guidelines have been proposed to improve diagnosis of genetic predisposition syndromes in cancer patients.¹⁷⁻²⁰ These guidelines do not include specific features of immunodeficiency/immunodysregulation. On the other hand, the warning signs developed by immunologists to help identify patients with a possible IEI do not fully integrate the importance of malignancies.²¹ We have brought together pediatric hematologists/oncologists, immunologists, diagnosticians and geneticists to create a shared approach to the identification of IEI in children presenting with malignancy. We aim to inform the pediatric hemato-oncologist about the necessity to think about an underlying IEI, review the differing risks of malignancy according to IEI subtypes and propose warning signs that can be used to identify pediatric cancer patients who

Table 1. Impact of IEI on the malignancy diagnosis, treatment and prognoses.

-
- Predisposition to malignancy (impaired cancer immunosurveillance and cancer immunoediting)/increased relapse and second malignancy risk
 - Increased incidence of rare and/or more aggressive malignancy in certain age groups
 - Increased chemotherapy toxicity
 - Increased morbidity and mortality based on underlying IEI
 - Indicated avoidance of radiotherapy in certain diagnostic groups (DSB group)
 - Risk of severe infection during cancer treatment, based on underlying IEI (CID v predominantly antibody deficiencies)
 - Possible prolonged recovery of hematological parameters after chemotherapy
 - IEI-based end organ damage (eg, bronchiectasis/granulomatous inflammation)
 - IEI-based autoimmunity (eg, cytopenias, inflammatory bowel disease)
 - IEI-based immunodysregulation
-

should be investigated for a possible IEL. We discuss the most common tests used in the work up of an IEL patient and their value in the setting of a newly diagnosed lymphoid malignancy.

Primary immunodeficiencies

The latest classification distinguishes 10 IEL categories: I - combined immunodeficiencies (CID); II - CID with associated or syndromic features; III - predominantly antibody deficiencies (PAD); IV - diseases of immune dysregulation; V - congenital defects of phagocytes; VI - defects in intrinsic and innate immunity; VII - autoinflammatory diseases; VIII - complement deficiencies; IX - bone marrow failure; X - phenocopies of inborn errors of immunity.¹⁰ The incidence of cancer varies between and within these categories of IEL.²¹ The prevalence of specific conditions also differs greatly and is influenced by ethnic background and rate of consanguinity but exact epidemiological data of these diseases are not available. There is substantial variation in the risk of malignancy among the various IEL.²² The majority of IEL-associated malignancies is recognized in children with a diagnosis of a combined immunodeficiency (CID) (groups I and II) or an immune dysregulation disorder particularly those associated with susceptibility to EBV and/or with lymphoproliferation (group IV). Group III is the most common IEL and malignancies in these patients manifest during adult age but also in childhood. Groups V, VI, IX are also related to an increased risk of cancer, in contrast to group VII and VIII.

Severe combined immunodeficiency (SCID), included within groups I and II and characterized by absent or severely diminished T cells, B cells and/or NK cells, is not commonly associated with malignancy as the disease is fatal without early HSCT. In contrast, patients with combined immunodeficiencies (CID), such as CD40L deficiency or cartilage hair hypoplasia, have a less severe immunological phenotype than SCID and have a substantially increased risk of developing malignancy.^{23,24} DNA repair disorders (ataxia telangiectasia (AT)) and Nijmegen breakage syndrome carry the greatest risk of malignancy with a cumulative incidence of malignancy of 22.6% by the early 20s in patients with AT and a crude incidence of 40% by the age of 20 years in patients with NBS.^{16, 25-28}

Immune dysregulation disorders (Group IV) are well known for their increased incidence of (especially EBV-associated) lymphoma. Examples include immune dysregulation disorders associated with lymphoproliferation such as autoimmune lymphoproliferative syndrome (ALPS) and CTLA4 deficiency, as well as disorders associated with susceptibility to EBV infections and hemophagocytic lymphohistiocytosis (HLH) such as X-linked lymphoproliferative disease (XLP), familial HLH syndromes, CD27 and CD70 deficiencies, and CTPS1 deficiency.²⁹⁻³¹ HLH itself usually manifests in childhood or adolescence. This disease, has many overlapping features with a spectrum of EBV-induced lymphoproliferative disorders and some genetic subtypes are associated with an increased risk of lymphoma.³²

PAD's (Group III) encompasses disorders with reduced or poorly functioning immunoglobulin as their main feature. Activated phosphoinositide 3-kinase delta syndrome (APDS), caused by mutations in *PIK3CD* (APDS1) or *PIK3R1* (APDS2), is classified

as a PAD but is also associated with features of immune dysregulation, lymphoid hyperplasia and lymphoma.³² Common variable immunodeficiency (CVID) is the most prevalent IEI. The diagnosis is made based on clinical criteria and the disease affects 0.6–3.8:100,000 children and adults.^{26,28,29,33} While the risk of malignancy in pediatric CVID patients is probably lower than in some other IEI disorders, there is a particular risk for the development of B-cell lymphomas such as extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas).^{34–36}

Congenital defects of phagocyte number or function (group V) such as severe congenital neutropenia (SCN), and GATA2 deficiency, as well as bone marrow failure disorders (group IX) such as those caused by SAMD9(L) mutations, are associated with an increased risk of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Severe congenital neutropenia is usually diagnosed at an early age and malignancy will not be the first symptom in these patients.^{37–40}

Defects in intrinsic and innate immunity (group VI) are, as a group, less commonly characterized by the occurrence of malignancies. However, certain subtypes of IEI within this group are associated with specific types of cancer, such as HPV-related skin cancer in patients with EVER1 and EVER2 deficiencies.⁴¹

Spectrum of malignancies in children with IEI

A diverse spectrum of lymphoid malignancies is seen in children with IEI including both precursor and peripheral malignancies of either B- or T-cell origin (Table 2) as recently summarized by Riaz et al.³² Most common are mature B-cell non-Hodgkin lymphomas.^{2,26} Notably, in contrast to sporadically presenting B-NHL, diffuse large B-cell lymphoma represents a substantially greater proportion of cases than Burkitt lymphoma. Another interesting feature is the relative abundance of rare lymphoma subtypes and destructive lymphoproliferative disorders falling short of lymphoma, with a recent study of 36 UK children with IEI referred for HSCT identifying 3 peripheral T cell lymphomas, 2 extranodal marginal zone lymphomas and 6 polymorphic lymphoproliferative disorders.³⁹ Recent EICNHL/BFM studies identified predisposing disorders in a high proportion of children diagnosed with peripheral T cell lymphomas (25%), extranodal marginal zone lymphomas (27%) and primary central nervous system lymphomas (19–29%), among which IEI were prevalent.^{42–44} Equally, some groups of IEI are associated with a higher prevalence of specific hematological malignancy subtypes including: NBS and T-cell leukemia/NHL, CVID and extranodal marginal zone lymphoma, and IL-2-inducible T cell kinase (ITK) deficiency and classic Hodgkin lymphoma.^{45–49} However, low patient numbers hamper the accurate association with specific lymphoid diagnoses in the majority of individual IEI cohorts. EBV-driven lymphoma is, as expected, more prevalent in children with underlying IEI.⁵⁰

As mentioned previously, the incidence of MDS and AML is increased in patients with phagocyte disorders (group V) and bone marrow failure disorders (group IX).⁵¹ Patients with severe congenital neutropenia (SCN) are particularly at risk for MDS and leukemia, mostly AML but also chronic myelomonocytic leukemia (CMML) and acute lymphoblastic leukemia (ALL). Carlsson et al estimated the cumulative incidence of MDS/leukemia in a Swedish cohort of SCN as high as 31%.³⁶

Table 2. Types of IEI to consider in a pediatric cancer patient.

Malignancy	IEI (according to IUIS classification, Tangye et al 2020)	Possible test	Possible outcome
ALL, non-Hodgkin lymphoma, classic Hodgkin lymphoma	<ul style="list-style-type: none"> - Hypomorphic SCID (I) - Combined immune deficiencies (CID) (I) - DNA repair disorders (I) - Primarily antibody disorders (III) - Disorders of immune dysregulation (IV) 	<ul style="list-style-type: none"> - Full blood count - Immunophenotyping - T cell function -NK cell function (CD107a release) - Immunoglobulin levels and subclasses - Radiosensitivity testing Molecular testing example (TREC's/Krec's) 	<ul style="list-style-type: none"> - Low neutrophils and/or lymphocytes - Low CD3, CD4 and or CD8 in CID or presence of double negative T cells (ALPS) and , low switched memory B cells (CVID) - Absent or diminished expression of specific proteins like WAS, CTLA4 <p>Diminished (SCID, CID) Diminished (XLP). Diminished (CVID, CID, DNARD) Diminished in DNARD Diminished (SCID, CID)</p>
MDS, AML	<ul style="list-style-type: none"> - Severe congenital neutropenia disorders - Shwachman-Diamond Syndrome - GATA2 deficiency - Bone marrow failure disorders 	<ul style="list-style-type: none"> - Full blood count 	<p>Low platelets, low red bloodcells (bone marrow failure) and/or neutrophils (SCN). Low monocytes (GATA2).</p>
Brain tumors Solid tumors	<ul style="list-style-type: none"> - DNA repair disorders - DNA repair disorders - Dyskeratosis congenita (telomeropathies) - PTEN deficiency (APDS-like syndrome) - CVID 	<ul style="list-style-type: none"> Radiosensitivity Full Blood counts Immunoglobulin levels Radiosensitivity 	<p>Diminished Low platelets, red bloodcells, Monocytes or neutrophils (Telomeropathies). Diminished (CVID, DNARD) Diminished (DNARD)</p>
EBV-associated smooth muscle tumors	<ul style="list-style-type: none"> - CID - Ataxia telangiectasia - GATA2 deficiency - RLTPR deficiency (<i>CARMIL2</i>) - ZAP70 deficiency 	<ul style="list-style-type: none"> Full Blood counts Immunophenotyping NK cell function (CD107 mobilisation) Immunoglobulin levels Full Blood counts Immunophenotyping 	<p>Low Monocytes (GATA2) Low lymphocytes (CID, AT) Low CD3, CD4 or CD8 cells (CID). Low CD8 cells (ZAP70 deficiency) Diminished Diminished Low Lymphocytes Low subsets (CD3, CD4 and or CD8, low switched memory B cells) Diminished</p>
Kaposi sarcoma	<ul style="list-style-type: none"> - Wiskott–Aldrich syndrome (<i>WAS</i>) - X-linked magnesium EBV and neoplasia (XMEN) (<i>MAGT1</i>) - IFN-γ receptor 1 deficiency (<i>IFNGR1</i>) - STIM1 deficiency - OX40 deficiency (<i>TNFRSF4</i>) 	<ul style="list-style-type: none"> Immunoglobulin levels Full Blood counts Immunophenotyping Immunoglobulin levels 	<p>Diminished Low Lymphocytes Low subsets (CD3, CD4 and or CD8, low switched memory B cells) Diminished</p>
Non-melanoma skin cancer	<ul style="list-style-type: none"> - EVER1 (<i>TMC6</i>) and EVER2 (<i>TMC8</i>) deficiencies - DOCK8 deficiency - Cartilage hair hypoplasia - Xeroderma pigmentosum 	<ul style="list-style-type: none"> Full Blood counts Immunoglobulin levels 	<p>Low neutrophils, (CHD), Elevated eosinophils (Dock8) High IgE in DOCK8</p>

Overview of the different malignancies, the different underlying IEI's and possible tests that can be performed by the pediatric hemato-oncologist. It should be stated that the different IEI's given in the table are examples and that the table is not complete. For all groups, genetic testing on germline DNA can be performed.

Data on brain and solid tumors in children with IEI are scarce. DNA repair disorders are the most notorious group of IEI with an increased risk of several childhood cancers including medulloblastoma, glioma, rhabdomyosarcoma, and osteosarcoma.⁵² CVID patients have a higher incidence of solid tumors, like gastric cancer especially in adults.⁹ Dyskeratosis congenital predisposes to various solid tumors, including EBV driven disorders.^{23,53} An increased incidence of non-melanoma skin cancers is seen in DOCK8, EVER1 and EVER2 deficiencies due to chronic cutaneous infections (eg, HPV), cartilage hair hypoplasia, and DNA repair disorders especially xeroderma pigmentosum and Rothmund Thomson syndrome.^{23,54–56} EBV-associated smooth muscle tumors are also seen in Ataxia Telangiectasia, and GATA2, CARMIL2 and ZAP70 deficiencies.⁸ Childhood Kaposi sarcoma, caused by human herpes virus-8 infection, is also quite unique to the setting of IEI and has been reported in patients with mutations in *WAS*, *IFNGR1*, *STIM1*, *MAGT1*, and *TNFRSF4*.⁵⁷

Clinical warning signs for the pediatric hemato-oncologist

While most IEI-associated lymphoproliferations/lymphoid malignancies affect children with known immunodeficiency, the identification of an underlying IEI in an apparently “sporadic” lymphoid malignancy can present a substantial diagnostic challenge. By definition, these children have not yet been referred for investigation of severe or recurrent infectious/immunological problems. Instead, careful review of the patient’s clinical history and physical examination combined with prudent use of routine laboratory investigations and pathological review can identify a group of patients who warrant additional investigation (Table 3).

Table 3. Warning signs for the pediatric hemato-oncologist.

Presenting history	Signs
Family history	<ol style="list-style-type: none"> 1. IEI case in the family 2. Multiple miscarriages 3. Unexplained infant death 4. Consanguinity
Past medical history	<ol style="list-style-type: none"> 5. Failure to thrive or unexplained diarrhea 6. Recurrent sinopulmonary infection (eg, pneumonia, sinusitis, otitis media) 7. Severe bacterial, viral or fungal infection (eg, septicemia, encephalitis, meningitis, osteomyelitis, mastoiditis) 8. Deep skin or inner organ abscesses 9. Persistent mucocutaneous fungal infection or deep mycotic infection 10. Multiple warts or mollusca of the skin 11. Severe eczema or dermatitis 12. Autoimmunity or multiple autoimmunity (eg, inflammatory bowel disease, type 1 diabetes mellitus, cytopenias, vasculitis) 13. Hepato/splenomegaly and/or benign lymphadenopathy 14. Recurrent parenteral antibiotic courses
Clinical features	<ol style="list-style-type: none"> 15. Microcephaly 16. Developmental delay 17. Cerebellar ataxia 18. Ocular or cutaneous telangiectasia 19. Short statue/osseous dysplasia 20. Facial dysmorphisms 21. Tonsil/lymph nodes atrophy 22. Bronchiectasis/granulomatous inflammation

One feature of pediatric cancer patients with IEI is the younger age at presentation compared with sporadic cases. National registry studies have shown a peak in excess childhood cancer diagnoses between 5 and 9 years of age, of which the majority are lymphoid malignancies.^{2,3} This younger age is further highlighted by the skew in histopathological diagnoses away from sporadic Burkitt lymphoma toward diffuse large B-cell lymphoma, classic Hodgkin lymphoma and marginal zone lymphomas, all of which pediatric oncologists would usually expect to see in the teenage and young adult population. Irrespective of age at presentation and diagnosis, routine clinical history in a child with known or suspected lymphoid malignancy should actively seek both a personal and family history of recurrent, persistent, severe and/or unusual infections, non-neoplastic lymphoproliferation, malignancy, autoimmunity, autoinflammation, or early childhood death in the family. Consanguinity should be documented as well.

Diagnosics

The suspicion of an underlying IEI in a patient presenting with malignancy warrants further investigation. The presence of an underlying IEI may have an important impact on the standard cancer therapy protocol, to reduce treatment-related toxicity (eg, dose modification of chemotherapy, avoidance of radiotherapy) or reduce the risk of relapse (eg, by performing HSCT after first remission).⁵⁸ We advise consulting an immunologist to assist with the diagnostic work-up. There are some pitfalls here. First, there usually is not much time. Especially in hematological malignancies, the oncologist may need to initiate treatment as soon as possible, leaving little time to perform further testing. Some tests, such as the evaluation of immunoglobulin levels or the presence of specific antibodies can be influenced by the chemotherapy as well as the supportive treatments such as blood transfusions. The recent implementation of immunotherapy in the standard of care of an increasing number of cancers will affect the immune system even more, sometimes leading to permanent secondary immunodeficiency. Hence it will be almost impossible to tell apart a primary (inborn) from a secondary (acquired) immunodeficiency.

Immunology

Diagnostic laboratory testing for IEI involves complete blood count (CBC), determination of serum immunoglobulin (Ig) classes and subclasses, evaluation of specific antibody response to polysaccharide and protein vaccine antigen, flow cytometry for extensive quantitation and phenotyping of T-, B- and NK-cell subsets, and various functional tests including lymphocyte proliferation assays, neutrophil function assays (eg, respiratory burst), cytokine secretion and/or intracellular cytokine production upon stimulation, NK cell cytotoxicity assay, assessment of cellular signaling pathways and protein expression analyses (Table 2).^{59,60} In patients with solid tumors, lymphocytopenia or neutropenia in the CBC could be an obvious warning sign. In patients with hematological malignancies, sometimes a parental blood sample can give additional information, for example by demonstrating monocytopenia in a relative of a GATA2

deficient proband. Lymphopenia should be further investigated by flow cytometry enabling detection and characterization of T-lymphopenia as seen in (S)CID and DNA repair disorders. Extended B-cell phenotyping is used in classification of CVID.⁶¹ Impaired NK cell function is a feature of HLH and EBV-related lymphoproliferation.⁶² Specific protein expression enables confirmation in certain IEI, like CD40L deficiency or IPEX.⁶³

To rule out antibody deficiency, measuring immunoglobulin levels including subclasses of IgG, is indispensable.^{59,60} The evaluation of specific antibodies such as iso-hemagglutinin and the titers of common postvaccination serum antibodies is crucial.⁶⁴ Specific postvaccination antibody responses to nonconjugated polysaccharide pneumococcal vaccine or *Salmonella enterica* subsp. Typhi Vi antigen capsular polysaccharide enables *in vivo* evaluation of specific antibody responses.⁵⁹ This test might be difficult at diagnosis. Finally, a variety of other tests can be helpful in well-selected cases, like the assessment of telomere length, neutrophil respiratory burst for chronic granulomatous disease (CGD), or *in vitro* evaluation of signaling pathways such as JAK-STAT or Toll like receptor pathways.

Radiosensitivity testing

Radiosensitivity assays have not yet found their way into a routine clinical setting, but can add important information if a DNA repair disorder is suspected. One option is to use the clonogenic cell survival assay which determines the ability of a cell to proliferate to form a large colony.⁶⁵ This test has already been used in the identification of several immunodeficiencies which are caused by double stranded DNA breaks such as Artemis deficiency.⁶⁶ Another approach is the use of the G2 micronucleus assay, which was used to identify Ataxia Telangiectasia in an atypical patient. This assay uses changes in the micronucleus following low doses of irradiation of peripheral white blood cells as a marker of increased DNA sensitivity.⁶⁷

Role of pathology

Often the first to diagnose a specific malignancy, pathologists have an early opportunity to consider the possibility of an underlying IEI in a child with cancer. This is facilitated by the examination of surgical biopsies. Lymphomas presenting in children with IEI are often histologically identical to those occurring sporadically, even if the age and tissue distribution of specific lymphoma types differs.⁶⁸ In contrast, some lymphoid neoplasms such as polymorphic lymphoproliferative disorders and EBV-associated mucocutaneous ulcers are highly associated with immunodeficiency, while the presence of certain morphological features in a common lymphoma type, such as polymorphic or Hodgkin-like features in a frank diffuse large B-cell lymphoma, may also suggest immunodeficiency.^{69–72} These lesions are typically EBV-positive, as are some immunodeficiency-associated marginal zone lymphomas and T/NK-cell lymphomas, and all pediatric lymphomas should be tested for EBV-association by *in situ* hybridization with positive findings correlated with peripheral blood EBV levels and serology.^{73,74} However, EBV-positivity alone is not sufficient to indicate an underlying IEI.

Any non-neoplastic lymphoid tissue sampled should be carefully examined for the abnormal constituent cell populations or altered lymphoid architecture described in several IEIs.^{75–78}

In contrast to the lymphoid neoplasms, histopathological features of carcinomas and other solid tumors that might suggest underlying IEI in children are poorly recognized.⁷⁹ Histopathologists must nevertheless be cognizant that cancer types rarely seen in children may be associated with IEI, especially when a specific link has been identified such as between squamous cell carcinoma and Fanconi anemia, or cholangiocarcinoma secondary to *Cryptosporidium*-induced sclerosing cholangitis in CD40L-deficient patients.^{80,81} Hematopathologists should be aware of morphological and immunophenotypic features that might suggest an atypical presentation of a bone marrow failure syndromes/phagocyte defect during diagnosis of AML/MDS, such as the bone marrow hypocellularity, prominent megakaryocytic dysplasia, and reduction in B-cells, NK cells, monocytes and hematogones characteristic of GATA2-deficient patients.⁸² If an IEI is suspected in a patient with a malignancy, the presence of EBV can be evaluated in the tumor and other relevant tissues and samples, especially peripheral blood. If underlying chronic EBV infection is suspected, immune phenotyping of B- and T-cells and NK-cell function should be performed before starting a therapy.

Molecular genetics

There have been very few studies on the genetic identification of preexisting IEI in pediatric cancer patients.^{26,37,83–86} Several studies have shown that about 10% of all childhood cancers are linked to an underlying germline mutation in a known cancer predisposition gene, but the contribution of germline mutations in IEI genes remains to be elucidated.^{83–85} Standard genetic testing is of very little added value here. Routine molecular diagnostic algorithms will only identify a small proportion of IEIs in cancer (Table 4). Chromothripsis might be a molecular indicator for an underlying Ataxia Telangiectasia but no other routine molecular diagnostic have been proposed as indicators for IEI in the current work-up for cancer.⁸⁷

High-throughput genetic sequencing technologies, like standardized next-generation sequencing (NGS) seem to be the future to screen germline DNA for a preexisting condition including IEI.¹⁵ Different NGS approaches are conceivable (Table 4). One common obstacle is the interpretation of variants with regard to their functional impact but understanding of this challenge is growing and will likely lead to improved computational approaches in due course. A cost-effective approach is the application of panel-sequencing to a defined set of targeted IEI genes. However, it needs to be taken into account that the number of newly identified IEI genes is still increasing, which makes it a challenge to keep a gene panel up to date and patient samples would need to be regularly re-sequenced if additional genes need to be analyzed. Applying WES and WGS overcomes these drawbacks since the analysis can be focused on a targeted set of IEI genes by applying *in silico* gene panels which can be easily adapted for new IEI genes. In combination with clinical data, additional analysis of the parents (trio-sequencing) can be beneficial for the interpretation of the inheritance and the functional impact of the variants.⁸⁸

Table 4. Variant detection methods.

Genetic method	Detects	Resolution	Pro	Con
Array CGH	DUP, DEL	≈0,07 Mbp	Routine diagnostic, cost efficient, available in many centers	Limited to copy number variation detection, does not detect most common IEI variants
SNP-array	DUP, DEL, CNN-LOH	≈0,05–0,1 Mbp	Routine diagnostic, cost efficient, available in many centers	Limited to copy number variation and LOH detection, does not detect most common IEI variants
Panel sequencing	SNV, indels	≈1 bp (SNV) ≈<20 bp indel	High throughput, cost-efficient, targeted panel	Limited set of genes that needs to be regularly updated and added to, no retrospective analyses of novel genes
Whole exome sequencing	SNV, indels	≈1 bp (SNV) ≈<20 bp indel	High throughput, covers most coding genes, in silico panels, retrospective analyses of newly identified genes possible	Limited to the coding genome, requires bioinformatics support
Whole genome sequencing	TRA (un/balanced), INV, DUP, DEL, SNV	≈1 bp (SNV) ≈0,01 Mbp SV	High throughput, covers most of the genome, in silico panels, retrospective analyses of newly identified genes possible	Requires bioinformatics support, available in few centers, large data storage capacities necessary
Sanger sequencing	SNV, indel	≈1 bp (SNV) ≈<40 bp (indel)	Routine diagnostic, cost efficient (if done for few specific variants), available in many centers	Low throughput

(bp, base pairs; CGH, comparative genomic hybridization; CNN-LOH, copy number neutral loss of heterozygosity; DEL, deletion; DUP, duplication; indel, insertion and deletion; INS, insertion; INV, inversion; SNV, single nucleotide variants; SV, structural variants; TRA, translocation.

Discussion

This article aims to support the PHO to recognize a possible underlying IEI in a child presenting with cancer. Along with a personal history of severe infections, a suggestive syndromic phenotype, or a family history of IEI, the type of cancer and age of the patient should be considered as warning signs. IEI patients have a high incidence of specific malignancies, especially leukemia and specific types of lymphoma at a relatively younger age. Another warning sign is an unexpected toxicity, such as prolonged bone marrow aplasia or lymphopenia which could be a sign of an underlying DNA repair disorder. Some baseline immunological tests are readily available to the PHO and can help. Measurement of immunoglobulin levels before the start of therapy is easy and cheap and will identify all patients with antibody deficiencies and sometimes other

IEIs. Lymphocyte subset analysis will give an idea of basic cellular immunological make-up and specific antibody testing an indication of immunological response. However results can be affected once therapy has started and it may be more difficult to tell the difference between an underlying primary immunodeficiency and a secondary immunodeficiency. In these cases, patients need to be monitored closely after cessation of therapy to see if immunological tests recover. Although there are not many data on that recovery after treatment for malignancy, stem cell transplantation excluded, this is believed to take up to 6 months or longer.⁸⁹ Ongoing studies will help to clarify the normal kinetics. Vaccination responses could be performed after the treatment is completed and the lymphocyte counts has normalized and routine re-vaccination is performed.⁹⁰ It should be noted that not all available functional immunological tests are discussed in this article and further more specialized testing may be recommended by an immunologist. Genetic testing of the child's germline DNA is becoming increasingly available and offers the ability to detect IEIs even during treatment.

It should be stressed that very often the diagnosis of an underlying IEI may be suspected, but not always be confirmed in time to allow treatment adaptation accordingly. Especially in these cases, the treatment and supportive care options should be considered, and careful balancing of treatment decisions is warranted.

Conclusion

The risk of malignancy is increased in many IEI patients. The presence of an underlying IEI in a child with cancer can have a significant impact on the treatment, including the need for dose reduction because of increased toxicity, or early use of HSCT. This article stresses the importance of recognizing these patients at an early stage. There are clinical warning signs, including the type of cancer and the age of onset, that could be used by the PHO to identify pediatric cancer patients with an underlying IEI. The analysis of immunoglobulins and lymphocyte subsets can give information but more specialized immunological testing may be required. Molecular genetic diagnostics, like NGS will play an increasing role in screening or confirming a diagnosis.

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Disclosure statement

The authors have no conflicts of interest to disclose.

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