ESPN Research Grant Application final report

Title of the project:	
Hypogammaglobulinemia in SDNS/FRNS children: incidence, risk factors for its development and effect of anti-CD20 treatment	
Principal investigator	**Year of award**
Alexandra Zurowska Manuela Colucci	2021

Please provide an overall summary of the project (max 500 words)

This multicenter European project focused on the evaluation of hypogammaglobulinemia, infectious complications, and vaccine competence in children affected by steroid-dependent or frequently relapsing nephrotic syndrome (SDNS/FRNS), treated with anti-CD20 monoclonal antibodies such as rituximab and obinutuzumab. The project was developed through the collaboration of several pediatric nephrology centers and combined both cross-sectional and prospective analyses in order to better characterize the immunological consequences of current treatment strategies used in difficult-to-treat nephrotic syndrome.

The cross-sectional cohort included 212 children with SDNS/FRNS receiving different immunosuppressive regimens, while the prospective cohort enrolled 111 patients undergoing new anti-CD20 treatment courses. Clinical, laboratory, and immunological data were systematically collected to assess serum immunoglobulin levels, infectious events, treatment exposure, and vaccine responses over time. The study demonstrated that long-term immunosuppressive therapy significantly increases the risk of hypogammaglobulinemia, with approximately 40% of patients presenting reduced serum IgG concentrations during follow-up. Previous treatment with cyclophosphamide emerged as the strongest risk factors associated with low IgG levels.

Although severe infections requiring hospitalization were relatively uncommon overall, they occurred more frequently in patients exposed to anti-CD20 therapies and were often associated with persistent or severe hypogammaglobulinemia. Respiratory infections represented the most common infectious complication. Prospective monitoring further showed that patients with pre-existing hypogammaglobulinemia before anti-CD20 administration had a significantly higher probability of developing prolonged or persistent hypogammaglobulinemia after treatment. Serum immunoglobulin concentrations generally remained stable over time, with only partial recovery in most patients. Importantly, a small subgroup developed severe persistent hypogammaglobulinemia requiring long-term immunoglobulin replacement therapy, particularly among children exposed to anti-CD20 therapy at younger age.

The project also demonstrated that anti-CD20 therapy induces long-lasting alterations in B-cell homeostasis and humoral immunity, persisting years after treatment. While total $CD19^{+}$, transitional and mature-naïve B cells progressively normalized during follow-up, memory B cells - especially IgG-producing switched memory subsets - remained persistently reduced in most patients, suggesting prolonged impairment of immunological memory despite apparent recovery of the overall B-cell compartment.

An additional objective of the project was the evaluation of vaccine competence after B-cell depletion therapy. The study showed that rituximab treatment significantly reduced anti-tetanus IgG titers, which was associated with a complete and persistent depletion of tetanus-specific IgG-producing memory B cells. Importantly, re-immunization strategies after B-cell recovery proved effective in restoring protective antibody and memory B-cell responses, supporting the feasibility and usefulness of post-treatment vaccination programs in this population.

Overall, the project provides important evidence regarding the immunological safety profile of anti-CD20 therapies in pediatric nephrotic syndrome and highlights the need for systematic monitoring of serum immunoglobulin levels, careful infection surveillance, and periodic assessment of vaccine protection in children receiving prolonged immunosuppressive treatment. These findings may contribute to the development of future clinical recommendations aimed at improving long-term safety and individualized management strategies for children with SDNS/FRNS.

How was the grant used for the project? (max 200 words)

The grant funding was used to support the laboratory and analytical activities required for the project. A total of 8000 € was allocated for the purchase of flow cytometry antibodies and reagents necessary for immunophenotyping and B-cell characterization analyses. An additional 4000 € was used for Fluorospot assay reagents to evaluate vaccine-specific immune responses and humoral competence. Finally, 8000 € was dedicated to database preparation, data management, and statistical analysis to ensure accurate collection, processing, and interpretation of the multicenter study data.

Please list all publications completed, with links to open access publications where available

- Zurowska A, Drozynska-Duklas M, Topaloglu R, Bouts A, Boyer O, Shenoy M, Vivarelli M; ESPN Glomerulonephritis Working Group. Rituximab-associated hypogammaglobulinemia in children with idiopathic nephrotic syndrome: results of an ESPN survey. Pediatr Nephrol. 2023;38(9):3035-3042. doi: 10.1007/s00467-023-05913-1. https://link.springer.com/article/10.1007/s00467-023-05913-1.
- Colucci M, Riganati M, Zotta F, Gargiulo A, Massella L, Ruggiero B, Cotugno N, Ricci G, Palma P, Emma F, Vivarelli M. Prolonged impairment of immunological memory after anti-CD20 treatment in pediatric idiopathic nephrotic syndrome: an extended follow-up. Front Immunol. 2025;16:1736921. doi: 10.3389/fimmu.2025.1736921. https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2025.1736921/full.

Are any other publications planned?

Yes. Two additional manuscripts are currently planned as part of the project outcomes.
The first manuscript, currently under revision, focuses on the impact of different immunosuppressive therapies, including anti-CD20 treatment, on immunological and vaccine competence in children with SDNS/FRNS from disease onset.
The second planned manuscript will report our results on the cross-sectional and perspective analyses of hypogammaglobulinemia development as well as on the effectiveness of re-immunization strategies following rituximab therapy in pediatric patients with nephrotic syndrome.
Both publications aim to further expand the translational and clinical implications of the project findings.

Please list all presentations relating to the project

- Magdalena Drozynska-Duklas. "Rituximab ESPN project". 56th Annual Meeting of the European Society for Paediatric Nephrology 24-27 September 2024, Valencia, Spain.
- Colucci Manuela. "Prospective evaluation of vaccine response in children with idiopathic nephrotic syndrome treated with rituximab". 57th Annual Meeting of the European Society for Paediatric Nephrology 15-18 October 2025, Athens, Greece.