(Paris), B. Mennesson (Pontoise), F. Michel (Meaux), A. Miton (Nancy), D. Monnier (Rennes), J-C. Robin (Montélimar), J. Nizard (Poissy), P. Perrier (Nancy), F. Pierre (Poitiers), H. Poissonnier (Paris), P. Poulain (Rennes), J.-C. Pons (Grenoble), S. Rouleau (Angers), T. Rousseau (Dijon), L. Salomon (Paris), A. Treisser (Monaco), M.-N. Varlet (Saint-Etienne), E. Verdy (Paris), E. Verspyck (Rouen), P. Vanlieferinghen (Clermont-Ferrand), D. Vauthier-Brouzes (Paris), and R. Wartanian (Angers).

Contribution: G.B. performed the research, analyzed data, and wrote the paper; R.P. contributed new samples; and C.K. designed the research.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Gérald Bertrand, INTS, Platelet Immunology Department, F-75015 Paris, France; e-mail: gerald.bertrand@live.fr.

References

1. Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. *Aust N Z J Obstet Gynaecol.* 2001; 41(1):45-55.
2. Berkowitz RL, Lesser ML, McFarland JG, et al. Antepartum treatment without early cordocentesis for standard-risk alloimmune thrombocytopenia: a randomized controlled trial. *Obstet Gynecol.* 2007;110(2 Pt 1): 249-255.
3. Bussel JB, Primiani A. Fetal and neonatal alloimmune thrombocytopenia: progress and ongoing debates. *Blood Rev.* 2008;22(1):33-52.
4. Pacheco LD, Berkowitz RL, Moise KJ Jr, Bussel JB, McFarland JG, Saade GR. Fetal and neonatal alloimmune thrombocytopenia: a management algorithm based on risk stratification. *Obstet Gynecol.* 2011;118(5): 1157-1163.
5. Bertrand G, Drame M, Martageix C, Kaplan C. Prediction of the fetal status in noninvasive management of alloimmune thrombocytopenia. *Blood.* 2011; 117(11):3209-3213.
6. Bertrand G, Bakchoul T, Javela K, Kjaer Killie M, Kaplan C; SSC of the ISTH. Interlaboratory workshop on anti-HPA-1a alloantibody quantification with the mAb-specific immobilization of platelet antigen technique. *J Thromb Haemost.* 2012;10(6):1172-1174.
7. Bertrand G, Jallu V, Gouet M, et al. Quantification of human platelet antigen-1a antibodies with the monoclonal antibody immobilization of platelet antigens procedure. *Transfusion.* 2005;45(8):1319-1323.

To the editor:

Identification of ITK deficiency as a novel genetic cause of idiopathic CD4$^+$ T-cell lymphopenia

Idiopathic CD4 lymphopenia represents a heterogeneous group of combined primary immunodeficiencies with markedly reduced CD4$^+$ T-cell counts. Although several genetic etiologies including MHC class II deficiency[1] or mutations in *RAG1*,[2] *MST1*,[3] or *LCK*[4] have been reported, the majority of patients remain genetically undetermined.

Here we describe a 17-year-old male Turkish patient of consanguineous background, referred to the hospital at 7 years of age. He suffered from recurrent pulmonary infections causing progressive immune reaction and bronchiectasia. Immunological analysis at the age of 10 showed persistently low CD4$^+$ T-cell counts (range: 8% to 16%; 286-714/μL) and increased CD8$^+$ T-cell counts (range: 50% to 59%; 1404-2950/μL, Figure 1A). T-cell proliferation responses to CD3/CD28 stimulation were reduced (not shown), whereas PHA stimulation led to normal proliferation of T-cells. B-cell counts and immunoglobulin levels were unremarkable (not shown); however, the patient showed reduced antipneumococcal antibody response.

To elucidate the underlying genetic cause, we combined homozygosity mapping (Figure 1B) and exome sequencing as previously reported, with minor modifications.[5] Unexpectedly, we identified a homozygous nonsense mutation located within the first exon of the *Interleukin-2-inducible T-cell kinase* (*ITK*) gene (c.C49T:p.Q17X) likely leading to nonsense-mediated decay of the corresponding gene product. The mutation was validated using capillary sequencing and showed perfect segregation under the assumption of autosomal-recessive inheritance (Figure 1C). ITK is a tyrosine kinase comprising 2 Src homology and a pleckstrin homology domain, respectively, acting downstream of the T-cell receptor complex and playing a critical role during thymic CD4$^+$/CD8$^+$ selection.[6] *Itk*$^{-/-}$ mice have reduced iNKT-cells and show an activated phenotype of peripheral CD4$^+$ T-cells.[7]

All ITK-deficient patients reported to date developed EBV-associated B-cell lymphoproliferation accompanied by hepato- and splenomegaly or Hodgkin lymphoma.[8-10] Decreased numbers of iNKT-cells are a hallmark feature of the disease.[8-11]

Surprisingly, this patient remained at sero-negative EBV status until the age of 17, although PCR-based copy-number analysis indicated borderline detectable EBV virus load (1000-2000 copies/mL). As all previously reported ITK-deficient patients were analyzed when they showed EBV-induced lymphoproliferation, we here had the unique opportunity to dissect EBV-dependent and EBV-independent ITK deficiency phenotypes. Consistent with previous findings in mouse[7] and human,[8-11] flow cytometry indicated an absence of iNKT-cells (Figure 1D), illustrating that the absence of iNKT-cells is a primary phenotype of ITK deficiency. Although CD4 lymphopenia has already been described in other ITK-deficient patients suffering from lymphoproliferative disease,[9,11] we here show that combined immunodeficiency with CD4 deficiency can be the predominant disease manifestation. Furthermore, defective T-cell proliferation has not been described in ITK-deficient patients, although it is concordant with the findings in *Itk*$^{-/-}$ mice. Of note, at the most recent follow-up, the patient presented with leiomyoma (not shown) and a high titer for EBV (23×10^6 copies/mL), in line with the characteristic, marked vulnerability to EBV infection in human ITK deficiency.[8-11]

In conclusion, in this case we identify ITK deficiency as a novel cause of idiopathic CD4 lymphopenia. Our analysis also sheds light on the immunophenotype of ITK deficiency in the absence of EBV-associated lymphoproliferation. Genetic assessment of patients with combined immunodeficiencies, in particular with predominant CD4 lymphophenia, should include mutational analysis of *ITK* even in the absence of EBV lymphoproliferation.

Nina Kathrin Serwas
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Deniz Cagdas
Division of Immunology, Hacettepe University Ihsan Doğramacı Children's Hospital, Ankara, Turkey

Sol A. Ban
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

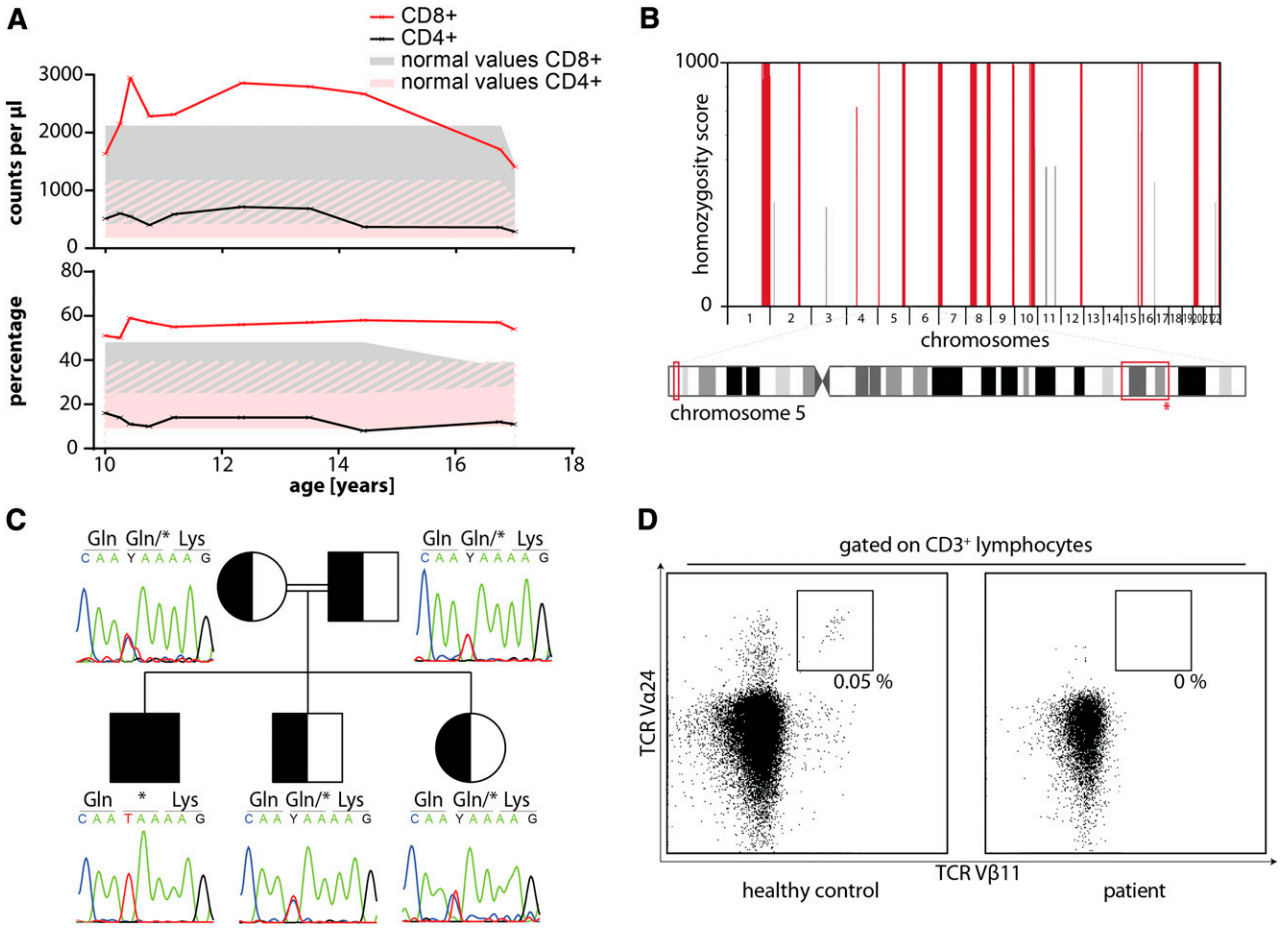

Figure 1. Immunological and genetic analysis. (A) The patient presented with persistently low numbers of $CD4^+$ T-cells and elevated numbers of $CD8^+$ T-cells throughout a period of 7 years. (B) Homozygosity mapping identified several homozygous regions. *ITK* (asterisk) was located inside a homozygous region on chromosome 5. (C) Identification of a homozygous nonsense mutation (c.C49T:p.Q17X) in the gene *ITK*. (D) The index patient shows complete absence of $TCRV\alpha24^+/TCRV\beta11^+$ iNKT-cells.

Kirsten Bienemann
Department of Paediatric Oncology, Haematology and Clinical Immunology, Center for Child and Adolescent Health, Heinrich Heine University, Düsseldorf, Germany

Elisabeth Salzer
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

İlhan Tezcan
Division of Immunology, Hacettepe University Ihsan Doğramacı Children's Hospital, Ankara, Turkey

Arndt Borkhardt
Department of Paediatric Oncology, Haematology and Clinical Immunology, Center for Child and Adolescent Health, Heinrich Heine University, Düsseldorf, Germany

Ozden Sanal
Division of Immunology, Hacettepe University Ihsan Doğramacı Children's Hospital, Ankara, Turkey

Kaan Boztug
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria

Contribution: N.K.P., E.S., and S.A.B. performed all experimental work; O.S., İ.T., and D.C. provided clinical care of the patient, provided clinical and immunological assessment, and were involved in critical scientific discussions; K. Bienemann and A.B. were involved in scientific discussions and helped with drafting the manuscript; K. Boztug conceived this study, provided laboratory resources and planned, designed and interpreted experiments; and N.K.P. and K. Boztug wrote the first draft and the revised version of the manuscript with input from K. Bienemann and A.B. Approval was obtained from the local institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Kaan Boztug, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, and Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Lazarettgasse 14 AKH BT 25.3, A-1090 Vienna, Austria; e-mail: kboztug@cemm.oeaw.ac.at.

References

1. de Préval C, Lisowska-Grospierre B, Loche M, Griscelli C, Mach B. A trans-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes. *Nature.* 1985;318(6043):291-293.
2. Kuijpers TW, Ijspeert H, van Leeuwen EM, et al. Idiopathic CD4+ T lymphopenia without autoimmunity or granulomatous disease in the slipstream of RAG mutations. *Blood.* 2011;117(22):5892-5896.
3. Nehme NT, Pachlopnik Schmid J, Debeurme F, et al. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood.* 2012;119(15):3458-3468.

4. Hauck F, Randriamampita C, Martin E, et al. Primary T-cell immunodeficiency with immunodysregulation caused by autosomal recessive LCK deficiency. *J Allergy Clin Immunol.* 2012;130(5):1144-1152, e1111.
5. Salzer E, Kansu A, Sic H, et al. Early-onset inflammatory bowel disease and common variable immunodeficiency-like disease caused by IL-21 deficiency. *J Allergy Clin Immunol.* 2014;133(6):1651, e12.
6. Berg LJ, Finkelstein LD, Lucas JA, Schwartzberg PL. Tec family kinases in T lymphocyte development and function. *Annu Rev Immunol.* 2005;23:549-600.
7. Liao XC, Littman DR. Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. *Immunity.* 1995;3(6):757-769.
8. Stepensky P, Weintraub M, Yanir A, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. *Haematologica.* 2011;96(3):472-476.
9. Mansouri D, Mahdaviani SA, Khalilzadeh S, et al. IL-2-inducible T-cell kinase deficiency with pulmonary manifestations due to disseminated Epstein-Barr virus infection. *Int Arch Allergy Immunol.* 2012;158(4): 418-422.
10. Huck K, Feyen O, Niehues T, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest.* 2009;119(5):1350-1358.
11. Linka RM, Risse SL, Bienemann K, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia.* 2012;26(5):963-971.

To the editor:

Myeloma cell sensitivity to bortezomib is associated with Dicer1 expression

Despite considerable progress of chemotherapeutic strategies and the introduction of the proteasome inhibitor bortezomib, multiple myeloma (MM) remains an incurable disease.[1] Mutations or loss of p53 occur in roughly 10% of untreated MM cells and are closely associated with resistance to bortezomib and dismal prognosis.[2] Although the inhibitory effect of bortezomib is well recognized, its downstream mechanisms of cytotoxicity remain largely elusive and at times controversial.

The discovery of microRNA (miR) has revealed a new level of regulation of cell signaling and homeostasis.[3] Deregulation of miR

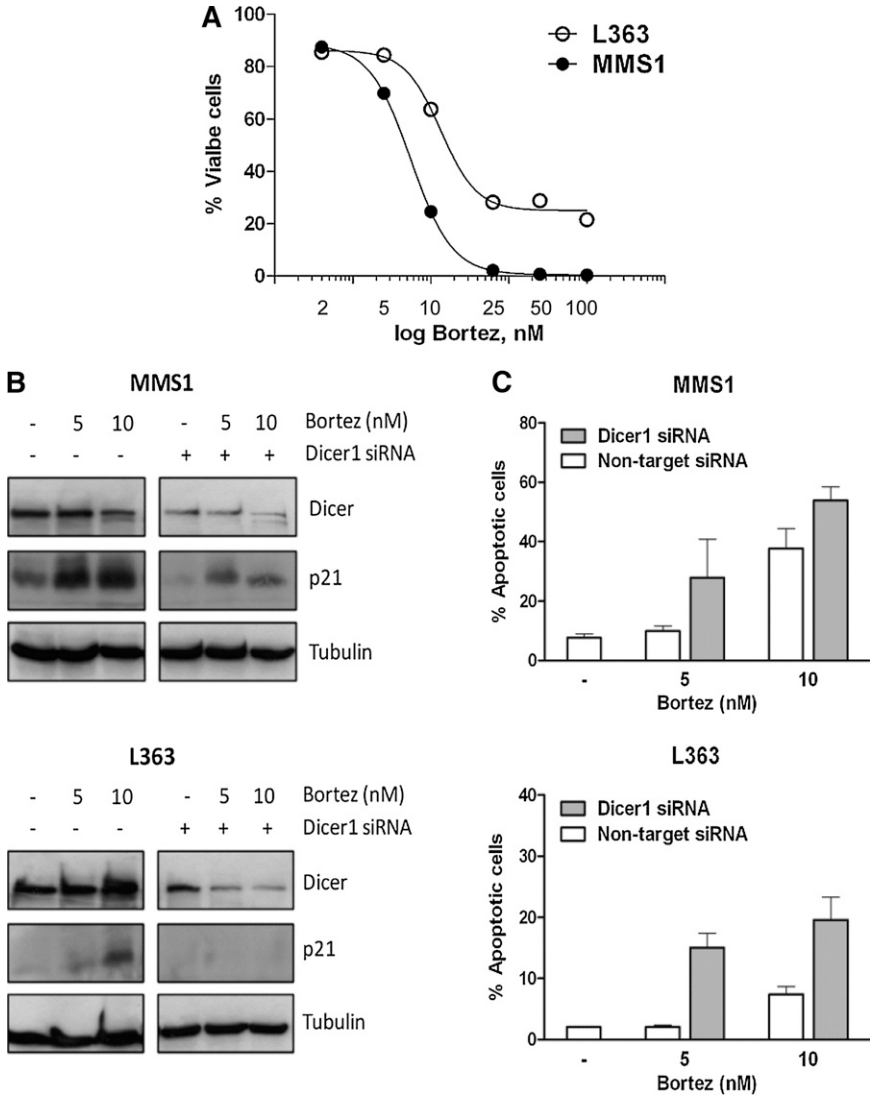

Figure 1. Effects of Dicer1 silencing on bortezomib-induced apoptosis in MM cells. (A) Representative dose-response curve of MMS1 and L363 cells after incubation for 24 hours with various bortezomib concentrations. Cell viability (%) was measured by annexin V/propidium iodide staining (AnnV/PI) (Invitrogen, Paisley, United Kingdom), and viable cells are defined as $AnnV^-/PI^-$. Results are shown as the mean of duplicates (n = 2). (B) Lysates were generated from Dicer1 knockdown with 100 nM short interfering RNA (siRNA) or nontarget siRNA control MMS1 and L363 cells treated with 5 to 10 nM bortezomib (Cell Signaling Technology, Danvers, MA) or vehicle for 24 hours, and the expression of Dicer1 (Cell Signaling Technology) and p21 (BD Transduction Laboratories, Heidelberg, Germany) proteins were analyzed by western blot. Tubulin served as a loading control. The image shows a representative of 3 independent experiments run on the same gel. Briefly, 4.5×10^5 MM cells were transiently transfected with Dicer1 siRNA (Cell Signaling Technology) or nontarget smart pool siRNA (Dharmacon, Lafayette, CO) using the TransIT-siQUEST transfection reagent (Mirus Bio, Madison, WI). MM cells were transfected with a solution containing 250 μL Opti-Mem medium (Invitrogen), 3.5 μL TransIT-siQUEST transfection reagent, and 100 nM Dicer1 siRNA or nontarget siRNA. Transfection efficiency was assessed by western blot. (C) Dicer1 knockdown increased apoptosis upon bortezomib treatment in MM cells. Dicer1 knockdown with 100 nM siRNA or nontarget siRNA control MMS1 and L363 cells was treated with 5 to 10 nM bortezomib or vehicle for 24 hours before an assessment of the percentage of $AnnV^+/PI^+$ cells by flow cytometry. Dicer1 knockdown potentiates the effect of bortezomib in L363 cells. The data presented are means ± SD of 3 experiments.