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LETTER TO THE EDITOR

Natural history of Ras-associated autoimmune leukoproliferative disorder: A 20-year follow-up of a *NRAS*-mutated patient excluding a malignant progression

Ras-associated autoimmune leukoproliferative disorder (RALD) is a rare condition resulting from a somatic gain-of-function (GoF) mutation in *NRAS* or *KRAS* genes that impairs leukocyte apoptosis and homoeostasis.[1] These patients show lymphadenopathy, splenomegaly, persistent leucocytosis with monocytosis/lymphocytosis and autoimmune manifestations.[1] Overlapping inborn error of immunity (IEI), autoimmune diseases and haematological malignancies, RALD is often difficult to define. Somatic mutations in *KRAS* or *NRAS* genes are also associated with juvenile myelomonocytic leukaemia (JMML), as well as various human cancer types.[2] Furthermore, certain patients with RALD may progress to JMML or acute myeloid leukaemia (AML),[1] whereas spontaneous improvement of the clinical symptoms has been observed in some *NRAS*/*KRAS*-mutated JMML patients.[3] Therefore, the proper diagnosis and follow-up of these rare patients is often challenging. Herein, we report the clinical history and the in-depth immune profile of a 22-year-old male with RALD.

The patient presented with splenomegaly, absolute mono-lymphocytosis and severe thrombocytopenia during the first month of life. Infectious causes of cytopenia were excluded. The bone marrow (BM) examination revealed trilineage hyperplasia, with mild left-shift myeloid maturation and no blast cells. Conventional karyotyping showed normal karyotype. Foetal haemoglobin was normal for age range. The patient was treated for the thrombocytopenia with high-dose intravenous immunoglobulins and steroids, obtaining a long-lasting partial response. The clinical course was uncomplicated until he was 12 years old, when he developed a chronic otitis with tympanic perforation. Subsequently, he maintained good clinical conditions, showing persistent splenomegaly, moderate but stable thrombocytopenia and increased absolute monocyte count.

The in-depth immunological assessment overtime (Table 1) showed a mild transient increase in double negative T cells. The immune profile, along with the clinical features, suggested autoimmune lymphoproliferative syndrome (ALPS), but the Fas-mediated apoptosis assay, performed at two time points, yielded normal results, and the plasmatic vitamin B12 level was within the normal range.

At the age of 16 years, a diagnostic Haloplex panel for IEIs was performed[4] on peripheral blood DNA, revealing a heterozygous somatic mutation c.35G>A p.G12D (rs121913237), in the *NRAS* gene (*NM_002524.5*). Sanger sequencing on buccal swab DNA resulted negative, confirming the somatic event. Considering ALPS-like phenotype and *NRAS* gene mutation, a diagnosis of RALD was made.

Regular blood evaluations showed total lymphocyte count in range, the monocyte count ranging between 710 and 1680/μL and platelets stable nearby 80 000/μL. Periodical BM evaluation excluded malignant transformation. Analysis of CD16, CD56 and CD10 expression on CD14+ monocytes did not find any abnormal expression, as previously described in some RALD patients.[5] Monocytosis was further evaluated through periodic in vitro cultures of the patient's peripheral blood mononuclear cells (PBMCs). As it occurs in patients with overt JMML, clonogenic assay showed spontaneous growth of granulocytic-macrophage progenitors (CFU-GM), abolished in adherent cell-depleted. However, unlike JMML cases, the growth of spontaneous CFU-GM was observed in a limited number of colonies and only at first evaluations. Indeed, clonogenic assays performed subsequently did not result in any 'spontaneous' growth. Targeted deep sequencing was studied at two different time points (at age of 13 and 20 years) on DNA extracted from PBMCs using the 30-gene myeloid solution panel by Sophia Genetics.[6] The *NRAS* mutation was confirmed with a stable variant allele frequency at first and second determination, 0.48 and 0.50 respectively, without any other somatic mutations.

Somatic mutations in *KRAS*/*NRAS* genes account for ALPS-like conditions classified in International Union of Immunological Societies as phenocopies of IEIs.[7,8] Our observation highlights the importance to explore somatic gene variants in patients with a specific clinical immunological picture. The assessment of apoptosis may be useful to properly convey the diagnostics. Differently from ALPS, somatic *KRAS*/*NRAS* mutations are responsible for defects of FAS-independent intrinsic apoptosis mechanism (activated cell-autonomous death).[9] In RALD, immunological alterations, as well as decreased *naïve* lymphocytes, the peripheral oligoclonal T- and B-cell expansion[10] and the intrinsic apoptotic defect[9] could contribute to a large spectrum of clinical and immunological manifestations (Figure 1).[1,6,11] JMML is a rare aggressive myelodysplastic/myeloproliferative disorder of early childhood

TABLE 1 Immunological characterization of the patient at different time points.

	1 Year	3 Years	13 Years	15 Years	16 Years	20 Years	21 Years
Total lymphocyte count 10^3/μL			2540 (1400–4200)	1990 (1400–4200)	1680 (1400–4200)	1980 (1400–4200)	1370 (1200–4100)
Total monocyte 10^3/μL			**1270**	880	**1060**	900	850
Platelet 10^3/μL			**114000**	**84000**	**71000**	**76000**	**86000**
Haemoglobin g/dL			13.4	13.4	14.7	15.9	15.1
CD3+	**19**	**41**	**47.6** (52–90)	**40** (52–90)	**47** (50–91)	51.4 (50–91)	51.4 (50–91)
CD3−CD16+CD56+	16.9	14.5	8.4 (4–51)	4.1 (4–51)	5.6 (5–49)	**4.8** (5–49)	**4.7** (5–49)
CD3+CD4+	**15**	**16.7**	24.9 (20–65)	21.8 (20–65)	26.1 (28–64)	30.2 (28–64)	31.8 (28–64)
CD3+CD8+	**6.9**	15.2	17.8 (14–40)	**13.4** (14–40)	16.1 (12–40)	17.7 (12–40)	14.5 (12–40)
CD19+	**59.6**	**39.5**	**41.8** (10.2–15.4)	**52** (10.2–15.4)	**46.6** (10.2–15.4)	**41.1** (10.2–15.4)	**43.1** (10.2–15.4)
% CD3 lymphocyte subsets							
TCRα/β+				91.5 (39–92)	94.7	93.7	95.7 (36–98)
TCRγ/δ+				4.1 (2–17)	4.8	5.8	3.9 (0.83–11)
CD3+CD4−CD8−				**3.1**	2.4	**2.9**	**3.8**
% CD4 lymphocyte subsets							
CD27+CD45RA+ naïve				31 (31–57)	**22.4**	**25.3**	**26.3** (31–57)
CD31+CD45RA+ (recent thymic emigrants, RTE)				**9.9** (37–62)	**7.7**	**6.7**	**7.2** (37–62)
CD27+CD45RA− (central memory)				**58** (10–27)	**68.6** (10–27)	**67.7** (10–27)	**69** (10–27)
CD27−CD45RA− (effector memory)				**10.6** (12–44)	**8.8** (12–44)	**6.8** (12–44)	**4.63** (12–44)
CD27−CD45RA+ (effector memory CD45RA+, EMRA)				**0.3** (4–12)	**0.17** (4–12)	**0.18** (4–12)	**0.09** (4–12)
CD25+CD127lowFOXP3+ (regulatory T cell, Treg)						**1.7** (3.7–9)	
CD45RO+CXCR5+ (follicular helper T-cell, Tfh)				10.5 (7–47)		6.6 (5–56)	**4.7** (5–56)
% CD8 lymphocyte subsets							
CCR7+CD45RA+ (naïve)				**13.4** (18–61)	23	20.5	24 (18–61)
CCR7+CD45RA− (central memory)				5.0 (3–12)	2.7 (3–12)	5.4 (3–12)	3.2 (3–12)
CCR7−CD45RA− (effector memory)				**61.0** (25–58)	50 (25–58)	54.2 (25–58)	47 (25–58)
CCR7−CD45RA+ (effector memory CD45RA+, EMRA)				19.8 (5–20)	**25.1** (5–20)	**20.3** (5–20)	**26** (5–20)
% B-lymphocyte subsets							
CD24++CD38++ (transitional)				**38.3** (3.9–7.8)	**23.8** (3.9–7.8)	**19.6** (3.9–7.8)	**17.1** (3.9–7.8)
CD27−IgD+IgM+ (naïve)				**93.9** (75.3–86.7)	**89.9** (75.2–86.7)	**80.8** (75.2–86.7)	**88.3** (75.2–86.7)

TABLE 1 (Continued)

	1 Year	3 Years	13 Years	15 Years	16 Years	20 Years	21 Years
CD27+IgD+IgM+ (unswitched memory)				**4.5** (4.6–10.2)	8.3 (4.6–10.2)	**17.2** (4.6–10.2)	9.9 (4.6–10.2)
CD27+IgD−IgM− (switched memory)				**2.03** (3.3–9.6)	**1.8** (3.3–9.6)	**2** (3.3–9.6)	**1.86** (3.3–9.6)
CD27−IgD−IgM−				3.2 (2.3–5.5)		**1** (2.3–5.5)	**1.1** (2.3–5.5)
CD21low CD38low				**0.2** (0.9–3.3)		**0.4** (0.9–3.3)	**0.2** (0.9–3.3)
% NK cell subsets							
CD56bright							19%
CD56dim							85%
CD56neg							3.5%
IgG (mg/dL)			1267 (604–1909)	1234 (604–1909)	1132 (604–1909)	879 (604–1909)	894 (604–1909)
IgA (mg/dL)			350 (61–301)	311 (61–301)	308 (61–301)	249 (61–301)	244 (61–301)
IgM (mg/dL)			**327** (59–297)	294 (59–297)	268 (59–297)	**303** (59–297)	**324** (59–297)
EBV (copies/mL)			Neg	Neg	1346	Neg	Neg
CMV (copies/mL)			Neg	Neg	Neg	Neg	Neg
T-cell proliferative response to PHA, OKT3							Normal
T-cell CD40/CD40L expression							Normal
B-lymphocyte proliferation, differentiation and Ig production							Normal

Note: Immunological assessment showed normal serum immunoglobulins, with slight IgM increase, normal IgA and IgG, increased CD19+ B cell and reduced CD4+CD45RA+ T cell subsets. The in-depth immunophenotype demonstrated low *naïve* CD45RA+ in both CD4+ and CD8+ T cells, low CD4+CD45RA+CD31+ recent thymic emigrant (RTE) and CD4+CD25+CD127lowFoxP3high (Treg), with floating double negative T cells CD3+CD4−CD8−αβ-TCR double negative T cells (DNTs, max 3.8% of CD3+ T cells). B-cell evaluation revealed an increase in CD19+ B cells, as well as CD24++CD38++ transitional B cells, and decreased CD27+IgD−IgM− memory switched B cells. These data remained stable over time. B-lymphocyte proliferation, differentiation and Ig production are upon response to T-independent stimuli as cytosine–phosphate–guanosine (CpG)-DNA. Range values in the bracket. The out-of-range values are reported in bold.

that shares clinical features with RALD. In 90% of JMML, a GoF somatic mutation in *NRAS*, *KRAS* or *PTPN11* is identified, or diagnosed in the context of RASopathies (germline mutations in RAS/MAPK pathway genes as *NF1* and *CBL*).[12] Allogeneic haematopoietic stem cell transplant (HSCT) is the only potentially curative treatment in JMML, with a 5-year disease free survival of 44% after HSCT, while is aggressive and fatal if untreated.[13,14] Nevertheless, in some patients with JMML and *KRAS*/*NRAS* mutation, a spontaneous improvement of haematological abnormalities has occasionally been reported.[3] Some authors proposed RALD and JMML in a wide phenotypic spectrum, from immune dysregulation to clonal disease, in which the addition of genetic or epigenetic events may contribute to malignant transformation.[15] Indeed, the progression from JMML to AML was reported in some RALD patients.[1] On the other side, in vitro T-cell apoptosis assays from *KRAS*/*NRAS*-mutated JMML patients displayed a normal levels of BIM expression (intrinsic apoptosis pathway) compared with RALD, thus the possibility to consider these conditions as separate entities.[9] The more frequent complications requiring therapies in RALD are autoimmune manifestations, necessitating HSCT in one reported patient.[1] Moreover, no data are available on a putative role of immunosuppressive therapies in the malignant progression.

The reported patient had not exhibited evidence of persistent autoimmunity and repeated clonogenic assay excluded JMML malignant progression. Furthermore, the longitudinal assessment for additional somatic mutations resulted negative, supporting the stability of clonal haematopoiesis. Currently, predicting which RALD patients are at higher risk of leukaemic progression or maintaining stability is uncertain. The identification of useful predictive markers for disease progression would enable a personalized approach to therapeutic strategy, including the HSCT eligibility. Our report stresses the importance of an integrated approach in which clinical parameters, along with functional assays and the longitudinal assessment for additional myeloid co-mutations, may allow to better predict the real risk of malignant progression. In conclusion, herein we reported the long-term follow-up of a patient with RALD. Expanding the cohort of patients with these rare conditions may increase the knowledge on disease pathogenesis and evolution, hopefully improving the monitoring strategies, as well as therapeutic management.

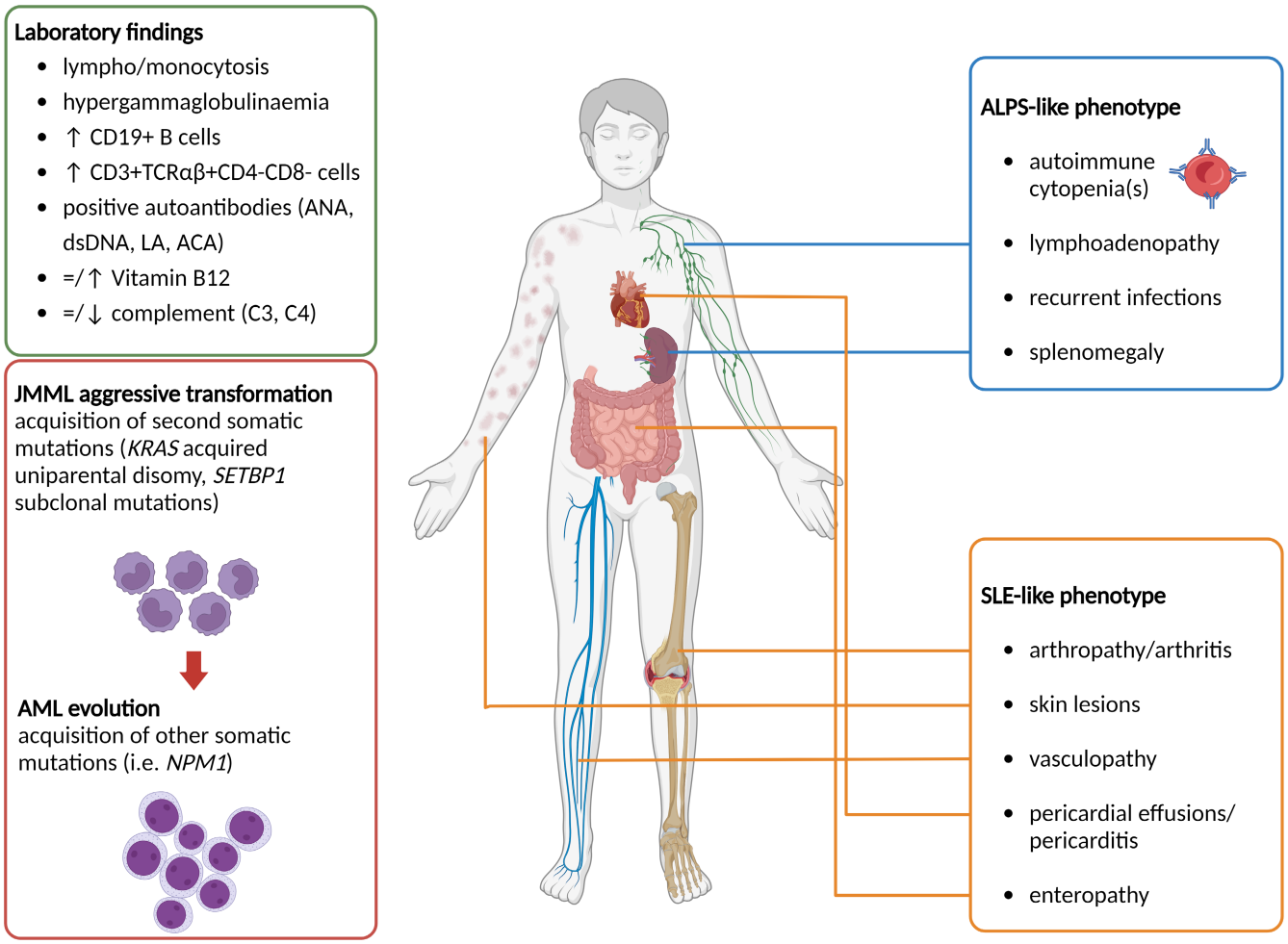

FIGURE 1 Clinical and immunological features associated with RALD. ACA, anticentromere antibodies; ALPS, autoimmune lymphoproliferative syndrome; AML, acute myeloid leukaemia; ANA, antinuclear antibody; JMML, juvenile myelomonocytic leukaemia; LA, lupus anticoagulant; SLE, systemic lupus erythematosus. The figure was created with Biorender.com.

AUTHOR CONTRIBUTIONS

B. Rivalta, E. Attardi, C. Cifaldi and C. Cancrini interpreted the data and wrote the manuscript; B. Rivalta, C. Cancrini, M. Luciani, G. Palumbo, M. Algeri, A. Finocchi, L. Pacillo and D. Amodio managed the patient; F. Locatelli, A. Aiuti and M. T. Voso edited the manuscript, helped in data interpretation and gave helpful intellectual insights during the study; C. Cifaldi, G. Di Matteo, S. Di Cesare, H. Hajrullaj, F. Barzaghi and V. Rosti performed experiments and participated in the analysis interpretation; C. Cancrini took responsibility for the integrity and the accuracy of the data presented; and all authors reviewed and approved the final version of this manuscript.

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DATA AVAILABILITY STATEMENT

Data used for this study are available upon request to the corresponding author.

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