LymphoSign Journal

The journal of inherited immune disorders

Volume 7, Number 3, 2020 EISSN 2292-5945

Immunodeficiency Canada

Created in 1999, Immunodeficiency Canada is a national organization working closely with diagnostic and treatment centres across Canada. It plays a vital role in being one of the first organizations in Canada to finance and encourage research in the field of primary immunodeficiency, as well as, providing education for patients and medical professionals. Programs and services are provided through the support of generous donors.

Vision

To cure Primary Immunodeficiency (PI)

Mission

To improve the lives of people with Primary Immunodeficiency (PI) by promoting early diagnosis and effective treatments through leadership in research, education and advocacy in partnership with health-care professionals, volunteers, industry and government

The Immunodeficiency Canada addresses its mission through four core programs:

- Network
- Education
- Research
- Patient Support

Programs and Services

- Emergency Financial Assistance
- Patient Support
- Primary Immunodeficiency Information
- Publications
- Resources
- Social Support

For Healthcare Professionals

Chaim Roifman Scholar Award for research

Supported by Baxter, CSL Behring, Grifols, Octapharma and Immunodeficiency Canada

- for Senior Investigators
- for Young Investigators

Conferences, seminars and symposiums
Immunodeficiency Canada-Grifols Fellow Training Program
Immunodeficiency Canada Network

- Consultations
- Education
- Immunologists network grants
- PI video conferences
- Healthcare facility sites and affiliates

Medical Advisory Board
Physicians Login (for diagnostic tools, case studies and resources)
PI Nurses Network
PI Social Workers Network

Additional Resources

Canadian, International resources and links to condition-specific information can be found at **www.immunodeficiency.ca**

Immunodeficiency Canada
Immunodéficience Canada

Providing patient support, education and research to cure Primary Immunodeficiency

Suite 848, 439 University Ave, Toronto, Ontario M5G1Y8
Tel 416-964-3434 **Fax** 416-964-6594
contactus@immunodeficiency.ca
charitable # 87276 0897 RR0001

www.immunodeficiency.ca

LymphoSign Journal

Volume 7, Number 3, 2020 EISSN 2292-5945

TABLE OF CONTENTS

Commentary

85 **Managing primary immunodeficiency during the COVID-19 pandemic**
Chaim M. Roifman

Review

90 **Dysregulated CARD11 signaling in the development of diffuse large B cell lymphoma**
Tyler A. Jones, Shelby M. Hutcherson, Jacquelyn R. Bedsaul, and Joel L. Pomerantz

Original Article

104 **Observational study of SARS-CoV-2 antibody immune response in a physician's practice**
Gerald Osher, Christopher C. Lamb, Yuliana Ibarra, and Deborah Erickson-Samson

Protocol, Practice, and Policy

109 **Report of the Canadian Expert Committee on the management of ADA deficiency**
Rae Brager, Parwinder Gill, Alison Haynes, Kyla Hildebrand, Vy H.D. Kim, Thomas B. Issekutz, Christine McCusker, Luis Murguia-Favela, Brenda Reid, Tamar Rubin, Julia Upton, and Chaim M. Roifman

LymphoSign Journal (ISSN electronic 2292-5945) is published quarterly in March, June, September, and December by LymphoSign Journal Limited Partnership, Toronto, Ontario, Canada; Tel.: 416-986-3508; Fax: 416-964-6594.

Service information

Scope

LymphoSign Journal publishes novel clinical, translational, and basic research in the fields of immunology, gastroenterology, neurology, dermatology, rheumatology, hematology, and infectious disease. The aim of the journal is to provide a forum for clinicians and scientists to discuss clinical observations, therapies, and insights into underlying disease mechanisms in immune disorders including immune deficiencies, auto-inflammatory disorders, allergy, bone marrow failure, and lymphoid malignancies. Special consideration will be given, but is not limited to, articles exploring adaptive and innate immunity, mucosal immunity, signal transduction, lymphocyte development and cell death, genomic medicine, gene regulation, DNA repair, and cell cytoskeletal networks. *LymphoSign Journal* publishes peer-reviewed original research articles, reviews, clinical trials, case reports, novel mutations, and imaging, as well as practice guidelines, algorithms, and protocols.

Subscriptions

Requests for subscriptions, renewals, and single or back issues, and all remittances should be addressed to: Subscription Office, Canadian Science Publishing, PO Box 361, Birmingham, AL 35283-0399, USA; Toll-free: 1-800-852-7404; Outside North America: 205-995-1567; Fax: 205-995-1588; Email: nrcresearchpress-csp@subscriptionoffice.com. 2020 subscriptions rates are as follows: Institution: electronic $525.00, Personal: electronic $150.00.

Submissions

To submit, use the journal's online submission system at http://mc06.manuscriptcentral.com/lpsn. The Instructions to Authors are available at http://lymphosign.com/page/lpsn-authors. All correspondence concerning submissions should be addressed to the Editorial Office at editorialoffice@lymphosign.com.

Offprints and reprints

Offprint and reprint requests should be directed to the Editorial Office at editorialoffice@lymphosign.com.

Permissions

For permission to reprint materials, contact the Editorial Office at editorialoffice@lymphosign.com.

Advertising sales

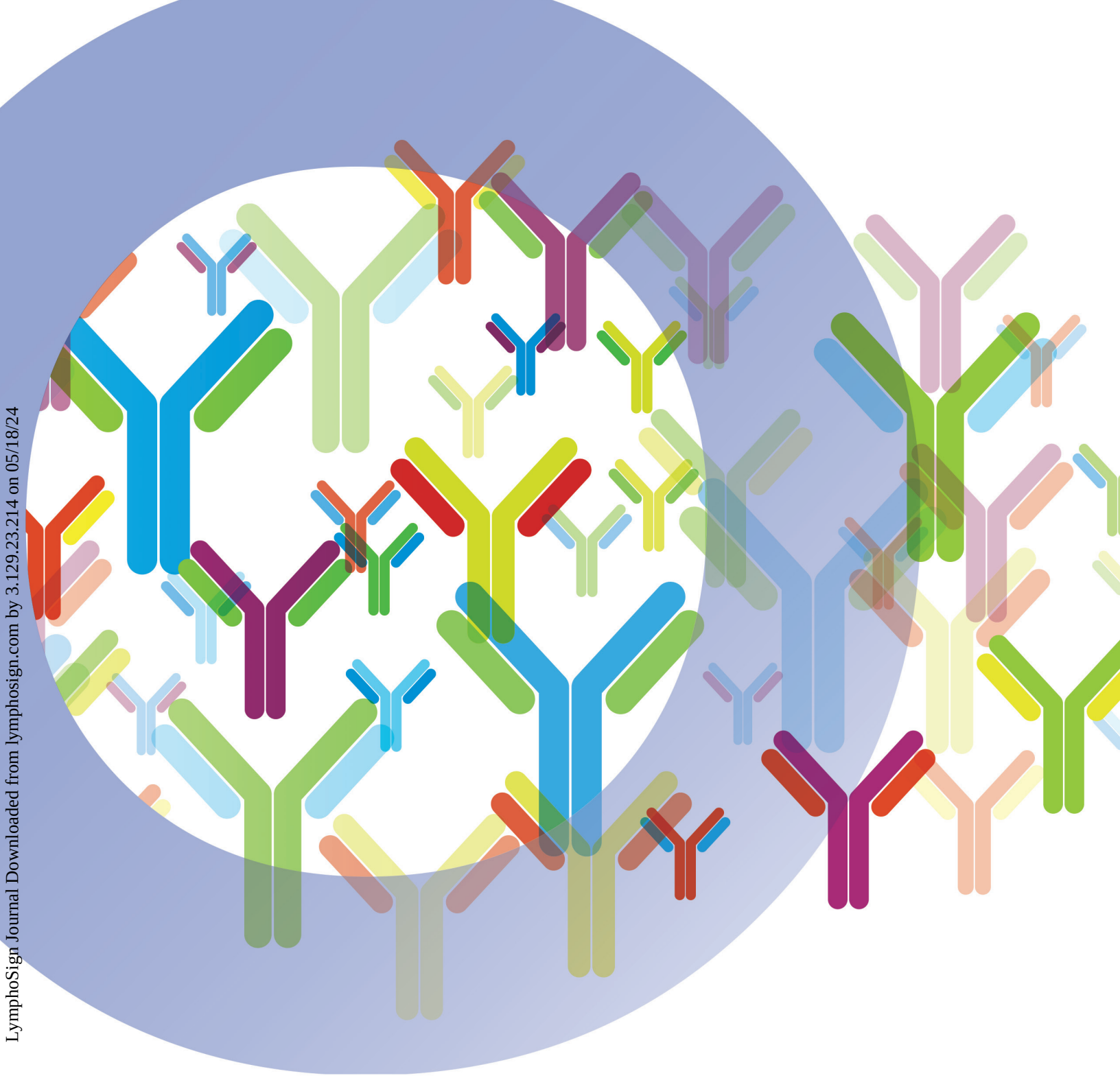
Advertising inquiries should be directed to the Editorial Office at editorialoffice@lymphosign.com. Acceptance of or the publishing of advertisements does not imply endorsement by either the Journal's Editors or the Publisher.

Publishing services

Publishing services are provided by Canadian Science Publishing, 1840 Woodward Drive, Suite 1, Ottawa, ON K2C 0P7 Tel: 613-656-9846; www.cdnsciencepub.com. Scientific Publishing Editor: Lianne Johnsen, lianne.johnsen@cdnsciencepub.com; Manager, Production: Judy Hum-Delaney, judy.hum-delaney@cdnsciencepub.com; Director, Publishing Operations: Judy Busnarda, judy.busnarda@cdnsciencepub.com.

Indexing

CrossRef, Google Scholar, HINARI, J-Gate, OCLC, ReadCube, Ulrich's, and Emerging Sources Citation Index.



LymphoSign Journal

Volume 7, Number 3, 2020 EISSN 2292-5945

Managing primary immunodeficiency during the COVID-19 pandemic

Chaim M. Roifman, CM, MD, FRCPC, FCACB[a,b*]

Introduction

The coronavirus disease 2019 (COVID-19) outbreak, which originated in Wuhan, China, is caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Li et al. 2020; Wong et al. 2020; Xu et al. 2020). To date, cases have been reported in all continents worldwide with the exception of Antarctica, and include over 25 million affected and 846 841 deaths (European Centre for Disease Prevention and Control 2020). The pandemic has led to a disproportionate loss of lives in our most vulnerable populations and widespread global economic downturn.

SARS-CoV-2 belongs to the same family of coronaviruses that cause the common cold, as well as the recent outbreaks of atypical viral pneumonia (severe acute respiratory syndrome, SARS; Middle East respiratory syndrome, MERS) (de Wit et al. 2016; Paules et al. 2020). Like all viruses, SARS-CoV-2 can multiply only in living cells. To enter living cells, viruses express proteins (ligands) on their surface which recognize complementary proteins on the surface of cells (receptors). Binding of viral ligands and cellular receptors allows entry of the virus into cells where they hijack its normal functions to reproduce. This take over by the virus damages the cells, and upon their death, multiple new copies of the virus are released into the bloodstream, infecting other cells and resulting in disease.

To maintain infectious ability, viruses must retain their complex structure which includes its genetic material, proteins, and fatty envelope. The infective ability of viruses on surfaces outside of the body and the precise mechanism whereby they propagate to people varies among viruses (Paules et al. 2020). Some can only be transmitted through blood transfusions, while others travel from person to person through droplets. Respiratory viruses such as influenza and SARS-CoV-2 rely on droplets produced by infected individuals (usually by coughing or sneezing). Direct exposure of the mouth, nose, or eyes to droplets in the air may result in infection (Carlos et al. 2020; Lu et al. 2020; Xia et al. 2020). Similarly, hand contact with contaminated surfaces and subsequent transfer to the airways or eyes may also lead to infection. SARS-CoV-2 appears infectious through large size droplets as well as fine mist (aerosol). Early reports also suggest evidence of transmission via the fecal-oral route (Gu et al. 2020; Hindson 2020).

SARS-CoV-2 does not stay infectious for extended periods on surfaces and varies from 24 hours on cardboard to several days or a week on plastic surfaces (Carraturo et al. 2020). Cold temperatures do not reduce the lifespan of SARS-CoV-2, and the virus remains viable for a long time in refrigerators and freezers. In contrast, high heat of more than 60 °C kills the virus. Copper and paper surfaces have antiviral activities due to emission of ions or chemical residues, respectively (Ren et al. 2020).

[a]Canadian Centre for Primary Immunodeficiency and the Division of Immunology & Allergy, Department of Paediatrics, The Hospital for Sick Children, Toronto, ON; [b]University of Toronto, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 23 August 2020
Accepted 31 August 2020
Available online 1 September 2020

LymphoSign Journal 7:85–89 (2020)
dx.doi.org/10.14785/lymphosign-2020-0009

Transmission of the virus to another individual may result in infection, which could lead to COVID-19 related disease. Those infected with SARS-CoV-2, like those infected with other coronaviruses or influenza viruses, may become sick or remain asymptomatic (Rothe et al. 2020). COVID-19 disease may be mild or severe, with components and symptoms related to damage inflicted directly by the virus or by a host exaggerated response to the infection (commonly called complications). Individuals sick with COVID-19 typically present with fever, cough, dyspnea, pneumonia, loss of smell and taste, and gastrointestinal symptoms (Jiang et al. 2020; Stawicki et al. 2020).

Mounting consistent experience indicates that the elderly and individuals with existing morbidities are at higher risk of developing more severe disease course (Chen et al. 2020; Huang et al. 2020; Li et al. 2020). Risk factors for critical illness include older age (>65 years), obesity, type II diabetes mellitus, males, cancer, chronic kidney disease, chronic obstructive pulmonary disease, immunodeficiency, serious cardiovascular disease, and sickle cell disease (Chen et al. 2020; Huang et al. 2020; Li et al. 2020). Complications and fatalities associated with COVID-19 are caused by poorly controlled inflammation triggered by the virus, including acute respiratory distress syndrome, coagulation disorders, acute kidney injury, and vascular injury (Chen et al. 2020).

Children and young people with no co-morbidities appear to experience less severe illness when infected (Yu and Chen 2020). Indeed, children make up about 2% of all reported cases in the world (Bialek et al. 2020; Stawicki et al. 2020; Wu and McGoogan 2020). Yet, some limited number of children with more serious complications of COVID-19, mainly multi-system inflammation, have been reported (Riphagen et al. 2020; Waltuch et al. 2020). Neither the true frequency of these events nor the long-term sequelae of COVID-19 in children have been determined.

Disease severity in children should not be confused with the frequency and ability of children to get infected and transmit the virus to others. This issue remains controversial, with some claiming children might be less contagious. Scientific analysis of SARS-CoV-2 transmission conducted by Christian Drosten, Germany's chief virologist, found no differences in coronavirus transmission across all age groups, including children (Jones et al. 2020). The study concluded that children can be as infectious as adults. From the beginning of the pandemic there have been case reports of young children who had high levels of SARS-CoV-2 virus detected in all body fluids tested. Further, a recent study indicated young children secrete more SARS-CoV-2 than sick adults (DeBiasi and Delaney 2020), suggesting children can be as contagious as adults.

Based on evidence and research collected thus far, the following are my recommendations for adults and children with primary immunodeficiency:

1. Keep physical distance from non-family members as much as possible.
2. Use masks when exposed to non-family members outside of your home—they protect you and others (medical masks were found superior).
3. Wash your hands with soap or hand sanitizer after you touch surfaces and especially before you touch your mouth, nose, or your eyes.
4. Avoid unnecessary and close contact with non-family members at all times, but especially in enclosed spaces.
5. Reduce the duration of stay in enclosed spaces which are shared by multiple non-family members.
6. If and when advised by your doctor, undertake schooling/learning from home.
7. Adults with immunodeficiency, if possible, are encouraged to work from home and reduce as much as possible exposure to workplace crowding.
8. Do not miss life saving tests and treatments because of the pandemic.
9. Outdoor activities are encouraged as long as they happen in a safe environment.
10. When in doubt, consult with your immunologist for more specific recommendations related to your condition.
11. These recommendations are case-specific sensitive and could be modified to take into account diverse needs.

COVID-19 related questions and answers for patients with primary immunodeficiency (PID)

1. Are patients with PID at increased risk of contracting COVID-19 illness?

There is little information regarding COVID-19 infections in children in general, or specifically in PID, likely

because such patients have been sheltered thus far. We should assume that children, adolescents, and adults with PID are equally susceptible to infection with this virus.

2. Could patients with PID experience more severe disease if they catch COVID-19?

Patients with PID are at increased risk of developing severe disease for multiple reasons:

1. Those with abnormalities in antibody production (hypogammaglobulinemia, agammaglobulinemia, CVID, others) or T cell dysfunction (SCID, CID) can get very sick when contracting respiratory viruses, and would likely be similarly affected by COVID-19. The degree of illness severity is dictated by the degree of immune competence (mild or profound T cell defect) and the efficacy of treatment (IVIG, BMT, gene therapy).
2. Patients with PID have co-morbidities known to be risk factors for severe COVID-19 illness, including chronic lung disease and diabetes (see Table 1).

We recommend these patients exercise extreme caution when making the decision whether to attend physical schools. It is best to have this specific situation evaluated by their immunologist.

3. How can I protect myself or my child at home?

The way of protecting from respiratory viral infections is to create a safe environment around them. Family members attending school or work should comply with COVID-19 prevention strategies, including physical distancing, hand hygiene, the use of masks, COVID-19 screening when possible, as well as testing if symptomatic.

4. How can I protect myself or my child in public?

1. Avoid enclosed spaces which are known to be a high transmission risk environment.
2. Outdoor activities are encouraged but with proper caution:
    - Avoid direct contact with surfaces, other individuals, or equipment
    - Use hand sanitizer if surfaces are touched after each contact
    - Avoid rubbing your eyes, or touching your mouth and nose
    - Use a face mask as much as possible

With all these measures in an outdoor setting the risk of transmission is very low.

Table 1: **Morbidity associated with PID which increases the risk for severe COVID-19.**

- Asthma
- Chronic lung disease
- Neurologic and neurodevelopmental conditions
- Endocrine disorders (such as diabetes mellitus)
- Heart disease (such as congenital heart disease, congestive heart failure, and coronary heart disease)
- Kidney diseases
- Liver disorders
- People with weakened immune system due to
○ Use of immunosuppressive drugs

5. What should I do if a family member develops respiratory symptoms?

The affected member should isolate themselves as best they can until tested for COVID-19. If possible, PID patients should be tested too, and monitored for symptoms. Importantly, if COVID-19 is suspected or confirmed, contact with other at-risk individuals (elderly family members, those with at-risk diseases) should be avoided.

6. What to do when a PID patient has respiratory symptoms?

Contact your family physician or specialist for instructions. Stay at home and avoid contact with others. Seek COVID-19 testing and monitor symptoms and report these to your doctor.

7. Are patients with PID who contracted COVID-19 contagious?

Patients with PID at all ages can transmit COVID-19 to others. Because many patients with PID have trouble clearing some viral infections and it takes them longer to recover, it is theoretically possible they might carry COVID-19 longer and potentially expose others to the virus for extended periods. It is therefore recommended that PID patients diagnosed with the virus should be

followed and tested repeatedly until clearance of the virus is substantiated.

REFERENCES

Bialek, S., Gierke, R., Hughes, M., McNamara, L.A., Pilishvili, T., and Skoff, T. 2020. Coronavirus disease 2019 in children—United States, February 12–April 2, 2020. Morb. Mortal. Wkly. Rep. **69**: 422–426. PMID: 32271728. doi: 10.15585/mmwr.mm6914e4.

Carlos, W.G., Dela Cruz, C.S., Cao, B., Pasnick, S., and Jamil, S. 2020. Novel Wuhan (2019-nCoV) coronavirus. Am. J. Respir. Crit. Care Med. **201**: P7–P8. PMID: 32004066. doi: 10.1164/rccm.2014P7.

Carraturo, F., Del Giudice, C., Morelli, M., Cerullo, V., Libralato, G., Galdiero, E., and Guida, M. 2020. Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces. Environ. Pollut. **265**: 115010. PMID: 32570023. doi: 10.1016/j.envpol.2020.115010.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., and Zhang, L. 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet, **395**: 507–513. PMID: 32007143. doi: 10.1016/S0140-6736(20)30211-7.

de Wit, E., van Doremalen, N., Falzarano, D., and Munster, V.J. 2016. SARS and MERS: Recent insights into emerging coronaviruses. Nat. Rev. Microbiol. **14**: 523–534. PMID: 27344959. doi: 10.1038/nrmicro.2016.81.

DeBiasi, R.L., and Delaney, M. 2020. Symptomatic and asymptomatic viral shedding in pediatric patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Under the surface. JAMA Pediatr. PMID: 32857158. doi: 10.1001/jamapediatrics.2020.3996.

European Centre for Disease Prevention and Control. 2020. COVID-19 situation update worldwide, as of 31 August 2020 [online]. Available from ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases [accessed 31 August 2020].

Gu, J., Han, B., and Wang, J. 2020. COVID-19: Gastrointestinal manifestations and potential fecal–oral transmission. Gastroenterology, **158**: 1518–1519. PMID: 32142785. doi: 10.1053/j.gastro.2020.02.054.

Hindson, J. 2020. COVID-19: Faecal–oral transmission? Nat. Rev. Gastroenterol. Hepatol. **17**: 259. PMID: 32214231. doi: 10.1038/s41575-020-0295-7.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., and Cao, B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, **395**: 497–506. PMID: 31986264. doi: 10.1016/S0140-6736(20)30183-5.

Jiang, F., Deng, L., Zhang, L., Cai, Y., Cheung, C.W., and Xia, Z. 2020. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). J. Gen. Intern. Med. **35**: 1545–1549. PMID: 32133578. doi: 10.1007/s11606-020-05762-w.

Jones, T.C., Mühlemann, B., Veith, T., Biele, G., Zuchowski, M., Hoffmann, J., Stein, A., Edelmann, A., Corman, V.M., and Drosten, C. 2020. An analysis of SARS-CoV-2 viral load by patient age. medRxiv. doi: 10.1101/2020.06.08.20125484.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K.S.M., Lau, E.H.Y., Wong, J.Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., Chen, C., Jin, L., Yang, R., Wang, Q., Zhou, S., Wang, R., Liu, H., Luo, Y., Liu, Y., Shao, G., Li, H., Tao, Z., Yang, Y., Deng, Z., Liu, B., Ma, Z., Zhang, Y., Shi, G., Lam, T.T.Y., Wu, J.T., Gao, G.F., Cowling, B.J., Yang, B., Leung, G.M., and Feng, Z. 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N. Engl. J. Med. **382**: 1199–1207. PMID: 31995857. doi: 10.1056/NEJMoa2001316.

Lu, C.W., Liu, X.F., and Jia, Z.F. 2020. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet, **395**: e39. PMID: :32035510. doi: 10.1016/S0140-6736(20)30313-5.

Paules, C.I., Marston, H.D., and Fauci, A.S. 2020. Coronavirus infections—More than just the common cold. JAMA, **323**: 707–708. PMID: 31971553. doi: 10.1001/jama.2020.0757.

Ren, S.-Y., Wang, W.-B., Hao, Y.-G., Zhang, H.-R., Wang, Z.-C., Chen, Y.-L., and Gao, R.-D. 2020. Stability and infectivity of coronaviruses in inanimate environments. World J. Clin. Cases, **8**: 1391–1399. PMID: 32368532. doi: 10.12998/wjcc.v8.i8.1391.

Riphagen, S., Gomez, X., Gonzalez-Martinez, C., Wilkinson, N., and Theocharis, P. 2020. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet, **395**: 1607–1608. PMID: 32386565. doi: 10.1016/S0140-6736(20)31094-1.

Rothe, C., Schunk, M., Sothmann, P., Bretzel, G., Froeschl, G., Wallrauch, C., Zimmer, T., Thiel, V.,

Janke, C., Guggemos, W., Seilmaier, M., Drosten, C., Vollmar, P., Zwirglmaier, K., Zange, S., Wolfel, R., and Hoelscher, M. 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N. Engl. J. Med. **382**: 970–971. PMID: 32003551. doi: 10.1056/NEJMc2001468.

Stawicki, S.P., Jeanmonod, R., Miller, A.C., Paladino, L., Gaieski, D.F., Yaffee, A.Q., De Wulf, A., Grover, J., Papadimos, T.J., Bloem, C., Galwankar, S.C., Chauhan, V., Firstenberg, M.S., Di Somma, S., Jeanmonod, D., Garg, S.M., Tucci, V., Anderson, H.L., Fatimah, L., Worlton, T.J., Dubhashi, S.P., Glaze, K.S., Sinha, S., Opara, I.N., Yellapu, V., Kelkar, D., El-Menyar, A., Krishnan, V., Venkataramanaiah, S., Leyfman, Y., Saoud Al Thani, H.A., B Nanayakkara, P.W., Nanda, S., Cioè-Peña, E., Sardesai, I., Chandra, S., Munasinghe, A., Dutta, V., Dal Ponte, S.T., Izurieta, R., Asensio, J.A., and Garg, M. 2020. The 2019–2020 novel coronavirus (severe acute respiratory syndrome coronavirus 2) pandemic: A joint American college of academic international medicine-world academic council of emergency medicine multidisciplinary COVID-19 working group consensus paper. J. Global Infect. Dis. **12**: 47–93. PMID: 32773996. doi: 10.4103/jgid.jgid_86_20.

Waltuch, T., Gill, P., Zinns, L.E., Whitney, R., Tokarski, J., Tsung, J.W., and Sanders, J.E. 2020. Features of COVID-19 post-infectious cytokine release syndrome in children presenting to the emergency department. Am. J. Emerg. Med. PMID: 32471782. doi: 10.1016/j.ajem.2020.05.058.

Wong, J.E.L., Leo, Y.S., and Tan, C.C. 2020. COVID-19 in Singapore-current experience: Critical global issues that require attention and action. JAMA, **323**: 1243–1244. PMID: 32077901. doi: 10.1001/jama.2020.2467.

Wu, Z., and McGoogan, J.M. 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA, **323**: 1239–1242. PMID: 32091533. doi: 10.1001/jama.2020.2648.

Xia, J., Tong, J., Liu, M., Shen, Y., and Guo, D. 2020. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J. Med. Virol. **92**: 589–594. PMID: 32100876. doi: 10.1002/jmv.25725.

Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Zhong, W., and Hao, P. 2020. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci. China Life Sci. **63**: 457–460. PMID: 32009228. doi: 10.1007/s11427-020-1637-5.

Yu, Y., and Chen, P. 2020. Coronavirus disease 2019 (COVID-19) in neonates and children from China: A review. Front. Pediatr. **8**: 287. PMID: 32574286. doi: 10.3389/fped.2020.00287.

Review

Dysregulated CARD11 signaling in the development of diffuse large B cell lymphoma

Tyler A. Jones, Shelby M. Hutcherson, Jacquelyn R. Bedsaul, and Joel L. Pomerantz*

ABSTRACT

CARD11 is a crucial scaffold protein that controls antigen-induced activation of lymphocytes. Upon antigen receptor signaling, CARD11 engages several signaling pathways, leading to the activation of NF-κB, mTOR, and JNK. CARD11 mutations are frequently found in patients with non-Hodgkin lymphoma and their ability to induce aberrant lymphocyte proliferation may be enhanced by mutations in regulators of CARD11 signal transduction. Here we describe how dysregulated CARD11 activity can promote lymphomagenesis through branched signaling pathways whose components and intermediates provide targets for novel diagnostic and therapeutic approaches.

Statement of novelty: This review discusses how gain-of-function CARD11 mutations promote lymphomagenesis by engaging branching signaling pathways and how these different pathways provide multiple targets for therapies.

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common lymphoid malignancy among adults and represents 30%–40% of non-Hodgkin lymphoma cases (Fisher and Fisher 2004). DLBCL is a fast-growing, aggressive type of non-Hodgkin lymphoma that has a median survival of <1 year if untreated (Rovira et al. 2015). Through gene expression profiling, DLBCL has been classified into activated B cell (ABC) and germinal center B cell (GCB) subtypes. The GCB DLBCL subtype expresses genes that define normal germinal center B cells, while the ABC DLBCL subtype exhibits a transcriptional profile similar to mature plasma cells (Alizadeh et al. 2000). The standard of care for ABC DLBCL is a combination of the anti-CD20 antibody rituximab and a 4-drug chemotherapy regimen of cyclophosphamide, doxorubicin, vincristine, and prednisone collectively referred to as R-CHOP; unfortunately, this treatment fails in more than 50% of ABC DLBCL patients (Coiffier and Sarkozy 2016). A thorough understanding of mutations that influence DLBCL disease progression will be necessary to innovate diagnostic tools and targeted therapies and improve outcomes for these patients.

One common signaling cascade that is dysregulated in several types of lymphoid malignancies is the nuclear factor kappa B (NF-κB) pathway. Nearly all cases of ABC DLBCL are characterized by constitutive activation of NF-κB, which has been shown to drive lymphomagenesis (Davis et al. 2001). NF-κB regulates the differentiation, cell cycle progression, proliferation, and survival of lymphocytes. Therefore, it is not surprising that certain mutations in signaling proteins upstream of NF-κB have been shown to cause aberrant lymphocyte activation and are associated with DLBCL cases. An obligate component of antigen receptor

Department of Biological Chemistry and Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD

*Corresponding author: Joel L. Pomerantz/joel.pomerantz@jhmi.edu

Submitted 14 June 2020
Accepted 2 August 2020
Available online 5 August 2020

LymphoSign Journal 7:90–103 (2020)
dx.doi.org/10.14785/lymphosign-2020-0006

signaling upstream of NF-κB activation is the scaffold protein CARD11. Approximately 10% of human ABC DLBCL biopsies exhibit gain-of-function (GOF) mutations in CARD11 that lead to enhanced NF-κB activation (Lenz et al. 2008). In addition to regulating NF-κB, CARD11 also engages branching signaling pathways leading to mammalian target of rapamycin (mTOR), Forkhead box protein O1 (FOX01), and c-Jun N-terminal kinases (JNK) activation through mechanisms that are not fully understood (Blonska et al. 2007; Oeckinghaus et al. 2007; Chen 2012; Wray-Dutra et al. 2018; Wei et al. 2019). Investigating the complex integration of multiple signaling pathways that may become dysregulated by mutant CARD11 is essential for understanding the role of CARD11 in the stages of lymphocyte oncogenesis. This review will summarize the molecular mechanisms by which mutations in both CARD11 and regulators of CARD11 drive lymphomagenesis and will provide supporting evidence that intermediates within the CARD11 signaling pathways serve as important therapeutic targets for treating DLBCL (Figure 1).

CARD11 signaling to NF-κB

Several studies have established the role of CARD11 in antigen-induced lymphocyte activation (Bedsaul et al. 2018). In the absence of antigen receptor signaling, CARD11 remains in an inactive conformation due to an inhibitory domain (ID) that contains 4 small Repressive Elements (REs) that prevent cofactor interaction (Jattani et al. 2016*a*, 2016*b*). Antigen receptor triggering leads to the phosphorylation of CARD11 serine residues 564, 567, 577, and 657 within the ID, in part by PKCβ in B cells and PKCθ in T cells, both of which are thought to phosphorylate S564 and S657 (Matsumoto et al. 2005; Sommer et al. 2005). Phosphorylation of CARD11 by other kinases also plays a role in promoting maximal CARD11 activity, including the phosphorylation of S567 by IKKβ (Shinohara et al. 2007). Though the mechanism remains unknown, phosphorylation of these serine residues in the ID is thought to neutralize the inhibitory function of the REs and allow CARD11 to undergo a conformational change to an open and active state. Once converted to an active scaffold, CARD11 is able to recruit several cofactors that bind to sites located in the CARD, LATCH, and Coiled-coil domains, such as Bcl10, MALT1, TRAF6, and HOIP (Ruland et al. 2001; Che et al. 2004; Sun et al. 2004; Yang et al. 2016). This multiprotein complex then activates the IKK complex, leading to the phosphorylation of the IκBα inhibitory protein and its subsequent ubiquitinylation and proteasomal degradation (Figure 1). NF-κB then translocates into the nucleus and regulates the transcription of pro-proliferative, pro-inflammatory, and anti-apoptotic genes that are important for lymphocyte function. Following activation, the signaling cofactors dissociate and CARD11 returns to its inactive state (Bedsaul et al. 2018).

Mutations that disrupt the autoinhibition of CARD11 have been linked to immunodeficiency and lymphoproliferative diseases. CARD, LATCH, and Coiled-coil domain mutations have been found in DLBCL biopsies, including C49Y, G123S, and G123D (Lenz et al. 2008; Compagno et al. 2009). Mutations in these domains disrupt autoinhibition by 4 REs (Jattani et al. 2016*b*) and increase signaling to NF-κB by 80- to 160-fold. This enhanced activity results in constitutive Bcl10 and HOIP association with CARD11, leading to the generation of polyubiquitinylated Bcl10 ($LinUb_n$-Bcl10), a key signaling intermediate that determines the quantitative output of CARD11 signaling (Lamason et al. 2010; Chan et al. 2013; Yang et al. 2016). Several studies have documented a critical role for hyperactive CARD11 signaling in promoting lymphomagenesis.

RNA interference and CRISPR/Cas9 screens have shown that most ABC DLBCL cell lines depend on CARD11-Bcl10-MALT1 (CBM) complex signaling (Ngo et al. 2006; Reddy et al. 2017; Phelan et al. 2018). Furthermore, DLBCL-derived CARD11 mutations introduced into activated B cells ex vivo and transplanted into *RAG1*−/− recipient mice were shown to induce aberrant B cell proliferation, antibody secretion, and plasmacyctic differentiation (Jeelall et al. 2012). In addition, mice expressing the strongly hyperactive CARD11 L225LI mutation under the control of the *Rosa26* promoter in the B cell lineage succumbed to aggressive B cell lymphoproliferation a few days after birth, demonstrating that heightened CARD11 signaling can be sufficient to cause a disease phenotype resembling ABC DLBCL (Knies et al. 2015). NF-κB signaling was found to be essential for CARD11 L225LI B cell survival because treating these cells with the IKK inhibitor Bay11-7082 prevented their survival and proliferation in vitro. Interestingly, other mouse models expressing GOF CARD11 alleles do not readily develop lymphoma, suggesting that the accumulation of additional mutations is necessary to drive

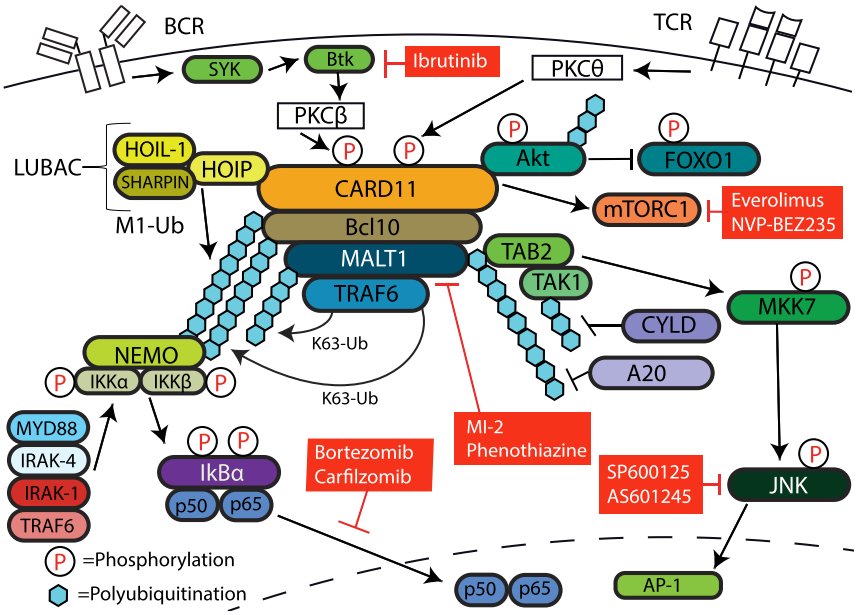


Figure 1: B cell receptor and T cell receptor signaling to CARD11 activates a network of signaling pathways that offer therapeutic targets. Therapeutics are indicated by the red boxes.

malignancy of B cells that express these GOF CARD11 mutations (Wray-Dutra et al. 2018; Wei et al. 2019). The molecular mechanisms that cause some GOF CARD11 mutations to promote lymphoma while others do not are still not well understood.

Certain GOF mutations found in DLBCL biopsy samples such as C49Y and G123S are also present in patients with B cell expansion with NF-κB and T cell anergy (BENTA) disease (Snow et al. 2012; Buchbinder et al. 2015). Occurring predominately in children, BENTA patients present with B cell expansion, splenomegaly, and susceptibility to recurrent ear, sinus, and infections of Epstein-Barr virus, molluscum contagiosum virus, or BK virus. BENTA patients have T cell numbers within normal pediatric ranges, but their T cells are hyporesponsive to ex vivo stimulation. Patients have reduced numbers of class-switched and memory B cells and their naive B cells have reduced capacity to differentiate into long-lived plasma cells (PCs) in vitro (Arjunaraja et al. 2017). Two BENTA patients have developed B cell tumors in adulthood, suggesting that the increased survival and expansion of BENTA B cells could allow for the acquisition of more mutations, and therefore predispose individuals to developing lymphoma (Lu et al. 2018). However, more studies are needed to identify and verify the other cooperating mutations that may be driving B cell lymphoma in BENTA patients.

GOF CARD11 mutations are not restricted to ABC DLBCL and BENTA disease and have also been identified in other DLBCL subtypes as well as in other malignancies including Acute T cell Leukemia/Lymphoma, Sézary syndrome, and Angioimmunoblastic T cell lymphoma (Compagno et al. 2009; da Silva Almeida et al. 2015; Kataoka et al. 2015; Wang et al. 2015; Vallois et al. 2016; Bedsaul et al. 2018). For example, the CARD11 variants F176C and F902C found in Angioimmunoblastic T cell lymphoma, and S615F and E262K found in Sézary syndrome, were confirmed to exhibit hyperactive CARD11 signaling to NF-κB (da Silva Almeida et al. 2015; Vallois et al. 2016). Determining the role of various CARD11 mutations in promoting these distinct lymphoid malignancies is an area of active investigation that may provide new insights to improve diagnostics and treatment options for CARD11-mediated diseases.

mTOR and Akt-FOXO1 signaling

In addition to signaling to NF-κB, CARD11 also engages the mTOR pathway, which is known to regulate genes involved in cell growth, survival, and proliferation

(Figure 1). CARD11 is thought to activate mTOR complex 1 (mTORC1) through direct interaction with ASCT2, by inducing ASCT2 gene expression, or both. ASCT2 then promotes glutamine uptake into the cell. Both CARD11 and MALT1 are required for optimal mTORC1 activation by antigen receptor signaling (Hamilton et al. 2014; Nakaya et al. 2014). Recently, it has been suggested that increased CARD11 signaling to mTORC1 could promote ABC DLBCL (Wray-Dutra et al. 2018). To elucidate the role of aberrant CARD11-mediated mTORC1 activation in ABC DLBCL, mice were engineered to express the L251P GOF CARD11 mutation from the *Rosa26* locus downstream of a lox-STOP-lox cassette and were then mated to Mb1-Cre mice to restrict Cre recombinase activity to B cells beginning at the pro-B cell stage (Mb1-aCard11). Germinal center (GC) B cells from the Mb1-a Card11 transgenic mice exhibited increased phosphorylation of S6 and 4E-BP1 as measured by flow cytometry, indicating increased mTOR signaling. Following sheep red blood cell (SRBC) immunization, GC B cells expressing CARD11 L251P also exhibited increased cell cycling and class switching, indicating enhanced terminal differentiation into short-lived PCs (Arjunaraja et al. 2017). Restricting aCARD11 expression to GC B cells using Cγ1-Cre also resulted in a smaller GC compartment and earlier PC production. Furthermore, the GC B cells also exhibited elevated levels of activation-induced-deaminase (AID) and decreased expression of the transcription factor FOXO1, which may help explain the aberrant phenotypes associated with GOF CARD11 in B cells (Wray-Dutra et al. 2018).

AID is the enzyme responsible for isotype switching and producing the diversity observed in the B cell receptor (BCR) repertoire through the process of somatic hypermutation. Overactive AID is associated with malignancies that have been attributed to hypermutations occurring in oncogenes as well as unintended genomic translocations (Maul and Gearhart 2010). FOXO1 is known to regulate levels of AID and the loss of FOXO1 has been shown to skew GC B cells toward the dark-zone phenotype (Dominguez-Sola et al. 2015; Sander et al. 2015). The dark zone is the compartment of the GC that predominately contains B cells undergoing proliferation and somatic hypermutation, while in the light zone B cells encounter and respond to selection signals (Victora et al. 2012). The results of Wray-Dutra et al. (2018) raised the possibility that some GOF CARD11 mutants promote ABC DLBCL lymphomagenesis through the enhanced differentiation of GC-derived B cells into PCs in concert with the accumulation of AID-mediated genetic aberrations that arise during the GC response. However, it appears that not all GOF CARD11 mutants regulate FOXO1 and B cell differentiation in the same way (Wei et al. 2019).

In B cells, phosphatidylinositol 3-kinase (PI3K) and mTORC2 can phosphorylate and activate the serine/threonine kinase Akt, which then phosphorylates FOXO1, suppressing its transcriptional activity and causing it to be excluded from the nucleus and degraded (Yusuf et al. 2004; Burgering 2008). FOXO1 is known to regulate cell cycle arrest through enhancing expression of the cyclin-dependent kinase inhibitor $p27^{kip1}$, down-regulating the D-type cyclins D1 and D2, and inactivating the S-phase repressor pRb (Medema et al. 2000; Schmidt et al. 2002). The suppression and degradation of FOXO1 allows for cell cycle progression, thereby increasing cell proliferation. It has been suggested that CARD11 signaling negatively regulates Akt activation through mechanisms that may involve Akt interaction with CARD11 via the Coiled-coil domain and K63-linked polyubiquitinylation of Akt, which affects Akt membrane recruitment and subsequent activation (Yang et al. 2009). Interestingly, Wei et al. (2019) determined that GOF CARD11 variants bind Akt with different affinities, leading to distinct outcomes of Akt activation and B cell differentiation.

To study how GOF CARD11 mutants differentially regulate the Akt/FOXO1 signaling pathway, Wei et al. (2019) used CRISPR/Cas9 genomic editing to generate knockin mice expressing mutations associated with BENTA disease, ABC DLBCL, and GCB DLBCL. Compared to wild-type CARD11, the GCB DLBCL variants K215M and L232LI were shown to enhance Akt interaction with CARD11, which suppressed Akt activation and enhanced FOXO1 protein and target gene expression. In contrast, the G123S variant found in both ABC DLBCL and BENTA patients exhibited reduced interaction with Akt, which led to enhanced Akt activation and reduced FOXO1 protein and target gene expression. These differences in Akt association and FOXO1-dependent gene expression correlated with changes in B cell differentiation. For example, the CARD11 K215M mutation further promoted GC B cell development in mixed bone marrow chimera experiments and K215M-expressing GC B cells displayed

increased levels of FOXO1 protein. In contrast, the CARD11 E134G mutation found in BENTA patients led to a markedly reduced GC compartment and GC B cells expressing this mutation exhibited lower levels of FOXO1 protein (Wei et al. 2019). The differential effects of GOF CARD11 mutations on Akt signaling to FOXO1, and the downstream impact on B cell differentiation, suggest that this property could influence the tendency of a particular CARD11 variant to promote one subtype of DLBCL or another in the context of lymphomagenesis. This notion warrants further investigation.

JNK signaling

CARD11 is also required for antigen receptor signaling to JNK, a kinase that controls cellular responses to stress stimuli and regulates apoptosis (Figure 1). CARD11 signaling specifically engages JNK2 in lymphocytes through a process that involves the CARD11 cofactors Bcl10, MALT1, and TAK1. Upon signal-induced Bcl10 oligomerization, the K63-linked polyubiquitinylation of MALT1, and the subsequent recruitment of TAB2 to ubiquitinylated MALT1, the kinases TAK1 and MKK7 phosphorylate and thereby activate JNK2 (Blonska et al. 2007; Oeckinghaus et al. 2007; Chen 2012) (Figure 1).

Knies et al. (2015) demonstrated the importance of JNK signaling in ABC DLBCL. The researchers found that the human ABC DLBCL cell lines HBL-1, OCI-Ly3, and OCI-Ly10, which exhibit constitutive JNK activation, were sensitive to SP600125, an ATP-competitive inhibitor of JNK2. Strikingly, they also found that 55% of human ABC DLBCL biopsies stained positive for phosphorylated JNK2, while all tested GCB DLBCL biopsies stained negative (Knies et al. 2015). Knies et al. (2015) also examined the role of JNK signaling in a mouse model in which the human DLBCL-derived mutation CARD11 L225LI was expressed from the *Rosa26* locus in a CD19-Cre-dependent manner. CARD11 L225LI-expressing B cells displayed high levels of c-Jun, constitutive JNK phosphorylation and activation, and increased concentrations of the phosphorylated forms of the AP-1 transcription factor subunits c-Jun and activating transcription factor 2 (ATF2). Autonomously proliferating CARD11 L225LI-expressing B cells isolated from these mice were susceptible to killing by SP600125 treatment in culture, similar to their susceptibility to the NF-κB pathway inhibitor Bay11-7082, indicating both pathways are important in sustaining proliferation by GOF CARD11.

AP-1 transcription factors are composed of heterodimeric complexes of Jun and ATF subfamilies, and it is not fully understood how the individual AP-1 family members regulate oncogenic B cell survival (Eferl and Wagner 2003). Juilland et al. (2016) showed that high expression of c-Jun, JunB, and ATF3 in ABC DLBCL cells is dependent on CARD11, MALT1, and the Toll-like receptor (TLR) signaling adaptor MyD88. Furthermore, blocking AP-1 function with a dominant-negative A-Fos construct or by silencing of ATF2 or ATF3 led to decreased viability in the HBL-1 ABC DLBCL cell line. Juilland et al. (2016) also observed that high nuclear protein expression of ATF3 appears to be a specific feature of non-GC/ABC DLBCL patient biopsies, further highlighting the importance of ATF3-containing AP-1 complexes downstream of chronic CARD11 signaling in ABC DLBCL. Taken together, these results indicate that the JNK signaling pathway is a potential therapeutic target that could be especially effective in DLBCL cases found to contain hyperactive CARD11 signaling.

Key role of E3 ligases and deubiquitinases

An important mechanism to attenuate CARD11 signaling is the removal of key polyubiquitinylated signaling intermediates. A20 is a ubiquitin editing enzyme that negatively regulates NF-κB signaling through a variety of mechanisms. For example, A20 can disrupt and cause steric hindrance of ubiquitin-mediated protein interactions and act as a deubiquitinase that cleaves K63-linked polyubiquitin chains on MALT1, thereby repressing NF-κB activation (Figure 1) (Düwel et al. 2009; Shembade et al. 2010; Srinivasula and Ashwell 2011). A20 is frequently mutated in DLBCL, with about 33% of ABC DLBCL patients having biallelic inactivation of A20 as a result of point mutations or epigenetic silencing (Compagno et al. 2009; Kato et al. 2009). Two different studies have shown that when A20 is introduced into the lymphoma cell lines SUDHL2, RC-K8, and KM-H2, which have biallelic *TNFAIP3* (A20) mutations causing inactivation and constitutive NF-κB signaling, these cell lines exhibit decreased NF-κB signaling, arrested cell growth, and eventually undergo apoptosis (Compagno et al. 2009; Kato et al. 2009).

Another negative regulator of NF-κB signaling is the cylindromatosis tumor suppressor protein CYLD, which suppresses IKK complex activation (Figure 1). CYLD cleaves K63-linked polyubiquitin from positive regulators of NF-κB signaling, such as IKKγ/NEMO, TRAF2, TRAF6, TRAF7, RIP1, and TAK1 (Brummelkamp et al. 2003; Kovalenko et al. 2003; Trompouki et al. 2003; Yoshida et al. 2005). CYLD also functions as a negative regulator of JNK and AP-1 by deubiquitinylating TAK1 (Reiley et al. 2007). Consistent with the notion that CYLD has the potential to function as a tumor suppressor in lymphomas that have constitutive CARD11 signaling, Xu (2019) found that ABC DLBCL cell lines HBL-1 and OCI-Ly10 and patients with non-GCB DLBCL exhibit constitutive phosphorylation of CYLD, a modification that represses its function. Both CYLD and A20 are cleaved by MALT1 to maximize JNK and NF-κB activation, respectively (Coornaert et al. 2008; Staal et al. 2011). MALT1 protease inhibitors may therefore be particularly effective at reducing constitutive CARD11 signaling by preventing MALT1 from silencing negative feedback mechanisms.

The E3 ubiquitin ligase RNF181 is another negative regulator of normal and oncogenic CARD11 signaling. Pedersen et al. (2016) showed that RNF181 limits the steady-state level of Bcl10 through K48-linked ubiquitinylation and degradation and in doing so, limits the proliferation of DLBCL cells that are dependent on active CARD11 signaling for growth and survival. Increasing the function or expression levels of the proteins that mediate K48-linked Bcl10 ubiquitinylation could provide a novel method to inhibit GOF CARD11 signaling.

Because the CARD11 signaling pathway depends on ubiquitinylation for downstream signaling, mutations in key E3 ubiquitin ligases that regulate CARD11 signaling could further increase NF-κB activation. The recruitment of Bcl10 and HOIP, the catalytic subunit of the Linear Ubiquitin Chain Assembly Complex (LUBAC), to CARD11 allows HOIP to conjugate linear ubiquitin chains to Bcl10 (Yang et al. 2016) (Figure 1). This $LinUb_n$-Bcl10 then binds to the IKK complex, promoting its activation. Mutations in the *RNF31* gene, which encodes HOIP, were found in 8% of ABC DLBCL samples (Y. Yang et al. 2014). CARD11 variants found in ABC DLBCL samples increased the association of CARD11 with both HOIP and Bcl10 in the absence of antigen receptor signaling (Chan et al. 2013; Yang et al. 2016). Because the level of NF-κB activation of each oncogenic CARD11 variant correlated with increased Bcl10 association and steady-state levels of $Lin(Ub)_n$-Bcl10, the ability of a CARD11 mutant to generate $Lin(Ub)_n$-Bcl10 likely determines its potential to promote lymphomagenesis. Therefore, $Lin(Ub)_n$-Bcl10 generation by CARD11 variants may serve as an effective tool for screening the signaling potency of future CARD11 mutations that are associated with disease.

Bridging BCR and TLR signaling

In addition to mutant CARD11 and the BCR signaling pathway, Toll-like receptors (TLRs) can also activate NF-κB in ABC DLBCL. MyD88, an adaptor protein for TLR signaling, is critical for NF-κB activation in response to pro-inflammatory stimuli such as IL-1, IL-18, and LPS (Adachi et al. 1998; Kawai et al. 1999). ABC DLBCL tumors have been shown to have co-occurring mutations in CD79B/A, A20, CARD11, and MYD88 (Ngo et al. 2011). Phelan et al. (2018) studied how hyperactive MyD88 can specifically interact with the CARD11 signaling pathway by performing BioID experiments using the L265P GOF MyD88 mutant. CARD11 and MALT1 were both biotinylated in ABC DLBCL cells expressing a L265P MyD88-BioID construct, providing evidence for close proximity of MyD88 to the CBM complex. Furthermore, CARD11/Bcl10 cytoplasmic puncta visualized by fluorescent proximity ligation assays were reduced by knockdown of TLR9 or MyD88 in double mutant cell lines. MALT1/MyD88 and Bcl10/MyD88 cytoplasmic puncta were reduced by knocking down CD79A, TLR9, and CARD11, which suggests the BCR and TLR9 may cooperate to control the assembly of a MyD88 and CBM supercomplex in ABC DLBCL cells. Additionally, knockdown of both MyD88 and CARD11 in HBL-1 cells via shRNA infection led to greater cell death compared to knockdown of either gene alone, suggesting that MyD88 and CARD11 signaling pathways cooperate to promote cell survival (Ngo et al. 2011). Silencing of MyD88 by shRNA in ABC DLBCL cell lines has also been shown to reduce expression of c-Jun, JunB, and ATF3, suggesting that hyperactive MyD88 has a role in mediating JNK signaling in ABC DLBCL (Juilland et al. 2016). If the crosstalk between MyD88 and CARD11 signaling pathways observed in ABC DLBCL is dependent on the co-occurrence of specific mutations in pathway components,

the targeting of this crosstalk may be a fruitful approach for precision treatment of lymphoma.

Therapies targeting CARD11 signaling

The prevalence of CARD11 mutations in lymphoma cells acquiring secondary resistance underscores the need to develop new therapeutic strategies that account for the specific mutations in each patient's lymphoid cancer. Some therapies that have shown promise in treating ABC DLBCL are specifically ineffective for patients harboring CARD11 mutations. A common target for treating B cell malignances is the non-receptor tyrosine kinase BTK, which acts downstream of the BCR to activate PKCβ (Figure 1). The BTK inhibitor ibrutinib has shown promise as a new therapy for treating ABC DLBCL, with Phase II trials showing a 40% objective response rate in patients (Aalipour and Advani 2014). However, ibrutinib was ineffective when tumors expressed *TNFAIP3* or GOF CARD11 mutations, as these mutations activate the pathway downstream of BTK (Aalipour and Advani 2014; Wilson et al. 2015).

A new strategy to treat DLBCL is to target the signaling cofactors of CARD11. As a central component of the CBM complex, MALT1 is an attractive target and inhibition of MALT1 shows promise to attenuate constitutive CARD11 signaling. The MALT1 inhibitor zVRPR-fmk was shown to inhibit the growth of ABC DLBCL cell lines in which CARD11 mutations drive constitutive MALT1 activation, but not to inhibit growth of GCB DLBCL cell lines in vitro (Ferch et al. 2009; Hailfinger et al. 2009). Other small molecule inhibitors of MALT1 have also shown good potential. Phenothiazines have elicited selective toxic effects for MALT1-dependent ABC DLBCL cells in vitro and in vivo (Nagel et al. 2012), while the MALT1 inhibitor MI-2 inhibited growth of ABC DLBCL cell lines in vitro and xenotransplanted ABC DLBCL tumors (Fontan et al. 2012). In addition, ABC DLBCL cells that harbor mutations in CD79 or CARD11 were treated with a combination of the BTK inhibitor ibrutinib and the MALT1 inhibitor S-mepazine and were found to exhibit markedly reduced MALT1 protease activity and NF-κB pro-survival factors that ultimately resulted in cell death (Nagel et al. 2015).

Another therapeutic strategy is to target signaling events downstream of the CBM complex such as the phosphorylation, ubiquitinylation, or degradation of IκBα, which sequesters NF-κB in the cytoplasm in the absence of signaling (Beg et al. 1992). For example, bortezomib blocks the proteolytic activity of the proteasome, thereby preventing signal-induced IκBα degradation (McConkey and Zhu 2008). When DLBCL patients received bortezomib in combination with chemotherapy, significantly higher overall survival was observed in patients with ABC DLBCL compared to patients with GCB DLBCL (Dunleavy et al. 2009). Furthermore, the second-generation proteasome inhibitor carfilzomib is an irreversible inhibitor, in contrast to the reversible binding of bortezomib, and is more selective for chymotryptic activity of the 20S proteasome than bortezomib (Demo et al. 2007). Carfilzomib has been shown to induce cell death in DLBCL lymphoma cell lines, including in rituximab-resistant lymphoma cell lines, and in primary B cell lymphoma patient samples (Gu et al. 2013). Since lymphoma cells with GOF CARD11 mutations can acquire resistance to common treatment options, inhibitors directly targeting CARD11 and signaling intermediates to NF-κB have the potential to be particularly effective at treating cancers with these specific CARD11 mutations.

Drugs targeting the other signaling pathways regulated by CARD11 are also currently being investigated. Targeting mTOR signaling proteins such as mTORC1 will likely improve outcomes for some patients, as it has been shown that DLBCL patients with an active mTORC1 signature have unfavorable responses to R-CHOP treatment compared to DLBCL patients who exhibit an inactive mTORC1 signature (Sebestyén et al. 2012). Inhibitors of mTOR signaling are under investigation for their effectiveness in treating DLBCL. The first generation mTOR inhibitors are called rapalogs because they are chemically derived from rapamycin. Rapalogs are allosteric inhibitors that block the interaction between mTOR and its intracellular receptor, FBPK12. Providing rapalogs as a monotherapy for cancer has had limited success, with modest response rates in major solid tumors (Don and Zheng 2010) that could be due to incomplete mTOR inhibition or their inability to prevent a negative feedback loop that activates Akt. Rapalogs have had success when combined with drugs that target other known oncogenic drivers. For example, the mTORC1 inhibitor everolimus and the anti-CD20 antibody rituximab were combined in a large Phase II study in relapsed/refractory DLBCL and showed an objective

response rate of 38% (Merli et al. 2015). Second generation mTOR inhibitors have been shown to overcome pro-survival feedback loops by inhibiting both mTORC1 and mTORC2 through targeting the ATP binding site in the mTOR kinase domain. The second generation mTOR inhibitor NVP-BEZ235 in combination with the immunomodulatory agent lenalidomide has been shown to effectively reduce cell proliferation in the non-GCB DLBCL derived cell lines OCI-Ly10, OCI-Ly3, and SUDHL2 and suppress tumor growth in an OCI-Ly10 xenograft mouse model (Jin et al. 2016). In addition, the combination of the BTK inhibitor ibrutinib and the ATP-competitive mTOR inhibitor AZD2014 was shown to strongly induce apoptosis in ABC-type DLBCL cell lines and suppress tumor growth in an OCI-Ly10 xenograft mouse model (Ezell et al. 2014). Clinical trials are still underway for a variety of cancer types to determine the efficacy and tolerability of second generation mTOR inhibitors, including NVP-BEZ235, PF-04691502, and GDC-0980 (Yang et al. 2019).

As alluded to previously, JNK is another therapeutic target for the treatment of lymphomas that express GOF CARD11 mutations. Two ATP-competitive JNK inhibitors, SP600125 and AS601245, have shown promise in preclinical studies to treat malignancies, such as T cell acute lymphoblastic leukemia (Cui et al. 2009). Both inhibitors are widely used for in vitro studies and results suggest that they could be used to treat a variety of other cancers, including colon and stomach cancers (Wu et al. 2020). However, there is a need to develop new JNK inhibitors that selectively inhibit specific isoforms of JNK to improve their efficacy on the distinct cancer types.

Conclusion

GOF CARD11 mutations are associated with an increasing number of lymphoid malignancies and B cell proliferative diseases, such as BENTA. DLBCL tumors can utilize chronically active forms of CARD11 or disrupt the negative regulation of CARD11 signaling to convert the transient and self-limiting antigen-dependent response that normally restricts lymphocyte growth into a sustained proliferative signal. Because CARD11 acts as a signaling hub for a variety of pathways, including NF-κB, mTOR, and JNK, there are ongoing efforts to elucidate all the branching effects of CARD11 signaling to provide insight into the nature of lymphoma development. CARD11 signal transduction becomes amplified when mutations occur that dysregulate the polyubiquitinylation of downstream signaling intermediates or when hyperactive TLR signaling cooperates with GOF CARD11 to drive oncogenesis. As discussed previously, certain drugs targeting the cofactors of CARD11 signaling are already showing promise as therapeutic strategies. The potential for pharmacological interference of CARD11 signaling remains to be fully explored, including the development of inhibitors that prevent the formation of the CBM complex by blocking the interactions between Bcl10 and CARD11 or MALT1 (C. Yang et al. 2014). New strategies to limit aberrant CARD11 activation without crippling the immune homeostasis role of the CBM complex will be needed to mitigate various lymphomas and lymphoproliferative diseases involving dysregulated CARD11. Examination of these future therapies in different lymphoma subsets and as part of novel combination therapies could reveal promising options for precision medicine in lymphoma treatment.

REFERENCES

Aalipour, A., and Advani, R.H. 2014. Bruton's tyrosine kinase inhibitors and their clinical potential in the treatment of B-cell malignancies: Focus on ibrutinib. Ther. Adv. Hematol. **5**(4): 121–133. PMID: 25360238. doi: 10.1177/2040620714539906.

Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., and Akira, S. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity, **9**(1): 143–150. PMID: 9697844. doi: 10.1016/S1074-7613(00)80596-8.

Alizadeh, A.A., Eisen, M.B., Davis, R.E., Ma, C., Lossos, I.S., Rosenwald, A., Boldrick, J.C., Sabet, H., Tran, T., Yu, X., Powell, J.I., Yang, L., Marti, G.E., Moore, T., Hudson, J., Jr., Lu, L., Lewis, D.B., Tibshirani, R., Sherlock, G., Chan, W.C., Greiner, T.C., Weisenburger, D.D., Armitage, J.O., Warnke, R., Levy, R., Wilson, W., Grever, M.R., Byrd, J.C., Botstein, D., Brown, P.O., and Staudt, L.M. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature, **403**(6769): 503–511. PMID: 10676951. doi: 10.1038/35000501.

Arjunaraja, S., Nosé, B.D., Sukumar, G., Lott, N.M., Dalgard, C.L., and Snow, A.L. 2017. Intrinsic plasma cell differentiation defects in B cell expansion with NF-κB and T cell anergy patient B cells. Front. Immunol. **8**: 913. PMID: 28824638. doi: 10.3389/fimmu.2017.00913.

Bedsaul, J.R., Carter, N.M., Deibel, K.E., Hutcherson, S.M., Jones, T.A., Wang, Z., Yang, C., Yang, Y.K., and Pomerantz, J.L. 2018. Mechanisms of regulated and dysregulated CARD11 signaling in adaptive immunity and disease. Front. Immunol. **9**: 2105. PMID: 30283447. doi: 10.3389/fimmu.2018.02105.

Beg, A.A., Ruben, S.M., Scheinman, R.I., Haskill, S., Rosen, C.A., and Baldwin, A.S., Jr. 1992. I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: A mechanism for cytoplasmic retention. Genes Dev. **6**(10): 1899–1913. PMID: 1340770. doi: 10.1101/gad.6.10.1899.

Blonska, M., Pappu, B.P., Matsumoto, R., Li, H., Su, B., Wang, D., and Lin, X. 2007. The CARMA1-Bcl10 signaling complex selectively regulates JNK2 kinase in the T cell receptor-signaling pathway. Immunity, **26**(1): 55–66. PMID: 17189706. doi: 10.1016/j.immuni.2006.11.008.

Brummelkamp, T.R., Nijman, S.M., Dirac, A.M., and Bernards, R. 2003. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-κB. Nature, **424**(6950): 797–801. PMID: 12917690. doi: 10.1038/nature01811.

Buchbinder, D., Stinson, J.R., Nugent, D.J., Heurtier, L., Suarez, F., Sukumar, G., Dalgard, C.L., Masson, C., Parisot, M., Zhang, Y., Matthews, H.F., Su, H.C., Durandy, A., Fischer, A., Kracker, S., and Snow, A.L. 2015. Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation. J. Allergy Clin. Immunol. **136**(3): 819–821.e1. PMID: 25930198. doi: 10.1016/j.jaci.2015.03.008.

Burgering, B.M.T. 2008. A brief introduction to FOXOlogy. Oncogene, **27**(16): 2258–2262. PMID: 18391968. doi: 10.1038/onc.2008.29.

Chan, W., Schaffer, T.B., and Pomerantz, J.L. 2013. A quantitative signaling screen identifies CARD11 mutations in the CARD and LATCH domains that induce Bcl10 ubiquitination and human lymphoma cell survival. Mol. Cell. Biol. **33**(2): 429–443. PMID: 23149938. doi: 10.1128/mcb.00850-12.

Che, T., You, Y., Wang, D., Tanner, M.J., Dixit, V.M., and Lin, X. 2004. MALT1/paracaspase is a signaling component downstream of CARMA1 and mediates T cell receptor-induced NF-κB activation. J. Biol. Chem. **279**(16): 15870–15876. PMID: 14754896. doi: 10.1074/jbc.M310599200.

Chen, Z.J. 2012. Ubiquitination in signaling to and activation of IKK. Immunol. Rev. **246**(1): 95–106. PMID: 22435549. doi: 10.1111/j.1600-065X.2012.01108.x.

Coiffier, B., and Sarkozy, C. 2016. Diffuse large B-cell lymphoma: R-CHOP failure—What to do? Hematology, **2016**(1): 366–378. PMID: 27913503. doi: 10.1182/asheducation-2016.1.366.

Compagno, M., Lim, W.K., Grunn, A., Nandula, S.V., Brahmachary, M., Shen, Q., Bertoni, F., Ponzoni, M., Scandurra, M., Califano, A., Bhagat, G., Chadburn, A., Dalla-Favera, R., and Pasqualucci, L. 2009. Mutations of multiple genes cause deregulation of NF-κB in diffuse large B-cell lymphoma. Nature, **459**(7247): 717–721. PMID: 19412164. doi: 10.1038/nature07968.

Coornaert, B., Baens, M., Heyninck, K., Bekaert, T., Haegman, M., Staal, J., Sun, L., Chen, Z.J., Marynen, P., and Beyaert, R. 2008. T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-κB inhibitor A20. Nat. Immunol. **9**(3): 263–271. PMID: 18223652. doi: 10.1038/ni1561.

Cui, J., Wang, Q., Wang, J., Lv, M., Zhu, N., Li, Y., Feng, J., Shen, B., and Zhang, J. 2009. Basal c-Jun NH_2-terminal protein kinase activity is essential for survival and proliferation of T-cell acute lymphoblastic leukemia cells. Mol. Cancer Ther. **8**(12): 3214–3222. PMID: 19996270. doi: 10.1158/1535-7163.MCT-09-0408.

da Silva Almeida, A.C., Abate, F., Khiabanian, H., Martinez-Escala, E., Guitart, J., Tensen, C.P., Vermeer, M.H., Rabadan, R., Ferrando, A., and Palomero, T. 2015. The mutational landscape of cutaneous T cell lymphoma and Sézary syndrome. Nat. Genet. **47**(12): 1465–1470. PMID: 26551667. doi: 10.1038/ng.3442.

Davis, R.E., Brown, K.D., Siebenlist, U., and Staudt, L.M. 2001. Constitutive nuclear factor κB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J. Exp. Med. **194**(12): 1861–1874. PMID: 11748286. doi: 10.1084/jem.194.12.1861.

Demo, S.D., Kirk, C.J., Aujay, M.A., Buchholz, T.J., Dajee, M., Ho, M.N., Jiang, J., Laidig, G.J., Lewis, E.R., Parlati, F., Shenk, K.D., Smyth, M.S., Sun, C.M., Vallone, M.K., Woo, T.M., Molineaux, C.J., and Bennett, M.K. 2007. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res. **67**(13): 6383–6391. PMID: 17616698. doi: 10.1158/0008-5472.CAN-06-4086.

Dominguez-Sola, D., Kung, J., Holmes, A.B., Wells, V.A., Mo, T., Basso, K., and Dalla-Favera, R. 2015. The FOXO1 transcription factor instructs the germinal center dark zone program. Immunity, **43**(6): 1064–1074. PMID: 26620759. doi: 10.1016/j.immuni.2015.10.015.

Don, A.S., and Zheng, X.F. 2010. Recent clinical trials of mTOR-targeted cancer therapies. Rev. Recent Clin.

Trials, **6**(1): 24–35. PMID: 20868343. doi: 10.2174/157488711793980147.

Dunleavy, K., Pittaluga, S., Czuczman, M.S., Dave, S.S., Wright, G., Grant, N., Shovlin, M., Jaffe, E.S., Janik, J.E., Staudt, L.M., and Wilson, W.H. 2009. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood, **113**(24): 6069–6076. PMID: 19380866. doi: 10.1182/blood-2009-01-199679.

Düwel, M., Welteke, V., Oeckinghaus, A., Baens, M., Kloo, B., Ferch, U., Darnay, B.G., Ruland, J., Marynen, P., and Krappmann, D. 2009. A20 Negatively regulates T cell receptor signaling to NF-κB by cleaving Malt1 ubiquitin chains. J. Immunol. **182**(12): 7718–7728. PMID: 19494296. doi: 10.4049/jimmunol.0803313.

Eferl, R., and Wagner, E.F. 2003. AP-1: A double-edged sword in tumorigenesis. Nat. Rev. Cancer, **3**(11): 859–868. PMID: 14668816. doi: 10.1038/nrc1209.

Ezell, S.A., Mayo, M., Bihani, T., Tepsuporn, S., Wang, S., Passino, M., Grosskurth, S.E., Collins, M., Parmentier, J., Reimer, C., and Byth, K.F. 2014. Synergistic induction of apoptosis by combination of BTK and dual mTORC1/2 inhibitors in diffuse large B cell lymphoma. Oncotarget, **5**(13): 4990–5001. PMID: 24970801. doi: 10.18632/oncotarget.2071.

Ferch, U., Kloo, B., Gewies, A., Pfänder, V., Düwel, M., Peschel, C., Krappmann, D., and Ruland, J. 2009. Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. J. Exp. Med. **206**(11): 2313–2320. PMID: 19841089. doi: 10.1084/jem.20091167.

Fisher, S.G., and Fisher, R.I. 2004. The epidemiology of non-Hodgkin's lymphoma. Oncogene, **23**(38): 6524–6534. PMID: 15322522. doi: 10.1038/sj.onc.1207843.

Fontan, L., Yang, C., Kabaleeswaran, V., Volpon, L., Osborne, M.J., Beltran, E., Garcia, M., Cerchietti, L., Shaknovich, R., Yang, S.N., Fang, F., Gascoyne, R.D., Martinez-Climent, J.A., Glickman, J.F., Borden, K., Wu, H., and Melnick, A. 2012. MALT1 small molecule inhibitors specifically suppress ABC-DLBCL in vitro and in vivo. Cancer Cell, **22**(6): 812–824. PMID: 23238016. doi: 10.1016/j.ccr.2012.11.003.

Gu, J.J., Hernandez-Ilizaliturri, F.J., Kaufman, G.P., Czuczman, N.M., Mavis, C., Skitzki, J.J., and Czuczman, M.S. 2013. The novel proteasome inhibitor carfilzomib induces cell cycle arrest, apoptosis and potentiates the anti-tumour activity of chemotherapy in rituximab-resistant lymphoma. Br. J. Haematol. **162**(5): 657–669. PMID: 23826755. doi: 10.1111/bjh.12452.

Hailfinger, S., Lenz, G., Ngo, V., Posvitz-Fejfar, A., Rebeaud, F., Guzzardi, M., Penas, E.M., Dierlamm, J., Chan, W.C., Staudt, L.M., and Thome, M. 2009. Essential role of MALT1 protease activity in activated B cell-like diffuse large B-cell lymphoma. Proc. Natl. Acad. Sci. U.S.A. **106**(47): 19946–19951. PMID: 19897720. doi: 10.1073/pnas.0907511106.

Hamilton, K.S., Phong, B., Corey, C., Cheng, J., Gorentla, B., Zhong, X., Shiva, S., and Kane, L.P. 2014. T cell receptor–dependent activation of mTOR signaling in T cells is mediated by Carma1 and MALT1, but not Bcl10. Sci. Signal. **7**(329): ra55. PMID: 24917592. doi: 10.1126/scisignal.2005169.

Jattani, R.P., Tritapoe, J.M., and Pomerantz, J.L. 2016*a*. Cooperative control of caspase recruitment domain-containing protein 11 (CARD11) signaling by an unusual array of redundant repressive elements. J. Biol. Chem. **291**(16): 8324–8336. PMID: 26884335. doi: 10.1074/jbc.M115.683714.

Jattani, R.P., Tritapoe, J.M., and Pomerantz, J.L. 2016*b*. Intramolecular interactions and regulation of cofactor binding by the four repressive elements in the caspase recruitment domain-containing protein 11 (CARD11) inhibitory domain. J. Biol. Chem. **291**(16): 8338–8348. PMID: 26884334. doi: 10.1074/jbc.M116.717322.

Jeelall, Y.S., Wang, J.Q., Law, H.D., Domaschenz, H., Fung, H.K., Kallies, A., Nutt, S.L., Goodnow, C.C., and Horikawa, K. 2012. Human lymphoma mutations reveal CARD11 as the switch between self-antigen-induced B cell death or proliferation and autoantibody production. J. Exp. Med. **209**(11): 1907–1917. PMID: 23027925. doi: 10.1084/jem.20112744.

Jin, Z., Qing, K., Ouyang, Y., Liu, Z., Wang, W., Li, X., Xu, Z., and Li, J. 2016. Low dose of lenalidmide and PI3K/mTOR inhibitor trigger synergistic cytoxicity in activated B cell-like subtype of diffuse large B cell lymphoma. J. Exp. Clin. Cancer Res. **35**: 52. PMID: 27009084. doi: 10.1186/s13046-016-0327-x.

Juilland, M., Gonzalez, M., Erdmann, T., Banz, Y., Jevnikar, Z., Hailfinger, S., Tzankov, A., Grau, M., Lenz, G., Novak, U., and Thome, M. 2016. CARMA1- and MyD88-dependent activation of Jun/ATF-type AP-1 complexes is a hallmark of ABC diffuse large B-cell lymphomas. Blood, **127**(14): 1780–1789. PMID: 26747248. doi: 10.1182/blood-2015-07-655647.

Kataoka, K., Nagata, Y., Kitanaka, A., Shiraishi, Y., Shimamura, T., Yasunaga, J., Totoki, Y., Chiba, K., Sato-Otsubo, A., Nagae, G., Ishii, R., Muto, S.,

Kotani, S., Watatani, Y., Takeda, J., Sanada, M., Tanaka, H., Suzuki, H., Sato, Y., Shiozawa, Y., Yoshizato, T., Yoshida, K., Makishima, H., Iwanaga, M., Ma, G., Nosaka, K., Hishizawa, M., Itonaga, H., Imaizumi, Y., Munakata, W., Ogasawara, H., Sato, T., Sasai, K., Muramoto, K., Penova, M., Kawaguchi, T., Nakamura, H., Hama, N., Shide, K., Kubuki, Y., Hidaka, T., Kameda, T., Nakamaki, T., Ishiyama, K., Miyawaki, S., Yoon, S.S., Tobinai, K., Miyazaki, Y., Takaori-Kondo, A., Matsuda, F., Takeuchi, K., Nureki, O., Aburatani, H., Watanabe, T., Shibata, T., Matsuoka, M., Miyano, S., Shimoda, K., and Ogawa, S. 2015. Integrated molecular analysis of adult T cell leukemia/lymphoma. Nat. Genet. **47**(11): 1304–1315. PMID: 26437031. doi: 10.1038/ng.3415.

Kato, M., Sanada, M., Kato, I., Sato, Y., Takita, J., Takeuchi, K., Niwa, A., Chen, Y., Nakazaki, K., Nomoto, J., Asakura, Y., Muto, S., Tamura, A., Iio, M., Akatsuka, Y., Hayashi, Y., Mori, H., Igarashi, T., Kurokawa, M., Chiba, S., Mori, S., Ishikawa, Y., Okamoto, K., Tobinai, K., Nakagama, H., Nakahata, T., Yoshino, T., Kobayashi, Y., and Ogawa, S. 2009. Frequent inactivation of A20 in B-cell lymphomas. Nature, **459**(7247): 712–716. PMID: 19412163. doi: 10.1038/nature07969.

Kawai, T., Adachi, O., Ogawa, T., Takeda, K., and Akira, S. 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity, **11**(1): 115–122. PMID: 10435584. doi: 10.1016/S1074-7613(00)80086-2.

Knies, N., Alankus, B., Weilemann, A., Tzankov, A., Brunner, K., Ruff, T., Kremer, M., Keller, U.B., Lenz, G., and Ruland, J. 2015. Lymphomagenic CARD11/BCL10/MALT1 signaling drives malignant B-cell proliferation via cooperative NF-κB and JNK activation. Proc. Natl. Acad. Sci. U.S.A. **112**(52): E7230–E7238. PMID: 26668357. doi: 10.1073/pnas.1507459112.

Kovalenko, A., Chable-Bessia, C., Cantarella, G., Israël, A., Wallach, D., and Courtois, G. 2003. The tumour suppressor CYLD negatively regulates NF-κB signalling by deubiquitination. Nature, **424**(6950): 801–805. PMID: 12917691. doi: 10.1038/nature01802.

Lamason, R.L., McCully, R.R., Lew, S.M., and Pomerantz, J.L. 2010. Oncogenic CARD11 mutations induce hyperactive signaling by disrupting autoinhibition by the PKC-responsive inhibitory domain. Biochemistry, **49**(38): 8240–8250. PMID: 20799731. doi: 10.1021/bi101052d.

Lenz, G., Davis, R.E., Ngo, V.N., Lam, L., George, T.C., Wright, G.W., Dave, S.S., Zhao, H., Xu, W., Rosenwald, A., Ott, G., Muller-Hermelink, H.K., Gascoyne, R.D., Connors, J.M., Rimsza, L.M., Campo, E., Jaffe, E.S., Delabie, J., Smeland, E.B., Fisher, R.I., Chan, W.C., and Staudt, L.M. 2008. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science, **319**(5870): 1676–1679. PMID: 18323416. doi: 10.1126/science.1153629.

Lu, H.Y., Bauman, B.M., Arjunaraja, S., Dorjbal, B., Milner, J.D., Snow, A.L., and Turvey, S.E. 2018. The CBM-opathies—A rapidly expanding spectrum of human inborn errors of immunity caused by mutations in the CARD11-BCL10-MALT1 complex. Front. Immunol. **9**: 2078. PMID: 30283440. doi: 10.3389/fimmu.2018.02078.

Matsumoto, R., Wang, D., Blonska, M., Li, H., Kobayashi, M., Pappu, B., Chen, Y., Wang, D., and Lin, X. 2005. Phosphorylation of CARMA1 plays a critical role in T cell receptor-mediated NF-κB activation. Immunity, **23**(6): 575–585. PMID: 16356856. doi: 10.1016/j.immuni.2005.10.007.

Maul, R.W., and Gearhart, P.J. 2010. Controlling somatic hypermutation in immunoglobulin variable and switch regions. Immunol. Res. **47**(1–3): 113–122. PMID: 20082153. doi: 10.1007/s12026-009-8142-5.

McConkey, D.J., and Zhu, K. 2008. Mechanisms of proteasome inhibitor action and resistance in cancer. Drug Resist. Updates, **11**(4–5): 164–179. PMID: 18818117. doi: 10.1016/j.drup.2008.08.002.

Medema, R.H., Kops, G.J., Bos, J.L., and Burgering, B.M. 2000. AFX-like forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through $p27^{kip1}$. Nature, **404**(6779): 782–787. PMID: 10783894. doi: 10.1038/35008115.

Merli, M., Ferrario, A., Maffioli, M., Arcaini, L., and Passamonti, F. 2015. Everolimus in diffuse large B-cell lymphomas. Future Oncol. **11**(3): 373–383. PMID: 25675120. doi: 10.2217/fon.14.264.

Nagel, D., Spranger, S., Vincendeau, M., Grau, M., Raffegerst, S., Kloo, B., Hlahla, D., Neuenschwander, M., Peter von Kries, J., Hadian, K., Dörken, B., Lenz, P., Lenz, G., Schendel, D.J., and Krappmann, D. 2012. Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. Cancer Cell, **22**(6): 825–837. PMID: 23238017. doi: 10.1016/j.ccr.2012.11.002.

Nagel, D., Bognar, M., Eitelhuber, A.C., Kutzner, K., Vincendeau, M., and Krappmann, D. 2015. Combinatorial BTK and MALT1 inhibition augments killing of CD79 mutant diffuse large B cell lymphoma.

Oncotarget, **6**(39): 42232–42242. PMID: 26540570. doi: 10.18632/oncotarget.6273.

Nakaya, M., Xiao, Y., Zhou, X., Chang, J.H., Chang, M., Cheng, X., Blonska, M., Lin, X., and Sun, S.C. 2014. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity, **40**(5): 692–705. PMID: 24792914. doi: 10.1016/j.immuni.2014.04.007.

Ngo, V.N., Davis, R.E., Lamy, L., Yu, X., Zhao, H., Lenz, G., Lam, L.T., Dave, S., Yang, L., Powell, J., and Staudt, L.M. 2006. A loss-of-function RNA interference screen for molecular targets in cancer. Nature, **441**(1): 106–110. PMID: 16572121. doi: 10.1038/nature04687.

Ngo, V.N., Young, R.M., Schmitz, R., Jhavar, S., Xiao, W., Lim, K.H., Kohlhammer, H., Xu, W., Yang, Y., Zhao, H., Shaffer, A.L., Romesser, P., Wright, G., Powell, J., Rosenwald, A., Muller-Hermelink, H.K., Ott, G., Gascoyne, R.D., Connors, J.M., Rimsza, L.M., Campo, E., Jaffe, E.S., Delabie, J., Smeland, E.B., Fisher, R.I., Braziel, R.M., Tubbs, R.R., Cook, J.R., Weisenburger, D.D., Chan, W.C., and Staudt, L.M. 2011. Oncogenically active MYD88 mutations in human lymphoma. Nature, **470**(7332): 115–119. PMID: 21179087. doi: 10.1038/nature09671.

Oeckinghaus, A., Wegener, E., Welteke, V., Ferch, U., Arslan, S.C., Ruland, J., Scheidereit, C., and Krappmann, D. 2007. Malt1 ubiquitination triggers NF-κB signaling upon T-cell activation. EMBO J. **26**(22): 4634–4645. PMID: 17948050. doi: 10.1038/sj.emboj.7601897.

Pedersen, S.M., Chan, W., Jattani, R.P., Mackie, d.S., and Pomerantz, J.L. 2016. Negative regulation of CARD11 signaling and lymphoma cell survival by the E3 ubiquitin ligase RNF181. Mol. Cell. Biol. **36**(5): 794–808. PMID: 26711259. doi: 10.1128/mcb.00876-15.

Phelan, J.D., Young, R.M., Webster, D.E., Roulland, S., Wright, G.W., Kasbekar, M., Shaffer, A.L., III, Ceribelli, M., Wang, J.Q., Schmitz, R., Nakagawa, M., Bachy, E., Huang, D.W., Ji, Y., Chen, L., Yang, Y., Zhao, H., Yu, X., Xu, W., Palisoc, M.M., Valadez, R.R., Davies-Hill, T., Wilson, W.H., Chan, W.C., Jaffe, E.S., Gascoyne, R.D., Campo, E., Rosenwald, A., Ott, G., Delabie, J., Rimsza, L.M., Rodriguez, F.J., Estephan, F., Holdhoff, M., Kruhlak, M.J., Hewitt, S.M., Thomas, C.J., Pittaluga, S., Oellerich, T., and Staudt, L.M. 2018. A multiprotein supercomplex controlling oncogenic signalling in lymphoma. Nature, **560**(7718): 387–391. PMID: 29925955. doi: 10.1038/s41586-018-0290-0.

Reddy, A., Zhang, J., Davis, N.S., Moffitt, A.B., Love, C.L., Waldrop, A., Leppa, S., Pasanen, A., Meriranta, L., Karjalainen-Lindsberg, M.L., Nørgaard, P., Pedersen, M., Gang, A.O., Høgdall, E., Heavican, T.B., Lone, W., Iqbal, J., Qin, Q., Li, G., Kim, S.Y., Healy, J., Richards, K.L., Fedoriw, Y., Bernal-Mizrachi, L., Koff, J.L., Staton, A.D., Flowers, C.R., Paltiel, O., Goldschmidt, N., Calaminici, M., Clear, A., Gribben, J., Nguyen, E., Czader, M.B., Ondrejka, S.L., Collie, A., Hsi, E.D., Tse, E., Au-Yeung, R.K.H., Kwong, Y.L., Srivastava, G., Choi, W.W.L., Evens, A.M., Pilichowska, M., Sengar, M., Reddy, N., Li, S., Chadburn, A., Gordon, L.I., Jaffe, E.S., Levy, S., Rempel, R., Tzeng, T., Happ, L.E., Dave, T., Rajagopalan, D., Datta, J, Dunson, D.B., and Dave, S.S. 2017. Genetic and functional drivers of diffuse large B cell lymphoma. Cell, **171**(2): 481–494.e15. PMID: 28985567. doi: 10.1016/j.cell.2017.09.027.

Reiley, W.W., Jin, W., Lee, A.J., Wright, A., Wu, X., Tewalt, E.F., Leonard, T.O., Norbury, C.C., Fitzpatrick, L., Zhang, M., and Sun, S.C. 2007. Deubiquitinating enzyme CYLD negatively regulates the ubiquitin-dependent kinase Tak1 and prevents abnormal T cell responses. J. Exp. Med. **204**(6): 1475–1485. PMID: 17548520. doi: 10.1084/jem.20062694.

Rovira, J., Valera, A., Colomo, L., Setoain, X., Rodríguez, S., Martínez-Trillos, A., Giné, E., Dlouhy, I., Magnano, L., Gaya, A., Martínez, D., Martínez, A., Campo, E., and López-Guillermo, A. 2015. Prognosis of patients with diffuse large B cell lymphoma not reaching complete response or relapsing after frontline chemotherapy or immunochemotherapy. Ann. Hematol. **94**(5): 803–812. PMID: 25501975. doi: 10.1007/s00277-014-2271-1.

Ruland, J., Duncan, G.S., Elia, A., del Barco Barrantes, I., Nguyen, L., Plyte, S., Millar, D.G., Bouchard, D., Wakeham, A., Ohashi, P.S., and Mak, T.W. 2001. Bcl10 is a positive regulator of antigen receptor-induced activation of NF-κB and neural tube closure. Cell, **104**(1): 33–42. PMID: 11163238. doi: 10.1016/S0092-8674(01)00189-1.

Sander, S., Chu, V.T., Yasuda, T., Franklin, A., Graf, R., Calado, D.P., Li, S., Imami, K., Selbach, M., Di Virgilio, M., Bullinger, L., and Rajewsky, K. 2015. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. Immunity, **43**(6): 1075–1086. PMID: 26620760. doi: 10.1016/j.immuni.2015.10.021.

Schmidt, M., Fernandez de Mattos, S., van der Horst, A., Klompmaker, R., Kops, G.J., Lam, E.W., Burgering,

B.M., and Medema, R.H. 2002. Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. Mol. Cell. Biol. **22**(22): 7842–7852. PMID: 12391153. doi: 10.1128/mcb.22.22.7842-7852.2002.

Sebestyén, A., Sticz, T.B., Márk, A., Hajdu, M., Timár, B., Nemes, K., Nagy, N., Váradi, Z., and Kopper, L. 2012. Activity and complexes of mTOR in diffuse large B-cell lymphomas—A tissue microarray study. Mod. Pathol. **25**(12): 1623–1628. PMID: 22899290. doi: 10.1038/modpathol.2012.141.

Shembade, N., Ma, A., and Harhaj, E.W. 2010. Inhibition of NF-κB signaling by A20 through disruption of ubiquitin enzyme complexes. Science, **327**(5969): 1135–1139. PMID: 20185725. doi: 10.1126/science.1182364.

Shinohara, H., Maeda, S., Watarai, H., and Kurosaki, T. 2007. IκB kinase β-induced phosphorylation of CARMA1 contributes to CARMA1-Bcl10-MALT1 complex formation in B cells. J. Exp. Med. **204**(13): 3285–3293. PMID: 18086859. doi: 10.1084/jem.20070379.

Snow, A.L., Xiao, W., Stinson, J.R., Lu, W., Chaigne-Delalande, B., Zheng, L., Pittaluga, S., Matthews, H.F., Schmitz, R., Jhavar, S., Kuchen, S., Kardava, L., Wang, W., Lamborn, I.T., Jing, H., Raffeld, M., Moir, S., Fleisher, T.A., Staudt, L.M., Su, H.C., and Lenardo, M.J. 2012. Congenital B cell lymphocytosis explained by novel germline CARD11 mutations. J. Exp. Med. **209**(12): 2247–2261. PMID: 23129749. doi: 10.1084/jem.20120831.

Sommer, K., Guo, B., Pomerantz, J.L., Bandaranayake, A.D., Moreno-García, M.E., Ovechkina, Y.L., and Rawlings, D.J. 2005. Phosphorylation of the CARMA1 linker controls NF-κB activation. Immunity, **23**(6): 561–574. PMID: 16356855. doi: 10.1016/j.immuni.2005.09.014.

Srinivasula, S.M., and Ashwell, J.D. 2011. A20: More than one way to skin a cat. Mol. Cell, **44**(4): 511–512. PMID: 22099299. doi: 10.1016/j.molcel.2011.11.004.

Staal, J., Driege, Y., Bekaert, T., Demeyer, A., Muyllaert, D., Van Damme, P., Gevaert, K., and Beyaert, R. 2011. T-cell receptor-induced JNK activation requires proteolytic inactivation of CYLD by MALT1. EMBO J. **30**(9): 1742–1752. PMID: 21448133. doi: 10.1038/emboj.2011.85.

Sun, L., Deng, L., Ea, C.K., Xia, Z.P., and Chen, Z.J. 2004. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. Mol. Cell, **14**(3): 289–301. PMID: 15125833. doi: 10.1016/S1097-2765(04)00236-9.

Trompouki, E., Hatzivassiliou, E., Tsichritzis, T., Farmer, H., Ashworth, A., and Mosialos, G. 2003. CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activation by TNFR family members. Nature, **424**(6950): 793–796. PMID: 12917689. doi: 10.1038/nature01803.

Vallois, D., Dobay, M.P., Morin, R.D., Lemonnier, F., Missiaglia, E., Juilland, M., Iwaszkiewicz, J., Fataccioli, V., Bisig, B., Roberti, A., Grewal, J., Bruneau, J., Fabiani, B., Martin, A., Bonnet, C., Michielin, O., Jais, J.P., Figeac, M., Bernard, O.A., Delorenzi, M., Haioun, C., Tournilhac, O., Thome, M., Gascoyne, R.D., Gaulard, P., and de Leval, L. 2016. Activating mutations in genes related to TCR signaling in angioimmunoblastic and other follicular helper T-cell-derived lymphomas. Blood, **128**(11): 1490–1502. PMID: 27369867. doi: 10.1182/blood-2016-02-698977.

Victora, G.D., Dominguez-Sola, D., Holmes, A.B., Deroubaix, S., Dalla-Favera, R., and Nussenzweig, M.C. 2012. Identification of human germinal center light and dark zone cells and their relationship to human B-cell lymphomas. Blood, **120**(11): 2240–2248. PMID: 22740445. doi: 10.1182/blood-2012-03-415380.

Wang, L., Ni, X., Covington, K.R., Yang, B.Y., Shiu, J., Zhang, X., Xi, L., Meng, Q., Langridge, T., Drummond, J., Donehower, L.A., Doddapaneni, H., Muzny, D.M., Gibbs, R.A., Wheeler, D.A., and Duvic, M. 2015. Genomic profiling of Sézary syndrome identifies alterations of key T cell signaling and differentiation genes. Nat. Genet. **47**(12): 1426–1434. PMID: 26551670. doi: 10.1038/ng.3444.

Wei, Z., Zhang, Y., Chen, J., Hu, Y., Jia, P., Wang, X., Zhao, Q., Deng, Y., Li, N., Zang, Y., Qin, J., Wang, X., and Lu, W. 2019. Pathogenic CARD11 mutations affect B cell development and differentiation through a noncanonical pathway. Sci. Immunol. **4**(41): eaaw5618. PMID: :31784498. doi: 10.1126/sciimmunol.aaw5618.

Wilson, W.H., Young, R.M., Schmitz, R., Yang, Y., Pittaluga, S., Wright, G., Lih, C.J., Williams, P.M., Shaffer, A.L., Gerecitano, J., de Vos, S., Goy, A., Kenkre, V.P., Barr, P.M., Blum, K.A., Shustov, A., Advani, R., Fowler, N.H., Vose, J.M., Elstrom, R.L., Habermann, T.M., Barrientos, J.C., McGreivy, J., Fardis, M., Chang, B.Y., Clow, F., Munneke, B., Moussa, D., Beaupre, D.M., and Staudt, L.M. 2015. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat. Med. **21**(8): 922–926. PMID: 26193343. doi: 10.1038/nm.3884.

Wray-Dutra, M.N., Chawla, R., Thomas, K.R., Seymour, B.J., Arkatkar, T., Sommer, K.M., Khim, S., Trapnell, C., James, R.G., and Rawlings, D.J. 2018. Activated CARD11 accelerates germinal center kinetics, promoting mTORC1 and terminal differentiation. J. Exp. Med. **215**(9): 2445–2461. PMID: 30127060. doi: 10.1084/jem.20180230.

Wu, Q., Wu, W., Jacevic, V., Franca, T.C.C., Wang, X., and Kuca, K. 2020. Selective inhibitors for JNK signalling: A potential targeted therapy in cancer. J. Enzyme Inhib. Med. Chem. **35**(1): 574–583. PMID: 31994958. doi: 10.1080/14756366.2020.1720013.

Xu, X. 2019. BTK inhibitors induce ABC-DLBCL cell apoptosis by inhibiting CYLD phosphorylation. Blood, **134**(Suppl. 1): 5046. doi: 10.1182/blood-2019-126763.

Yang, C., David, L., Qiao, Q., Damko, E., and Wu, H. 2014. The CBM signalosome: Potential therapeutic target for aggressive lymphoma? Cytokine Growth Factor Rev. **25**(2): 175–183. PMID: 24411492. doi: 10.1016/j.cytogfr.2013.12.008.

Yang, J., Nie, J., Ma, X., Wei, Y., Peng, Y., and Wei, X. 2019. Targeting PI3K in cancer: Mechanisms and advances in clinical trials. Mol. Cancer, **18**(1): 26. PMID: 30782187. doi: 10.1186/s12943-019-0954-x.

Yang, W.L., Wang, J., Chan, C.H., Lee, S.W., Campos, A.D., Lamothe, B., Hur, L., Grabiner, B.C., Lin, X., Darnay, B.G., and Lin, H.K. 2009. The E3 ligase TRAF6 regulates Akt ubiquitination and activation. Science, **325**: 1134–1138. PMID: 19713527. doi: 10.1126/science.1175065.

Yang, Y., Schmitz, R., Mitala, J., Whiting, A., Xiao, W., Ceribelli, M., Wright, G.W., Zhao, H., Yang, Y., Xu, W., Rosenwald, A., Ott, G., Gascoyne, R.D., Connors, J.M., Rimsza, L.M., Campo, E., Jaffe, E.S., Delabie, J., Smeland, E.B., Braziel, R.M., Tubbs, R.R., Cook, J.R., Weisenburger, D.D., Chan, W.C., Wiestner, A., Kruhlak, M.J., Iwai, K., Bernal, F., and Staudt, L.M. 2014. Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. Cancer Discov. **4**(4): 480–493. PMID: 24491438. doi: 10.1158/2159-8290.CD-13-0915.

Yang, Y.K., Yang, C., Chan, W., Wang, Z., Deibel, K.E., and Pomerantz, J.L. 2016. Molecular determinants of scaffold-induced linear ubiquitinylation of B cell lymphoma/leukemia 10 (Bcl10) during T cell receptor and oncogenic caspase recruitment domain-containing protein 11 (CARD11) signaling. J. Biol. Chem. **291**(50): 25921–25936. PMID: 27777308. doi: 10.1074/jbc.M116.754028.

Yoshida, H., Jono, H., Kai, H., and Li, J.D. 2005. The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll-like receptor 2 signaling via negative cross-talk with TRAF6 and TRAF7. J. Biol. Chem. **280**(49): 41111–41121. PMID: 16230348. doi: 10.1074/jbc.M509526200.

Yusuf, I., Zhu, X., Kharas, M.G., Chen, J., and Fruman, D.A. 2004. Optimal B-cell proliferation requires phosphoinositide 3-kinase-dependent inactivation of FOXO transcription factors. Blood, **104**(3): 784–787. PMID: 15069012. doi: 10.1182/blood-2003-09-3071.

Original Article

Observational study of SARS-CoV-2 antibody immune response in a cohort of patients at a North Suburban Chicago, Illinois, in a physician's practice

Gerald Osher[a], Christopher C. Lamb[b,c,d*], Yuliana Ibarra[a], and Deborah Erickson-Samson[a]

ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic. The application of point of care serological testing can help determine past infection and assist healthcare workers assess patient risk.

Method: An observational study of 114 subjects in North Suburban Chicago, Illinois, was performed using the Clungene® lateral flow immunoassay (LFI). Patients' PCR test results and clinical symptoms were used to compare the seroconversion rate of this patient population with the surrounding community.

Results: Excluding 1 aberrant result, there was 100% positive agreement (10) between PCR and antibody (IgG or IgM) test results. There were 7 patients who did not have a prior PCR test who were positive for IgG; 5 of the 7 had clinical symptoms consistent with possible exposure and 2 were asymptomatic. There was 1 person with a suspected exposure to an infected person who was IgM positive. Ninety-five asymptomatic patients were seronegative. The overall rate of 15.9% seroconversion (IgG or IgM) is consistent with other community-based testing results in the North Suburban Chicago, Illinois area.

Conclusion: Rapid screening tests to identify antibody positive patients recovered from coronavirus disease-2019 can be a useful tool for healthcare professionals to determine or confirm past infection.

Statement of novelty: Limited data is available on the use of point of care serological testing to assist healthcare professionals with the assessment of their patient population regarding past SARS-CoV-2 infectivity and seroconversion. The present study successfully investigated the use of a point of care antibody test in a physician's office to determine which patients have developed antibodies, indicating an immune response to SARS-CoV-2, and to assist with decisions on whether patients should pursue normal social and workplace activities.

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic. The percentage of infected individuals who seroconvert is unknown. Serological tests can help determine whether a person or population of people have developed antibodies indicative of an adaptive immune response to SARS-CoV-2. In April and May of 2020, 2 internist gerontology general practitioner's offices in

[a]North Shore Medical Associates, Bannockburn, IL; [b]BioSolutions Services LLC, Englewood Cliffs, NJ; [c]Department of Management and Entrepreneurship, Silberman College of Business, Fairleigh Dickinson University, Teaneck, NJ; [d]Weatherhead School of Management, Case Western Reserve University, Cleveland, OH

*Corresponding author: Christopher C. Lamb/ccl48@case.edu

Submitted 3 July 2020
Accepted 5 August 2020
Available online 6 August 2020

LymphoSign Journal 7:104–108 (2020)
dx.doi.org/10.14785/lymphosign-2020-0007

North Suburban Chicago, Illinois, tested a cohort of patients to determine their SARS-CoV-2 antibody immune response. North Suburban Chicago consists of communities north of Chicago, bordering the shores of Lake Michigan. Previously reported SARS-CoV-2 results by a North Suburban Chicago, Illinois community hospital suggested an 18% infectivity rate during this same period (Seidenberg 2020).

Introduction

SARS-CoV-2 has caused over 162 748 infections and 7301 deaths in Illinois State at the time of writing this report (Illinois Department of Health 2020). To avoid spread of SARS-CoV-2 and to help standardize definitions of clearance, it is important to understand the development of antibodies to infection (Ragnesola et al. 2020). Serology (antibody) tests may detect different types of antibodies. The most common are IgM and IgG. Published data suggests that 95% of patients with a confirmed PCR test (either documented result or self-reported) have a positive IgG antibody (Wajnberg et al. 2020). Another study found 3 types of IgM seroconversion: synchronous seroconversion of IgG and IgM, IgM seroconversion earlier than that of IgG, and IgM seroconversion later than that of IgG (Long et al. 2020).

Positive antibody results from appropriately validated serology tests that are designed to be very specific to the SARS-CoV-2 virus can indicate whether a patient has had recent or prior SARS-CoV-2 infection (Long et al. 2020). In addition, while there is uncertainty with this new virus, it is possible that the use of antibody tests and clinical follow-up can provide the medical community with more information on whether or not, and how long, a person who has recovered from the virus is at lower risk of infection if they are exposed to the virus again (FDA 2020).

Methods

Two physician practices in North Suburban Chicago, Illinois, tested 114 study participants: 11 of whom had a positive PCR test of which 10 had some reported coronavirus disease-2019 (COVID-19)-like symptoms, 6 were symptomatic or had exposure risk, and 97 were asymptomatic. The age of the subjects ranged from 3 to 84. Serological testing was performed between 22 April 2020 and 26 May 2020. Inclusion criteria included patients routinely managed by the practicing physicians who consented to a serological test. Using a whole blood sample drawn from a lancet, subjects were tested for the detection of SARS-CoV-2 antibodies (IgG and IgM) using the point of care CLUNGENE® SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid Test Cassette lateral flow immunoassay (LFI). Previously, the manufacturer of the LFI validated this immunoassay for the qualitative detection of antibodies to SARS-CoV-2 and the data was submitted to the US FDA consistent with the requirements of the Emergency Use Authorization (FDA 2020). See Figure 1, a schematic diagram showing the location of the sample well, buffer well, and control and test lines of the Rapid Test Cassette.

The tests were performed by a trained health professional. The results were visually analyzed according to the manufacturers' instructions for use.

Results

Eleven patients had a confirmed PCR test indicating prior infection. The common clinical manifestation of these PCR patients was fever and fatigue. Serological testing was performed between 27 and 42 days post PCR SARS-CoV-2 positive result for 10 of the patients. See Table 1. Ten of the 11 PCR positive patients were IgG (10) or IgM (1) antibody positive (91%).

The 1 PCR positive patient who was seronegative (antibody negative) was evaluated again by a second serological test and was also IgG and IgM negative (CLUNGENE 2020; Quest Diagnostics 2020). Based on an overall clinical evaluation, it was determined that the patient was SARS-CoV-2 infected but had Waldenstrom's macroglobulinemia (WM), a cancer of the immune system in which antibodies are over produced in an unregulated fashion. Individuals with this type of disease do not respond in a normal fashion to immunologic stimuli. The fact that this patient did not produce measurable antibodies to the coronavirus is not surprising and consistent with this disease. Therefore, this WM patient was excluded from the result data.

There were 6 patients who did not have a prior PCR test but were symptomatic or had exposure risk; all were positive for IgG or IgM. The rate of seroconversion for the symptomatic, exposed or PCR positive subpopulation was 100% (16/16). There were 2 IgG positive asymptomatic patients and 95 asymptomatic seronegative

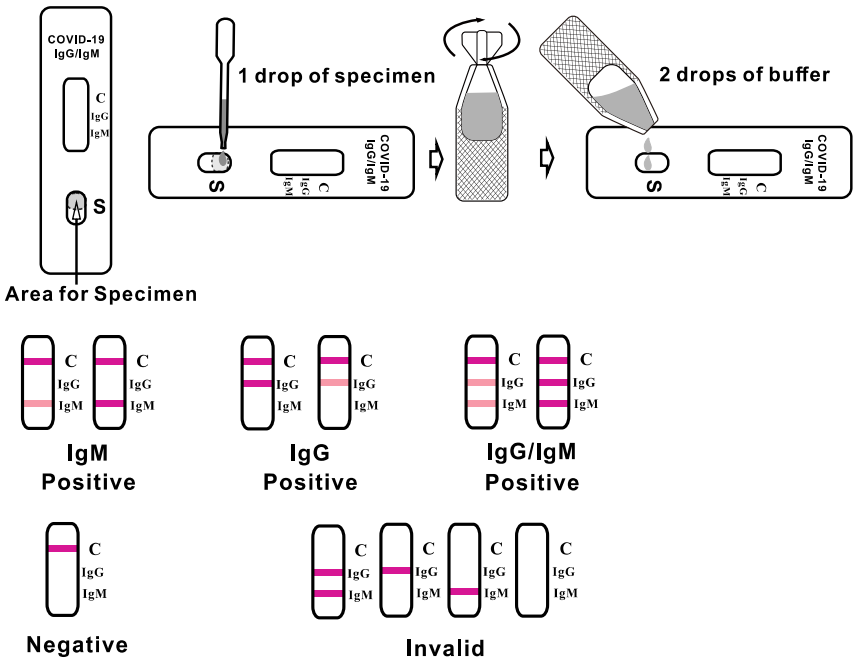


Figure 1: A schematic diagram showing the location of the sample well, buffer well, and control and test lines of the Rapid Test Cassette.

Table 1: **PCR positive patients.**

Date of PCR test	Date of serological test	No. of days between tests	Antibody results
3/27/2020	4/22/2020	27	IgG+
3/24/2020	4/22/2020	29	IgG+
4/5/2020	4/23/2020	19	IgG+
3/26/2020	4/24/2020	29	IgG+
3/23/2020	4/24/2020	31	IgG- (2x)[a]
3/26/2020	4/27/2020	32	IgG+
3/16/2020	4/27/2020	42	IgG+
3/28/2020	4/27/2020	31	IgG+
3/19/2020[b]	4/24/2020	35[b]	IgG+
3/19/2020[b]	4/27/2020	38[b]	IgG+
Unknown	4/28/2020	Unknown	IgM+

[a]Patient was SARS-CoV-2 infected but had Waldenstrom's macroglobulinemia and, as expected, did not produce measurable antibody when tested with 2 different antibody test systems.
[b]Exact date of PCR test unknown; estimated number of days between tests.

patients. The rate of seroconversion for the asymptomatic subpopulation was 2% (2/97). The overall rate of seroconversion for the entire population (IgG or IgM) was 15.9% (18/113). See Table 2. This overall rate is consistent with other community-based testing results in the North Suburban Chicago, Illinois area.

Discussion

Our study analyzed whole blood samples for SARS-CoV-2 from a cohort of patients presenting at 2 physician offices during April and May of 2020. The patients were tested to determine antibodies related to potential infection and recovery from SARS-CoV-2. The CLUNGENE® SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid Test Cassette was reportedly simple to implement with adequately trained clinical staff. Results were obtained within 15 minutes and the red procedural control line confirmed that adequate specimen volume, sufficient membrane wicking, and error-free procedural technique were used. With the exception of 1 unusual patient, clinically documented infected patients with a confirmed SARS-CoV-2 PCR test were 100% in positive agreement with the presence

Table 2: **Summary of results.**

Measure	Value
PCR positive, symptomatic, or exposed	
Positive PCR/IgG or IgM positive	10
Clinically suggestive positive/IgG positive	5
Exposed patient: IgM positive	1
Subtotal (symptomatic)	16/16 (100%)[a]
Asymptomatic	
Asymptomatic/IgG positive	2
Asymptomatic IgG/IgM negative	95
Subtotal (asymptomatic)	2/97 (2.1%)
Overall seroconversion rate	18/113 (15.9%)

[a]Waldenstrom's macroglobulinemia patient excluded from results.

of the IgG antibody. These results are consistent with the manufacturer's clinical performance data showing 97.4% positive IgG agreement with SARS-CoV-2 infected patients with a known positive PCR test as reported in the Instructions for Use, and 100% sensitivity when confirmed PCR infected patients were tested 15–42 days following infection in a separate study submitted to the US FDA (FDA 2020). Consistent with recently published data, IgM can persist more than 23 days after symptom onset (Wajnberg et al. 2020).

There have been 3 prior publications evaluating the CLUNGENE® test. One showed lower sensitivity (Vásárhelyi et al. 2020); however the cassettes used in this study were a previous generation assay with lower sensitivity and the PCR test used to determine positive agreement had "logistical problems during the PCR analysis and the suppliers of reagents used for certain component changed"[1]. Therefore, the results are not comparable. A second study analyzed blood samples from COVID-19 convalescent plasma donors and determined that the CLUNGENE® test possessed high sensitivity and specificity for COVID-19 antibodies (Ragnesola et al. 2020). A third evaluated CLUNGENE®'s diagnostic performance and reported a specificity of 99% and sensitivity of 97.4% for IgM or IgG 14 days following infection which are consistent with this observational study (Van Elslande et al. 2020).

Limitations

Our study has several limitations. Samples were not tested for virus neutralization; therefore neutralizing activities of the detected IgG antibodies are not known. The small sample size of patients makes it difficult to draw a definitive conclusion or determine the relationship between antibody response and clinical course. It should be emphasized that the CLUNGENE® test is only a qualitative serological test and not quantitative. At the time of this publication there are no approved quantitative serological tests (CDC 2020).

Conclusion

Rapid screening tests to identify antibody positive people recovered from coronavirus disease-2019 can be a useful tool for healthcare professionals. Confirming suspected antibody formation in SARS-CoV-2 cases with the help of serological testing can reduce exposure risk. This study highlights the relevance of serological testing to assess patient immunity by antibody detection and assist with decisions on whether to pursue normal social and workplace activities.

Disclosures

Christopher C. Lamb, PhD, worked with the manufacturer of the Clungene® test on the Emergency Use Authorization submission to the US FDA.

Funding

No external funding was received for this work.

Acknowledgements

The author acknowledges Ryan Dagenais for assistance with literature review and editorial support with the manuscript; his efforts were funded by BioSolutions Services.

REFERENCES

CDC. 2020. Interim guidelines for COVID-19 antibody testing [online]. Available from https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html#anchor_1590280385631.

CLUNGENE. 2020. CLUNGENE COVID-19 IgG/IgM Rapid Test Kit EUA submission # EUA201121.

FDA. 2020. FAQs on testing for SARS-CoV-2 [online]. Available from https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-testing-sars-cov-2.

[1]Translated from original language of Hungarian to English via Google Translate.

Illinois Department of Health. 2020. COVID-19 statistics [online]. Available from https://www.dph.illinois.gov/covid19/covid19-statistics.

Long, Q.X., Liu, B.Z., Deng, H.J., Wu, G.C., Deng, K., Chen, Y.K., Liao, P., Qiu, J.F., Lin, Y., Cai, X.F., Wang, D.Q., Hu, Y., Ren, J.H., Tang, N., Xu, Y.Y., Yu, L.H., Mo, Z., Gong, F., Zhang, X.L., Tian, W.G., Hu, L., Zhang, X.X., Xiang, J.L., Du, H.X., Liu, H.W., Lang, C.H., Luo, X.H., Wu, S.B., Cui, X.P., Zhou, Z., Zhu, M.M., Wang, J., Xue, C.J., Li, X.F., Wang, L., Li, Z.J., Wang, K., Niu, C.C., Yang, Q.J., Tang, X.J., Zhang, Y., Liu, X.M., Li, J.J., Zhang, D.C., Zhang, F., Liu, P., Yuan, J., Li, Q., Hu, J.L., Chen, J., and Huang, A.L. 2020. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat. Med. **26**(6): 845–848. PMID: 32350462. doi: 10.1038/s41591-020-0897-1.

Quest Diagnostics. 2020. IgG antibody serology testing to indicate SARS-CoV-2 exposure [online]. Available from https://www.questdiagnostics.com/home/Covid-19/HCP/antibody/.

Ragnesola, B., Jin, D., Lamb, C.C., Shaz, B.H., Hillyer, C.D., and Luchsinger, L.L. 2020. COVID 19 antibody detection using lateral flow assay tests in a cohort of convalescent plasma donors. BMC Res. Notes, **13**(1): 372. PMID: 32762746. doi: 10.1186/s13104-020-05212-0.

Seidenberg, B. 2020. NorthShore moving to convert Glenbrook Hospital into temporary site for COVID-19 patients [online]. Available from https://evanstonroundtable.com/Content/COVID-19/RT-Articles/Article/NorthShore-Moving-to-Convert-Glenbrook-Hospital-Into-Temporary-Site-for-Covid-19-Patients/41/237/17760.

Van Elslande, J., Houben, E., Depypere, M., Brackenier, A., Desmet, S., André, E., Van Ranst, M., Lagrou, K., and Vermeersch, P. 2020. Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. Clin. Microbiol. Infect. **26**(8): 1082–1087. PMID: 32473953. doi: 10.1016/j.cmi.2020.05.023.

Vásárhelyi, B., Kristóf, K., Ostorházi, E., Szabó, D., Prohászka, Z., and Merkely, B. 2020. The diagnostic value of rapid anti IgM and IgG detecting tests in the identification of patients with SARS CoV-2 virus infection. Orv. Hetil. **161**(20): 807–812. PMID: 32364358. doi: 10.1556/650.2020.31859.

Wajnberg, A., Mansour, M., Leven, E., Bouvier, N. M., Patel, G., Firpo, A., Mendu, R., Jhang, J., Arinsburg, S., Gitman, M., Houldsworth, J., Baine, I., Simon, V., Aberg, J., Krammer, F., Reich, D., and Cordon-Cardo, C. 2020. Humoral immune response and prolonged PCR positivity in a cohort of 1343 SARS-CoV 2 patients in the New York City region. medRxiv. doi: 10.1101/2020.04.30.20085613.

Report of the Canadian Expert Committee on the management of ADA deficiency

Rae Brager[a], Parwinder Gill[b], Alison Haynes[c], Kyla Hildebrand[d,e], Vy H.D. Kim[f,g], Thomas B. Issekutz[h], Christine McCusker[i], Luis Murguia-Favela[j], Brenda Reid[f,g], Tamar Rubin[k], Julia Upton[f,g], and Chaim M. Roifman[f,g,l*]

ABSTRACT

Adenosine deaminase (ADA) deficiency is a form of severe combined immunodeficiency. Aberrant mutations in the *ADA* gene result in loss of ADA activity and the toxic accumulation of metabolites that damage both immune and non-immune organs. While patients with complete ADA deficiency present during infancy with failure to thrive, recurrent bacterial, viral and fungal infections, those with incomplete (partial) deficiency may present at a later age with milder symptoms associated with reduced T, B, and NK cell subpopulations. Based on experience in Canadian centres, we provide management guidelines for patients with ADA deficiency, including a treatment algorithm for use of hematopoietic stem cell transplantation, gene therapy, and enzyme replacement therapy.

Statement of novelty: Herein, we define guidelines for the management and treatment of patients with ADA deficiency.

Introduction

Adenosine deaminase (ADA) is a ubiquitously expressed enzyme of the purine salvage pathway which catalyzes the irreversible deamination of adenosine and deoxyadenosine. It is essential for normal lymphoid development, with particularly high levels found in the thymus (Adams and Harkness 1976; Poliani et al. 2009). Deficiency of ADA, caused by mutations in the *ADA* gene and subsequent impairment of ADA activity, leads to an autosomal recessive form of severe combined immunodeficiency (SCID) (Sauer et al. 2012; Bradford et al. 2017). The toxic accumulation of ADA substrates and metabolites interferes with downstream metabolic pathways, including inhibition of ribonucleotide reductase and subsequent blockade of DNA synthesis, as well as S-adenosylhomocysteine hydrolase-dependent transmethylation (Flinn and Gennery 2018). The detrimental effects are most pronounced in pathways regulating lymphocyte

[a]Division of Rheumatology, Immunology, and Allergy, Department of Paediatrics, McMaster Children's Hospital, McMaster University, Hamilton, ON; [b]Division of Clinical Immunology, St. Joseph's Hospital, Western University, London, ON; [c]Division of Pediatrics, Faculty of Medicine, Memorial University, St John's, NL; [d]Division of Allergy & Immunology, Department of Pediatrics, University of British Columbia, Vancouver, BC; [e]Child & Family Research Institute, Vancouver, BC; [f]Division of Immunology & Allergy, Department of Paediatrics, The Hospital for Sick Children, Toronto, ON; [g]University of Toronto, Toronto, ON; [h]Division of Immunology, Department of Pediatrics, IWK Health Centre, Dalhousie University, Halifax, NS; [i]Division of Pediatric Allergy Immunology and Dermatology, Department of Pediatrics, McGill University Health Center, Montreal, QC; [j]Section of Hematology/Immunology, Department of Pediatrics, University of Calgary, Calgary, AB; [k]Winnipeg Children's Hospital, Division of Pediatric Allergy and Immunology, University of Manitoba, Winnipeg, MB; [l]Canadian Centre for Primary Immunodeficiency, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 21 August 2020
Accepted 24 August 2020
Available online 30 August 2020

LymphoSign Journal 7:109–115 (2020)
dx.doi.org/10.14785/lymphosign-2020-0008

maturation and function, although non-immunological organ systems, including the hepatic, renal, pulmonary, skeletal, peripheral and central nervous systems, can also be affected (reviewed by(Whitmore and Gaspar (2016)).

ADA deficiency affects 1:200 000 live births (Blackburn and Kellems 2005), with a higher frequency reported in Canadian Inuit and Mennonite populations (Grunebaum et al. 2013). Without treatment it is usually fatal within the first year of life. Genotype-phenotype correlations have revealed greater metabolic disturbance in those with more severe biallelic defects in *ADA* (Arredondo-Vega et al. 1998; Cagdas et al. 2018). By contrast, hypomorphic mutations result in somewhat reduced ADA activity and are associated with less severe or late-onset phenotypes. Prior to the advent of newborn screening, patients with complete ADA deficiency traditionally presented during infancy with recurrent bacterial, viral and fungal infections and failure to thrive, while laboratory evaluations revealed severe lymphopenia, hypogammaglobulinemia, and neutropenia. Skeletal abnormalities, cognitive impairment, and hearing loss are common (Albuquerque and Gaspar 2004; Titman et al. 2008; Sauer et al. 2009; Manson et al. 2013). Those with incomplete (partial) deficiency may present with milder but gradually worsening manifestations, associated with reduced populations of T, B, and NK cells (Santisteban et al. 1993; Shovlin et al. 1993). In either case, measurement of ADA enzyme activity and associated levels of ADA substrates/metabolites (adenosine, 2′deoxyadenosine (dAXP), deoxyadenosine triphosphate) is often the first step in identifying ADA deficiency.

The implementation of newborn screening for SCID in Canada (first introduced in Ontario in 2013 and now routine across Nova Scotia, New Brunswick, Prince Edward Island, and Alberta (Biggs et al. 2017; Reid et al. 2017)) has resulted in the early detection of ADA deficient patients as well as improved survival of this cohort (Scott et al. 2019). Management currently relies on immediate enzyme replacement therapy (ERT), immunoglobulin replacement, protective isolation procedures, and antibiotic prophylaxis until definitive treatment is initiated (Figure 1). Patients require close monitoring to reduce the risk of infectious and non-infectious complications (Table 1).

ERT using polyethylene glycol-modified bovine ADA has proved to be effective in correcting the immune and some non-immune abnormalities conferred by ADA deficiency, however, in some patients immune function declined over time (Booth et al. 2007). The bovine-derived ADA product was replaced in 2019 by a recombinant form of the enzyme (elapegademase) which has shown similar in-vitro and possibly better in-vivo activity compared to its predecessor (Murguia-Favela et al. 2020). ERT remains a costly treatment. Thus, it is considered a bridging modality before curative treatment is initiated. To date, hematopoietic stem cell transplantation (HSCT) is the treatment of choice when a human leukocyte antigen (HLA)-matched related donor (MRD) is available (Griffith et al. 2008). Another option that has been shown to correct for the absence of ADA is ex vivo-modified autologous hematopoietic stem cell gene therapy (HSC-GT) (Kohn et al. 1995; Aiuti et al. 2002; Ferrua et al. 2010; Cicalese et al. 2016). Experiences with HSCT with matched unrelated donors (MUD) or haploidentical donors have shown lower success rates (Hassan et al. 2012), although these options can still be considered if MRD HSCT or HSC-GT are not available.

Herein we, the Canadian Expert Committee, define guidelines on the management of ADA deficiency based on our collective experience and available literature in managing this group of patients.

Diagnosis of ADA deficiency

Complete deficiency

Mandatory findings[1]

(1) <1% of normal ADA activity in red blood cells or peripheral blood lymphocytes[2]
(2) Severe lymphopenia
(3) Low to absent responses to mitogens
(4) Biallelic pathogenic mutations in the ADA gene

[1]It is acknowledged that mandatory findings are ideal criteria to follow. However, in some circumstances where a sibling has been diagnosed with ADA deficiency or when all mandatory findings cannot be met but administration of treatment is urgent, criteria No. 4 plus one of the other findings could be sufficient.
[2]In patients who received blood transfusion, ADA level measurement in peripheral blood lymphocytes should be performed.

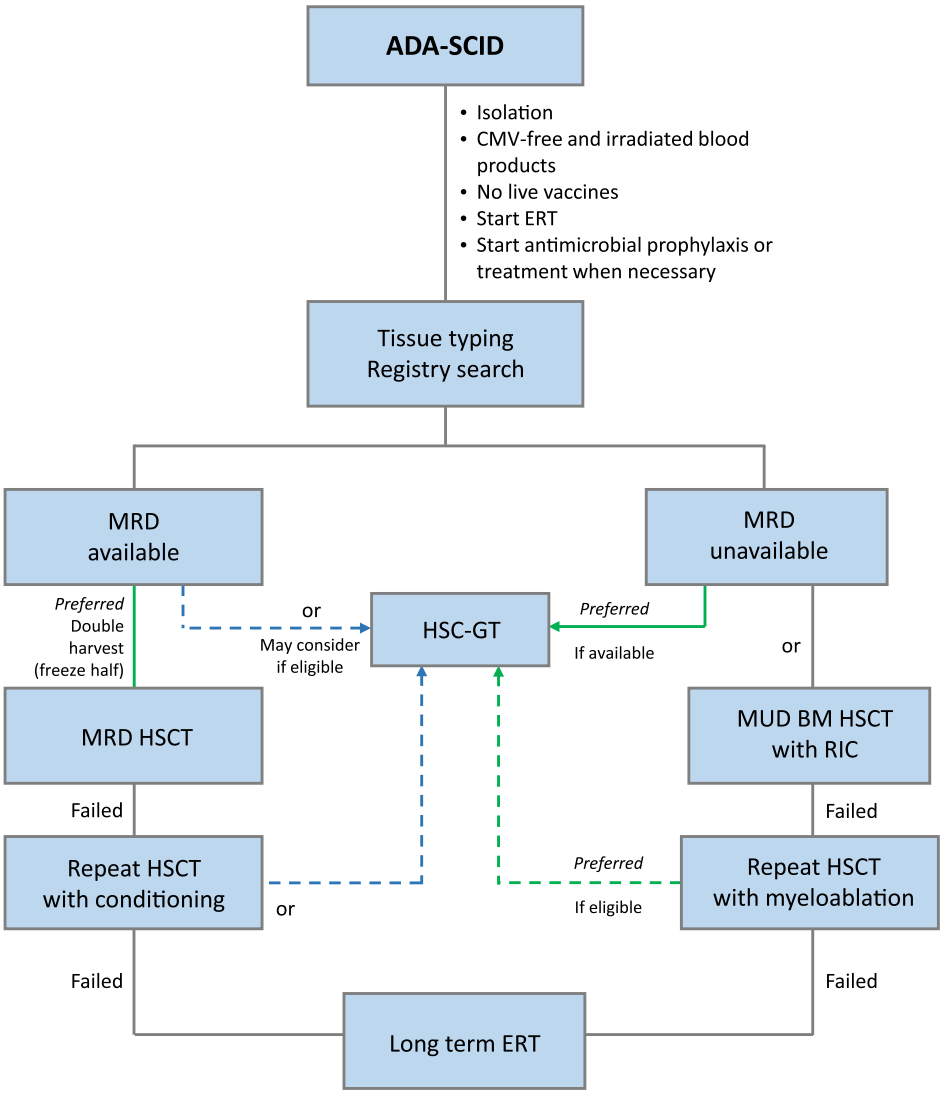


Figure 1: Algorithm for management of patients with ADA deficiency. ADA, adenosine deaminase; BM, bone marrow; CMV, cytomegalovirus; ERT, enzyme replacement therapy; HSC-GT, hematopoietic stem cell gene therapy; HSCT, hematopoietic stem cell transplantation; MRD, matched related donor; MUD, matched unrelated donor; RIC, reduced intensity conditioning; SCID, severe combined immunodeficiency.

Table 1: **Recommended immune evaluation while on ERT.**[a]

Time after starting ERT	First 6 mo or until immune reconstitution	6–18 mo	18–24 mo	>24 mo
Trough plasma ADA activity levels and deoxyadenosine levels[b]	At 3 and 6 mo	Q 6 mo	Q 6 mo	Q 12 mo
CBC, differential	At 2 wk, 1, 3, and 6 mo	Q 12 mo	Q 12 mo	Q 12 mo
Lymphocyte immunophenotyping	At 6 mo	Q 6 mo	Q 12 mo	Q 12 mo
IgG, IgA, IgM	At 6 mo	Q 6 mo	Q 12 mo	Q 12 mo
Mitogen stimulation	Q 6 mo	Q 6 mo	Q 12 mo	Q 12 mo
TCR-Vbeta—adjunct testing for consideration	Q 6 mo	Q 6 mo	Q 12 mo	Q 12 mo
CD45Ra/Ro—adjunct testing for consideration	Q 6 mo	Q 6 mo	Q 12 mo	Q 12 mo

[a]For elapegademase (Revcovi) naïve patients, guidelines in the product monograph may be followed.
[b]Testing for antibodies to elapegademase (Revcovi) should be performed if a persistent fall in plasma ADA activity trough levels below 15 mmol/h/L occurs.

Additional but not mandatory findings

(5) Increased deoxyadenosine in urine, plasma, or dried blood spots
(6) <1% of normal ADA activity in T cells

Additional clues to aid in diagnosis

(7) Recent/persistent infections consistent with SCID
(8) Neurological, skeletal, lung and liver anomalies, and
(9) Hematopoietic anomalies (most commonly neutropenia)

Incomplete (partial) deficiency

Mandatory findings

(1) Low ADA activity in red blood cells
(2) 20%–75% of normal ADA activity in lymphocytes
(3) T cell lymphopenia
(4) Low responses to mitogens

Additional but not mandatory findings

(5) Increased deoxyadenosine in urine, plasma, or dried blood spots

Additional clues to aid in diagnosis

(6) Can have delayed and (or) milder onset of clinical features listed under complete deficiency

Clinical and laboratory features

Non-immune features of ADA deficiency

Skeletal

- Rib and small bone anomalies

Neurological

- Developmental delay, hearing anomalies, seizures

Lungs

- Bronchiectasis, alveolar proteinosis

Liver

- Increased liver enzymes, autoimmune hepatitis, hepatic failure

Renal

- Hemolytic uremic syndrome

Hematopoietic and immune anomalies

Lymphoid cells

- Decreased number and function of T, B cells, NK cells

Lymphoid tissues

- Dysplastic thymus and lymph nodes

Hematopoietic

- Neutropenia, myeloid dysplasia

Management of patients with ADA deficiency

General

- Protective isolation at home or in hospital until reconstituted immune function[3]
- Immunoglobulin replacement
- Prophylaxis against *Pneumocystis jirovecii* pneumonia (trimethoprim-sulfamethoxazole) at 1 month of age
- Avoid breast feeding if CMV IgG is positive in the mother
- Use CMV negative and irradiated or leuko-reduced blood products, when needed, discuss with local blood bank as per policy for immunosuppressed patients
- Avoid live vaccines (MMRV, rotavirus, BCG)

Specific treatment

Enzyme replacement (ERT)

- For newly diagnosed patients it is recommended to start recombinant PEG-ADA, elapegademase (Revcovi) at a total weekly dose of 0.4 mg/kg, divided and administered twice a week (0.2 mg/kg per injection) intramuscularly, as per product insert.
- Reassessment of dosing is recommended 6 months from treatment initiation and could be gradually reduced and (or) consolidated to 1 injection per week, based on immune reconstitution and (or) ADA activity of more than 30 mmol/h/L and dAXP level under 0.02 mmol/L.
- For patients already on pegademase (Adagen) treatment, we recommend changing to

[3]The decision to isolate at home or in the hospital usually depends on the patient's medical condition and home compliance.

elapegademase (Revcovi) with a starting dose of 0.2 mg/kg once a week while periodically increasing or decreasing doses by increments of 0.033 mg/kg weekly to monitor immune reconstitution; ADA activity above 30 mmol/h/L and (or) dAXP below 0.02 mmol/L.

Treatment duration

- For most patients ERT should be a temporary option until definitive treatment such as HSCT or HSC-GT are available.
- ERT should be stopped just prior or shortly after HSCT[4].
- Before HSC-GT, ERT should be administered according to the approved GT protocol used.
- Long term ERT can be used when HSCT or HSC-GT cannot be performed, and can be continued in patients who were treated for long durations (>10 years) and have no other option or where the risk of HSCT is deemed to be too high.
- Long term ERT can be effective in patients with partial ADA-deficiency

Dosing adjustment

- Doses should be adjusted over time according to ADA activity trough levels and dAXP levels.
- Antibodies to elapegademase (Revcovi) should be tested in cases where a persistent decline in plasma ADA activity is recorded.

REFERENCES

Adams, A., and Harkness, R.A. 1976. Adenosine deaminase activity in thymus and other human tissues. Clin. Exp. Immunol. **26**: 647–649. PMID: 1009688.

Aiuti, A., Slavin, S., Aker, M., Ficara, F., Deola, S., Mortellaro, A., Morecki, S., Andolfi, G., Tabucchi, A., Carlucci, F., Marinello, E., Cattaneo, F., Vai, S., Servida, P., Miniero, R., Roncarolo, M.G., and Bordignon, C. 2002. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science, **296**: 2410–2413. PMID: 12089448. doi: 10.1126/science.1070104.

Albuquerque, W., and Gaspar, H.B. 2004. Bilateral sensorineural deafness in adenosine deaminase-deficient severe combined immunodeficiency. J. Pediatr. **144**: 278–280. PMID: 14760277. doi: 10.1016/j.jpeds.2003.10.055.

Arredondo-Vega, F.X., Santisteban, I., Daniels, S., Toutain, S., and Hershfield, M.S. 1998. Adenosine deaminase deficiency: Genotype-phenotype correlations based on expressed activity of 29 mutant alleles. Am. J. Hum. Genet. **63**: 1049–1059. PMID: 9758612. doi: 10.1086/302054.

Biggs, C.M., Haddad, E., Issekutz, T.B., Roifman, C.M., and Turvey, S.E. 2017. Newborn screening for severe combined immunodeficiency: A primer for clinicians. CMAJ, **189**: E1551–E1557. PMID: 29255099. doi: 10.1503/cmaj.170561.

Blackburn, M.R., and Kellems, R.E. 2005. Adenosine deaminase deficiency: Metabolic basis of immune deficiency and pulmonary inflammation. Adv. Immunol. **86**: 1–41. PMID: 15705418. doi: 10.1016/S0065-2776(04)86001-2.

Booth, C., Hershfield, M., Notarangelo, L., Buckley, R., Hoenig, M., Mahlaoui, N., Cavazzana-Calvo, M., Aiuti, A., and Gaspar, H.B. 2007. Management options for adenosine deaminase deficiency; proceedings of the EBMT satellite workshop (Hamburg, March 2006). Clin. Immunol. **123**: 139–147. PMID: 17300989. doi: 10.1016/j.clim.2006.12.009.

Bradford, K.L., Moretti, F.A., Carbonaro-Sarracino, D.A., Gaspar, H.B., and Kohn, D.B. 2017. Adenosine deaminase (ADA)-deficient severe combined immune deficiency (SCID): Molecular pathogenesis and clinical manifestations. J. Clin. Immunol. **37**: 626–637. PMID: 28842866. doi: 10.1007/s10875-017-0433-3.

Cagdas, D., Gur Cetinkaya, P., Karaatmaca, B., Esenboga, S., Tan, C., Yilmaz, T., Gumus, E., Baris, S., Kuskonmaz, B., Ozgur, T.T., Bali, P., Santisteban, I., Orhan, D., Yuce, A., Cetinkaya, D., Boztug, K., Hershfield, M., Sanal, O., and Tezcan, I. 2018. ADA deficiency: Evaluation of the clinical and laboratory features and the outcome. J. Clin. Immunol. **38**: 484–493. PMID: 29744787. doi: 10.1007/s10875-018-0496-9.

Cicalese, M.P., Ferrua, F., Castagnaro, L., Pajno, R., Barzaghi, F., Giannelli, S., Dionisio, F., Brigida, I., Bonopane, M., Casiraghi, M., Tabucchi, A., Carlucci, F., Grunebaum, E., Adeli, M., Bredius, R.G., Puck, J.M., Stepensky, P., Tezcan, I., Rolfe, K., De Boever, E., Reinhardt, R.R., Appleby, J., Ciceri, F., Roncarolo, M.G., and Aiuti, A. 2016. Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. Blood, **128**: 45–54. PMID: 27129325. doi: 10.1182/blood-2016-01-688226.

[4]Some suggest continuing ERT for 2 weeks.

Ferrua, F., Brigida, I., and Aiuti, A. 2010. Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency. Curr. Opin. Allergy Clin. Immunol. **10**: 551–556. PMID: 20966749. doi: 10.1097/ACI.0b013e32833fea85.

Flinn, A.M., and Gennery, A.R. 2018. Adenosine deaminase deficiency: A review. Orphanet J. Rare Dis. **13**: 65. PMID: 29690908. doi: 10.1186/s13023-018-0807-5.

Griffith, L.M., Cowan, M.J., Kohn, D.B., Notarangelo, L.D., Puck, J.M., Schultz, K.R., Buckley, R.H., Eapen, M., Kamani, N.R., O'Reilly, R.J., Parkman, R., Roifman, C.M., Sullivan, K.E., Filipovich, A.H., Fleisher, T.A., and Shearer, W.T. 2008. Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: Current status and critical needs. J. Allergy Clin. Immunol. **122**: 1087–1096. PMID: 18992926. doi: 10.1016/j.jaci.2008.09.045.

Grunebaum, E., Cohen, A., and Roifman, C.M. 2013. Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies. Curr. Opin. Allergy Clin. Immunol. **13**: 630–638. PMID: 24113229. doi: 10.1097/ACI.0000000000000006.

Hassan, A., Booth, C., Brightwell, A., Allwood, Z., Veys, P., Rao, K., Honig, M., Friedrich, W., Gennery, A., Slatter, M., Bredius, R., Finocchi, A., Cancrini, C., Aiuti, A., Porta, F., Lanfranchi, A., Ridella, M., Steward, C., Filipovich, A., Marsh, R., Bordon, V., Al-Muhsen, S., Al-Mousa, H., Alsum, Z., Al-Dhekri, H., Al Ghonaium, A., Speckmann, C., Fischer, A., Mahlaoui, N., Nichols, K.E., Grunebaum, E., Al Zahrani, D., Roifman, C.M., Boelens, J., Davies, E.G., Cavazzana-Calvo, M., Notarangelo, L., and Gaspar, H.B. Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation and European Society for Immunodeficiency. 2012. Outcome of hematopoietic stem cell transplantation for adenosine deaminase-deficient severe combined immunodeficiency. Blood, **120**: 3615–3624; quiz 3626. PMID: 22791287. doi: 10.1182/blood-2011-12-396879.

Kohn, D.B., Weinberg, K.I., Nolta, J.A., Heiss, L.N., Lenarsky, C., Crooks, G.M., Hanley, M.E., Annett, G., Brooks, J.S., El-Khoureiy, A., Lawrence, K., Wells, S., Moen, R.C., Bastian, J., Williams-Herman, D.E., Elder, M., Wara, D., Bowen, T., Hershfield, M.S., Mullen, C.A., Blaese, R.M., and Parkman, R. 1995. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. Nat. Med. **1**: 1017–1023. PMID: 7489356. doi: 10.1038/nm1095-1017.

Manson, D., Diamond, L., Oudjhane, K., Hussain, F.B., Roifman, C., and Grunebaum, E. 2013. Characteristic scapular and rib changes on chest radiographs of children with ADA-deficiency SCIDS in the first year of life. Pediatr. Radiol. **43**: 589–592. PMID: 23179487. doi: 10.1007/s00247-012-2564-2.

Murguia-Favela, L., Min, W., Loves, R., Leon-Ponte, M., and Grunebaum, E. 2020. Comparison of elapegademase and pegademase in ADA-deficient patients and mice. Clin. Exp. Immunol. **200**: 176–184. PMID: 31989577. doi: 10.1111/cei.13420.

Poliani, P.L., Facchetti, F., Ravanini, M., Gennery, A.R., Villa, A., Roifman, C.M., and Notarangelo, L.D. 2009. Early defects in human T-cell development severely affect distribution and maturation of thymic stromal cells: Possible implications for the pathophysiology of Omenn syndrome. Blood, **114**: 105–108. PMID: 19414857. doi: 10.1182/blood-2009-03-211029.

Reid, B., Ovadia, A., and Dinur Schejter, Y. 2017. Managing newborn screening for SCID in a referral centre. LymphoSign J. **4**: 77–79. doi: 10.14785/lymphosign-2017-0005.

Santisteban, I., Arredondo-Vega, F.X., Kelly, S., Mary, A., Fischer, A., Hummell, D.S., Lawton, A., Sorensen, R.U., Stiehm, E.R., Uribe, L., Weinberg, K., and Hershfield, M.S. 1993. Novel splicing, missense, and deletion mutations in seven adenosine deaminase-deficient patients with late/delayed onset of combined immunodeficiency disease. Contribution of genotype to phenotype. J. Clin. Invest. **92**: 2291–2302. PMID: 8227344. doi: 10.1172/JCI116833.

Sauer, A.V., Mrak, E., Hernandez, R.J., Zacchi, E., Cavani, F., Casiraghi, M., Grunebaum, E., Roifman, C.M., Cervi, M.C., Ambrosi, A., Carlucci, F., Roncarolo, M.G., Villa, A., Rubinacci, A., and Aiuti, A. 2009. ADA-deficient SCID is associated with a specific microenvironment and bone phenotype characterized by RANKL/OPG imbalance and osteoblast insufficiency. Blood, **114**: 3216–3226. PMID: 19633200. doi: 10.1182/blood-2009-03-209221.

Sauer, A.V., Brigida, I., Carriglio, N., and Aiuti, A. 2012. Autoimmune dysregulation and purine metabolism in adenosine deaminase deficiency. Front. Immunol. **3**: 265. PMID: 22969765. doi: 10.3389/fimmu.2012.00265.

Scott, O., Brager, R., Dent, P., Siu, V., Reid, B., Kim, V.H.-D., and Roifman, C.M. 2019. Survival of patients with severe combined immunodeficiency in the newborn screening era: A single-centre experience 2013–2019 (in Abstracts from the Immunodeficiency Canada—7th SCID Symposium, Montreal, QC,

24 October 2019). LymphoSign J. **6**: 148–163. doi: 10.14785/lymphosign-2019-0015.

Shovlin, C.L., Hughes, J.M., Simmonds, H.A., Fairbanks, L., Deacock, S., Lechler, R., Roberts, I., and Webster, A.D. 1993. Adult presentation of adenosine deaminase deficiency. Lancet, **341**: 1471. PMID: 8099155. doi: 10.1016/0140-6736(93)90910-9.

Titman, P., Pink, E., Skucek, E., O'Hanlon, K., Cole, T.J., Gaspar, J., Xu-Bayford, J., Jones, A., Thrasher, A.J., Davies, E.G., Veys, P.A., and Gaspar, H.B. 2008. Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies. Blood, **112**: 3907–3913. PMID: 18645040. doi: 10.1182/blood-2008-04-151332.

Whitmore, K.V., and Gaspar, H.B. 2016. Adenosine deaminase deficiency—More than just an immunodeficiency. Front. Immunol. **7**: 314. PMID: 27579027. doi: 10.3389/fimmu.2016.00314.

LymphoSign Journal

Working together
for patients with PI

Immunodeficiency Canada
Immunodéficience Canada

jmcn Jeffrey Modell Centers Network

Canadian Science Publishing