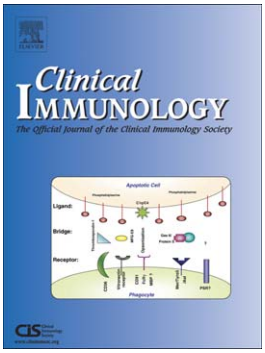

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Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase δ syndrome

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Key words: activated phosphoinositide 3-kinase δ syndrome; sirolimus, autoimmune hemolytic anemia.

To the editor,

Primary immunodeficiencies (PIDs) are a heterogeneous group of rare diseases that clinically manifest with infections, autoimmunity, inflammation, and malignancy as a result of impairment of one or more effector mechanisms of the immune system. Identification of the molecular cause underlying PID informs clinicians on management and best practise in these complex cases [1]. Without a molecular diagnosis clinicians are often required to be reactive rather than proactive in treatment decisions, which can lead to an increased morbidity in PID patients. Knowledge of the aberrant molecular pathways in cases of PID is even more apparent now as the field of precision medicine expands within the discipline [2]. We highlight the utility of a non-bias investigational approach using whole exome sequencing to reach a genetic diagnosis in a case activated phosphoinositide 3-kinase δ syndrome (APDS) (OMIM 615513) that led to long-term clinical stability with a precision medicine approach.

A 4-year-old boy presented to a secondary care pediatric service with a three-day history of fever, abdominal pain, grunting and breathlessness. He had an unremarkable past medical history apart from three brief hospital admissions for rash and fever which were attributed to viral infections, and was fully immunised according to the UK childhood vaccination schedule. His parents are non-consanguineous and he is an only child. The family history was unremarkable. On physical examination he was tachypneic and febrile with decreased breath sounds at the lung left base and firm non-tender splenomegaly. Blood cultures isolated *Streptococcus pneumoniae* 23F and the child was treated for a community-acquired pneumonia with intravenous ceftriaxone. However the splenomegaly persisted and the patient suffered further bacterial sino-pulmonary infections which required antibiotic treatment in the community. The patient then developed a chronic relapsing autoimmune hemolytic anemia (AIHA) with a positive direct antiglobulin test (DAT) IgG++++, C3d++++, a nadir hemoglobin of 44 g/L, haptoglobulin of <0.06g/L, and elevated lactate dehydrogenase of 1464iu/L. Immunological investigations revealed T cell lymphopenia and impaired pneumococcal polysaccharide antibody responses despite boosters (Supplementary data Tables 1 and

2). Computed tomography imaging confirmed splenomegaly with florid lymphadenopathy in the small bowel mesentery, splenic hilum, periportal region and pre-aortic area (Supplementary Figure 1). An excision lymph node biopsy showed a partial loss of normal architecture with a polyclonal CD8+ reactive T cell infiltrate with no evidence of malignant disease (Figure 1). Bone marrow biopsy was normal.

Initial management with subcutaneous immunoglobulin replacement at a dose of 0.1g/kg/week (due to impaired specific polysaccharide responses) and prophylactic clarithromycin controlled the infections, but the AIHA required rituximab, prednisolone, and mycophenolate. Despite these treatments recurrent DAT positive relapses of hemolysis rendered him steroid dependent. The lymphoid bulk did not respond to the immunosuppressive regimes and he began to suffer intermittent colicky abdominal pains that culminated in bowel obstruction requiring emergency laparotomy. Intraoperatively, the cause of the bowel obstruction was found to be gross intramural thickening within the small bowel with resultant luminal compression. Histological examination of the resected thickened segment of small bowel showed dense infiltration of polyclonal CD8+ T cells, similar to those observed in the enlarged lymph node (Supplementary data Figure 2).

Whole exome sequencing was performed and data was analysed against a virtual panel of immunodeficiency genes (Supplementary data Table 4), which identified the heterozygous variant *PIK3CD* NM_005026: exon24: c.3061G>A: p.(Glu1021Lys). This variant was subsequently confirmed by Sanger sequencing (Supplementary data Figure 3). This variant in *PIK3CD* has previously been reported to be pathogenic resulting in APDS [3, 4]. Patients with APDS suffer frequent sinopulmonary infections as a result of impaired humoral immunity as well as autoimmune phenomena and lymphoid hyperplasia due to immune dysregulation [3].

Due to refractory AIHA, and the genetic results supporting over activity of the PI3K-AKT-mTOR pathway within the patient's lymphocytes, the mTOR inhibitor sirolimus was commenced as a precision medicine with the aim of controlling the hemolysis and reducing the lymphoid bulk [4]. Previously sirolimus has also been reported to be successful in treating refractory autoimmune

cytopenias in PID conditions including common variable immunodeficiency and autoimmune lymphoproliferative syndrome [5, 6].

After the initiation of sirolimus, in contrast to previous immunosuppressive regimes, there has been no relapse of hemolysis, infectious complications, and prednisolone has been withdrawn. His DAT has remained negative since commencing sirolimus. We have observed a reduction in lymphadenopathy volume, resolution in splenomegaly, and an improvement in the T cell senescence pattern with an increase in naive T cell percentages (Supplementary data Table 1). Sirolimus trough levels have fluctuated from 4.3 – 11.8ng/ml (Supplementary data Table 5) with no clinical impact, suggesting the target dose range for sirolimus in APDS may be lower than in solid organ transplant recipients (10-15ng/ml). He has now remained well for over 18 months with no relapse of hemolysis and continues with sirolimus 2mg daily and immunoglobulin replacement treatment.

New genomic technologies, such as whole exome sequencing by next generation sequencing, are now available and affordable [7]. In primary immunodeficiency cases, knowledge of the molecular diagnosis and subsequent impacts on cellular pathway allow the identification of precision medicines targets that can significantly improve a patient's quality of life [8]. As with all medical investigations, genomic tests require careful selection of the test methodology and assessment of pre-test probability. When undertaking whole exome sequencing in a patient specific consideration of informed consent, data analysis complexities, the reporting of incidental findings and impacts on the wider family is needed. The integration of genomic tests into routine clinical work has many challenges ahead, but cases such as this demonstrate the powerful impact that the integration of genomic medicine can have on the diagnosis and treatment for patients across medical specialities [9].

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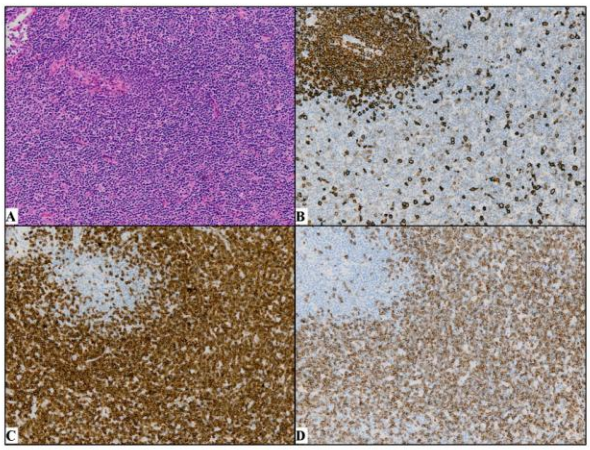
Figure 1

Lymph node biopsy. A) The H&E demonstrates a residual primary B cell follicle in the top left of the image. B) The follicle is demonstrated on staining with CD20. C) Surrounding the follicle there is an expansion of CD3+ T cells within the paracortex. D) The majority of the T cells express CD8+.



Highlights:

- Activated phosphoinositide 3-kinase δ syndrome may be complicated by refractory autoimmune hemolytic anemia.
- Sirolimus presents a precision therapeutic for the PI3Kδ-AKT-mTOR pathway in these cases.
- Sirolimus can induce long term remission of autoimmune hemolytic anemia in activated phosphoinositide 3-kinase δ syndrome.
- Sirolimus can improve the T cell immunosenescent phenotype in activated phosphoinositide 3-kinase δ syndrome.