Genetics of Sjogren's Syndrome and Colorectal Cancer

Thomas M. Flagg '25 and Michael J. Wolyniak

Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943

Introduction

Sjogren's Syndrome is a systemic autoimmune disease affecting an estimated 4 million Americans. Characterized by inflammation of the salivary and lacrimal glands, patients with Sjogren's often experience dryness of the eyes and mouth. Additional symptoms include fatigue, joint pain, and dysfunction of organs including the kidneys, lungs, G.I. tract, liver, pancreas, nervous system, and blood vessels [1]. In Sjogren's Syndrome, glandular tissues such as the lacrimal and salivary glands are attacked by lymphocytes. T cells collect in these glands, releasing cytokines such as interleukin-17, interleukin-21, interferon gamma, and tumor necrosis factor-alpha. This causes chronic inflammation and renders the tissues inoperable, leading to dysfunction in the glands that produce saliva and tears. Furthermore, hyperactivity of B cells is sometimes found in ectopic germinal centers, formed within the affected glandular tissues [1-3].

Due to this dysfunction of the salivary glands, patients with Sjogren's Syndrome are at an increased risk of developing dental caries. The oral cavity is home to over 700 species of bacteria, and this diverse microbiome exists in a precarious state of homeostasis [4]. Due to dryness in the mouth caused by salivary gland dysfunction, patients with Sjogren's Syndrome experience a proliferation of bacteria. Normal oral commensals such as *Fusobacterium nucleatum, Streptococcus mucans,* and *Porphyromonas gingivalis*, can then overgrow and spread to the gastrointestinal tract via swallowing of saliva or through the bloodstream [5-6]. Once in the gut, these oral pathogens can infiltrate the weakened mucosa barrier, infiltrating the epithelial lining of the GI tract. This introduction of new species causes a shift in the balance that exists within the gut microbiome, resulting in a state of dysbiosis. Dysbiosis in the gut microbiome is seen to cause chronic inflammation from the immune system response. This state of chronic inflammation results in tumorigenesis due to DNA damage, cell proliferation, the inhibition of apoptosis, and other virulence factors (Figure 1) [5-7]. Because patients with Sjogren's Syndrome are susceptible to gut dysbiosis and experience higher populations of these bacteria [7], we expect that by comparing patients with early-onset and aggressive colorectal cancer with genes that are associated with Sjogren's Syndrome, patients with genes associated with Sjogren's Syndrome will have a higher instance of early-onset and metastatic colorectal cancer.

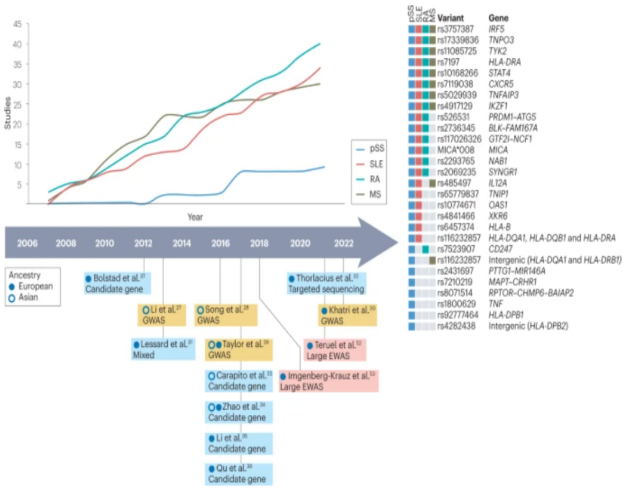

Figure 1: A graph from Thorlacius et al.'s study on the genetics of Sjogren's Syndrome, highlighting the rise in research on autoimmune disorders. However, Sjogren's Syndrome research lags behind other conditions. The gene list on the right links specific genes to autoimmune disorders, with blocks indicating associations. Abbreviations: pSS (primary Sjogren's Syndrome), SLE (systemic lupus erythematosus), RA (rheumatoid arthritis), and MS (multiple sclerosis). Many genes linked to Sjogren's Syndrome are shared with other autoimmune diseases [8].

Genetics of Sjogren's Syndrome

Patients with Sjogren's Syndrome are at an increased risk of developing some cancers. Large scale studies on the genetics of Sjogren's Syndrome have shown that there are certain genes that can be associated with the disease and its development. Despite it being one of the larger autoimmune diseases, there is much still unknown about the disease. The disease predominantly affects women, and it is thought that environmental factors along with genetic factors play a role in its development. Recent studies have outlined the role of genetics in Sjogren's Syndrome, and associated certain risk loci in gene sequences. (Figure 2) [8].

Many genes associated with Sjogren's Syndrome belong to the Human Leukocyte Antigen (HLA) locus, specifically class II. HLA Class II genes include HLA-DR, HLA-B, HLA-DQ,and HLA-DP. These genes are tasked with aiding in the body's adaptive immune system response to stimuli presenting as a threat often in the form of viruses and vaccines. HLA genes contain information for cell surface molecules that present antigenic peptides to receptors on T cells. Multiple studies have indicated that this locus has the strongest genetic association

with Sjogren's Syndrome and therefore includes the majority of the genes in question [8-9].

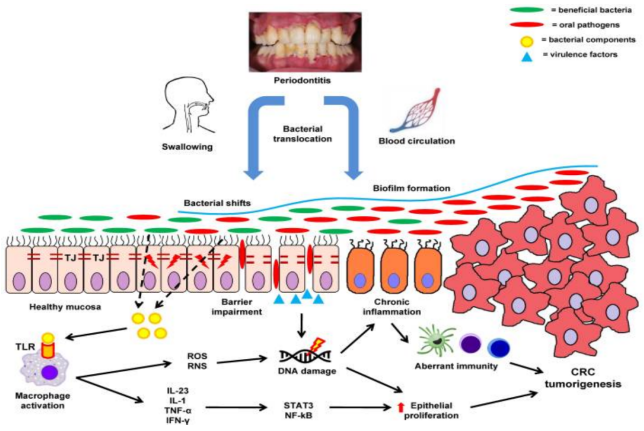

Figure 2: The path bacteria take from the oral cavity of a patient with periodontitis to the intestine resulting in colorectal cancer tumorigenesis. Bacteria such as *Fusobacterium nucleatum*, normally abundant in the oral cavity, travel to the intestines either through swallowing or through the bloodstream. Once in the intestines, they offset the balance of microbiota, leading to intestinal dysbiosis. Macrophage activation then occurs through the secretion of bacterial components and biofilm formation. Inflammation occurs, along with the proliferation of epithelial cells, and DNA damage and mutation. This eventually leads to tumorigenesis and the formation of colorectal cancer [5].

Studies also indicate that there are other genes associated with Sjogren's Syndrome. These genes are non-antigen presenting. This includes genes associated with interferon signature, Interferon Regulatory Factor 5 (IRF5), Signal Transducer and Activator of Transcription 4 (STAT4), Interleukin 12A (IL12A), and Natural Cytotoxicity Triggering Receptor 3 (NCR3). Interferon signature genes are any genes induced during an interferon response, which the immune system uses against viral infections [9].

Genes associated with lymphocyte regulation include B-Lymphocyte Kinase (BLK), B-cell activating factor (BAFF), Early B-cell factor 1 (EBF1), General Transcription Factor IIi (GTF2I), C-X-C chemokine receptor type 5 (CXCR5), Tumor Necrosis Factor Superfamily Member 4 (TNFSF4), TNF Alpha Induced Protein 3 (TNFAIP3), TNFAIP3-interacting protein 1(TNIP1), Lymphotoxin-alpha (LTA), and C-C motif chemokine 11 (CCL11). These genes help the body regulate the functions of B and T cells, which specialize in recognizing antigens and destroying pathogens [9].

Data

Three studies from the Memorial Sloan Kettering Center for Cancer Research were referred to when compiling research, Colorectal Cancer (MSK, JNCI 2021) [12], Colorectal Cancer (MSK, Gastroenterology 2020) [13], and Metastatic Colorectal Cancer (MSK, Cancer Cell 2018) [14]. These independent studies were accessed through cBioPortal, a cancer genomics database. The genes IRF5, TNPO3, TYK2, HLA-DRA, STAT4, CXCR5, TNFAIP3, IKZF1, BLK, PRDM1, GTF2I, MICA, NAB1, SYNGR1, IL12A, TNIP1, OAS1, XKR6, HLA-B, HLA-DQA1, HLA-DQB1, CD247, PTTG1, MAPT, RPTOR, HLA-DPB1, and HLA-DPB2 were entered in separate queries o three different studies. Colorectal Cancer (MSK, JNCI 2021) [12] contained 1516 samples. Queried genes were altered in 9% (131/1516) of samples (Figure 3A). Not all of the samples found altered were positively oncogenic, however, some are known to be oncogenic [cBioPortal]. Most mutations found simply had unknown oncogenic effects. In the study, Colorectal Cancer (MSK, Gastroenterology 2020) [13], queried genes were found altered in 9% of samples (43/471) (Figure 3B). In a study of Metastatic Colorectal Cancer (MSK, Cancer Cell 2018) [14] queried genes were found in 8% (95/1134) of samples (Figure 3C).

The largest single study on Colorectal Cancer in the database contained 1,516 samples. The queried genes found to be altered in the study had various effects (Figure 3A). 31 out of 1,516 samples included a mutation of the tumor necrosis factor-a-induced-protein 3 (TNFAIP3) gene. Part of the tumor necrosis factor (TNF) family, TNFAIP3 encodes for an enzyme that is involved in regulating the NF-kB pathway. This pathway is proinflammatory and expresses genes for cytokines, and chemokines [15]. The mutations include amplifications, truncating, and missense mutations. In 10 of the patients whose samples were collected, mutations were likely oncogenic and consisted primarily of frameshift deletions or insertions. Missense mutations had unknown oncogenic effects. Another gene, Ikaros family zinc finger 1 (IKZF1), was mutated in 46 out of 1516 samples. IKZF1 encodes a transcription factor that regulates differentiation of lymphocytes. It is often found mutated in B-cell lymphocytic leukemia [cBioPortal]. However, in all the mutated samples, none were definitive drivers with oncogenic effects. PR domain zinc finger protein 1 (PRDM1) was found altered in 26/1516 samples. PRDM1 encodes a protein tasked with the regulation of various processes. This protein is a transcriptional repressor involved in the cellular response to viral infections [16].

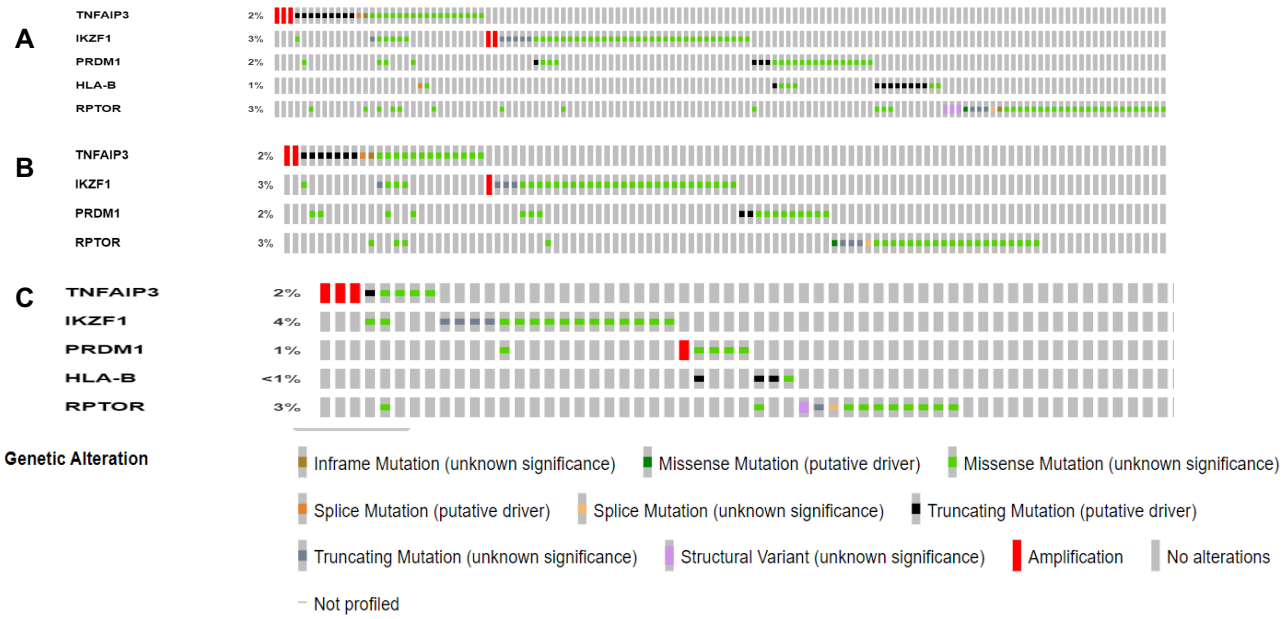

Figure 3. The above figures are onco-prints from the cBioPortal online cancer genomics database. The onco-prints depict a search of 27 genes associated with Sjogren's Syndrome, and what frequency they are found to be altered in the study. The studies above include A) Colorectal Cancer (MSK, JNCI 2022), B) Metastatic Colorectal Cancer (MSK, Cancer Cell 2019), and C) Colorectal Cancer (MSK, Gastroenterology 2020). The tracks directly to the right of the percentages depict what kind of mutation is found for each gene. Listed below the onco-prints is a figure legend detailing which type of mutation is represented by each color [cBioPortal].

Because these mutations are truncating in a tumor suppressor gene, they are likely oncogenic [cBioPortal]. Human Leukocyte Antigen- B (HLA-B) was found to have 18 mutations, 11 of which being likely oncogenic. 100% of mutated samples were in patients who suffered from early onset colorectal cancer (Figure 4). HLA-B was the only member of the Human Leukocyte Antigen complex featured in the query to include alterations. The regulatory associated protein of MTOR complex 1 (RPTOR) was found altered in 45/1516 samples. However, mutations were found to be likely oncogenic in only one of the samples. The mTORC1 signaling pathway is responsible for regulating growth in the body in response to different internal and external cues [cBioPortal]. The alterations of this gene found in the study also had unknown effects.

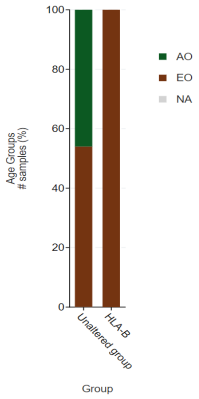

Figure 4. Generated by the cBioPortal online cancer genomics database depicts a comparison of patients with HLA-B gene mutations and cancer onset. The unaltered group represents all samples in the study Colorectal Cancer (MSK, JNCI 2022), with just under half of the patients suffering from average onset colorectal cancer. Patients with mutations of the HLA-B gene all suffer from early onset colorectal cancer [cBioPortal].

Discussion

Patients with autoimmune diseases often have an increased risk of cancer [17]. Sjogren's Syndrome is an autoimmune disorder affecting 4 million Americans involving glandular tissue [1]. A set of 27 genes has been identified with the development of Sjogren's Syndrome. Using cBioPortal these genes were cross-referenced with samples from 3 different colon cancer studies, encompassing thousands of samples. Analysis revealed that the genes TNFAIP3, IKZF1, PRDM1, HLA-B, and RPTOR were found to be mutated in 8.6% (269/3121) of samples, and 8.5% (264/3086) of patients. TNFAIP3 was altered in 2% (64/3121) of samples. IKZF1 was mutated in 3.3% (102/3121) of samples. PRDM1 was mutated in 1.6% (50/3121) of samples. HLA-B was mutated in 0.6% (20/3121) of samples. RPTOR was mutated in 2.8 (88/3121) of samples. While not all of these mutations were oncogenic, their effects require further study. Based on these findings, I propose a gene panel for patients with Sjogren's Syndrome that would include these five genes. Patients with Sjogren's Syndrome may be at risk of aggressive colorectal cancer. Patients with the HLA-B gene may be at greater risk of early-onset colorectal cancer. While over half of the patients in the colorectal cancer (MSK, JNCI 2022) study had average onset cancer, 100% of patients with mutations in the HLA-B gene had early-onset colorectal cancer (Figure 5). Furthermore, almost half of patients with mutations in these 5 genes suffered from Signet Cell and Mucinous adenocarcinoma of the colon and rectum. Based on this, I would include screening for Signet Cell and Mucinous adenocarcinoma of the colon and rectum.

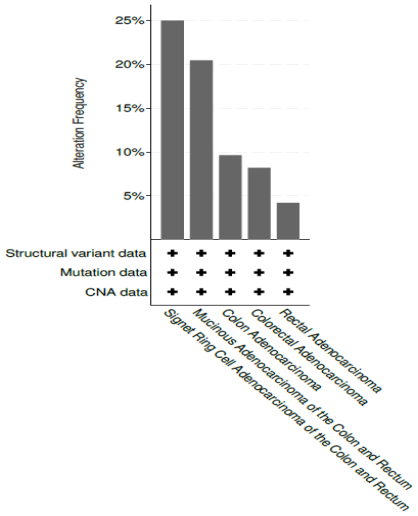

Figure 5. The above graph from the cBioPortal online cancer genomics database depicts a comparison between alteration frequency of 5 genes associated with Sjogren's Syndrome. These genes include TNFAIP3, IKZF1, PRDM1, HLA-B, and RPTOR. The graph shows the frequency of each detailed type of cancer of the patients who also have the 5 Sjogren's Syndrome associated genes [cBioPortal].

Future Study

In future studies, the inclusion of wet lab experiments may prove to be beneficial. The nature of this research is strictly remote, as there is no wet lab access. All data compiled came from the cBioPortal online cancer genomics database and from published scientific journals. A lack of a wet lab to perform experiments sets this research apart. Wet labs are expensive and difficult to access, however, online databases are free and relatively simple to navigate. As research and technology change, so does the need for immense amounts of data to be stored and sorted. Genetic research is relatively new but already includes mass amounts of data points. The vast amount of clinical research being completed and recorded in the United States allows researchers to have access to many datasets. Ultimately research is conducted by professionals in an attempt to better the health of the nation and the individual. Data concluded from this research can be utilized for disease prevention, early identification, improving the longevity of life, and improving quality of life.

Acknowledgments

I would like to thank Dr. Wolyniak for his help and oversight over the course of the summer, Dr. Irene Reed of St. Joseph's for her help getting started from the beginning, and to Hampden-Sydney College for allowing me to conduct this research this summer.

References

1. Arthritis Foundation. (n.d.). *Sjogren's*. Arthritis. https://www.arthritis.org/diseases/sjogrens-syndrome
2. Manfrè V, Chatzis LG, Cafaro G, Fonzetti S, Calvacchi S, Fulvio G, Navarro Garcia IC, La Rocca G, Ferro F, Perricone C, Bartoloni E, Baldini C. Sjögren's syndrome: one year in review 2022. Clin Exp Rheumatol. 2022 Dec;40(12):2211-2224. doi: 10.55563/clinexprheumatol/43z8gu. Epub 2022 Dec 20. PMID: 36541236.
3. U.S. Department of Health and Human Services. (2024, June 26). *Sjögren's disease*. National Institute of Arthritis and Musculoskeletal and Skin Diseases. https://www.niams.nih.gov/health-topics/sjogrens-disease
4. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019 Jan-Apr;23(1):122-128. doi: 10.4103/jomfp.JOMFP_304_18. PMID: 31110428; PMCID: PMC6503789.
5. Huang X, Huang X, Huang Y, Zheng J, Lu Y, Mai Z, Zhao X, Cui L, Huang S. The oral microbiome in autoimmune diseases: friend or foe? J Transl Med. 2023 Mar 22;21(1):211. doi: 10.1186/s12967-023-03995-x. PMID: 36949458; PMCID: PMC10031900.
6. Zepeda-Rivera M, Minot SS, Bouzek H, Wu H, Blanco-Míguez A, Manghi P, Jones DS, LaCourse KD, Wu Y, McMahon EF, Park SN, Lim YK, Kempchinsky AG, Willis AD, Cotton SL, Yost SC, Sicinska E, Kook JK, Dewhirst FE, Segata N, Bullman S, Johnston CD. A distinct Fusobacterium nucleatum clade dominates the colorectal cancer niche. Nature. 2024 Apr;628(8007):424-432. doi: 10.1038/s41586-024-07182-w. Epub 2024 Mar 20. PMID: 38509359; PMCID: PMC11006615.
7. Verstappen GM, Pringle S, Bootsma H, Kroese FGM. Epithelial-immune cell interplay in primary Sjögren syndrome salivary gland pathogenesis. Nat Rev Rheumatol. 2021 Jun;17(6):333-348. doi: 10.1038/s41584-021-00605-2. Epub 2021 Apr 28. PMID: 33911236; PMCID: PMC8081003.
8. Thorlacius GE, Björk A, Wahren-Herlenius M. Genetics and epigenetics of primary Sjögren syndrome: implications for future therapies. Nat Rev Rheumatol. 2023 May;19(5):288-306. doi: 10.1038/s41584-023-00932-6. Epub 2023 Mar 13. PMID: 36914790; PMCID: PMC10010657.
9. Teos LY, Alevizos I. Genetics of Sjögren's syndrome. Clin Immunol. 2017 Sep;182:41-47. doi: 10.1016/j.clim.2017.04.018. Epub 2017 May 3. PMID: 28476436; PMCID: PMC5660941.
10. Koliarakis I, Messaritakis I, Nikolouzakis TK, Hamilos G, Souglakos J, Tsiaoussis J. Oral Bacteria and Intestinal Dysbiosis in Colorectal Cancer. *International Journal of Molecular Sciences*. 2019; 20(17):4146. https://doi.org/10.3390/ijms20174146
11. Schoggins JW. Interferon-Stimulated Genes: What Do They All Do? Annu Rev Virol. 2019 Sep 29;6(1):567-584. doi: 10.1146/annurev-virology-092818-015756. Epub 2019 Jul 5. PMID: 31283436.
12. Cercek A, Chatila WK, Yaeger R, Walch H, Fernandes GDS, Krishnan A, Palmaira L, Maio A, Kemel Y, Srinivasan P, Bandlamudi C, Salo-Mullen E, Tejada PR, Belanfanti K, Galle J, Joseph V, Segal N, Varghese A, Reidy-Lagunes D, Shia J, Vakiani E, Mondaca S, Mendelsohn R, Lumish MA, Steinruecke F, Kemeny N, Connell L, Ganesh K, Markowitz A, Nash G, Guillem J, Smith JJ, Paty PB, Zhang L, Mandelker D, Birsoy O, Robson M, Offit K, Taylor B, Berger M, Solit D, Weiser M, Saltz LB, Aguilar JG, Schultz N, Diaz LA, Stadler ZK. A Comprehensive Comparison of Early-Onset and Average-Onset Colorectal Cancers. J Natl Cancer Inst. 2021 Nov 29;113(12):1683-1692. doi: 10.1093/jnci/djab124. PMID: 34405229; PMCID: PMC8634406.
13. Mondaca S, Walch H, Nandakumar S, Chatila WK, Schultz N, Yaeger R. Specific Mutations in APC, but Not Alterations in DNA Damage Response, Associate With Outcomes of Patients With Metastatic Colorectal Cancer. Gastroenterology. 2020 Nov;159(5):1975-1978.e4. doi: 10.1053/j.gastro.2020.07.041. Epub 2020 Jul 27. PMID: 32730818; PMCID: PMC7680360.
14. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, Jayakumaran G, Middha S, Zehir A, Donoghue MTA, You D, Viale A, Kemeny N, Segal NH, Stadler ZK, Varghese AM, Kundra R, Gao J, Syed A, Hyman DM, Vakiani E, Rosen N, Taylor BS, Ladanyi M, Berger MF, Solit DB, Shia J, Saltz L, Schultz N. Clinical Sequencing Defines the Genomic Landscape of Metastatic Colorectal Cancer. Cancer Cell. 2018 Jan 8;33(1):125-136.e3. doi: 10.1016/j.ccell.2017.12.004. PMID: 29316426; PMCID: PMC5765991.
15. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009 Dec;1(6):a001651. doi: 10.1101/cshperspect.a001651. Epub 2009 Oct 7. PMID: 20457564; PMCID: PMC2882124.
16. Smith MA, Maurin M, Cho HI, Becknell B, Freud AG, Yu J, Wei S, Djeu J, Celis E, Caligiuri MA, Wright KL. PRDM1/Blimp-1 controls effector cytokine production in human NK cells. J Immunol. 2010 Nov 15;185(10):6058-67. doi: 10.4049/jimmunol.1001682. Epub 2010 Oct 13. PMID: 20944005; PMCID: PMC3864810.
17. Nancy Volkers, Do Autoimmune Diseases Raise The Risk of Cancer?, *JNCI: Journal of the National Cancer Institute*, Volume 91, Issue 23, 1 December 1999, Pages 1992–1993, https://doi.org/10.1093/jnci/91.23.1992