2020

BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: MOLECULAR FEATURES, EPIDEMIOLOGICAL RISK FACTORS, AND EVIDENCE-BASED CLINICAL RECOMMENDATIONS

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Digital Object Identifier: https://doi.org/10.13023/etd.2020.174

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Medicine
at the University of Kentucky

By

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2020

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ABSTRACT OF DISSERTATION

BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: MOLECULAR FEATURES, EPIDEMIOLOGICAL RISK FACTORS, AND EVIDENCE-BASED CLINICAL RECOMMENDATIONS

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging cancer of the immune system that can form around textured-surface breast implants. In this dissertation, the cellular and molecular mechanisms of BIA-ALCL are reviewed with a focus on the role of oncogenic JAK-STAT3 signaling in BIA-ALCL tumorigenesis and progression. Herein, the epidemiology of BIA-ALCL is systematically studied to better define the risk of BIA-ALCL and to determine the oncologic safety of smooth surface devices relative to BIA-ALCL formation. Next, a systematic review is conducted which critically appraises current clinical guidelines in order to establish an evidence base to better inform diagnosis and treatment. Finally, a molecular investigation is undertaken to determine the biological mechanisms of the disease which revealed pervasive upregulation of the JAK-STAT3 pathway as a key pathogenic feature in BIA-ALCL tumorigenesis. Herein, a novel mechanism of tumorigenesis via the JAK-STAT3 pathway is proposed—highlighting its potential mechanistic role. Collectively, the clinical research studies that comprise this dissertation demonstrate the oncologic safety of smooth-devices while illustrating substantial knowledge gaps in the risk of BIA-ALCL for commercially available textured breast devices in the U.S. market. This work also provides evidence-based recommendations and updates on diagnosis and treatment. Finally, this dissertation shows that BIA-ALCL tumorigenesis likely occurs through a novel mechanism that facilitates malignant transformation from a chronic inflammatory state through the JAK-STAT3 pathway.

KEYWORDS: Breast Implants; Lymphoma, Epidemiology, Systematic Review, JAK-STAT3

Ryan C. DeCoster

03/30/2020

Date

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DEDICATION

To Rachel, Easton, and Darren.
Thank you for your love, encouragement, and support.

ACKNOWLEDGMENTS

Timothy A. Butterfield, PhD. Arguably one of the most intelligent people I have met in my life. There is honestly nothing this guy can't do, including rewiring ignition switches on my kids' go kart—it still runs—thank you for that. More importantly, thank you for being an incredible mentor. You have pushed me to think critically and independently. You have taught me that if you put your mind to it, that there isn't anything that you can't accomplish. I will miss "feed Ryan Wednesdays (thank you Cindy)," our long talks in the lab, discussing good bourbon, and quartering out venison in your garage, all while enjoying a cold glass of Oberon.

B. Mark Evers, MD, FACS. I can't thank you enough for believing in me and allowing me to be a part of the T32 program. Having you as a mentor opened up doors that I could have never imagined, and none of the successes I've had would have been possible without you. You have inspired me to become a better surgeon, scientist, husband, and father, and I will forever be grateful.

Henry C. Vasconez, MD, FACS. You once told me that your brother, Luis Vasconez, would not say an unkind word about the devil. You, sir, are cut from that same cloth. The quintessential surgeon, I have learned so much from you about how a surgeon should conduct themselves, both personally and professionally. I can't thank you enough for the opportunity to have worked with you throughout medical school and graduate school. I wouldn't be where I am today without your unwavering support and mentorship.

Brian Rinker, MD, FACS. Thank you for all of your support and encouragement over the last few years. You are the model surgeon and father. It truly amazes me how you are able to achieve such incredible career success while continuing to put your family first. It

is one of the reasons that I sought you out as a mentor and I can only hope to replicate what you have done so well in the coming years.

J. Matthew Webster, PhD. It has truly been a pleasure having you as a mentor. I always enjoyed swinging by your office unannounced to discuss all things science and life over a cup of joe. You played an instrumental role in teaching me how to become a scientist, especially with grant writing, and it paid off big time. I won't forget about those Lakers tickets.

Mark W. Clemens, MD, FACS. The work that is highlighted throughout this dissertation would not have been possible without your collaboration and expertise. Thank you.

Lesley Wong, MD, FACS. You are one of the kindest and most hard-working surgeons I have ever met. Thank you for agreeing to be my outside examiner.

Evan B. Lynch, MD, PhD. It has truly been a pleasure working with you over the last year, and I greatly appreciate all of the hard work that you put into the projects described in this dissertation. You have an incredible mind, and you have pushed me to become a better scientist. It will be fun to watch you in the coming years, my friend.

Alisha R. Bonaroti, MD, MS. You are a real go-getter, and it has been so much fun working with you over the last few years on this project and others. I really value our friendship, and I will greatly miss all of the time that we have spent together at Starbucks discussing research projects, career, and all things life. I hope that you and Marc have an incredible wedding and I wish you all the very best.

William D. North, MD, a.k.a, the "Sherriff." Thank you for encouraging me to pursue my dream of becoming a plastic surgeon.

Betsy F. Fink. You are an incredible resource that I used to my full advantage over the last three years. Thank you so much for all of your help with IRB and IACUC protocols, collecting specimens, teaching me microsurgery, and listening to me rant. I will miss Winchester water (aka Ale-8), the smell of bacon in the lab in the mornings, and the food that you always brought me. Also, thanks for always letting me raid your ridge; more importantly, thank you for your friendship. Although I will miss our fireside chats, I know that I will always have my lifelong friend, just a phone call or text away.

Rachel. To my beautiful and amazing wife, thank you so much for supporting my dreams at each and every turn. Whether I wanted to be a professional baseball player or Stanford-trained plastic surgeon, you have always been there encouraging and supporting me every step of the way. Hands down, you are the best decision I have ever made. Well, it is actually you who decided on me, and for that, I will forever be grateful. You are one of the strongest women I know. Thank you for being such an inspiration and for being such an incredible wife and mother to our two beautiful children.

Easton. My kind of sweet-loving boy. You have the warmest heart. I can't wait to see the man that you become.

Darren. To my strong-willed, fearless bundle of joy. Your laughter and energy are infectious. I only wish that you and your brother wouldn't grow up so fast. I am so proud of you, and I can't wait to watch you both grow into fine young men.

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It is not the critic who counts; not the man who points out how the strong man stumbles, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly; who errs, who comes short again and again, because there is no effort without error and shortcoming; but who does actually strive to do the deeds; who knows great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and who at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who neither know victory nor defeat.

Theodore Roosevelt

"The Man in the Arena" Excerpt from speech "Citizen in a Republic"

April 23rd, 1910

Later popularized by the U.S. Navy Seals

PREFACE

Chapters two through six are based on the following manuscripts:

DeCoster, RC, Lynch, EB, Bonaroti, AR, Miranda, RN, Hunt, KK, Clemens, MW (2020) Breast Implant-Associated Anaplastic Large Cell Lymphoma: A Clinical Update *Clinics in Plastic Surgery* (under review)

DeCoster, RC, Clemens, MW, Di Napoli, A, Lynch, EB, Bonaroti, AR, Rinker, BD, Butterfield, TA, Vasconez, HC (2020) Cellular and Molecular Mechanisms of Breast Implant-Associated Anaplastic Large Cell *Plastic and Reconstructive Surgery* (accepted)

DeCoster, RC, Lynch, EB, Miranda, RN, Medeiros, LJ, Fink, BF, Rinker, BD, Butterfield, TA, Vasconez, HC, Clemens, MW (2020) Aberrant JAK-STAT3 Signaling is a Key Molecular Feature of Breast Implant-Associated Anaplastic Large Cell Lymphoma *Plastic and Reconstructive Surgery* (under review)

DeCoster, RC, Rinker, BD, Webster, JM, Butterfield, TA, Vasconez, HC, Clemens, MW (2020) Current Risk of Breast Implant-Associated Anaplastic Large Cell Lymphoma: A Systematic Review of Epidemiological Studies (targeting *JAMA Surgery*)

DeCoster, RC, Lynch, EB, Bonaroti, AR, Webster, JM, Butterfield, TA, Evers, BM, Vasconez, HC, Clemens, MW (2020) Breast Implant-Associated Anaplastic Large Cell Lymphoma: An Evidence-Based Systematic Review *Annals of Surgery* (under review)

This dissertation is based upon stand-alone manuscripts. Therefore, there may be some redundancy in the introduction sections of chapters two through six.

CHAPTER 1. INTRODUCTION

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a novel T-cell lymphoma associated with textured-surface breast implants. Since the sentinel event, which was first reported in the mid-to-late 90s, over 800 cases and 30 deaths have been confirmed worldwide. Now provisionally classified as a unique clinical entity by the World Health Organization, the disease has commanded significant attention from both the scientific and clinical communities as well as regulatory agencies. In 2019, the U.S Food and Drug Administration (FDA) conducted hearings on breast implant safety, which included BIA-ALCL. Although the FDA concluded that textured-surface devices did not meet the criteria to issue a ban, they later issued a class 1 recall, the strongest type of recall, on all Allergan (Dublin, Ireland) textured breast devices which account for greater than 90% of cases worldwide. Although relatively rare and despite the removal of high-risk devices, the number of cases continues to rise as a result of increased physician awareness and diagnostic advances, indicating the emerging nature of this disease.

The current risk of BIA-ALCL ranges between 1:355-1:200,000. However, when considering risk profiles according to implant or patient specificity, manufacturer type, and geographic status, there is significant variation. Further complicating the interpretation of these data are the lack of well-defined study populations and considerable variation in the reporting of epidemiologic parameters. Furthermore, with the removal of Allergan devices, much of the available epidemiological data does little to mitigate risk for patients and providers considering the use of a commercially available textured device. Therefore, the current risk of BIA-ALCL is not well-defined and should be examined further.

After two decades of investigation, the biological mechanisms responsible for BIA-ALCL tumorigenesis and progression remain poorly understood. Early reports suggested that a subclinical, peri-prosthetic biofilm infection may facilitate T-cell clonal expansion. Lending credibility to this theory is the concept that capsular contracture, a major complication associated with breast implants, arises from an infectious agent, suggesting that capsular contracture and BIA-ALCL may share a common origin. Nevertheless, more recent investigations have failed to establish a connection between the breast microbiome and the malignant transformation of BIA-ALCL. Other studies have used high-throughput genetic sequencing to focus on oncogenic changes in order to better understand the pathogenesis of BIA-ALCL. Molecular studies have identified activating mutations in TP53, DNMT3A, and the JAK-STAT3 pathway. While this provides some insight into the pathophysiology of the disease, it remains unclear if these oncogenic mutations lead to downstream events that facilitate malignant transformation. As such, the molecular mechanisms responsible for BIA-ALCL tumorigenesis remain largely unknown.

The majority of cases present as an acute-onset seroma greater than one-year following implantation with a textured device. In order to establish a diagnosis, the seroma should be drained and sent for cytopathology, which includes CD30 immunohistochemistry and flow cytometry to determine the presence of a clonal T-cell gene rearrangement. National Comprehensive Cancer Network (NCCN) consensus guidelines were established to guide the diagnosis and treatment of BIA-ALCL. Current expert recommendations support complete surgical resection as the standard of care while adjuvant therapy is reserved for advanced disease or cases refractory to surgical excision.

Despite the establishment of consensus guidelines and expert recommendations, the evidence supporting those has not been systematically studied.

In summary, although the risk of BIA-ALCL has been previously determined, limitations and differences in study design and reporting have failed to provide an accurate risk estimate for currently available textured breast devices. Moreover, the lack of knowledge regarding the molecular mechanisms and evidence guiding current treatment recommendations is concerning. As such, further investigation into the molecular features, epidemiological risk factors, and evidence supporting current clinical recommendations is warranted.

The specific aims of this dissertation are as follows:

1) To determine if NCCN consensus guidelines and current treatment recommendations are supported by evidence-based practices of BIA-ALCL for complete surgical resection, adjuvant therapy, and breast reconstruction following complete resolution.
2) To better define the current risk of BIA-ALCL in the U.S. breast implant population
3) To determine the oncologic safety of smooth-surface breast implants with respect to the malignant transformation of BIA-ALCL
4) To elucidate the molecular mechanisms of BIA-ALCL

Chapter two focuses on relevant clinical background information on BIA-ALCL, while chapter three provides a narrative review on the cellular and molecular basis of the disease while suggesting a novel mechanism of lymphomagenesis. The first specific aim

is addressed in chapter four. This chapter provides a comprehensive overview of BIA-ALCL and critically appraises current expert recommendations and guidelines in order to better inform best evidence-based practices on diagnosis and treatment. The second and third aims of this dissertation are examined in chapter five, which systematically reviews the epidemiology of BIA-ALCL and assesses the oncology safety of smooth surface devices. The fourth aim is addressed in chapter six. A molecular investigation using hybridization-based transcriptional profiling was conducted in order to determine the biological mechanisms responsible for BIA-ALCL. The final chapter of this dissertation concludes by summarizing all pertinent findings in the context of the field while establishing a research agenda for current and future investigations.

Collectively, the objectives and specific aims are to critically appraise the evidence regarding the diagnosis and treatment of BIA-ALCL while simultaneously assessing the oncologic safety of smooth surface devices, determining an accurate risk profile for commercially available textured devices and elucidating the molecular drivers of BIA-ALCL.

CHAPTER 2. BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: A CLINICAL UPDATE

Synopsis

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging cancer of the immune system that is exclusively associated with textured-surface breast implants. This clinical review provides an update on the diagnosis and management of BIA-ALCL with an emphasis on major advances. The epidemiology and pathophysiology of the disease are also reviewed, focusing on current paradigm shifts and highlighting current controversies related to disease classification. Finally, we conclude by discussing medicolegal and ethical issues surrounding BIA-ALCL while establishing a future basic science and clinical research agenda that is central to improving patient safety.

Background

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a non-Hodgkin lymphoma subtype that arises around textured-surface breast implants.[1] Since first being described in the mid-to-late '90s, over 800 cases have been pathologically confirmed worldwide.[2] Cases of BIA-ALCL vary widely by both geography and textured device characteristics, suggesting a complex individual risk profile.[3,4] Allergan (Dublin, Ireland) Biocell textured implants, which are responsible for over 90% of reported cases worldwide when the device history was known, are now subject to a worldwide recall. Despite the removal of these high-risk devices from the global market, other textured devices remain commercially available. Given the emerging nature of the disease and the millions of patients still at risk for developing the disease, heightened awareness and a thorough knowledge of BIA-ALCL is required.

Over the past several years, plastic surgeons, together with oncologists and pathologists, have standardized the current guidelines on diagnosis and treatment—as failure to appropriately identify or manage BIA-ALCL cases can lead to patient demise. This evidence-based narrative review aims to provide clinical updates on the epidemiology and pathophysiology of BIA-ALCL while placing a particular emphasis on major advances in diagnosis and treatment. Herein, we highlight evidence-based surgical and therapeutic strategies for achieving complete remission. Finally, we discuss regulatory oversight issues surrounding textured devices and breast implants in general and conclude with establishing future research priorities.

Epidemiology

Since first being described, the epidemiological knowledge of BIA-ALCL has evolved considerably.[3–5] Current data suggest that the risk of developing the disease is higher than previously thought. Recently, Cordeiro et al. estimated a 26-year cumulative incidence of 1:355 patients with an Allergan Biocell implant and a patient-specific incidence rate of 0.311 cases per 1,000 person-years (95% CI: 0.018-0503).[4] They also demonstrated an implant-specific risk of BIA-ALCL at 1:602 devices. However, with the FDA removing Allergan devices from the market, it is unclear what the risk of BIA-ALCL is for commercially available textured devices. Data from Doren et al.[3] provide a risk estimate of Mentor (Mentor Worldwide LLC, Irvine, Calif.) Siltex implants at 1:51428. Calobrace et al. estimate a global combined risk of BIA-ALCL for Sientra (Santa Barbara, Calif.) and Silimed (Rio de Janeiro, Brazil.) implants at 1:200000.[6] Magnusson and colleagues challenged this calculation and found Silimed implants to have the highest risk of BIA-ALCL at 1:2832 compared to Allergan Biocell 1:3345 and Mentor Siltex at 1:86029.[7] From both a methodologic and epidemiologic standpoint, the Calobrace study was not designed to determine the risk of BIA-ALCL. Many epidemiological studies of BIA-ALCL, including the Doren study, have been limited by inadequate post-market surveillance. Without the knowledge of global sales data standardized across different populations (which may carry different nonequivalent, unmodifiable risks), actual comparisons cannot be evaluated.

Importantly, the only currently modifiable risk factor identified to date remains surface texturization of the implanted device. Although previously confined to the breast implant pocket, gluteal implant-associated anaplastic large cell lymphoma associated

with textured devices have now been reported, providing strong evidence that anatomic location does not play a significant role in tumor development.[8] Moreover, recently described cases in the transgender breast implant population indicate that the disease does not show a predilection for gender.[9] The U.S. FDA currently acknowledges a risk of developing ALCL from a textured device at 1:3817-1:30000.[10] However, heterogeneity in the worldwide literature, along with differences in regulatory agency estimates, underscores the significant geographic variation in the reported risk. Although the risk of BIA-ALCL remains relatively low, the increasing number of global cases emphasizes the emerging nature of the disease.

Pathogenesis

This section provides a cursory overview of the pathogenesis of BIA-ALCL relevant to practicing clinicians. An in-depth analysis of the cellular and molecular mechanisms is outside of the scope of this article and has been reviewed in detail elsewhere.[11]

2.1.1 Bacterial wall lipopolysaccharide hypothesis

Early investigations into the pathogenesis of BIA-ALCL suggested that subclinical, periprosthetic biofilms drive BIA-ALCL tumorigenesis.[12] This attractive hypothesis has medical precedent, as a causal link between some gastric-associated cancers and bacterial invasion (*Helicobacter pylori*) is well-established.[13] Loch-Wilkinson et al. demonstrated that the risk of BIA-ALCL increases as the surface area increases, a major predictor of bacterial load on implants, with the highest risk of BIA-ALCL residing in implants with the most aggressive surface characteristics.[5] These

researchers implicated the gram-negative bacillus *Ralstonia pickettii* in altering the implant pocket microbiome and causing oncogenic transformation of BIA-ALCL.[12] However, Ralstonia is a commonly identified pathogen in water sources, and the initial studies linking Ralstonia have not been replicated, suggesting this once-promising hypothesis may be inaccurate. In a subsequent study using 16S RNA sequencing, Walker et al. demonstrated that the microbiome has no apparent role in BIA-ALCL formation.[14] To that end, the microbiome in the non-diseased breast has yet to be established and is currently the focus of federally-funded research. The biofilm hypothesis has since been adapted into a lipopolysaccharide-driven carcinogenesis, which has been shown in oral squamous cell carcinoma and colon cancers.[15] However, a mechanism by which LPS facilitates malignant transformation in even well-described cancers remains incompletely understood. In a prospective study of BIA-ALCL at the senior author's institution, 24 patients included intraoperative technique details at time of original breast implant placement. BIA-ALCL patients had received betadine irrigation (12 patients: six 50% Strength, four 25% Strength, two "tea-colored") and seven patients had received antibiotic irrigation: (five Bacitracin/Cefazolin/Gentamicin and two Polymyxin/Bacitracin) and still went on to develop disease (**Figure 1**). To date, no operative strategy has been shown to decrease the future risk of BIA-ALCL. Worldwide clusters of disease represent heightened disease awareness and excellent long-term surveillance, and misattributing clusters to "poor breast implant technique" without any supportive data, only shames surgeons and discourages the reporting of cases.

2.1.2 Cancer genetics

Recent molecular investigations have provided novel insights into the biological mechanisms responsible for BIA-ALCL lymphomagenesis, but much work remains. Over the last five years, high throughput genetic sequencing technologies have enabled the identification of somatic mutations in DNMT3A and the JAK-STAT3 pathway, as well as germline mutations in TP53.[16] Data from Di Napoli and colleagues[17] has identified the JAK-STAT3 pathway as a key component of disease progression. Unpublished studies from our laboratory mechanistically corroborate these data and suggest JAK-STAT3 as a potential actionable therapeutic target with candidate drugs (e.g., JAK inhibitors), which may prove to be beneficial in patients with advanced or surgically unresectable disease. The prevailing hypothesis behind aberrant JAK-STAT3 pathway activation considers an overactivated immune system driving the malignant transformation of capsular lymphocytes. Interestingly, the JAK-STAT3 pathway mechanistically links chronic inflammation and other cancers, including lymphomas.[18] Comprehensive *in vitro* and *ex vivo* studies utilizing BIA-ALCL tissues are required to identify the inciting event responsible for driving JAK-STAT activation and is the current focus of our research group.

2.1.3 The role of chronic inflammation and implant surface characteristics

The link between chronic inflammation and cancer has been well established.[13] Our group has provided evidence that a chronically overactivated immune system predisposes to errors in DNA replication and subsequent driver gene mutations (e.g., STAT3).[19,20] Alternatively, some have postulated that chronic trauma to the breast pocket

induces a chronic inflammatory state that cultivates a microenvironment that favors tumorigenesis.[21] However, a low incidence of BIA-ALCL despite millions of patients with textured implants indicates a host-specific immune susceptibility for developing the disease. The foreign body reaction has been an attractive area of research in establishing a chronic inflammatory state, suggesting investigation in host-implant interactions may elucidate pathogenic signaling. Work by Turner and colleagues has investigated the role of aryl hydrocarbons, a conserved chemical structure found on the textured implant surfaces, to drive cellular proliferation of capsular lymphocytes through their associated receptor.[22] An alternative hypothesis by Kadin et al. suggests an allergen-driven etiology from a chronic allergic response to the implant itself. However, a unifying hypothesis linking immune responses and carcinogenesis remains elusive[23], and underscores the critical need for comprehensive genetic studies to identify patient-specific risk profiles.

2.1.4 BIA-ALCL: lymphoproliferative disorder vs. lymphoid neoplasm

The debate over the classification of BIA-ALCL as a "benign condition", as opposed to a lymphoid malignancy has served to limit the initiation of surveillance and definitive treatment. Some authors have argued that BIA-ALCL is a lymphoproliferative disorder that encompasses a broad spectrum of CD30+ benign seromas, malignant seromas, and distant metastasis.[24] Recently, experts from the World Health Organization (WHO) have provisionally classified BIA-ALCL as a unique lymphoid neoplasm, and specifically not a lymphoproliferative disorder.[25] Advanced disease is the end of the spectrum of cancer stages and substantiates the WHO classification of BIA-ALCL as a lymphoma rather than benign or lymphoproliferative. Untreated BIA-ALCL leads to

invasive, metastatic disease—and misclassification or overt failure to diagnose this disorder can lead to patient death.[26]

Diagnosis and Treatment

2.1.5 Clinical presentation

BIA-ALCL typically presents as an acute-onset periprosthetic fluid collection greater than one year following implantation in approximately 80% of cases. Patients may also present with lymphadenopathy (4-12%) or a palpable mass (8-24%). Less often (<5%), patients may present with capsular contracture or cutaneous involvement. The median time to presentation is 7-10 years (range, 1-28 years). BIA-ALCL is equally distributed between cosmetic and reconstructive patients, suggesting that history of a previous malignancy such as breast cancer does not predispose to the subsequent development of the disease. Previously confined to the breast implant pocket, reports of gluteal implant-associated anaplastic large cell lymphoma associated with textured breast devices have now surfaced, giving the impression that anatomic location may not play a significant role in tumor development.[8] Moreover, recently described cases in the transgender breast implant population indicate that the disease does not show a predilection for gender.[9]

2.1.6 Diagnostic workup

The presentation of any delayed seroma should raise immediate clinical suspicion for BIA-ALCL (**Figure 2-1**). It is important to note that all implants contain a trace amount (5-10 mL) of fluid in the periprosthetic space, which is normal and does not

warrant further screening. Obtaining a detailed clinical history and performing a thorough physical exam is paramount. After ruling out other causes of late seromas (e.g., infection), a diagnostic workup should commence with fine-needle aspiration (>50mL) of the seroma under ultrasound guidance or in consultation with interventional radiology. The aspirate should be sent for cytopathology with the request to *rule-out BIA-ALCL*. The order set should include CD30 immunohistochemistry and flow cytometry to determine the presence of a clonal T-cell gene rearrangement. In order to establish a diagnosis of BIA-ALCL, three criteria must be met: Monoclonal expansion of and strong expression of CD30+ T-cells, and the presence of large, anaplastic lymphoma cells. Importantly, CD30 positivity alone is not a pathognomonic feature and does not constitute a diagnosis of BIA-ALCL, as benign seromas have been found to harbor CD30+ lymphocytes. The other pathogenic features must also be present for a definitive diagnosis. Specific protocols for pathologic diagnosis have been established.[27,28] In order to identify 95% of randomly distributed lesions in specimens without grossly identifiable lesions, 12 capsular biopsies should be taken, two from each side of the face of a cube.[28]

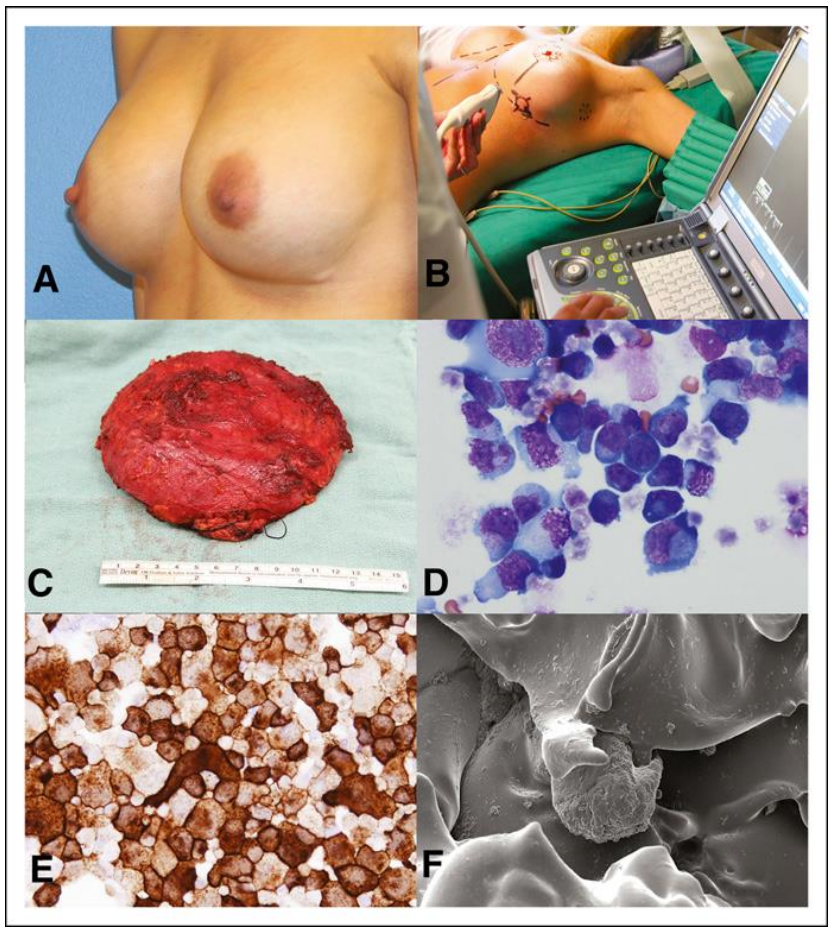


Figure 2-1. Patient Example and Surgical Treatment
This woman presented 7 years after bilateral cosmetic breast augmentation with swelling of the left breast and palpable lymphadenopathy (A). She underwent an incisional biopsy of the capsule, drainage of the effusion, and subsequent complete surgical excision that included implant removal and total capsulectomy with lymph node excisional biopsy by ultrasound guidance (B and C). Effusion demonstrated large cells (D: Wright Giemsa,31000; E: Anti CD30 immunocytochemistry,31000) capsule and excised lymph nodes were negative for lymphoma. The diagnosis rendered was breast implant–associated anaplastic large-cell lymphoma, Ann Arbor stage IE, MD Anderson Cancer Center stage 1A. Scanning electron microscopy demonstrates the textured surface of the involved breast implant with attached cells. (F; magnification, 31,000) The patient did not receive radiation or chemotherapy and underwent surveillance by positron emission tomography–computed tomography scan every 3 months the first year and every 6 months after the first year. Patient is disease free after 2 years of follow-up. Reprinted with permission. Clemens et al: Complete Surgical Excision Is Essential for the Management of Patients With Breast Implant-Associated Anaplastic Large-Cell Lymphoma *J Clin Oncol* Vol. 34 (2), Year: 2016 160-168.

Hanson et al. recently developed a novel, low-cost screening test which can be deployed in the clinical setting.[29] Using a commercially available (R&D Systems, Minneapolis, MN), CD30-specific enzyme-linked immunosorbent assay (ELISA), they standardized and validated the ability of the assay to reliable detect BIA-ALCL in seroma fluid. The authors demonstrated that the assay could effectively be used to evaluate suspicious seromas, presenting a reliable and more rapid alternative to standard CD30 immunohistochemistry. Nevertheless, it is important to reinforce that this should only be employed as an office-based screening tool. Definitive diagnosis still requires further pathologic evaluation.

2.1.7 Oncologic resection and adjuvant therapy

One of the most significant advances highlighted in this update has come from seminal work by Clemens et al. on the surgical treatment of the disease.[30,31] When diagnosed and treated in accordance with NCCN guidelines, BIA-ALCL carries an excellent prognosis, with five-year overall and event-free survival rates approaching 91% and 46%, respectively.[32] With complete surgical excision, event rates are reduced to 0% (Stages T1, T2) and 14.3% for Stage T3. A TNM staging system has since been proposed and validated, which replaces the previously used Ann Arbor Lugano Classification for BIA-ALCL. The MD Anderson TNM staging system of BIA-ALCL is summarized in **Table 2-1** and an illustration is provided in **Figure 2-2**. Collectively, these data demonstrate the clinical superiority of complete surgical resection over adjuvant therapy. This is reflected in NCCN guidelines, which highlight en bloc resection as the standard of care while adjuvant therapy is reserved for MD Anderson Stages IIB-IV.

Table 2-1. MD Anderson Staging System for BIA-ALCL

TNM Classification	Description
Primary tumor (T)	
T1	Confined to effusion or a layer on luminal side of capsule
T2	Early capsule infiltration
T3	Cell aggregates or sheets infiltrating the capsule
T4	Lymphoma infiltrates beyond the capsule
Regional lymph nodes (N)	
N0	No lymph node involvement
N1	One regional lymph node (+)
N2	Multiple regional lymph nodes (+)
Distant metastasis (M)	
M0	No distant spread
M1	Spread to other organs/sites
Stage	
1A	T1N0M0
1B	T2N0M0
1C	T3N0M0
IIA	T4N0M0
IIB	T1-3N1M0
III	T4N1M0
IV	T (any) N (any) M1

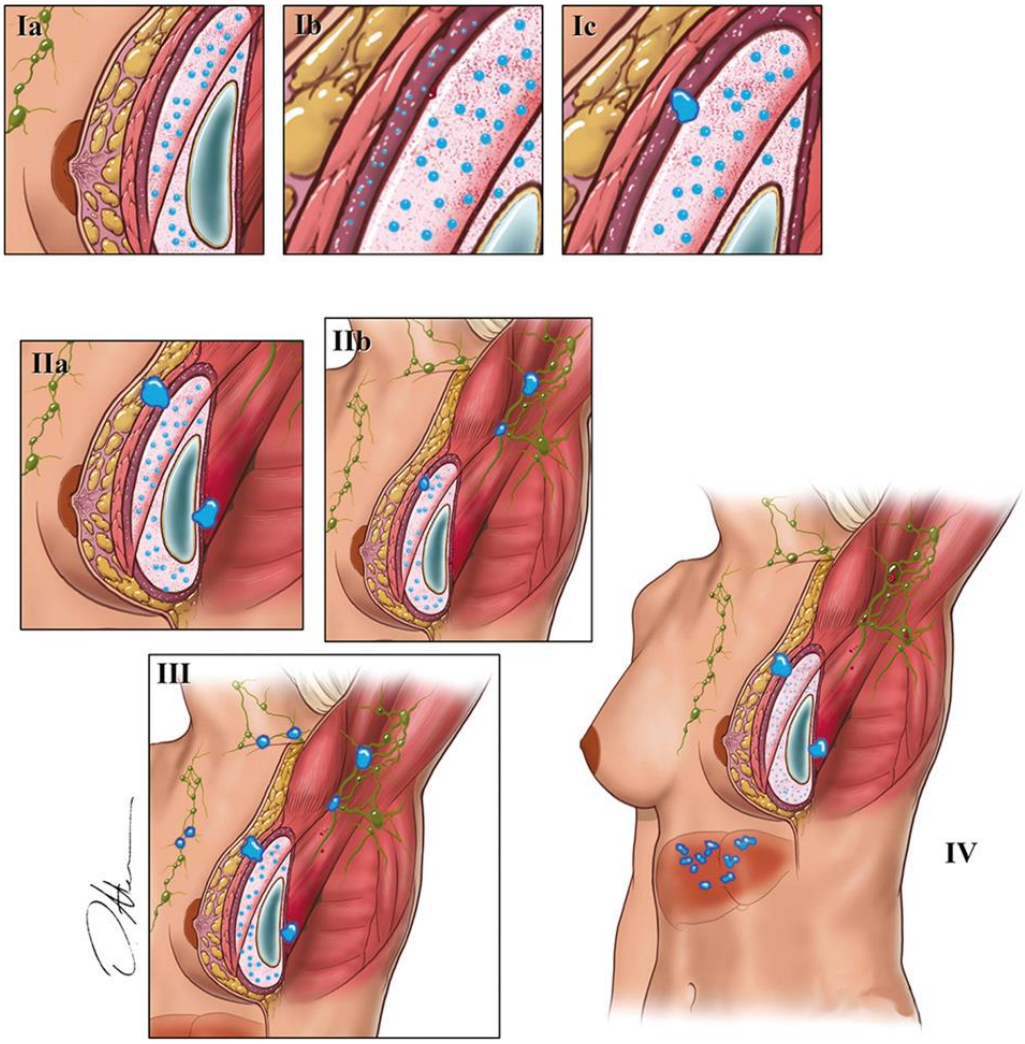


Figure 2-2. This TNM system was modeled after the American Joint Committee on Cancer TNM staging system for solid tumors.
Reprinted with permission. © (2016) American Society of Clinical Oncology. All rights reserved. Clemens et al: Complete Surgical Excision Is Essential for the Management of Patients With Breast Implant-Associated Anaplastic Large-Cell Lymphoma *J Clin Oncol* Vol. 34 (2), Year: 2016 160-168.

The preoperative workup should include several laboratory tests, which are summarized in **Table 2-2**. PET-CT may be used to assess for the presence of capsular masses or chest wall extension and may be helpful in guiding the surgical approach. It is highly recommended that oncologic resection should be done by, or in collaboration with a surgical oncologist in order to minimize the risk of locoregional recurrence. En bloc resection should proceed in the standard oncologic fashion, which includes placement of orientation sutures, deployment of surgical clips within the tumor bed, and utilizing sterilized instruments if performing a contralateral explantation.[33] Because BIA-ALCL does not involve the breast parenchyma, mastectomy is not indicated.

Table 2-2. Recommended Preoperative Laboratory Testing

Preoperative test	Notes
CBC w/ diff	
CMP	
LDH and Hep B	Order LDH and Hep B if chemotherapy is being considered
Bone marrow biopsy	Consider if high suspicion of advanced disease (locally aggressive or lymph node metastasis)
PET-CT	Used to assess for chest wall involvement and to guide surgical resection

CBC w/ diff Complete Blood Count with Differential; CMP, Complete Metabolic Panel; LDH, Lactate Dehydrogenase; Hep B, Hepatitis B; PET-CT, Positron Emission Tomography-Computed Tomography.

For asymptomatic patients, the FDA, ASPS, and the authors do no suggest prophylactic implant removal at this time. Complete capsulectomy encompasses the removal of the entire capsule. Conversely, en bloc resection is an oncologic procedure with the goal of achieving clear margins. This marks a clear distinction between complete capsulectomy and en bloc resection. Therefore, in a patient without clinically proven disease, complete capsulectomy alone is insufficient as a risk-reducing operation.

2.1.8 Oncologic surveillance

Per NCCN guidelines, patients should be followed closely by an oncologist every 3-6 months for two consecutive years in order to monitor for disease recurrence. PET-CT is the preferred imaging modality used to monitor for locoregional recurrence or distant metastasis.

2.1.9 Breast reconstruction after treatment for BIA-ALCL

Given the relatively low recurrence rate of 4% at five years,[34] breast reconstruction can be offered after definitive oncologic treatment for BIA-ALCL. The senior author has proposed a treatment algorithm based upon the MD Anderson TMN staging classification whereby patients with surgically resectable disease (stage IA-IC) are offered either immediate reconstruction or delayed reconstruction following surveillance PET-CT at 3-6 months. Patients with advanced disease (stage IIA-IV) are offered delayed reconstruction following surveillance imaging at 6-12 months after any adjuvant therapy. The approach to breast reconstruction may include implant replacement, which should proceed using a smooth device, as discussed below. The possibility of device-induced recurrence may deter patients from implant-based

reconstruction, and alternative methods include mastopexy, autologous tissue transfer, or serial fat grafting. Patient satisfaction has been shown to be excellent after reconstruction following BIA-ALCL treatment.

Medicolegal and Ethical Considerations

2.1.10 Breast Implant Safety and Regulatory Oversight

Significant concerns over the safety of breast implants have reemerged at the forefront of plastic and reconstructive surgery. Recently, a controversial paper resurrected the age-old theory that silicone breast implants may be associated with an increased risk of rare harms.[35] Collectively, these concerns prompted the FDA to conduct public advisory hearings on breast implant safety in March 2019. The evidence presented resulted in newly proposed black box warnings for all breast implants (**Table 2-3**). While it is important to note that these warnings have yet to be finalized, plastic surgeons should expect to see some iteration of these warnings on package inserts for all breast devices in the very near future.

Table 2-3. Proposed U.S. Food and Drug Administration Breast Implant Label Warnings

Description
Breast implants are not considered lifetime devices. The longer people have them, the greater the chances are that they will develop complications, some of which will require more surgery
Breast implants have been associated with the development of a cancer of the immune system called breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). This cancer occurs more commonly in patients with textured breast implants than smooth implants, although rates are not well defined. Some patients have died from BIA-ALCL
Patients receiving breast implants have reported a variety of systemic symptoms such as joint paint, muscle aches, confusion, chronic fatigue, autoimmune diseases and others. Individual patient risk for developing these symptoms has not been well established. Some patients report complete resolution of symptoms when the implants are removed without replacement

2.1.11 Implant Screening and Patient Education

The most recent FDA guidance also specifically addresses screening for implant rupture. The draft calls for updated screening, which would supersede the prior recommendation for MRI evaluation at two years post-implantation and every three years thereafter. However, screening is not widely adopted as it is not covered by many insurance policies. This is just one of many considerations that should be discussed with patients during the informed consent process. All patients receiving an implant should be made aware of the existence of BIA-ALCL, current incidence, common presenting symptoms, and general screening recommendations. The informed consent process is paramount to the preservation of patient autonomy and raises the question of how best to retrospectively inform patients whose implants were placed prior to current knowledge of BIA-ALCL.

Disease Reporting

All suspected or confirmed cases of BIA-ALCL should be reported to the Patient Registry and Outcomes For breast Implants and anaplastic large cell Lymphoma (ALCL) etiology and Epidemiology (PROFILE) registry (https://www.thepsf.org/research/registries/profile). PROFILE is a collaboration between the Plastic Surgery Foundation, the American Society of Plastic Surgeons, and the FDA. PROFILE currently acknowledges 871 cases of BIA-ALCL and two cases occurring solely with exposure of a textured-surface tissue expander followed by smooth-only implants. Surgeons should consider the risks and benefits of tissue expander breast reconstruction with a textured vs. smooth expander. For this reason, we strongly suggest

that expansion of the breast pocket should proceed using a smooth surface expander. Echoing this concept, recent data have demonstrated both the safety and efficacy of smooth expander reconstruction.[36]

Insurance Coverage and Diagnostic Codes

Insurance coverage of BIA-ALCL has expanded in recent years. ASPS has provided further guidance on insurance coverage for third-party payers at the following link: https://www.plasticsurgery.org/documents/Health-Policy/Reimbursement/Insurance-2017-BIA-ALCL.pdf. A detailed list of relevant diagnostic and procedural codes is summarized in **Table 2-4**.

Table 2-4. Diagnostic and Procedural Codes for BIA-ALCL

Code	Description	Numeric definition
ICD		
	Anaplastic large cell lymphoma kinase-negative, extranodal, solid organ sites	C84.79
	Unspecified lump in breast, nodule, mass, or swelling of the breast	N63
	Enlarged lymph node	R59.9
	Other specified disorders of the breast	N64.4
CPT		
	Fine needle aspiration with imaging guidance	10022
	Breast biopsy, open, incisional	19101
	Excision of chest wall tumor	19260
	Removal intact mammary implant	19328
	Breast periprosthetic capsulectomy	19371
	Biopsy/excision, lymph node; open or deep axilla	38525

Source: American Society of Plastic Surgeons. ICD, International Classification of Diagnostic Codes-10th Revision; CPT, Current Procedural Terminology Codes.

Defining Research Priorities

Stakeholders, including The Plastic Surgery Foundation and the Aesthetic Surgery Education and Research Foundation, have recently prioritized the funding of projects related to breast implant safety. Due to its uncommon incidence, investigation into the pathogenesis of BIA-ALCL has been limited to *in vitro* and *ex vivo* models. Future basic science research initiatives should focus on the development of an animal model of BIA-ALCL to answer questions about implant texturization, chronic inflammation driving carcinogenesis, and to clarify immune susceptibility profiles. These studies not only expose the complex etiology of the disease but also suggest areas for novel treatment in metastatic disease.

In addition to basic science investigation, several clinically relevant questions remain. Understanding population-based risk profiles will allow plastic surgeons to better inform patients with textured devices about their specific risk. Only then can a complete discussion about prophylactic implant removal occur. Attempts to streamline diagnosis, through point-of-care testing in the office,[29] would improve time to treatment and should be actively explored. While effective treatment strategies exist, plastic surgeons have the opportunity to use BIA-ALCL as a model to uncover scientific truths broadly applicable to all ALK- ALCLs. Thus, research efforts focused on BIA-ALCL have the potential to impact a larger subset of lymphoma patients, not just breast-implant associated lymphomas.

Conclusions

Major advances in the diagnosis and treatment of BIA-ALCL in recent years have led to significant improvements in overall and disease-free survival. Most notably, en bloc resection alone is capable of achieving complete remission for the majority of cases and is now the standard of care. Despite the removal of the high-risk devices from the U.S. and other markets around the world, the number of cases will continue to rise for the foreseeable future. Given that a significant number of patients worldwide still live with these devices, research to understand the etiology of the disease and clarify individual risk profiles must continue. Only through these efforts can plastic surgeons have informed discussions with their patients about BIA-ALCL.

CHAPTER 3. CELLULAR AND MOLECULAR MECHANISMS OF BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA

Abstract

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging and highly treatable cancer of the immune system that can form around textured-surface breast implants. While the underlying etiology has yet to be elucidated, an emerging theme—linking pathogenesis to a chronic inflammatory state–-continues to dominate the current literature. Specifically, the combination of increasing mutation burden and chronic inflammation leads to aberrant T-cell clonal expansion—however the impetus remains largely unknown. Proposed mechanisms include a lipopolysaccharide endotoxin response, oncogenic transformation related to viral infection, associated trauma to the breast pocket, particulate matter digestion by capsular macrophages, chronic allergic inflammation, and genetic susceptibility. The JAK/STAT3 pathway is a major signaling pathway that regulates a variety of intracellular growth and survival processes. Constitutive activation of JAK/STAT3 has been implicated in several malignancies including lymphomas and has recently been identified as a potential key mediator in BIA-ALCL. The purpose of this article is to review the cellular and molecular mechanisms of BIA-ALCL with a focus on the role of oncogenic JAK/STAT3 signaling in BIA-ALCL tumorigenesis and progression. Selected experimental work from our group on aberrant JAK/STAT3 signaling in BIA-ALCL is also included. We will discuss how an inflammatory microenvironment may facilitate malignant transformation through the JAK/STAT3 pathway—highlighting its potential mechanistic role. Our hope is that further investigation of this signaling pathway will reveal avenues for utilizing

JAK/STAT3 signaling as a prognostic indicator and novel therapeutic target in the case of advanced disease.

Background

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging, CD30+, anaplastic lymphoma kinase (ALK)-negative, non-Hodgkin's lymphoma associated with textured-surface breast implants.[1,37–41] Since first being described in the mid to late '90s, over 800 cases have been reported worldwide.[2,42,43] The current average lifetime risk is estimated between 1:355 – 1:30,000 persons with a textured surface breast implant which further varies according to manufacturer specific risks.[3,44] The disease remains equally distributed among cosmetic and reconstructive patients,[3,45] suggesting that a history of previous malignancy (e.g., breast cancer) is not an independent risk factor for the development of BIA-ALCL.

After nearly two decades of research, the molecular mechanisms responsible for aberrant T-cell clonal expansion in BIA-ALCL remain poorly understood.[46] General consensus implicates the induction of a chronic inflammatory state in in a genetically susceptible host that leads to subsequent malignant transformation. The exact cause of the chronic inflammation, whether it is a response to a lipopolysaccharide (LPS) endotoxin,[12] trauma to the breast pocket,[21] viral infection,[17] allergen-driven,[23] or particulate matter digestion from the textured-implant surface remains highly debated and is the focus of our research group and several others around the world.[47–50] Interestingly, recent molecular studies have identified novel, activating mutations in the Janus kinase (JAK),

and signal transducer and activator of transcription factor three (*STAT3*) pathway as a major risk factor for the development of BIA-ALCL.[16,20,51–54]

The JAK/STAT signaling pathway is a major intracellular signaling pathway that regulates a variety of biochemical processes.[55] In humans, there are four members of the JAK family of kinases: JAK 1–3 and Tyrosine Kinase 2. The STAT protein family is comprised of seven members: STAT 1–4, 5a, 5b, and 6. External cues, cytokines, growth factors, and interleukins, bind to JAK receptors located in the cytoplasm and activate STAT via phosphorylation (**Figure 3-1**). Phosphorylated *STAT* receptors dimerize and translocate into the nucleus to regulate genes that are crucial for cancer inflammation in the tumor microenvironment. Interestingly, aberrant *STAT3* signaling has been established as a mechanistic link between chronic inflammation in non-BIA-ALCL cancers, including B and T cell lymphomas, and among the latter systemic anaplastic large cell lymphomas.[18,56–60] Persistent *STAT3* activation has been definitively linked to improved tumor survival and cell proliferation, increased angiogenesis and tumor metastasis. A clearer understanding of the direct link between JAK/STAT signaling and BIA-ALCL is required. This study aims to critically review the cellular and molecular mechanisms of BIA-ALCL with a focus on the current evidence supporting the critical role that JAK/STAT3 plays in the malignant transformation of BIA-ALCL and offers several novel hypotheses for future investigation.

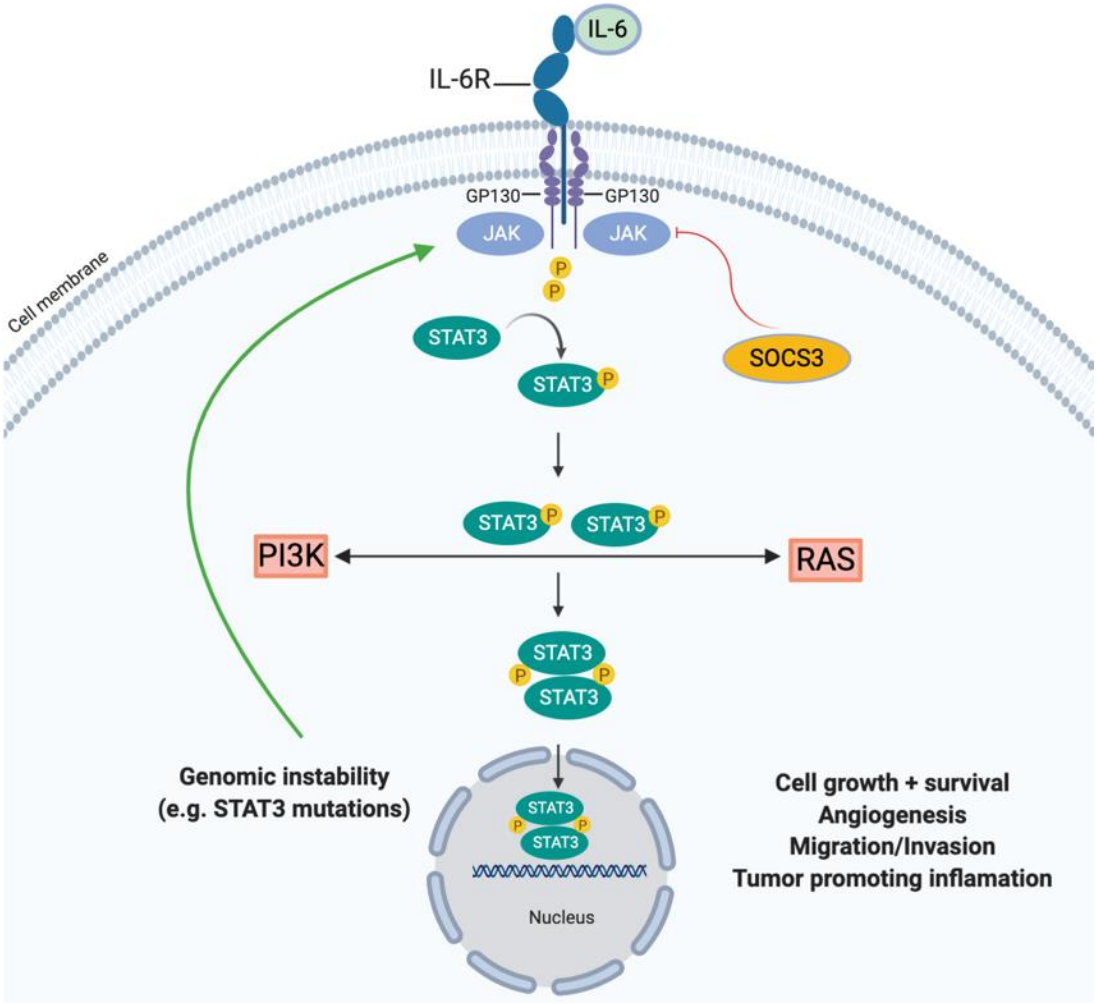


Figure 3-1. Overview of JAK/STAT3 signaling pathway
The binding of an extracellular ligand (e.g. IL-6) to its receptor (e.g. IL-6R), activates JAK via intrinsic tyrosine kinase activity. Activated JAK receptors transfer a phosphate group to the SH2 domain of cytoplasmic STAT3 proteins resulting in STAT3 activation. Phosphorylated STAT3 forms a homodimer that translocates into the nucleus to regulate genes that are critical for tumor promoting inflammation, as well as tumor cell growth and survival, migration and invasion, and angiogenesis. Constitutively activated JAK-STAT3 pathway facilitates genetic instability (e.g. activating STAT3 mutations) and promotes tumorigenesis through a feed forward loop. Suppressor of cytokine signaling three (SOCS3) is an important inhibitor of JAK.

Foreign Body Response to Implantable Devices

Breast augmentation or reconstruction with an implantable device, including tissue expanders or breast implants initiates a complex immunobiologic cascade known as a "foreign body reaction."[61] Briefly, the human body utilizes a coordinated local and systemic immune response to the biologic components of the implant in an attempt to phagocytize and eliminate detected foreign antigens. In instances where phagocytosis of the offending agent is unsuccessful, macrophages and giant cells accumulate and lay down collagen networks to develop a fibrous capsule around the source (**Figure 3-2**). Following biomaterial implantation, host reactions include injury, blood-material interactions, provisional matrix formation, acute inflammation, chronic inflammation, granulation tissue development, foreign body reaction, and fibrous capsule development.[61] Initially, local inflammatory signaling drives non-specific protein adsorption, fibrin-predominate provisional matrix formation and trafficking of immune cells to the site of injury.[62] Neutrophil infiltration and mast cell degranulation causes a local increase in the concentration of interleukin-4 (IL-4) and interleukin-13 (IL-13), cytokines typically associated with a Th2 or allergy-mediated immune response. Recently, Kadin et al identified IL-13 in BIA-ALCL specimens which led them to speculate that BIA-ALCL may occur in response to an allergen, from either the breast implant surface or an LPS endotoxin.[63,64]

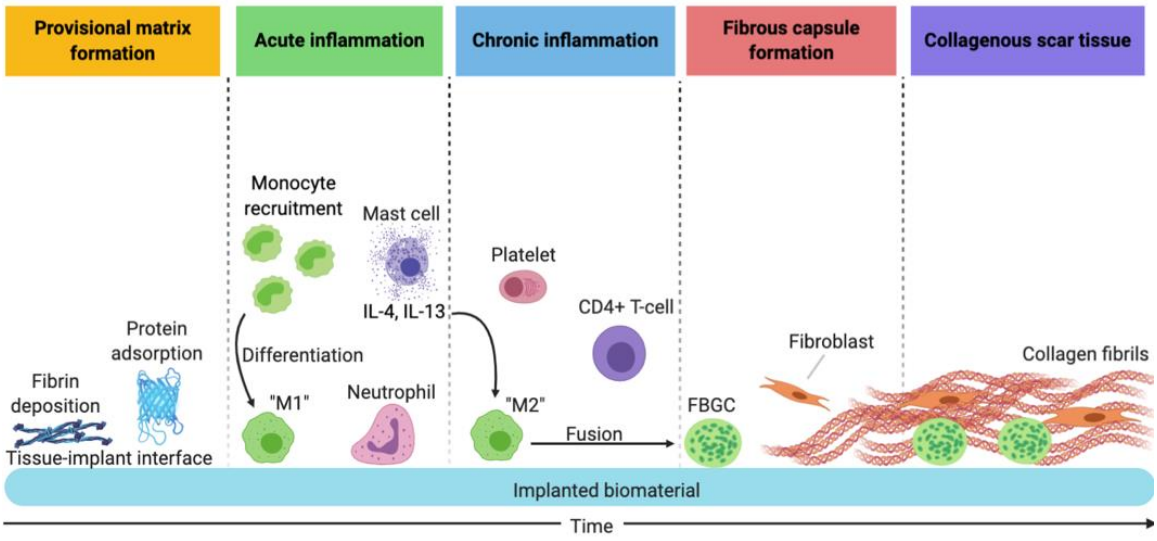


Figure 3-2. Foreign body response to implantable devices
Immediately after implantation, fibrin deposition and non-specific protein adsorption leads to provisional matrix formation at the tissue-implant interface. Following provisional matrix formation, monocytes and neutrophils infiltrate the implant space, characterizing the acute inflammatory process. Monocytes differentiate into proinflammatory M1-macrophages. Simultaneously, mast cell degranulation with histamine release regulates the acute inflammatory response to implantable devices. Interluekin-4 (IL-4) and interleukin-13 (IL-13) also released by mast cells modulates the magnitude of the foreign body response. Following acute inflammation, macrophages and lymphocytes invade the biomaterial interface, marking the beginning of the chronic inflammatory response. IL-4 and IL-13 released from mast cells and Th2 lymphocytes (not shown) activates M2 macrophages that regulate wound healing through generation of a collagen-based extracellular matrix (ECM) in conjunction with fibroblasts. Coalescence of M2-macrophages leads to the formation of foreign body giant cells. Over time, the ECM matures to form the fibrous capsule with a low abundance of immune cells and fibroblasts.

Following recruitment of monocytes/macrophages and other mononuclear cells to the implant site, persistent *frustrated phagocytosis*[65] ultimately leads to coalescence of macrophages into multinucleated giant cells, infiltration of fibroblasts and extracellular matrix protein deposition, followed by formation of the peri-implant fibrous capsule.[61] Nuclear Factor κB (NF-κB), transforming growth factor β (TGF-β) and matrix metalloproteinase (MMP) signaling pathways figure prominently in this transition. At this point, the characteristics of the chronic immune reaction change—the implant is essentially *walled off* from the rest of the body in a protected, immune-privileged, and relatively hypoxic environment—but the chronic immune response to the implant remains. As long as the immune cascade remains activated, the risk of DNA alteration in overstimulated cells increases and chronic stimulation could lead to the activating JAK/STAT mutations in BIA-ALCL. Establishing chronicity in the acquisition of novel genetic mutations in BIA-ALCL remains a nascent area of research but could offer new avenues for diagnosis and treatment of this complex disease.

Implant Texturization and Capsular Morphology

The introduction of implant texturization, a known modifiable risk factor for the development of BIA-ALCL, improved implant stabilization on the chest wall while similarly diminishing the rate of capsular contracture specifically in subglandular augmentation. However, texturization brought unique challenges and complications, including late seromas and a "double" capsule phenomenon not previously identified in smooth textured implant counterparts.[66] Given that BIA-ALCL is thought to arise from the implant capsule, understanding the capsular biology is essential to understanding the

pathogenesis of BIA-ALCL. Histologically, the capsule has relatively low cellularity and consists of sparse inflammatory cells including macrophages and lymphocytes interspersed with thick fibrous bands of collagen. Katzin et al. showed that benign effusions and implant capsules from patients with textured-surface breast implants were T-cell predominant, expressing CD3+ CD4+ CD29+ CD45RO-. CD29 (integrin beta-1) is a cell surface receptor responsible for cellular adhesion and leukocyte homing.[67] They also found that implant-associated lymphocytes were commonly accompanied by silicone laden foamy macrophages, which the authors argued provided strong evidence to support the hypothesis that silicone-laden macrophages act as the antigen presenting cell to CD4+ T-cells, driving the immune response, cytokine release, further T-cell chemotaxis, and cellular trafficking to implanted devices. Katzin et al. also observed that T-cell activation occurred as early as one year after implantation and persisted up to 9 years, well within median time to presentation of BIA-ALCL (8-10 years).[37]

Wolfram et al. characterized the cellular and molecular composition of benign and contracted capsules from patients with silicone breast implants.[68,69] While surface texturization characteristics were not specified, their studies provided additional evidence that silicone breast implants are capable of eliciting a strong Th1/Th17–weighted T-cell immune response, with FoxP3+/CD25+ T regulatory cells (Treg) found within the frontier layer of the fibrous capsule among the population of T effector cells. Of note, Lechner found FoxP3 expression in the TLBR cell lines, whereas Di Napoli et al. found that a proportion of BIA-ALCL showed a FoxP3+/CD25+ phenotype and a significant enrichment in RORC1 and IL-17A transcripts, suggesting that BIA-ALCL tumor cells may retain a phenotypical plasticity between Treg and Th17 cells.

Although the cause of the T-cell activation in BIA-ALCL remains an area of intense focus and debate, these data demonstrate that textured silicone breast implants are capable of generating an early and sustained T-cell response which may be activated by antigen-presenting, silicone-laden capsular macrophages (**Figure 3-3**). Taken together, this may serve as the inciting event that promotes an inflammatory milieu and facilitates malignant transformation.

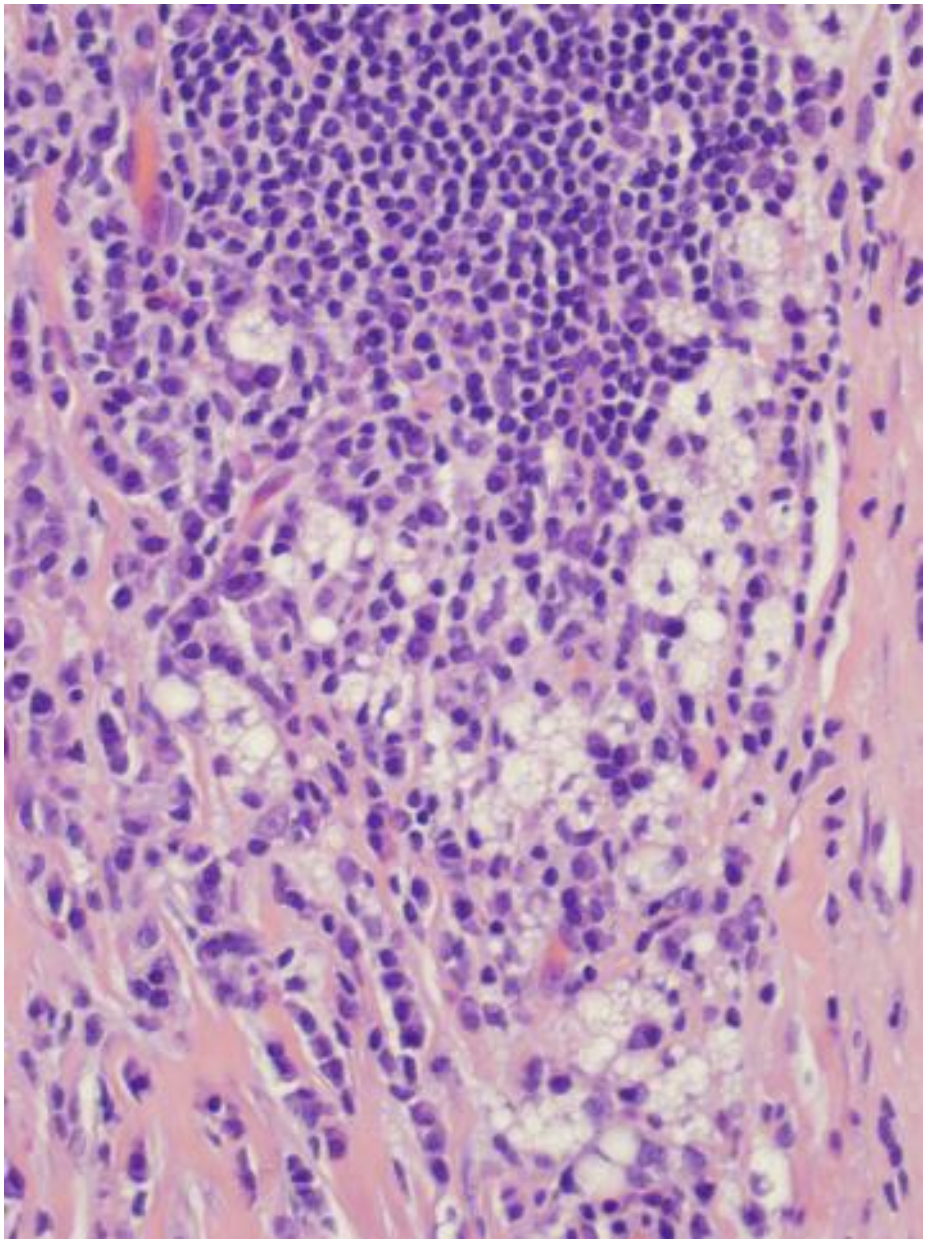


Figure 3-3. Silicone-laden "foamy macrophages" contained within the capsule of a patient with a textured-surface breast implant.

Emerging Theories on Pathogenesis

3.1.1 Biofilm theory

Previous research has implicated an LPS endotoxin from gram negative bacteria inciting malignant transformation as a number of sarcomas and B cell lymphomas originate from cancer-promoting bacterial-associated inflammatory pathways.[70] Specifically, gastric MALT B-cell lymphoma arising from an inflammatory reaction to *H. pylori* as an example. LPS endotoxin leading to BIA-ALCL, however, is an emerging area of investigation. Research from Deva and colleagues has implicated breast implant contamination with the development of capsular contracture[71]—an enhanced fibrotic response to implanted foreign material. These data led Hu et al. to hypothesize BIA-ALCL tumorigenesis may occur as in response to any gram negative bacteria's LPS coat.[12] However, LPS induced T-cell lymphomagenesis has no reported precedence. Early investigations into the microbiome of BIA-ALCL implicated *Ralstonia pickettii*. Interestingly, *Ralstonia pickettii* is a biofilm generator commonly found in water sources, but it also occurs as a common laboratory contaminant, the latter of which has been discussed in detail in the orthopedic literature.[72,73] Walker et al. used 16S rRNA sequencing to test the *Ralstonia* hypothesis and better define the microbiome of BIA-ALCL specimens (n = 8) and benign breast implant capsules from the contralateral breast.[14] Their study failed to replicate the *Ralstonia spp.* data previously described by Hu and colleagues.[12] Walker et al. demonstrated a gram-positive predominance and that BIA-ALCL does not appear to have a distinct microbiome in comparison to normal

capsules. Collectively, these data challenge the notion of a gram-negative shift, and whether that has any role in pathogenesis.

Jacombs et al. studied breast implant surface characteristics and bacterial loads in smooth versus textured-surface implants inoculated with *Staphylococcus epidermidis* and found that texturized devices carried a bacterial load (1.8×10^8 bacteria/g) 20-times greater than smooth (5.75×10^6 bacteria/g) in a porcine model.[74] Intuitively, a greater surface area of a textured implant holds a greater number of bacteria as a mere consequence of higher physical capacity. While this finding highlights an important difference between smooth and textured breast devices, it fails to provide a mechanism by which an increased bacterial load could lead to BIA-ALCL formation. Although evidence for a specific bacterial pathogen remains elusive, that does not preclude involvement of an infectious agent. *STAT3* activation as a result of a bacterial infection has been shown to drive infection-associated cancers.[75] In fact, aberrant *JAK1/STAT3* signaling is highly involved in progression of *H. pylori*-induced gastric cancer.[75] While microbial data are conflicting, future investigations should seek to determine the ability of opportunistic, breast implant-associated pathogens to induce JAK/STAT activation.

BIA-ALCL and JAK/STAT3

3.1.2 BIA-ALCL harbors oncogenic JAK/STAT3 mutations

Brody was among the first to suggest that genetics without a biofilm potentiator may be central to the pathogenesis of BIA-ALCL and theorized that it may also partially explain susceptibility to the disease.[21] To date, oncogenic JAK-STAT3 pathway

mutations have been described in 43.8% of successfully tested cases (**Table 3-1**). In a landmark study, Blombery et al. utilized whole exome sequencing on DNA extracted blood and peri-prosthetic effusions in two patients with pathologically confirmed BIA-ALCL.[52] In the first case, the authors identified a somatic, oncogenic mutation in *STAT3*. The *STAT3* missense variant (p.S614R) leads to increased transcription of *STAT3* which has also been shown in other T-cell and NK cell lymphoproliferative disorders. In the second case, somatic and germline missense variants were identified in *JAK1* (G1079V) and *JAK3* (V772I), respectively. Interestingly, amino acid substitutions in *JAK1* G1079V have been observed in ALK-negative systemic ALCL. The *JAK3* variant has been observed in other peripheral-TCLs and NK cell lymphomas as well; however, the significance of the variant is unclear as it has been shown to occur in the general population at a frequency of 0.5-1% without an associated pathologic phenotype.

Table 3-1. Next-generation sequencing data summary of JAK/STAT3 pathway mutations in breast implant-associated anaplastic large cell lymphomas

Year	Author	Sequencing type	Gene panel size	Utilization of corresponding healthy controls	No. of specimens tested successfully	No. of specimens harboring a JAK/STAT3 pathway mutation
2018	Blombery et al.	Targeted-NGS	180	No	9/9 (disregarding two repeat patients)	7/9 (77.8)
2018	Oishi et al.	Targeted-NGS	5	No	15/15	4/15 (26.7)
2018	Letourneau et al.	Targeted-NGS	26	No	1/1	1/1 (100)
2016	Di Napoli et al.	Targeted-NGS	465	Yes	5/7	1/5 (20)
2016	Blombery et al.	WES	20,000	Yes	2/2	2/2 (100)

JAK/STAT3, Janus kinase and Signal Transducer and Activator of Transcription Factor Three; NGS, Next-Generation Sequencing; WES, Whole Exome Sequencing; No., Number.

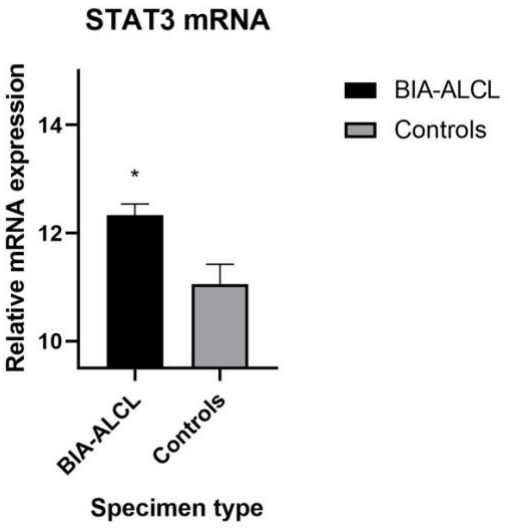
Other investigators have been able to use similar genetic sequencing techniques to independently converge on candidate mutations in JAK/STAT implicated in the development of BIA-ALCL. First, Di Napoli and colleagues utilized targeted-next generation sequencing (NGS) (465-gene panel) on seven BIA-ALCL specimens and identified oncogenic mutations in two separate cases.[53] In the first case, dual oncogenic *STAT3* and suppressor of cytokine signaling 1 (*SOCS1*) mutations were discovered. *SOCS1* is an important negative regulator of the pathway that is capable of directly inhibiting JAK.[76] The observed *SOCS1* mutation occurred as a result of a frame-shift mutation that led to a premature stop codon in *SOCS1* (p.P83Rfs*20). In their second case, Di Napoli described the same missense *STAT3* variant (p.S614R) first described by Blombery and colleagues.[52] In a follow-up study, Blombery et al. also performed targeted-NGS (180-gene panel) on 11 BIA-ALCL specimens.[51] Ten of the 11 cases harbored a JAK-STAT3 pathway genetic variant. Seven out of the 11 cases contained a *STAT3* variant. Two cases with wild-type *STAT3* contained an *SOCS1* or an activating *JAK1* mutation. Oishi et al. used targeted-NGS (5-gene panel) on BIA-ALCL tumor specimens (n = 15).[16] Oncogenic *JAK/STAT3* mutations were found in 26.7% (4/15) of specimens. The same *STAT3* variant (p.S614R) previously described by Blombery and others was found in another case.[52,53,77,78] Oishi et al. also discovered a novel *STAT3* missense variant (Y640F) in two other cases.

While outside the scope of this review, the authors acknowledge that other non-JAK/STAT3 pathway genetic variants in *TP53*[53,79–81] and *DNMT3A*[53] have been described in BIA-ALCL.

3.1.3 BIA-ALCL tumors express STAT3 transcripts and activated STAT3

Although 73.3% of the specimens lacked a JAK/STAT mutation in the study by Oishi et al., 100% of BIA-ALCL specimens tested to date have exhibited activated *STAT3* on immunohistochemistry. Lechner and colleagues developed and characterized the first BIA-ALCL cell lines (TLBR 1-3).[82,83] Immunoblotting revealed activation of *STAT3* across all cell lines, with the greatest activation in the most clinically advanced case. These finding were confirmed in xenograft models showing similar gene expression profiles. Di Napoli et al. investigated the gene-expression profiles of BIA-ALCL ($n = 6$) compared to normal T-cells and other peripheral T-cell lymphomas.[17] Gene set enrichment analysis revealed that similar to systemic ALCL, BIA-ALCL tumor specimens showed activation of *STAT3* signaling and downregulation of the T-cell receptor (TCR) pathway, suggesting the acquisition of an antigen-independent, constitutively activated state. Our group compared the transcriptional profiles of BIA-ALCL tumor specimens using hybridization-based transcriptional profiling and found that *STAT3* was also differentially expressed in BIA-ALCL relative to healthy controls (**Figure 3-4**).

A.



B.

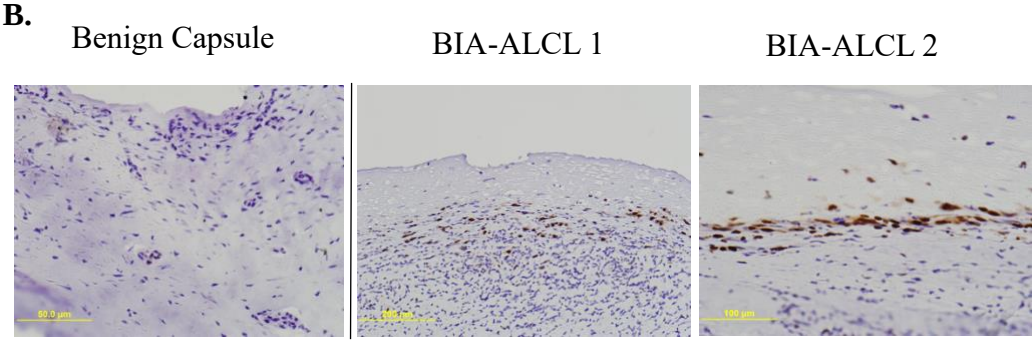


Figure 3-4. Differential STAT3 expression in BIA-ALCL.
(A) Upregulation of STAT3 mRNA expression in BIA-ALCL vs. benign breast implant capsule; * $p < 0.014$ (B) Immunohistochemistry of phosphorylated STAT3 in BIA-ALCL vs. benign breast implant capsules.

3.1.4 STAT3 drives Th1/Th2/Th17 polarization and allergic inflammation

Kadin et al. and Di Napoli et al. suggest that BIA-ALCL tumors likely derive from CD4+ memory activated T-cells with features of Th1/Th17.[84] Importantly, Th1/Th17 cells are antigen-driven memory T-cells that have been implicated in other chronic inflammatory conditions including rheumatoid arthritis and psoriasis, as well as cancer.[85] Kadin and colleagues also reported that BIA-ALCL cells produce the Th2 cytokine IL-13, and variably express the Th2 transcription factor GATA3. These findings support an antigenic stimulant in BIA-ALCL allergic in nature. Interestingly, constitutively active *STAT3* is known to induce a Th17 phenotype, but it is also required for the expression of Th2-associated cytokines and transcription factors and the development of allergic inflammation.[86] Rastogi et al. recently integrated the biofilm hypothesis with JAK/STAT signaling, claiming that a subclinical biofilm infection can elicit chronic inflammation resulting in oncogenic transformation through a modified Th1/Th17 cellular response or through oncogenic mutations in JAK-STAT.[87] The authors of the current paper tend to agree that malignant transformation in BIA-ALCL *must* progress from chronic inflammation, thereby inducing a Th1/Th17 and Th2 response which facilitates aberrant T-cell clonal expansion (**Figure 3-5**).

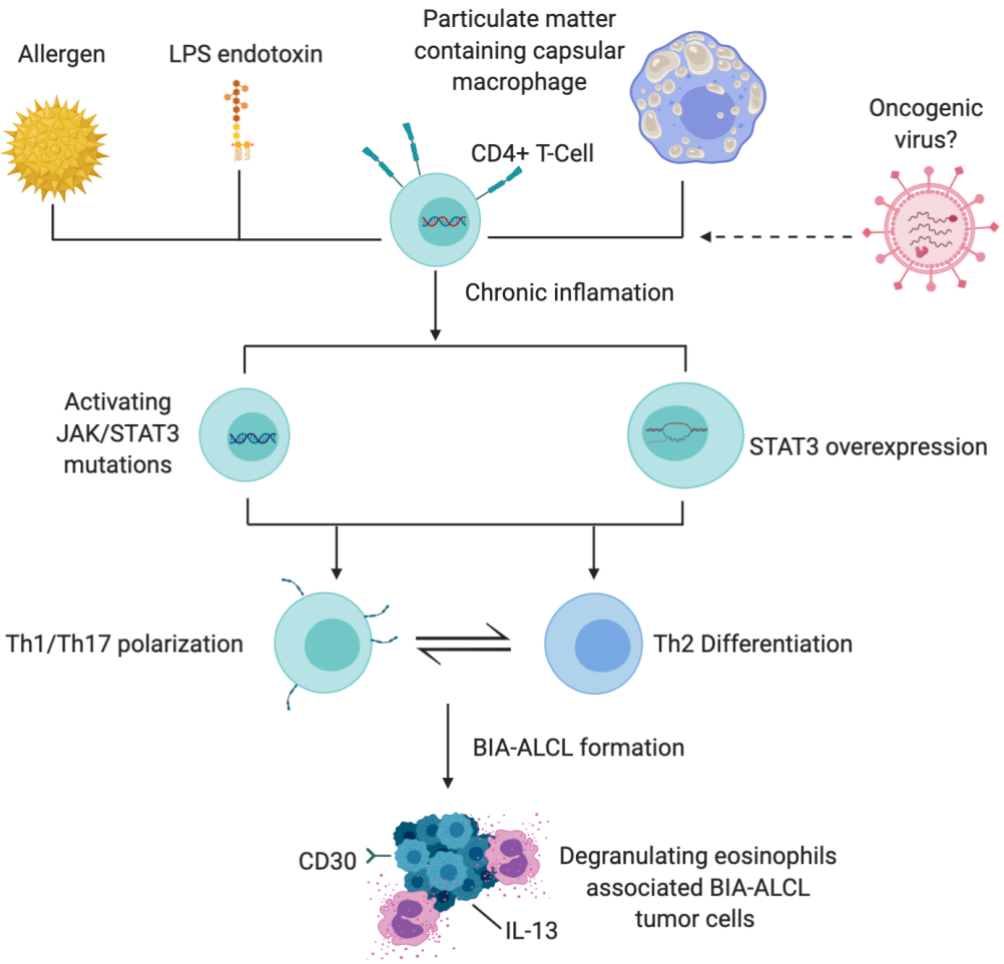


Figure 3-5. Proposed mechanisms of BIA-ALCL tumorigenesis.
Interaction of an allergen, LPS endotoxin, particulate matter, or possibly on oncogenic virus through an antigen presenting cell interacting with a naive CD4+ T-cell leads to a chronic inflammatory state. The chronic immune response results in aberrant STAT3 signaling that may or may not facilitate activating STAT3 mutations in a feed forward loop. An overabundance of STAT3 promotes the differentiation of Th1/Th17 as well as Th2 lymphocyte phenotypes, ultimately leading to unregulated T-cell clonal expansion and BIA-ALCL formation.

3.1.5 IL-6/JAK/STAT3 signaling axis in BIA-ALCL

The IL-6/JAK/STAT3 signaling axis may explain pathogenesis in cases without a driver gene mutation. As previously mentioned, IL-6 is the main cytokine activator of the JAK/STAT3 pathway. While Lechner et al. showed that BIA-ALCL tumors highly expressed IL-6, neutralization experiments failed to inhibit tumor proliferation (unpublished data).[82] The authors speculated that this may have occurred as a result of high levels of IL-6 produced by tumor cells. Chen et al. interrogated cell lines from several ALK-negative lymphoma subtypes including BIA-ALCL (TLBR 1/2).[88] Abrogation of GP130, a subunit of IL-6R, induced tumor cell death even in the presence of activating *JAK1*/*STAT3* mutations in all cell lines except for BIA-ALCL. Despite high levels of IL-6 and IL-6R expression, TLBR 1/2 cell lines remained viable. This suggests that while cytokine receptor signaling is critical for most ALK-negative lymphomas, BIA-ALCL may have other independent mechanisms for stimulating JAK/STAT3 expression, perhaps through growth factor mediated signaling or underlying genetic mutations.

3.1.6 JAK/STAT inhibition induces tumor cell death

Lechner et al. showed that TLBR cell lines are subject to JAK-STAT inhibition.[82] When treated *in vivo* with sunitinib, a JAK-STAT inhibitor, tumor cell death was induced in a dose-dependent manner. Although more recent evidence-based treatment recommendations call for complete surgical excision and implant removal in most cases, JAK-STAT may serve as a novel therapeutic target in cases of advanced disease.[30–32,89] A list of potential therapies targeting the JAK/STAT3 pathway is summarized in **Table 3-2**.

Table 3-2. Potential therapies targeting IL-6/JAK/STAT3 pathway in BIA-ALCL

Drug Name	FDA Status	Indication	Mechanism of Action	Notes
JAK Inhibitors				
Ruxolitinib	FDA-approved	Primary myelofibrosis, Polycythemia vera, GVHD	JAK 1,2 inhibition	Trials for HNSCC and BC
Tofacitinib	FDA-approved	RA	JAK 3 inhibition	Studied in IBD and psoriasis, increase risk of lymphoma
Baricitinib	FDA-approved	Moderate to severe RA	JAK 1,2 inhibition	
Upadacitinib	FDA-approved	Moderate to severe RA unresponsive to MTX	JAK 1 inhibition	
Fedratinib	FDA-approved	Primary or secondary myelofibrosis	JAK 2 inhibition	Black box warning for serious encephalopathy
STAT3 Inhibitors				
Atovaquone	FDA-approved	Antimicrobial for malaria, toxoplasmosis, PCP	Downregulates GP130	
Pyrimethamine	FDA-approved	Antimicrobial for malaria and toxoplasmosis	Competitively inhibits DHF-reductase	Attenuated breast cancer tumor growth in murine model, ongoing phase 2 trial for CLL and SLL
Cetuximab	FDA-approved	HNSCC, NSCLC, colorectal cancer	Monoclonal antibody against EGFR	
Pimozide	FDA-approved	Antipsychotic	Dopamine antagonist	Anticancer effects on osteosarcoma, leukemia, breast cancer, melanoma
TTI-101	Phase 1 trial	BC, HNSCC, NSCLC, HCC, CRC, Gastric cancer, Melanoma	Direct STAT3 inhibitor	
IMX-110	Phase 2a trial	Solid tumors, Pancreatic cancer, Breast cancer, Ovarian cancer	Nanoparticle encapsulating STAT3 inhibitor and low-dose doxorubicin	
IL-6 Inhibitors				
Bazedoxifene	FDA-approved	Osteoporosis prevention	SERM, IL-6 and IL-11 inhibitor	Synergistic effect with temsirolimus in treatment of osteosarcoma
Tocilizumab	FDA-approved	RA, Juvenile idiopathic arthritis, Giant cell arteritis, Cytokine release syndrome	Humanized monoclonal antibody to IL-6R	Studied in ankylosing spondylitis and systemic lupus erythematosus
Siltuximab	FDA-approved	Castleman's disease	Chimeric monoclonal antibody to IL-6	Phase 2 studies for prostate and ovarian cancer
Sarilumab	FDA-approved	RA	Human monoclonal antibody to IL-6R	
Other				

Brentuximab vedotin	FDA-approved	ALCL, Hodgkin lymphoma, mycosis fungoides	Chimeric monoclonal antibody to CD30	Antibody-drug conjugate that delivers MMAE to CD30+ cells
Crizotinib	FDA-approved	NSCLC	Inhibits ALK	Multiple clinical trials for ALCL and advanced solid tumors

IL-6, Interleukin six; IL-11, Interleukin eleven; BC, breast cancer; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung carcinoma; SCC, squamous cell cancer; HCC, hepatocellular carcinoma; CRC, colorectal cancer, IL-6R, IL-6 receptor; RA, Rheumatoid Arthritis; SERM, selective estrogen receptor modulator, PCP, pneumocystis carinii pneumonia; MMAE, monomethyl auristatin E.

Conclusions

Recent molecular studies have expanded the concept that aberrant JAK/STAT3 signaling may be a critical component in BIA-ALCL tumorigenesis and progression and may provide a novel therapeutic target for select patients. As such, larger, comprehensive oncogenomic studies are needed to better define the genetic landscape of BIA-ALCL, the frequency at which JAK/STAT3 pathway mutations occur, and their functional significance.

CHAPTER 4. BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: AN EVIDENCE-BASED SYSTEMATIC REVIEW

Abstract

Objective: The authors introduce breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) as a relevant, emerging disease through the epidemiology, pathophysiology, and clinical outcomes that have come to inform the current federal regulatory climate surrounding textured breast devices. This evidence-based systematic review synthesizes and critically appraises current clinical recommendations and advances in the diagnosis and treatment of BIA-ALCL. This review also aims to broaden physician awareness across diverse specialties, particularly among general practitioners, breast surgeons, surgical oncologists, and other clinicians who may encounter patients with breast implants in their practice.

Background: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging and treatable immune cell cancer definitively linked to textured-surface breast implants. Although National Comprehensive Cancer Network (NCCN) consensus guidelines and other clinical recommendations have been established, the evidence supporting these guidelines has not been systematically studied. The purpose of this evidence-based systematic review is to synthesize and critically appraise current clinical guidelines and recommendations while highlighting advances in diagnosis and treatment and raising awareness for this emerging disease. **Methods**: This evidence-based systematic review evaluated primary research studies focusing on the diagnosis and treatment of BIA-ALCL that were published in PubMed, Google Scholar, and other scientific databases through March 2020. **Results and Conclusions:** The clinical

knowledge of BIA-ALCL has evolved rapidly over the last several years with significant advances in diagnosis and treatment, including en bloc resection as the standard of care. Despite a limited number of high-quality clinical studies comprised mainly of Level III and Level V evidence, current evidence aligns with established NCCN consensus guidelines. When diagnosed and treated in accordance with NCCN guidelines, BIA-ALCL carries an excellent prognosis.

Background

Breast implants are used extensively in the United States and throughout the world for breast augmentation and breast reconstruction. Textured-surface breast implants, a common type of breast implant, have been linked to breast implant-associated anaplastic large cell lymphoma (BIA-ALCL), an emerging non-Hodgkin type T-cell lymphoma.[90] While BIA-ALCL shares morphologic and immunophenotypic characteristics similar to other anaplastic large cell lymphomas, specifically anaplastic lymphoma kinase-negative anaplastic large cell lymphoma (ALK- ALCL), its presentation, diagnosis, and clinical course represent a novel clinical entity with unique challenges for medical practitioners.

Since first being described in the mid to late '90s,[2,47,91,92] over 800 cases have been confirmed worldwide.[42] The majority of cases present with an acute onset, unilateral periprosthetic effusion, and follow an indolent clinical course when diagnosed and treated promptly.[37] When practitioners misdiagnose, fail to diagnose, or do not adhere to clinical guidelines, disseminated disease and death have resulted.[93] Reported cases of BIA-ALCL stratify equally between cosmetic and reconstructive patients, suggesting that history of a

previous malignancy, such as breast cancer, is not an independent risk factor for the development of the disease. However, reports of implant-associated blood cancers continue to surface following reconstructive or cosmetic surgeries with textured devices,[8,94] implicating textured implants in the pathogenesis of this rare disease, while similarly raising concerns about the long-term safety of textured devices.[35,95] Despite some of these concerns, Tandon et al. found that the use of textured breast implants for cosmetic indications is increasing.[96] In 2017, approximately 70,000 textured breast implants were placed in the U.S., accounting for 12.5% of the total market share.[97] In contrast, textured breast implants accounted for nearly 90% of device preference throughout Europe and Australia.[6] As such, there are currently millions of women worldwide with textured-surface breast implants, which poses a significant health risk for patients exposed to this type of device.

In 2011, the U.S. Food and Drug Administration (FDA) issued a safety communication about the possible association between breast implants and BIA-ALCL.[98] Shortly thereafter, the World Health Organization provisionally classified BIA-ALCL as a distinctly challenging clinical entity.[25] Out of that concern, nearly forty different countries have banned the use of Allergan Biocell (Dublin, Ireland) textured-surface breast implants, and France has banned the use of macrotextured devices altogether.[99] Following worldwide bans, the U.S. FDA called for a Class 1 device recall. Subsequently, Allergan issued a voluntary, worldwide recall of their textured-surface breast implants and textured-surface tissue expanders.[44,100] Allergan's "salt-loss" manufacturing technique creates an exceptionally coarse macrotextured surface that maximizes tissue ingrowth in order to maintain breast pocket stability and improve

aesthetic outcomes. However, this same process has come under scientific scrutiny, as Allergan carries the highest manufacturer-specific risk (1:355 - 2,207 patients) for the development of BIA-ALCL.[4,101] Other device companies employ different texturing techniques that result in less rugged surfaces, including the Mentor corporation (Irvine, CA), which have allowed textured breast devices to remain commercially available in the U.S., despite their association with BIA-ALCL. Mentor specifically uses a negative-imprint stamping technique that carries significantly lower risk estimates (1:86,029 implants; 95% CI: 15,440 – 1,301,759) for the development of lymphoma in the Australia-New Zealand cohort which translates to an increased risk of 27.1:1 for Allergan Biocell implants compared to Mentor Siltex implants.[3] At this time, considerable clinical debate exists over the best course of action to both identify at-risk individuals with textured devices and adequately protect these patients from disease development while further preventing all future cases of BIA-ALCL. Despite recognition as a distinct clinical entity, BIA-ALCL remains underdiagnosed given its subtle clinical presentation and lack of physician awareness of the disease.

Evidence-based medicine is an applied methodology that utilizes the best, currently available evidence to guide clinical decision-making and care of individual patients in order to optimize patient outcomes. Although consensus guidelines and clinical recommendations have been put forth regarding diagnosis and treatment, the evidence supporting those recommendations has not been systematically studied. The purpose of this evidence-based systematic review is to detail and critically evaluate current practice recommendations for the effective diagnosis and management of BIA-ALCL in order to improve missed or misdiagnoses, increase reporting of affected

individuals, and to determine if current treatment guidelines are supported by high-quality evidence. This study also aims to increase physician awareness of this emerging disease, particularly among breast surgeons, surgical oncologists, and other clinicians who may encounter patients with breast implants in their practice.

Methods

4.1.1 Search strategy

A systematic review of PubMed (MEDLINE), EMBASE, Google Scholar, Web of Science, the Cochrane library, and the grey literature was conducted between March 1-15, 2020. The following search terms and Medical Subject Headings (MeSH) were used in combinations with Boolean operators: *breast implant associated-anaplastic large cell lymphoma, breast implant, breast implants, lymphoma, treatment,* and *diagnosis.*

4.1.2 Inclusion and exclusion criteria

Study inclusion criteria consisted of patient-oriented primary research related to the diagnosis and treatment of BIA-ALCL. Review articles were included on a case-by-case basis dependent on the ability to provided novel insights, including advancements or changes in diagnosis and treatment not discussed in a primary article. Editorials, discussions, and case reports were excluded. Citation chaining was performed on articles that met inclusion criteria using Web of Science. Two independent reviewers screened (R.C.D., M.W.C.) titles, abstracts, and the text of identified articles. Disagreement between reviewers was handled through discussion until there was 100% agreement. Only articles in the English language were reviewed. The search strategy was designed to

capture articles focused on the diagnosis and treatment of BIA-ALCL. The list of references was reviewed for relevant studies, and no additional articles were discovered as a result. Each study was assessed for potential sources of bias. Levels of evidence were assigned, and articles related to current treatment recommendations (e.g., en bloc resection, chemotherapy, radiation therapy, breast reconstruction) were ranked using the American Society of Plastic Surgeons (ASPS) Evidence-Based Rating Scales for Therapeutic Studies (**Table 4-1**). This systematic review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Table 4-1. American Society of Plastic Surgeons Evidence-Based Rating Scale for Therapeutic Studies

Level of Evidence	Description
I	High-quality, multi-centered or single-centered, randomized controlled trial with adequate power; or systematic review of these studies
II	Lesser-quality, randomized controlled trial; prospective cohort or comparative study; or systematic review of these
III	Retrospective cohort or comparative study; case-control study; or systematic review of these studies
IV	Case series with pre-/post-test; or only post-test
V	Expert opinion developed via consensus process; case report or clinical example; or evidence based on physiology, bench research or “first principles”

Results

An overview of the search strategy is provided in **Figure 4-1**. The initial search yielded 511 articles. No other articles were identified from other sources. After removing duplicates found in the search (*n* = 3), 508 articles remained. Titles and abstracts were reviewed (*n* = 508) for relevance, and as a result, 501 articles were excluded on the basis of study design and lack of primary evidence related to diagnosis or treatment. The remaining articles (*n* = 7) were reviewed in their entirety and met inclusion criteria (**Table 4-2**). Studies were comprised of level III (*n* = 3) and level V (*n* = 4) evidence that focused on the diagnosis and treatment of BIA-ALCL. The limited number of available studies and heterogeneity in reported data precluded any meta-analysis.

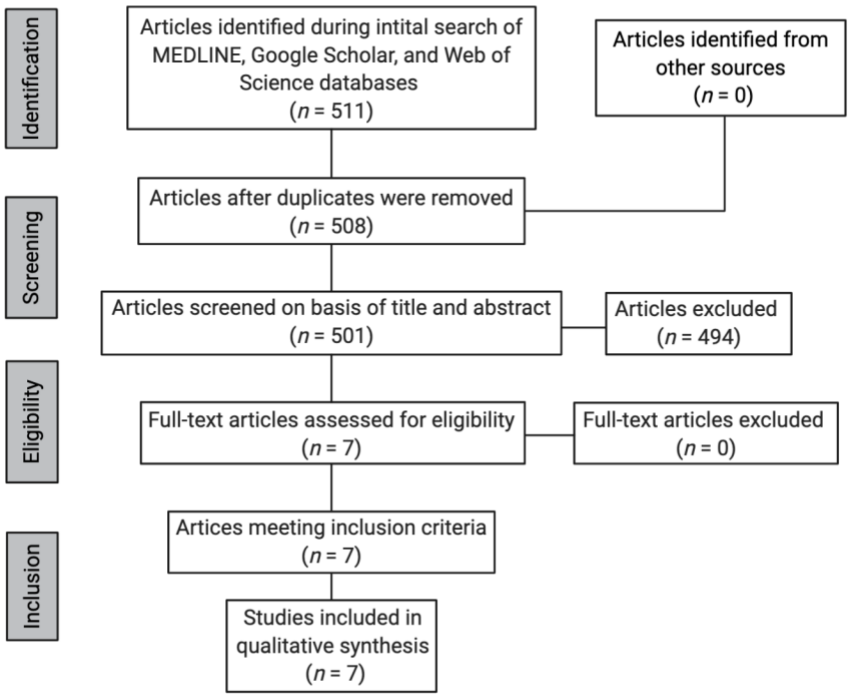


Figure 4-1. Schematic of search strategy

Table 4-2. Cohort Studies and Consensus Guidelines of Breast Implant-Associated Anaplastic Large Cell Lymphoma

Authors	Reference	Year	Study design	Focus of article	Level of evidence
Clemens et al	Complete Surgical Excision Is Essential for the Management of Patients with Breast Implant–Associated Anaplastic Large-Cell Lymphoma	2016	Retrospective	Surgical Resection/ Adjuvant Therapy	III
Tevis et al	Stepwise En Bloc Resection of Breast Implant-Associated Anaplastic Large Cell Lymphoma with Oncologic Considerations	2019	Retrospective cohort	Surgical Resection	III
Lamaris et al	Breast Reconstruction Following Breast Implant-Associated Anaplastic Large Cell Lymphoma	2019	Retrospective cohort	Breast Reconstruction	III
Clemens et al	How to Diagnose and Treat Breast Implant Associated Anaplastic Large Cell Lymphoma	2018	CME	Diagnosis and Treatment	V
Mehta-Shah et al	How I Treat Breast Implant Associated Anaplastic Large Cell Lymphoma	2018	Review	Diagnosis and Treatment	V
Clemens et al	2019 NCCN Consensus Guidelines on the Diagnosis and Treatment of Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL)	2019	Expert Consensus	Diagnosis and Treatment	V
Clemens et al	Finding Consensus After Two Decades of Breast Implant-Associated Anaplastic Large Cell Lymphoma	2019	Review	Diagnosis and Treatment	V

Epidemiology

Historically, rare diseases present epidemiological challenges for investigators; precisely estimating the true incidence of disease remains an elusive task. With respect to BIA-ALCL, existing epidemiological studies are limited by a lack of global reporting and incomplete breast implant sales data, making it similarly difficult to quantify an accurate risk assessment.[39] The current lifetime risks associated with the development of BIA-ALCL vary significantly according to geography and are also manufacturer specific.[102] The Australian Therapeutic Goods Administration estimates a lifetime risk of 1:2,500 - 1:25,000 patients with a textured breast implant.[45] More recent work by Doren et al. estimates an average lifetime prevalence across manufacturers of 1:30,000 patients with a textured breast implant in the U.S.[3] Interestingly, the authors' reported a nearly six-fold increase in the lifetime prevalence of BIA-ALCL cases attributable to Allergan textured devices compared to textured devices from other manufacturers. These data were later cited by the FDA as partial reasoning for issuing the Class 1 recall.[103] Allergan's unique manufacturing process highlights the texturing process as a critical regulator of disease pathogenesis. As such, investigators have focused on understanding the innate and adaptive immune response to implanted devices in hopes that their efforts will yield a clearer understanding of the cellular and molecular mechanisms driving disease development.

Pathophysiology

BIA-ALCL is a subset of systemic anaplastic large cell lymphomas (sALCL), which are a class of non-Hodgkin type peripheral T-cell lymphomas. Investigators

stratify sALCLs by cellular and molecular markers that carry either favorable or less favorable clinical outcomes. The presence of anaplastic lymphoma kinase (ALK^+) occurs in 60-80% of sALCLs and carries a favorable 5-year progression-free survival. The other 20-40% of ALK^- sALCLs are characterized by specific gene rearrangements—*Dusp22*, *TP63*, or *Triple Negative* (ALK^-, *Dusp22*-, *TP63*-) and carry an overall survival rate of ≤ 50%.[104] BIA-ALCL cells isolated from patients are classified as *Triple Negative* ALCLs.[16] Although, reports exist of diffuse large B-cell lymphomas, marginal zone B-cell lymphoma, and plasmacytomas occurring adjacent to textured-surface breast implants, suggesting that the disease may have a broad spectrum of genotypic and phenotypic variations.[105,106] BIA-ALCL cells also carry the CD30 cell surface marker that traditionally marks activated B- and T-cells. Therefore, BIA-ALCL cells are pathologically classified as $CD30^+$, ALK^- lymphoma cells.

After two decades of investigation, the biological basis of the disease remains poorly understood.[19] Current evidence suggests BIA-ALCL arises from a novel antigenic stimulus that induces a chronic inflammatory state.[1,11] Consistent exposure to inflammatory cytokines in a genetically susceptible individual ultimately leads to unregulated immune-cell clonal expansion and lymphomagenesis. However, the specific antigenic stimulus remains a controversial topic and is the focus of our laboratory and others. Early investigations identified a gram-negative bacillus, *Ralstonia pickettii,* in establishing a subclinical, periprosthetic biofilm, leading to a lipopolysaccharide (LPS) endotoxin-induced carcinogenesis.[12] After a more careful examination, the Ralstonia data have since been refuted, and currently, no clear association between the breast microbiome and BIA-ALCL pathogenesis exists.[14] Other investigators have focused on

allergen-driven carcinogenesis, either from particulate matter from the operating suite landing on implant surface or aberrant activation of the aryl hydrocarbon receptor by the contaminants residing on the implant surface itself.[22,63,64] Genetics, in combination with other factors, is also thought to be a major risk factor for the disease,[20,107] with oncogenic mutations in *TP53*,[53,79,80,108] *DNMT3A*[53], and the JAK-STAT3 pathway being described.[16,51–54] Other proposed oncogenic drivers may include viruses or chronic trauma to the breast pocket.[17,21] Nevertheless, evidence to support a unifying theory has remained elusive, and the complex interplay between these factors remains largely unknown.

Natural History and Spectrum of Disease

Early reports suggested two distinct histologic subtypes of BIA-ALCL, *in situ* disease and *infiltrative* disease, each of which carried a significantly different prognosis. Over time, the knowledge of the disease has evolved to encompass a spectrum of disease that spans multiple diverse disease environments, including effusion-limited disease, superficial capsular involvement, a grossly identifiable lesion, lymph node extension, and finally, distant metastasis. *In situ* or effusion-limited disease is confined within the breast implant capsule and is characterized by a lymphomatous cell layer on the luminal capsular surface with or without suspension of anaplastic lymphoma cells in the serous fluid. The infiltrative subtype extends into or beyond the fibrous capsule and may be associated with locoregional or distant metastasis. The infiltrative subtype carries an inferior prognosis.

Clinical Presentation

The majority (80%) of patients present with a spontaneous delayed seroma formation (greater than 1-year following implantation) but can also present with lymphadenopathy (4 -12%) or a palpable mass (8 – 24%). Less frequently (< 5%), the disease may present with local or systemic symptoms, including fever, capsular contracture, or cutaneous manifestations. The median interval time to presentation is 7-10 years (range 1-28 years) following textured device implantation for breast augmentation or reconstruction. Left untreated, scant $CD30^{+}$, ALK^{-} cells contained within the seroma fluid may coalesce and acquire characteristics of solid tumors[26,40]—including distant metastasis—underscoring the importance of early diagnosis and intervention.

Diagnosis

The National Comprehensive Cancer Network (NCCN) guidelines for the diagnosis and treatment of BIA-ALCL were established based on the current understanding of the literature. In the subsequent paragraphs, we will discuss and critically appraise the clinical data that coalesced to form these essential guidelines while highlighting advances in diagnostic and therapeutic strategies and addressing current controversies not covered in NCCN guidelines.

4.1.3 Differential diagnosis and diagnostic work-up

Generally, BIA-ALCL follows an indolent clinical course and has an excellent prognosis when diagnosed and treated promptly. A proposed diagnostic algorithm is outlined in **Figure 4-2**. Briefly, suspicious seromas should be drained using ultrasound-

guided fine-needle aspiration or in consultation with interventional radiology. It is important to note that the peri-implant space around most implants contains only a trace amount (5-10 mL) of fluid. Thus, an independent finding in an otherwise asymptomatic patient does not warrant further investigation. After excluding other differential diagnoses of delayed seroma (e.g., infection, isolated trauma to the chest wall), aspirate (minimum 50 mL) should be sent for cytopathology with the request to "rule out BIA-ALCL." A BIA-ALCL rule out requires three specific areas of investigation— $CD30^+$ cells by immunohistochemistry, cellular atypia as assessed with microscopy and flow cytometry to assess for T-cell clonality.[32,89,109,110] Positive samples must typically satisfy all of the three requirements: $CD30^+$ cells in the aspirate; noted cellular atypia; and T-cell clonality (**Figure 4-3**).

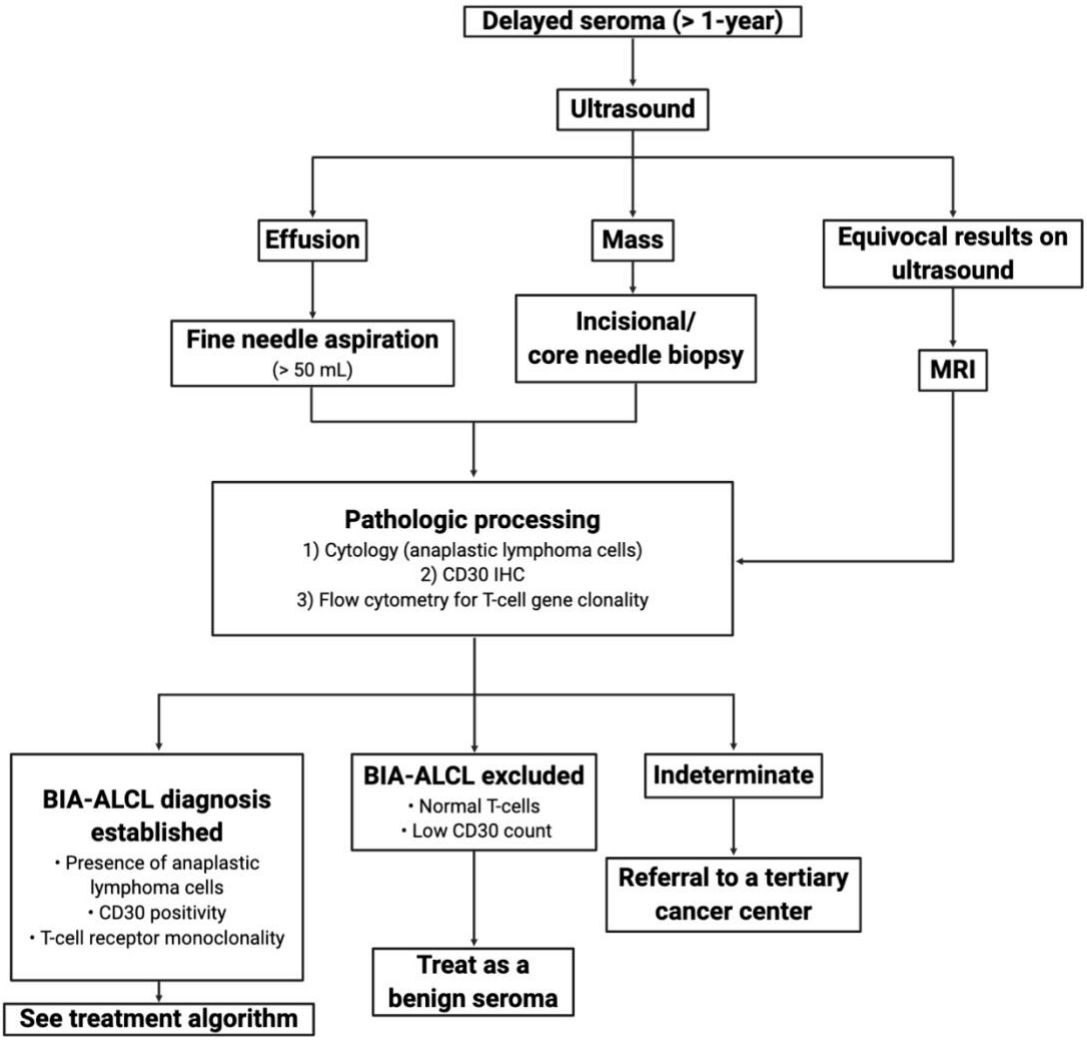


Figure 4-2. Evidence-based diagnostic algorithm for BIA-ALCL.

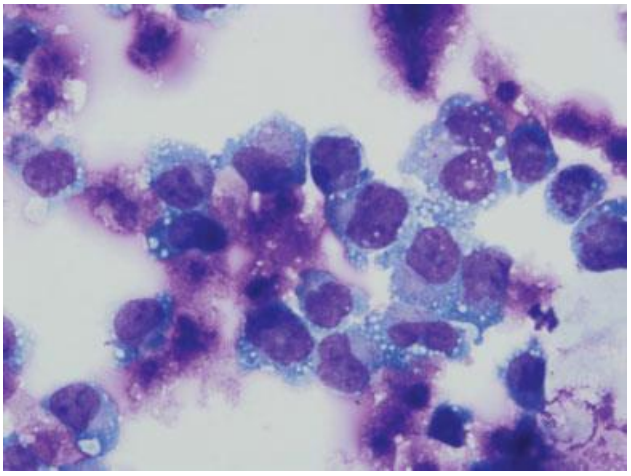


Figure 4-3. BIA-ALCL Lymphoma Cells
A malignant effusion in a BIA-ALCL patient demonstrates large pleomorphic anaplastic cells with prominent horseshoe-shaped nuclei and nuclear folding. (hematoxylin stain, 500X magnification) Positive anaplastic cytology, CD30 immunohistochemistry expression, and single T cell clonality demonstrated on flow cytometry are required for BIA-ALCL diagnosis. Reprinted with permission by Clemens, MW, DeCoster, RC, Fairchild, et al., 2019. Finding Consensus After Two Decades of Breast Implant-Associated Anaplastic Large Cell Lymphoma *Semin Plast Surg.* 33(4):270-278, Thieme Medical Publishers, Georg Thieme Verlag KG.

While CD30 expression is a fundamental diagnostic element of BIA-ALCL, isolated expression is not pathognomonic for establishing the diagnosis, as CD30 is also expressed on other immune cells, including activated T- and B-cells, eosinophils, and macrophages. Thus, $CD30^+$ lymphocytes with otherwise normal morphology do not require further investigation. Histologic experiments to assess cellular atypia focuses on identifying cells with anaplastic features—pleomorphic nuclei, either heterochromic or hyperlobulated, and abundant cytoplasm presenting in dense cellular sheets. These cells are often "hallmark" cells of ALCL. T-cell clonality suggests T-cell receptor (TCR) gene rearrangement in response to a single antigenic stimulus. Thus, if a single peak appears in $CD30^+$ flow cytometry, further investigation is warranted. As referenced earlier, the combination of these three characteristics, $CD30^+$ cells, exhibiting cellular atypia, and TCR clonality, is highly suspicious for BIA-ALCL and should prompt clinical intervention.

4.1.4 Diagnostic imaging

Ultrasound remains the imaging modality of choice for detecting a BIA-ALCL related effusion or mass. Adrada et al. found that ultrasound conveys an 84% sensitivity and a 75% specificity for detecting an effusion and is 46% sensitive and 100% specific for detecting a mass.[111] Equivocal results on ultrasound should be further investigated with magnetic resonance imaging. The role of positron emission tomography-computed tomography (PET-CT) for preoperative workup and tumor surveillance is discussed in further detail below (see oncologic surveillance).

4.1.5 Pathologic processing of specimens

In a proposed update to the College of American Pathologist policy on surgical specimen collection, Lyapichev et al. recently developed a standardized protocol for handling and processing BIA-ALCL tumor specimens.[28] Using mathematical modeling, the authors formulated an equation [minimum number of samples = 3.6 + 106.8/ (coverage%)] that can be used to determine the minimum number of sections required to identify 95% of randomly distributed lesions in patients that do not have grossly identifiable lesions. The formula translates into a requirement of 12 biopsies per capsule, two for each side of the face of a cube. A more standardized protocol for the handling, sampling, and reporting of BIA-ALCL cases will continue to improve *diagnostic* accuracy and advance the collective understanding of the mechanisms underpinning this complex disease by providing more generalizability and statistical power to future studies.

4.1.6 Pathologic staging and prognosis

Although the Lugano modification (Ann Arbor staging system) has traditionally been used to stage non-Hodgkin lymphomas, BIA-ALCL displays behaviors most similar to solid tumors. Clemens et al. demonstrated that the TNM staging system more accurately predicted overall survival and recurrence of BIA-ALCL than the Ann Arbor staging system ($p = 0.01$).[30] The TNM staging system for BIA-ALCL is summarized in **Table 4-3**. Furthermore, Clemens et al. demonstrated a 91% five-year overall survival rate and a five-year event-free survival rate of 49%.[30] As previously mentioned, overall and event-free survival increase with the use of complete surgical excision when

compared to other treatment modalities ($p < 0.001$). Additionally, when comparing prognosis according to stage, patients receiving complete surgical resection had an event rate of 0% for stages T1, T2, and 14% at stage T4 ($p < 0.001$). Taken together, these data strongly suggest that en bloc resection combined with early detection yields a better early-term prognosis accompanied by a substantial survival benefit.

Table 4-3. TNM Staging System for BIA-ALCL

TNM/Stage Classification	Description
Primary tumor (T)	
T1	Confined to effusion or a layer on luminal side of capsule
T2	Early capsule infiltration
T3	Cell aggregates or sheets infiltrating the capsule
T4	Lymphoma infiltrates beyond the capsule
Regional lymph nodes (N)	
N0	No lymph node involvement
N1	One regional lymph node (+)
N2	Multiple regional lymph nodes (+)
Distant metastasis (M)	
M0	No distant spread
M1	Spread to other organs/sites
Stage	
1A	T1N0M0
1B	T2N0M0
1C	T3N0M0
IIA	T4N0M0
IIB	T1-3N1M0
III	T4N1M0
IV	T (any) N (any) M1

Treatment

Due to the emerging nature of this complex disease, a multidisciplinary team of plastic surgeons, surgical oncologists, and pathologists should be assembled following a definitive diagnosis of BIA-ALCL. The subsequent sections outline in detail evidence-based treatment strategies for achieving complete resolution. An overview of the treatment algorithm is provided in **Figure 4-4.**

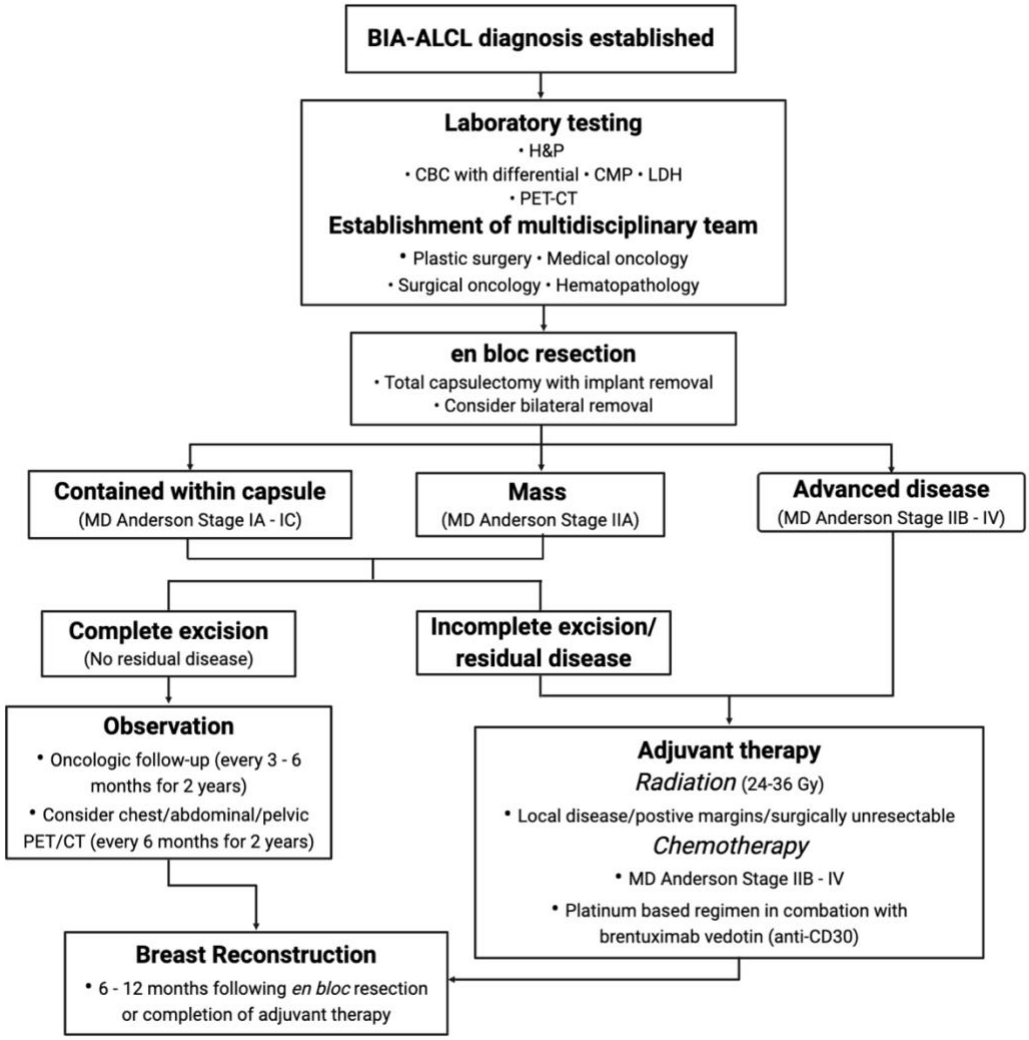


Figure 4-4. Evidence-based treatment algorithm for BIA-ALCL

4.1.7 Surgical management

4.1.7.1 Preoperative workup

Following the establishment of a BIA-ALCL diagnosis, a team of multidisciplinary experts consisting of a medical oncologist, surgical oncologist, radiation oncologist, pathologist, and the plastic surgeon should be assembled. A list of suggested laboratory testing based on the current understanding of the reported cases is summarized in **Table 4-4**. PET/CT should be considered preoperatively to assess for capsular masses or extension into the chest wall and can serve as a "roadmap" to guide oncologic resection. However, the role of PET-CT in evaluating local disease immediately following (2 -3 months) tumor extirpative surgery may be diminished as a result of surgery-induced inflammation.

Table 4-4. Suggested preoperative laboratory testing

Test	Comments
Complete blood count with differential	
Complete metabolic panel	
Lactate dehydrogenase (LDH)	Order LDH and Hep B if chemotherapy is being considered
Hepatitis B (Hep B)	
Bone marrow biopsy	Order if high suspicion of advanced disease (locally aggressive or lymph node metastasis)
PET/CT	Used to assess for chest wall involvement and to guide surgical resection

4.1.7.2 En Bloc resection

Clemens et al. compared different therapeutic approaches and assessed oncologic outcomes in 87 patients with BIA-ALCL.[30] The authors found that complete surgical excision (e.g., complete capsulectomy) demonstrated long-term, disease-free survival compared to all other therapeutic modalities ($p = 0.001$). As a result, current National Comprehensive Cancer Network (NCCN) consensus guidelines call for en bloc surgical resection of the surrounding capsule and removal of the implant (**Figure 4-5**).[32] It is important to note that 2 - 4% of BIA-ALCL cases present with bilateral disease. Therefore, removal of the contralateral implant with complete capsulectomy should be considered should symptoms warrant. Tevis et al. outlined the steps for en bloc resection and processing with all relevant oncologic considerations.[33] Given that BIA-ALCL does not involve the breast parenchyma, mastectomy is not indicated.

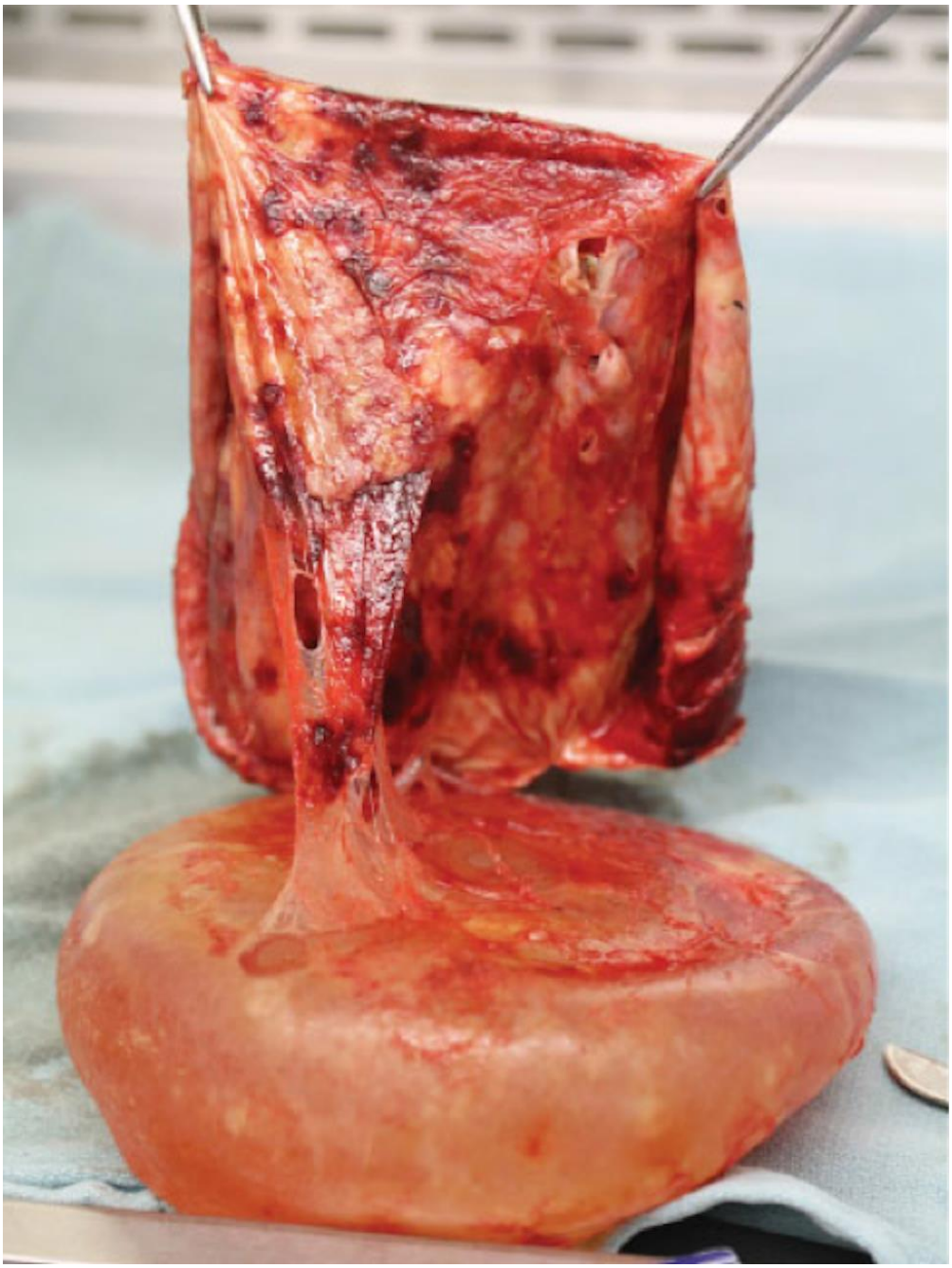


Figure 4-5. En bloc surgical resection and device explantation.
The capsule and implant of a BIA-ALCL patient are shown during evaluation by pathology. Note the thickened surface of the capsule which had developed into a mass. Reprinted with permission by Clemens, MW, DeCoster, RC, Fairchild, et al., 2019. Finding Consensus After Two Decades of Breast Implant-Associated Anaplastic Large Cell Lymphoma *Semin Plast Surg.* 33(4):270-278, Thieme Medical Publishers, Georg Thieme Verlag KG.

For asymptomatic patients concerned about the potential risk of developing BIA-ALCL, there is currently no evidence to support prophylactic implant removal as the risks associated with the required surgical procedure outweighs the current risk of BIA-ALCL development. This does, however, bring up an important issue. In the asymptomatic patient with a textured surface implant who wants the device removed out of concern of developing BIA-ALCL, is a total capsulectomy warranted? Complete capsulectomy remains an exceedingly challenging surgical procedure, which carries its own risks, such as additional bleeding and an increased risk of pneumothorax—specifically due to the strong adherence of the posterior wall of the capsule to the chest wall. Currently, there is insufficient clinical evidence to suggest the selection of subtotal versus total capsulectomy. Although the evidence supports a capsular origin of BIA-ALCL, there is not enough evidence at this time to definitively establish complete capsulectomy as a risk-reducing procedure in the asymptomatic patient. This concept marks an important distinction between complete capsulectomy and en bloc resection, where the goal of the latter is to achieve clear margins, something not obtainable in the patient where the disease is not clinically evident. Nevertheless, the patient and surgeon should engage in a meaningful discussion to consider the patient's desire as well as the specific risks and benefits for each approach on a case-by-case basis.

4.1.8 Indications for adjuvant therapies

The use of adjuvant chemotherapy or radiation in surgically unresectable or advanced disease is backed by Level III evidence.[30] Current NCCN guidelines advocate for the use of brentuximab vedotin, a monoclonal antibody directed against CD30 or a combination anthracycline-based chemotherapeutic regimen, CHOP (cyclophosphamide,

adriamycin, vincristine, and prednisone), which is reserved for cases of residual or disseminated disease (MD Anderson Stage IIB-IV).[32] Radiation therapy (24 – 36Gy) should be considered for patients with local residual disease, positive margins, or surgically unresectable disease with chest wall extension and carries the same level clinical of evidence.

As mentioned, the current therapeutic regimen was born out of necessity to handle cases where en bloc resection is not achievable. The role of targeted therapies remains under consideration. For example, recent work from our group and others has identified aberrant JAK-STAT3 pathway involvement, which may serve as a novel therapeutic target for JAK-STAT inhibitors in the future. To that end, prospective studies are needed to further delineate the most effective chemotherapeutic regimen in the case of disseminated disease.

4.1.9 Breast reconstruction after BIA-ALCL

Practitioners can reasonably offer immediate or delayed breast reconstruction after oncologic resection for BIA-ALCL to most patients, given the favorable prognosis of the disease with appropriate management. Methods of breast reconstruction after device explantation and complete surgical resection include implant replacement, autologous tissue transfer, mastopexy, or serial fat grafting. Given the known association of ALCL with textured implants, it is strongly recommended that implant-based reconstruction proceeds with smooth, round silicone implants should implant reconstruction, so be desired. Although patients may be reluctant to pursue implant-based reconstruction given the anxiety of device-induced recurrence, evidence has consistently demonstrated that all confirmed cases of BIA-ALCL have only occurred

with textured devices.[34] However, psychologic fear should be explored preoperatively as the aforementioned options of autologous tissue transfer, mastopexy, or fat grafting demonstrate similar patient satisfaction and clinical outcomes in breast reconstruction and should remain as viable reconstructive options.

The timing of reconstruction after treatment has been highly debated and depends on disease severity at presentation. Lamaris et al. proposed a treatment algorithm based on their experience reconstructing 18 consecutive patients after treatment for BIA-ALCL.[34] Patients with surgically resectable disease (stage IA-IC) can be offered either immediate reconstruction or delayed reconstruction after surveillance PET/CT in 3-6 months. Complete capsulectomy can result in devascularized tissue and must be considered in any patient undergoing immediate reconstruction. Those patients with advanced disease (stage IIA-IV) should be offered delayed reconstruction after surveillance imaging, which generally occurs 6-12 months after completion of surgical resection and any adjuvant chemotherapy.

4.1.10 Oncologic surveillance

Patients that have been successfully treated should be followed by an oncologist every 3-6 months for a period of two years.[31] Follow up should include a physical examination, and physicians may elect to use CT or PET/CT of the chest/abdomen/pelvis to monitor for tumor recurrence.

Insurance coverage

Insurance coverage for BIA-ALCL is provided by some major carriers, including Blue Cross and Aetna. Coverage includes removal of the implant with capsulectomy out of medical necessity, one indication being BIA-ALCL. A comprehensive list of ASPS Insurance Coverage Criteria for Third-Party Payers-BIA-ALCL may be found on the following website: (https://www.plasticsurgery.org/documents/Health-Policy/Reimbursement/Insurance-2017-BIA-ALCL.pdf). Relevant diagnostic and procedural codes are included in **Table 4-5**.

Table 4-5. Relevant International Classification of Diseases, Tenth Revision, and Current Procedural Terminology Codes for Suspected and Confirmed BIA-ALCL Cases

Code	Description
ICD-10 diagnostic codes	
C84.79	Anaplastic large cell lymphoma kinase-negative, extranodal, solid organ sites
N63	Unspecified lump in breast, nodule, mass, or swelling of the breast
R59.9	Enlarged lymph node
N64.4	Other specified disorders of the breast
CPT procedural codes	
10022	Fine needle aspiration with imaging guidance
19101	Breast biopsy, open, incisional
19260	Excision of chest wall tumor
19328	Removal intact mammary implant
19371	Breast periprosthetic capsulectomy
38525	Biopsy/excision, lymph node; open or deep axilla

International classification of disease-tenth Revision, ICD-10; Current procedural terminology, CPT.

Disease reporting

All suspected or confirmed cases should be reported to the American Society of Plastic Surgeons/Plastic Surgery Foundation Patient Registry and Outcomes For Breast Implants and anaplastic large cell lymphoma (ALCL) etiology and Epidemiology (PROFILE) registry (https://www.thepsf.org/research/registries/profile/case-submission). The PROFILE registry now recognizes 288 confirmed or suspected cases in the U.S., bringing the total worldwide cases to 871 as of December 6th, 2019.[112]

Conclusions

The clinical knowledge of BIA-ALCL has advanced rapidly over the last several years. This evidence-based systematic review critically evaluated current NCCN consensus guidelines and clinical recommendations while highlighting advances related to the diagnosis and treatment of BIA-ALCL, including en bloc resection as the standard of care in the majority of cases. Despite a limited number of high-quality studies, current clinical recommendations and NCCN consensus guidelines are supported by evidence and represent best clinical practices. As reinforced throughout this article and in conjunction with NCCN guidelines, early diagnosis, and strict adherence to clinical guidelines maintain an excellent prognosis for patients diagnosed with the disease. For the asymptomatic patient with a textured breast implant, there is currently no evidence to support prophylactic removal, as performing removal in conjunction with complete capsulectomy may not be a risk-reducing procedure. As the incidence of BIA-ALCL continues to increase, prospective studies are needed to further delineate the most effective diagnostic algorithms and treatment strategies. Finally, well-designed

epidemiologic studies are needed to more accurately quantify the risk of BIA-ALCL for patients considering breast augmentation or breast reconstruction with a textured-device in order to better understand both the risk and benefits in order to improve patient safety.

CHAPTER 5. CURRENT RISK OF BREAST-IMPLANT ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA: A SYSTEMATIC REVIEW OF EPIDEMIOLOGICAL STUDIES

Abstract

Background: Recent epidemiological studies have attempted to accurately determine the risk of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). However, comparisons of previously published works are difficult due to widespread variations in reporting. We systematically review the epidemiology in order to better define the current risk of BIA-ALCL. Herein, we report the global epidemiology with an emphasis on the U.S. breast implant population while simultaneously assessing the oncologic safety of smooth-surface devices. **Methods:** A systematic review of PubMed and other scientific databases, as well as the grey literature, was conducted for epidemiologic studies on BIA-ALCL. Using analytical and descriptive epidemiology, we estimated the cumulative incidence and incidence rate of BIA-ALCL using a standardized approach. Cumulative incidence was reported at implant and patient-specific levels. **Results:** The patient-specific cumulative risk within the U.S. market ranges from 1.79 per 1000 (1:559) to 2.82 per 1000 (1:355) patients with a textured implant. The implant-specific risk of Allergan textured devices ranges from 1:602-871 to 1:8500, while the risk of commercially available Mentor Siltex implants is 1:50000. No epidemiological study or regulatory agency reported a case of BIA-ALCL occurring exclusively with a smooth device. **Conclusions:** With the removal of Allergan textured breast devices, this study demonstrates substantial gaps in the epidemiological knowledge of BIA-ALCL, including the current risk of commercially available textured breast implants in the U.S. market.

Although the risk of BIA-ALCL is low, surgeons should exercise extreme caution when considering the use of a textured breast device for cosmetic or reconstructive purposes.

Background

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a novel T-cell lymphoma associated with textured-surface breast implants.[1,41,90] Under most circumstances, BIA-ALCL presents as an acute-onset, periprosthetic fluid collection greater than 1-year following device implantation.[37] National Comprehensive Cancer Network consensus guidelines advocate for device explantation and complete surgical excision as the standard of care.[30,32] Adjuvant therapy, including chemotherapy and radiation, are reserved for patients with advanced or refractory disease. Although the biological mechanisms remain largely unknown, texturization is thought to play a critical role in the malignant transformation of the disease.[102] Some authors have suggested that the aggressive surface texturing characteristics may induce a chronic inflammatory milieu through implant-host interactions that result from constant mechanical shear forces on the surrounding breast parenchyma.[21] Other potential avenues of pathogenesis include lipopolysaccharide-induced carcinogenesis resulting from higher loads of bacteria found in subclinical biofilms relative to smooth surface devices,[12] as well as aberrant genetic changes (e.g., JAK-STAT, *TP53*) as a result of an over-activated immune system.[11]

In theory, the solution should be as simple as discounting further use of all textured breast devices; however, it is much more complex. Many authors cite decreased local complications and adverse outcomes, including lower rates of capsular contracture, implant malposition, and re-operation that favor the selection of textured devices over

smooth.[6] Therefore, textured breast devices remain attractive in many countries throughout the world, including the U.S. and recent evidence has suggested that their use is increasing.[96]

In 2019, the U.S. Food and Drug Administration (FDA) conducted hearings on breast implant safety, ultimately concluding that the evidence to ban textured devices was insufficient.[113] Nevertheless, governmental regulatory bodies, including FDA, continue to debate the scientific validity of an exclusive association between textured surface breast implants and BIA-ALCL, citing a lack of evidence as well as reports documenting a history of smooth devices. The culmination of BIA-ALCL, along with other issues surrounding breast implants (e.g., breast implant illness),[35,114] led the FDA to mandate *black box warnings* on all breast implants, regardless of filling (saline vs. silicone) or surface (textured vs. smooth).[10] The proposed warnings are outlined in **Table 5-1**. As such, there is an immediate need to determine the oncologic safety of both smooth surface and textured breast devices using high-quality epidemiological studies.

Table 5-1. U.S. Food and Drug Administration Proposed Warnings for Breast Implants

Description
Breast implants are not considered lifetime devices. The longer people have them, the greater the chances are that they will develop complications, some of which will require more surgery
Breast implants have been associated with the development of a cancer of the immune system called breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). This cancer occurs more commonly in patients with textured breast implants than smooth implants, although rates are not well defined. Some patients have died from BIA-ALCL
Patients receiving breast implants have reported a variety of systemic symptoms such as joint paint, muscle aches, confusion, chronic fatigue, autoimmune diseases and others. Individual patient risk for developing these symptoms has not been well established. Some patients report complete resolution of symptoms when the implants are removed without replacement

Cancer epidemiology plays an essential role in identifying and quantifying risk factors of a disease in order to guide the development of effective prevention strategies. Previous epidemiological studies have attempted to quantify the risk of BIA-ALCL accurately;[3–5,7] however, comparisons of published studies are difficult due to a lack of well-defined study populations and widespread variations in the reporting of epidemiological parameters. The purpose of this study is to better define the risk of BIA-ALCL by systematically reviewing the epidemiological literature on the disease. Determining an accurate risk estimate for commercially available devices is essential for both patients and providers when considering the risks and benefits of using a textured breast device. This study also aims to definitively establish an exclusive association between textured-surface breast implants and foreign-body carcinogenesis that is BIA-ALCL while simultaneously demonstrating the oncologic safety of smooth devices. Herein, we report the global epidemiology of BIA-ALCL with a focus on the U.S. breast implant population while simultaneously assessing the possible association with smooth-surface devices.

Methods

5.1.1 Search strategy

A systematic review of epidemiological population-based cohort studies on BIA-ALCL was conducted in PubMed, Google Scholar, and EMBASE databases between March 9-20, 2020 using a combination of BIA-ALCL and epidemiological-related search terms. Search parameters included the terms and Medical Subject Headings (MeSH) *breast implant-associated anaplastic large cell lymphoma, breast implant(s), lymphoma,*

epidemiology, *cancer epidemiology*, *incidence*, and *cancer incidence*. A search of the grey literature was also performed. Two independent reviewers screened titles, abstracts, and full texts of identified articles (RCD, MWC). Disagreement between reviewers was resolved via discussion until there was 100% agreement. Citation chaining was performed using Web of Science. Critical appraisal of the evidence was conducted using a modified STrengthening Reporting of OBservational studies in Epidemiology (STROBE) checklist that was developed within the aims of the present study. The modified checklist was comprised of key quality factors including a risk-of-bias assessment and consisted of 10 total items. A single-point system was used to score each item. Quality scores were calculated for each article and taken out of 10 (max score) in order to facilitate comparisons of the relative quality of each study. Higher scores were indicative of higher overall quality, while lower scores did not necessarily reflect poor study quality, but rather a lower relative quality assessment compared to other included studies. Global regulatory agency data were reviewed for epidemiological data related to BIA-ALCL that were not captured in the main search.

5.1.2 Inclusion and exclusion criteria

Inclusion was limited to primary epidemiological research on BIA-ALCL reported in prospective cohort studies, case-series, case-control studies, conference proceedings, and abstracts. Articles comparing the risk of BIA-ALCL to other lymphomas[115] were excluded, as were articles in which the epidemiology of a previously described cohort had been recently published.[7,116] Only articles in the English language were reviewed. This systematic review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

5.1.3 Data abstraction and quality assessment

Abstracted data included author, journal, year of publication, country, study period, number of incident cases, study design, study period, patient-specific cumulative incidence, implant-specific cumulative incidence, incidence rate (per 100 000 person-years). In cases where the incidence rate was reported differently (e.g., per 1000 person-years), rates were standardized per 100 000 person-years, which is the conventional method for reporting cancer incidence rates.[117] Analytical and descriptive epidemiology was used to estimate the cumulative incidence (i.e., risk) of BIA-ALCL according to patient and implant specificity. Cumulative incidence was reported at implant and patient-specific levels. Levels of evidence were ranked from highest to lowest according to the American Society of Plastic Surgeons evidence-based rating scales for prognostic/risk studies (**Table 5-2**). Regulatory agency-specific epidemiologic data were collected from Australia, Canada, the Netherlands, the U.S., and the U.K.

Table 5-2. American Society of Plastic Surgeons Evidence Rating Scale for Prognostic/Risk Studies

Level of Evidence	Description
I	High-quality, multi-centered or single-centered, prospective cohort or comparative study with adequate power; or a systematic review of these studies
II	Lesser-quality prospective cohort or comparative study; retrospective cohort or comparative study; untreated controls from a randomized controlled trial; or a systematic review of these studies
III	Case-control study; or systematic review of these studies
IV	Case series with pre/post test; or only post test
V	Expert opinion developed via consensus process; case report or clinical example; or evidence based on physiology, bench research or “first principles”

To investigate the possible association between smooth surface devices and BIA-ALCL, the FDA's Manufacturer User Facility Device Experience database, and the American Society of Plastic Surgeons (ASPS) Patient Registry and Outcomes For breast Implants and anaplastic large cell lymphoma (ALCL) etiology and Epidemiology (PROFILE) registry were queried for reports of BIA-ALCL. MAUDE collects medical device reports on data related to suspected device-associated deaths, serious injuries, and malfunctions, and the limitations of MAUDE with regard to breast implant safety and BIA-ALCL have been previously described.[39,118] PROFILE is a prospectively maintained database that collects data regarding breast implants and ALCL.

Results

An overview of the search is shown in **Figure 5-1**. The initial search generated 81 articles. One additional article was identified in a conference proceeding. Titles and abstracts from 12 articles were further reviewed to assess for study eligibility. The full text from nine articles were reviewed. After meeting study inclusion criteria, eight articles underwent quality assessment and data abstraction (**Table 5-3**). Disease incidence was reported in seven studies while incidence rates were described in two studies. Included studies differed in two main ways: study design and the reporting of incidence and incidence rates.

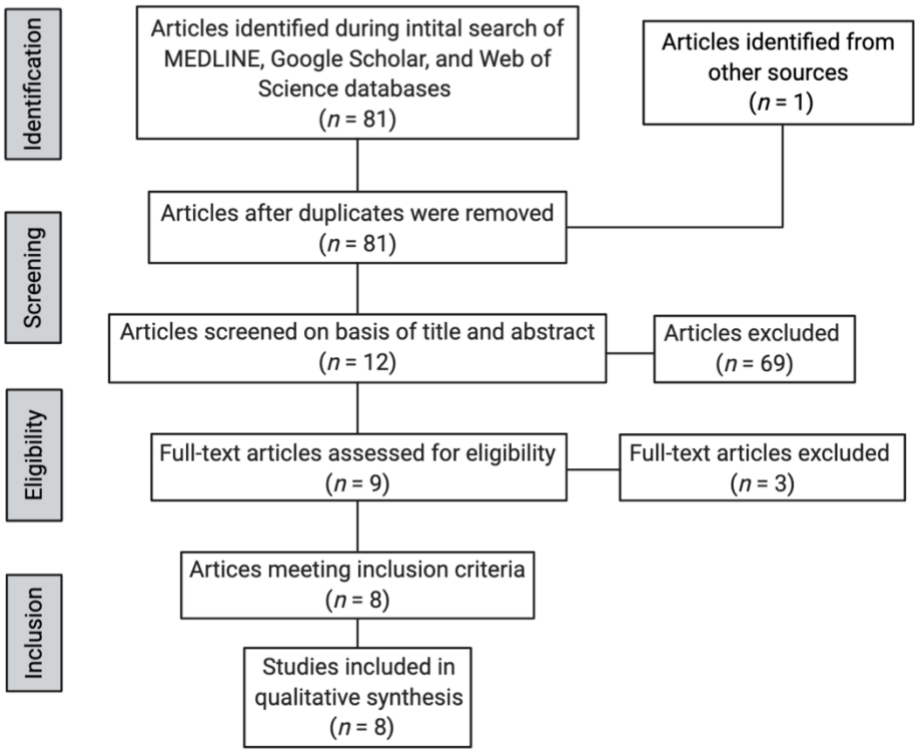


Figure 5-1. PRISMA flow diagram

Table 5-3. Epidemiological Studies of BIA-ALCL

Author	Year	Country	Study design	Study period	Level of evidence	Incident BIA-ALCL cases	Sample size	Patient specific incidence	Implant specific incidence	Incidence rate (person-years)
Largent et al	2011	USA	Retrospective	1994-2007	II	3	NR	1.46 per 100,000 person-years (Allergan)	NR	1.46 per 100,000
McGuire et al	2016	USA	Prospective Cohort	-2014	II	4 initially (now 8)	17,656	1:2,207 (Allergan)	NR	NR
Cordeiro et al	2020	USA	Retrospective Cohort	1992-2019	III	10	3456	1/355	1/602 (Allergan)	NR
Nelson et al	2020	USA	Retrospective Cohort	1991-2017	III	11	9373	1:559 (1.79 per 1000)	1:871 (1.15 per 1000) (Allergan)	NR

De Boer et al	2018	Netherlands	Retrospective Cohort	1990-2016	III	43	3000	1:6920 at 75 years of age (reported as NNH)	NR	NR
Campanale et al	2018	Italy	Retrospective Cohort	2015-2017	III	22	10,000,000	2.8 per 100,000	NR	NR
Loch-Wilkinson et al	2019	Australia	Retrospective	2015-2019	III	104		NR	1:1947 (Silimed) 1:36,730 (Mentor Siltex)	NR
Doren et al	2018	USA	Case Series	1996-2015	IV	100	3,000,000	NR	1:8500 (Allergan) 1:50,000 (Mentor)	2.03 per 1,000,000 1.86 per 1,000,000 (Allergan) 0.33 per 1,000,000 (Mentor)

5.1.4 U.S. epidemiology of BIA-ALCL

5.1.4.1 Patient-specific risk

Two studies have examined the incidence of BIA-ALCL within the U.S. breast implant population. Both studies are exclusive to the reconstructive cohort, which introduces selection bias. The patient-specific cumulative risk within the U.S. ranges from 1.79 per 1000 (1:559)[119] to 2.82 per 1000 (1:355)[4] patients with a textured surface implant. This translates to an overall cumulative risk estimate for patients in the U.S. of 0.003% to 0.29% at 20 years and 26 years, respectively. When considering the cumulative risk from the time of implantation, proportions ranged from 0.00 at 5 years, 0.002 at 10 years, 0.007 at 15 years, and 0.011 at 20 years,[4] while other estimates suggest a cumulative risk estimate of 4.4 per 1000 patients at 10-12 years and 9.4 per 1000 patients at 14-16 years.[119]

5.1.4.2 Implant-specific risk

Using analytical and descriptive epidemiology and the data provided in Doren et al.,[3] we calculated manufacturer specific risks in the U.S. breast implant population. U.S. implant-specific risks are less heterogeneous than global risk estimates with incidences ranging from 1:602-871 to 1:8500 textured implants, which are exclusive to Allergan (Dublin, Ireland) textured devices.[3,4,101] The risk estimate for Mentor (Mentor Worldwide LLC, Irvine, Calif.) Siltex implants is 1:51 000. Implant-specific risks for other currently available textured devices (e.g., Sientra, Santa Barbara, Calif.) in the U.S. market are not reported.

5.1.4.3 Incidence rate

U.S. specific incidence rates vary from 0.311 cases per 1000 person-years (95% CI: 0.118-0.503)[4] to 1.46 per 100000 person-years (95% CI: 0.30-0.43)[120] to 2.03 cases per 1 million person-years [1.86 per million (Allergan); 0.33 per million (Mentor)].[3] Following conversion, the standardized incidence rate determined by the present study of BIA-ALCL in the U.S. ranges from 0.203 per 100000 person-years to 31.1 per 100 000 person-years, indicating that the cumulative risk of BIA-ALCL is higher than previously thought. When considering incidence rates according to U.S. manufacturer specificity, a 5.67-fold difference for Allergan Biocell (1.87per 1 million person-years) compared to Mentor Siltex (0.33 per 1 million person-years) implants was reported ($p<0.001$).[3] The incidence rate for Sientra implants was not reported.

5.1.5 Global epidemiology of BIA-ALCL

5.1.5.1 Patient-specific risk

Global risk estimates of BIA-ALCL, according to international regulatory agencies, are summarized in **Table 5-4**. In the Netherlands, the age-adjusted incidence of BIA-ALCL from a textured device is approximately 1:6920 patients with a textured implant at 75 years of age.[121] The Australian Therapeutic Goods Administration previously reported a risk estimate of 1:1000-1:10000 patients; however, the risk has widened to 1:2500 to 1:25000 patients with a textured breast implant.[45] The Italian-specific incidence is 2.8 per 100000 patients.[122] A global heat map is used to illustrate the geographic distribution of BIA-ALCL cases worldwide (**Figure 5-2**). This distribution is

reinforced by showing the breakdown of country-specific cases and related deaths in **Table 5-5**.

Table 5-4. Summary of Global Regulatory Agency Risk Estimates of BIA-ALCL

Country	Source	Risk
Australia	Australian Therapeutic Good Administration	1:2500-1:25 000 patients
Canada	Health Canada	Overall: 1:24 177 1:3565 (Allergan) 1:16 703 (Mentor)
United Kingdom	Medicines and Healthcare Products Regulatory Agency	1:24 000 (implants)
United States	Food and Drug Administration	1:3817-1:30 000

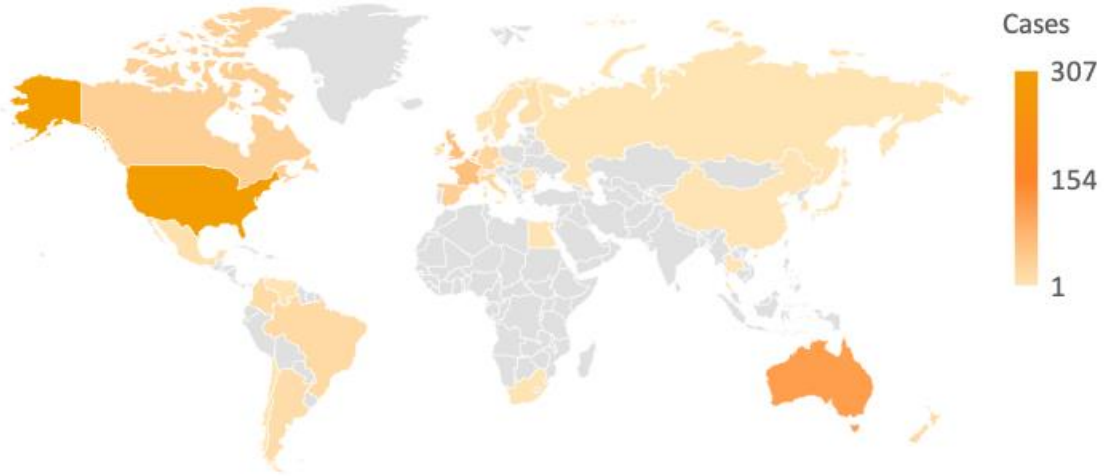


Figure 5-2. Geographic distribution of BIA-ALCL cases worldwide

Table 5-5. Global Distribution of BIA-ALCL Cases and Attributed Deaths

Country	Cases	Deaths
Argentina	13	1
Australia	112	4
Belgium	12	
Brazil	19	1
Bulgaria	1	
Canada	34	1
Chile	2	
China	1	
Colombia	17	1
Czech Republic	1	
Denmark	9	
Egypt	1	
Finland	11	
France	58	3
Germany	24	
Ireland	1	
Israel	8	
Italy	50	1
Japan	1	
Mexico	7	
Netherlands	60	1
New Zealand	16	1
Norway	3	
Romania	1	
Russia	2	
Singapore	1	
South Africa	2	
South Korea	2	
Spain	35	
Sweden	8	2
Thailand	1	
Venezuela	2	
United Kingdom	61	1
United States	307	7
Total	885	34

5.1.5.2 Implant-specific risk

Manufacture-specific implant risks are outlined in **Table 5-6**. In Australia, the current implant-specific risk of BIA-ALCL varies widely, ranging from 1:2832 – 1:86 029 implants.[7,102] When considering risk according to manufacturer specificity, the highest risk was in Silimed polyurethane implant (1:2832 95% CI: 1582-5673), followed by Allergan Biocell (1:3345 95% CI: 2475-4642) and finally Mentor Siltex (1:86029 95% CI: 15 440-1 301759) implants. Health Canada, the Canadian equivalent of the U.S. FDA, currently estimates an overall risk of 1:24177 implants.[123] This distills down to a manufacturer-specific risk of 1:3565 (Allergan Biocell) and 1:16703 (Mentor Siltex) in the Canadian breast implant population, which translates to a 16.52 increased risk of Biocell implants. In the United Kingdom, the risk of BIA-ALCL is 1:24000 implants inserted.[124]

Table 5-6. Manufacturer-specific Global Risk Estimates of BIA-ALCL

Manufacturer	Textured implant type	Texturization method	Global risk
Allergan	Biocell	Salt loss	1:602 to 1:8500
Mentor	Siltex	Negative imprint	1:6703-1:86 029
Sientra		Proprietary method	1:200 000*
Silimed	Polyurethane	Foam-coated	1:2832

*Non-epidemiologic study by Calobrace et al.

5.1.6 BIA-ALCL is exclusively associated with textured-surface breast implants

U.S. and non-U.S. population-based, and case-control studies, in combination with a review of government databases, consistently revealed an association between textured-surface breast implants and the incidence of BIA-ALCL. Importantly, not a single epidemiological study or government database reported a case of BIA-ALCL occurring solely in the context of a smooth surface breast implant.

Discussion

This systematic review provides a detailed examination of existing epidemiologic data on the global risk of BIA-ALCL. In lieu of a conventional systematic review based on randomized clinical trials, this comprehensive review is comprised of epidemiological observational studies of BIA-ALCL in the breast implant population. The heterogeneity of reported data precluded meta-analysis and limited the calculation of combined risk estimates. Irrespective of this, we were able to draw comparisons between studies by standardizing epidemiological parameters whenever possible.

As demonstrated, the risk of BIA-ALCL varies substantially, especially when considering incidence according to manufacturer type. In the U.S. market, the average lifetime risk of BIA-ALCL ranges from 1:355 – 1:51 000 patients with a textured surface breast implant. Allergan's Biocell implants carry that highest manufacturer-specific risk at 1:2207-1:8500,[3,101] followed by Mentor Siltex implants at 1:51 000. This translates into a nearly six-fold increase in the risk of BIA-ALCL when comparing Allergan Biocell to Mentor Siltex breast implants ($p<0.001$). These data, among others, weighed heavily on the decision for the U.S. FDA to issue a Class 1 recall, the most serious type of recall, on

all Allergan textured breast devices. With the removal of Allergan textured devices from the U.S. and other markets worldwide, much of the currently available epidemiologic data does little to mitigate risks for patients currently considering the use of a textured breast device for breast reconstruction or cosmetic augmentation. As such, there is a paucity of epidemiological data for commercially available textured breast implants which is highly concerning from a patient safety perspective.

As shown in the present study, the increased risk of BIA-ALCL associated with Allergan textured breast implants in the U.S. is well-established. Conversely, risk estimates for Mentor products (1:51 000) are less well-established, and the risk of Sientra implants has gone virtually unreported in the U.S. literature. A single non-epidemiologic U.S. based study reported a combined 20-year, worldwide risk of BIA-ALCL for Sientra and Silimed (Rio de Janeiro, Brazil) of 1:200 000 implants.[6] Therefore, based on the findings of the present study, the current risk of BIA-ALCL for commercially available devices likely resides somewhere between 1:51 000-1:200 000. However, it is unclear how the upper limit of that risk estimate stratifies according to manufacturer type or if it is generalizable to the U.S. population given the methodologies used to arrive at that calculation. Combined with the removal of Allergan devices from the U.S market, these data, along with a limited number of other risk estimates, do little to guide implant selection for patients considering breast augmentation or breast reconstruction with a textured surface device. Future risk assessment studies on currently available breast devices are warranted.

The present study also identified clustering of cases in the U.S., Australia and New Zealand, the U.K., the Netherlands, and France, with widespread geographic

variation in global risk estimates. The highest number of cases occurred in the U.S., which accounts for 1 out of every 2.6 cases (38.4%) worldwide. These data coincide with recent risk estimates suggesting the highest incidence of BIA-ALCL within the U.S. breast implant population. These data are somewhat surprising, given that textured breast devices account for less than 10% of sales in the U.S. market.[6] Conversely, Australia is predominantly a textured device market, yet it only accounts for 1:7 cases (14.3%). These differences in clustering and subsequent risk profiles are likely a result of increased awareness, improved surveillance, access to care, and long-term follow-up, rather than epidemiologic or pathologic phenomena. Unfortunately, there is a misconception held by few that clustering of BIA-ALCL cases is indicative of poor breast implant technique. Given that no evidence currently exists to support an association between surgical technique and BIA-ALCL tumorigenesis, the authors of the current study vehemently oppose such a notion that threatens to undermine the reporting of future cases amongst surgeons. Previous studies have also suggested that genetics may account for differences in worldwide incidence, citing the lack of clustering in the Asian breast implant population as evidence.[6,22,102] This concept has recently been challenged with reports of BIA-ALCL emerging in this population.[125] While genetics, more specifically epigenetics, may account for geographic variations in cumulative risk found in the present study, the current evidence does not support such a concept at this time.

Texturization plays a critical role in the malignant transformation of BIA-ALCL. Yet, regulatory agencies remain reluctant to acquit smooth surface devices. Importantly, we did not find a single case of BIA-ALCL that had been reported to PROFILE where a patient had a pure history of a smooth implant. As of July 2019, FDA's MAUDE

database acknowledged 457 unique medical device reports with a BIA-ALCL diagnosis, of which 26 are recognized as occurring with a smooth device.[126] Of those, 12 have an unknown prior implant history, 7 have a history of a prior textured implant, and in 7 cases, surface characteristics were unknown. Contradicting these reports, this systematic review found no published reports of the disease occurring exclusively with a smooth-surface device. Moreover, this study failed to identify a single case of BIA-ALCL associated with a smooth device in any registry or government database where a patient had not already been exposed to a textured device, which includes exposure to a textured tissue expander. FDA currently denies any association between textured expanders and BIA-ALCL; however, it is important to note that PROFILE does recognize two cases of ALCL have occurred in patients receiving tissue expander breast reconstruction with a textured-surface expander followed by permanent implant exchange with smooth surface implants.[112] While the sample size is small, this reinforces the concept of texturization and the role it plays in malignant transformation.

5.1.7 Limitations

The current study is only as strong as the quality of data that were abstracted during the search. Retrospective designs have limited previous epidemiological studies of BIA-ALC, along with extrapolated denominators based on inaccurate implant sales figures, incomplete clinical data, and a lack of long-term follow-up, all of may act as potential sources of bias in the present study. This systematic review also limited inclusion criteria to articles exclusively disseminated in the English language. As such, it is possible, although highly improbable, that epidemiological studies on BIA-ALCL may exist in other languages. Additionally, the lack of reported cases of BIA-ALCL with

smooth devices precluded a calculation of the relative risk of smooth vs. textured devices. Finally, differing methodologies combined with the heterogeneity of data, we were unable to standardize all epidemiological parameters across studies or assess temporal trends in the risk of the disease.

Conclusions

This is the first systematic review on the epidemiology of BIA-ALCL in the breast implant population. Of great concern, this systematic review identified substantial gaps in the epidemiological knowledge of BIA-ALCL that have resulted from a dearth of high-quality epidemiological evidence and widespread differences in reporting which hinder the interpretation and generalization of risk estimates. These differences highlight the importance of standardized reporting of age-adjusted epidemiological parameters to allow for more reliable comparisons across various breast implant populations. Specifically, the present study demonstrated significant global geographic and manufacturer-specific variation in the risk of the disease. Further investigation of demographic, epigenetic, and environmental risk factors, including implant surface characteristics, may account for these differences and is therefore warranted. With the removal of Allergan textured devices, this study also found that the current risk of commercially available textured-surface breast implants, specifically in the U.S. market, is not well-defined and impairs the ability to provide a thorough informed consent thereby threatening patient safety. Patients and providers should exercise extreme caution when considering the use of a textured breast device for cosmetic or reconstructive purposes. Finally, this systematic review demonstrated that there is no evidence to

support the hypothesis that BIA-ALCL is associated with smooth-surface breast implants at this time. Although these data suggest that smooth-surface breast implants are oncologically safe, more extensive prospective studies are needed before definitive conclusions may be drawn.

CHAPTER 6. ABERRANT JAK-STAT3 SIGNALING IS A KEY MOLECULAR FEATURE OF BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA

Abstract

Background: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging lymphoma linked to textured-surface breast implants. The molecular mechanisms responsible for lymphomagenesis remain poorly understood. This study utilizes transcriptional profiling to mechanistically investigate the molecular pathogenesis of BIA-ALCL. **Methods**: Purified RNA isolates from BIA-ALCL and benign breast implant capsule specimens underwent hybridization-based transcriptional profiling using a 770-gene panel (Nanostring) comprising 13 known cancer pathways. Global significance scoring of differential expression profiles was used to identify pathways of interest and guide gene selection. Genes of interest were further selected based on statistical significance ($p < 0.05$) with a Benjamini-Yekutieli correction to control the false discovery rate. Immunohistochemistry was used to validate gene expression. **Results**: BIA-ALCL tumors showed a 2.26-fold upregulation of *STAT3* gene expression ($p < 0$ .014) as well as upregulation of other JAK-STAT3 pathway genes relative to controls. Global significance scoring revealed highest pathway activation in BIA-ALCL occurring in JAK-STAT. Furthermore, pathways involved in apoptosis avoidance and cell-cycle progression were differentially upregulated compared to pathways involved in cell growth and differentiation that were downregulated. Immunohistochemistry revealed BIA-ALCL samples had a significantly higher average of pSTAT3+ cells per high power field (68.65±31.57) than benign capsular tissues (23.93±16.93; $p < 0.031$). **Conclusion**: BIA-ALCL tumors employ pervasive JAK-STAT pathway activation. Involvement of

JAK-STAT represents an attractive area for therapeutic intervention in patients with advanced-stage disease and provides avenues for future investigation that might lead to an increased understanding of the mechanisms of lymphomagenesis in BIA-ALCL and potentially other types of ALK- ALCL.

Background

Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging type of T-cell lymphoma that can form around saline or silicone-filled textured-surface breast implants.[37,40,41,79] These tumors uniformly and strongly express CD30, are negative for anaplastic lymphoma kinase (ALK) and carry clonal T-cell receptor gene rearrangements. Since the index case of this disease, which was first reported in the mid to late 1990s,[2,91,92] over 800 cases have been reported worldwide.[42] The average lifetime risk ranges from 1:355 – 1:30,000 persons with a textured-surface breast implant, but can vary significantly when stratifying risk according to manufacturer type.[4,7,102] In general, BIA-ALCL cases typically present as an acute-onset periprosthetic fluid collection occurring at least one year after device implantation.[27,40] Although most cases typically follow an indolent clinical course when diagnosed and treated promptly, gaps in the understanding of disease development and progression have limited early diagnosis and treatment—leading to poor clinical outcomes including metastatic disease and death in a subset of patients.[30,31,109] The present study remains one of the first clinical research studies utilizing BIA-ALCL patient samples to better discern the factors contributing to the development of this disease.

The central obstacle to advancing BIA-ALCL therapies for patients presenting with aggressive disease remains the overall lack of understanding of molecular drivers of this malignancy. Clinicians and scientists have developed multiple viable hypotheses regarding disease etiology, including pathogen-mediated oncogenesis occurring from lipopolysaccharide-induced tumorigenesis,[12,87] oncogenic viruses,[17] chronic trauma to the breast pocket leading to malignant transformation,[21] chemical structures from the textured device that facilitate aryl hydrocarbon receptor-mediated T-cell proliferation[22] and allergen-driven chronic inflammation.[63,64] Despite this wide swath of proposed theories, all of the factors mentioned above point toward the development of a chronic inflammatory state within the breast pocket that ultimately results in an unregulated T-cell clonal expansion.[20,46] After almost two decades of continued investigation, the scientific data supporting the *exact* mechanisms driving BIA-ALCL tumorigenesis and progression remain largely underdeveloped.[1] Demanding growth requirements for BIA-ALCL tumor cell lines (TLBR 1-4) and the lack of a validated animal model[19] have further impeded scientific progress, but of more pressing concern remains the deficiency in well-designed primary research studies utilizing patient tissue samples for scientific discovery; the present study hopes to address this need.

Overwhelming evidence implicates aberrant JAK-STAT activation in almost every type of T-cell specific malignancy—ranging from T-cells transformed by human T-cell lymphotrophic virus 1 (HTLV-1), acute lymphoblastic leukemia (ALL) and including ALK+ and ALK- anaplastic large cell lymphoma.[56,127,128] In ALK- ALCL, like BIA-ALCL, anywhere from 47-80% of known genetic mutations occur within the JAK-STAT pathway, leading to increased $STAT3^{Y705}$ phosphorylation and nuclear

localization.[56] Despite these findings, only one study has investigated the transcriptional profile of BIA-ALCL.[17]

The current study aims to conduct transcriptional profiling of BIA-ALCL tumor specimens compared to benign breast implant capsules to better understand the molecular pathogenesis of BIA-ALCL. It is hoped these insights will better inform the diagnosis and treatment of this emerging disease. Inquiry into this specific molecular activating pathway is likely to yield clinically useful results, as specific JAK inhibitors have shown promise in blocking both cytokine-receptor activation and receptor-phosphorylation events in other T-cell malignancies. We hypothesize that BIA-ALCL samples, like other ALK- ALCLs, will harbor genetic mutations within the JAK-STAT pathway representing aberrant signaling activation that directly leads to increased nuclear localization of pSTAT3 within affected tissues.

Materials and Methods

6.1.1 Ethics statement

A pilot case (BIA-ALCL)-control (benign breast implant capsules) study was conducted to investigate the molecular profiles of BIA-ALCL. Tumor samples were collected under a protocol approved by The University of Texas MD Anderson Cancer Center institutional review board (IRB) protocol, and healthy tissue samples were collected under an approved IRB protocol at the University of Kentucky Markey Cancer Center.

6.1.2 Patients and samples

After obtaining informed consent, BIA-ALCL tumor specimens (n = 4) were collected from adults undergoing oncologic resection at The University of Texas MD Anderson Cancer Center. Two independent pathologists from the Department of Hematopathology confirmed the diagnoses. Investigators obtained informed consent from each patient, and samples were coded before analysis. Healthy control tissue (n = 8) consisted of breast implant capsules obtained during breast implant exchange or from patients undergoing tissue expander to permanent implant exchange. All specimens were fixed in 10% neutral-buffered formalin overnight and embedded in paraffin (FFPE) for future use. Clinicopathologic data, including age at diagnosis or implant removal, the median time from initial implantation to presentation, tumor stage (where applicable), and implant surface characteristics (smooth vs. textured) were collected for both cohorts.

6.1.3 RNA extraction and transcriptional profiling

Messenger RNA (mRNA) was extracted from tumor (n = 4) and healthy control (n = 8) FFPE specimens per the manufacturer's protocol using a QIAGEN Allprep DNA/RNA Mini Prep Kit (Hilden, Germany). mRNA was stored at -80°C. mRNA quality was determined by visualization of 18S and 28S bands using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). Three BIA-ALCL tumor specimens (ALCL 3 excluded) and eight healthy breast implant capsule specimens met RNA quality thresholds and were utilized for quantitative analysis. Next, mRNA was subjected to the Nanostring nCounter® Sprint Profiler system (Nanostring Technologies, Seattle, WA) using the Pancancer Pathways Codeset for human tissue. Nanostring uses

multiplex hybridization technology with a panel of over 770 cancer-associated human genes, representing 13 canonical cancer-associated pathways as well as six housekeeping genes.[129] Raw counts were generated using the human PanCancer pathways panel and normalized using endogenous controls. Directed global significance scoring (GSS) was used to identify pathways of interest and guide gene selection. Genes of interest were further selected based on statistical significance ($p < 0.05$).

6.1.4 Immunohistochemistry

Tissues were fixed in 4% neutral buffered formalin overnight, processed through paraffin, and sectioned at 5μm before analysis as above. Samples were stained with pSTAT3$_{Y705}$ (Cell Signaling, Danvers, MA). Briefly, paraffin sections were serially hydrated through graded alcohols before antigen retrieval with citrate buffer (pH 6.0) in a decloaking chamber (Biocare Medical, Concord, CA). Sections were then incubated in primary antibody (1:250) overnight at 4°C, washed briefly in buffer before secondary antibody incubation with an anti-rabbit peroxidase-labeled polymer (Dako, Carpinteria, CA). Treated sections were then developed using a 3,3'-diaminobenzidine tetrahydrochloride chromagen (Dako) and counterstained with hematoxylin before imaging. Additional sections were subjected to Hematoxylin and Eosin (H&E) staining.

6.1.5 Assessment of pSTAT3 Activation

pSTAT3 activation was defined as nuclear positivity of pSTAT3 antibody within the tissue sample prepared as detailed above. Four independent ALCL and four independent benign capsule tissues were analyzed initially at low power magnification and representative images—where sufficient cellular positivity with minimal background

was evident—were obtained at high power (400X) magnification. At least ten representative images were obtained per sample. From these representative images, between 5-10 high power fields (HPF) for each sample were used for scoring. All images were processed using Image J software (National Institutes of Health, Bethesda, MD), and positive cells were marked using program tools to improve precision. Scoring criteria consisted of counting positive cells per HPF in a blinded fashion. Two investigators participated in the scoring (RCD, EBL) and submitted their counts independently for statistical analysis.

6.1.6 Statistical analysis

Gene expression analyses were conducted using the Nanostring nSolver (Version 4.0) and Nanostring nCounter Advanced Analysis software (Version 2.0.115). A Benjamini-Yekutieli correction was used to control the false discovery rate. An unpaired Student's t-test with Welch correction was used to assess pSTAT3 cell counts. All non-gene expression analyses were conducted using GraphPad Prism (Version 8.2.1, San Diego, CA). Clinicopathologic characteristics were described using descriptive statistics. Alpha was set at $p < 0.05$ a priori.

Results

6.1.7 Clinicopathologic characteristics

Clinicopathologic characteristics, therapies, and outcomes are described in **Table 6-1**. The median age of patients diagnosed with BIA-ALCL was 58.5 years (range: 41-76 years). All patients (100%) were exposed to an Allergan Biocell textured-surface breast

implant. The median interval time to diagnosis following implantation was 9.0 years (range: 6-13 years). Twenty-five percent of patients had TNM Stage IA, while the remainder of patients presented as Stage IIA (50%) and Stage IB (25%). Half of patients (50%) received combination therapy while the other 50% received oncologic resection exclusively. The median follow-up was 51.5 months (range: 38-140 months). All patients (100%) achieved complete resolution at long-term follow-up. For the controls (data not shown), all patients (100%) had a surgical indication of breast reconstruction and were exposed to an Allergan textured-surface tissue expander.

Table 6-1. Clinicopathologic features, therapy, and outcomes of patients with breast implant-associated anaplastic large cell lymphoma (*n* = 4)

Variable	ALCL 1	ALCL 2	ALCL 3	ALCL 4
Patient age (years)	54	63	76	41
Surgical indication	Cosmetic	Reconstruction	Reconstruction	Cosmetic
Clinical presentation	Effusion	Effusion	Effusion	Mass
Therapy	CCaps,CHOP,Rad	CCaps	CCaps	CCaps,CHOP,Rad
Implant surface	Textured Biocell	Textured Biocell	Textured Biocell	Textured Biocell
Interval time to diagnosis (years)	8	13	6	10
TNM stage at presentation	IB	IIA	IA	IIA
Follow up (months)	38	140	55	48
Clinical outcome	CR	CR	CR	CR

6.1.8 JAK-STAT3 signaling is differentially upregulated in BIA-ALCL tumors

In order to determine which pathway(s) plays a predominant role in BIA-ALCL tumorigenesis, a panel of 13 canonical cancer pathways was selected to perform hybridization-based transcriptional profiling using the Nanostring nCounter Sprint system. Overall, unadjusted differential expression of the 40 most statistically significant gene transcripts is shown in **Figure 6-1.** After adjusting p-values to control the false discovery rate, 44 statistically significant differences in gene expression were identified between BIA-ALCL samples and benign capsule tissue. (**Figure 6-2**). Twenty-eight were found to be upregulated in BIA-ALCL samples, whereas 16 genes were down-regulated compared to benign capsules. The resulting heat map shows strong hierarchal clustering of BIA-ALCL tumor specimens, indicating a relatively homogenous molecular expression profile among these tumors. Using pathway scoring and directed GSS,[130] we identified pathways with the highest upregulation (**Figure 6-3**). Areas of high scoring included pathways related to chromatin modification, DNA damage and repair, cell cycle progression and apoptosis, transcriptional dysregulation, oncogenic driver genes, and the JAK-STAT3 pathway. According to GSS, BIA-ALCL samples showed the highest activation of JAK-STAT3, indicating aberrant expression of the pathway. *STAT3* expression specifically exhibited a 2.26-fold-change in BIA-ALCL tumors ($p < 0.013$) (**Figure 6-4**). A KEGG signaling pathway demonstrating downstream events in aberrant JAK-STAT signaling in BIA-ALCL is shown in **Figure 6-5**. The KEGG pathway highlights the important role that aberrant JAK-STAT3 pathway activation plays in driving 12 other canonical cancer pathways (**Figure 6-6**).

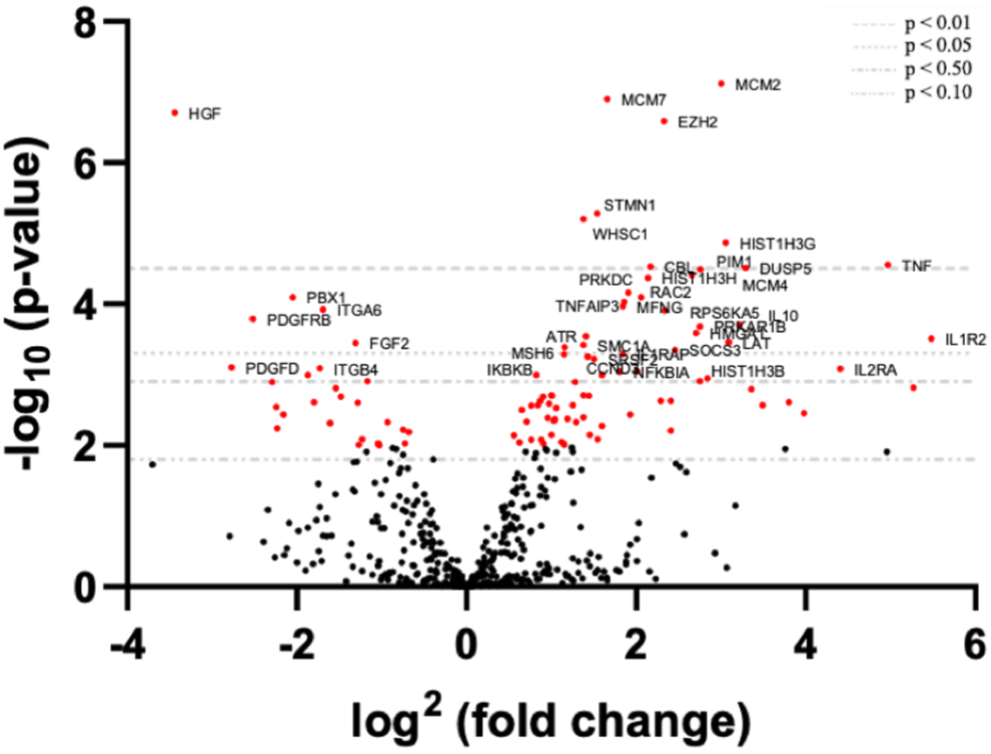


Figure 6-1. Volcano plot of differential gene expression in BIA-ALCL compared to benign breast implant capsules.
Horizontal lines represent various false discovery rate thresholds.

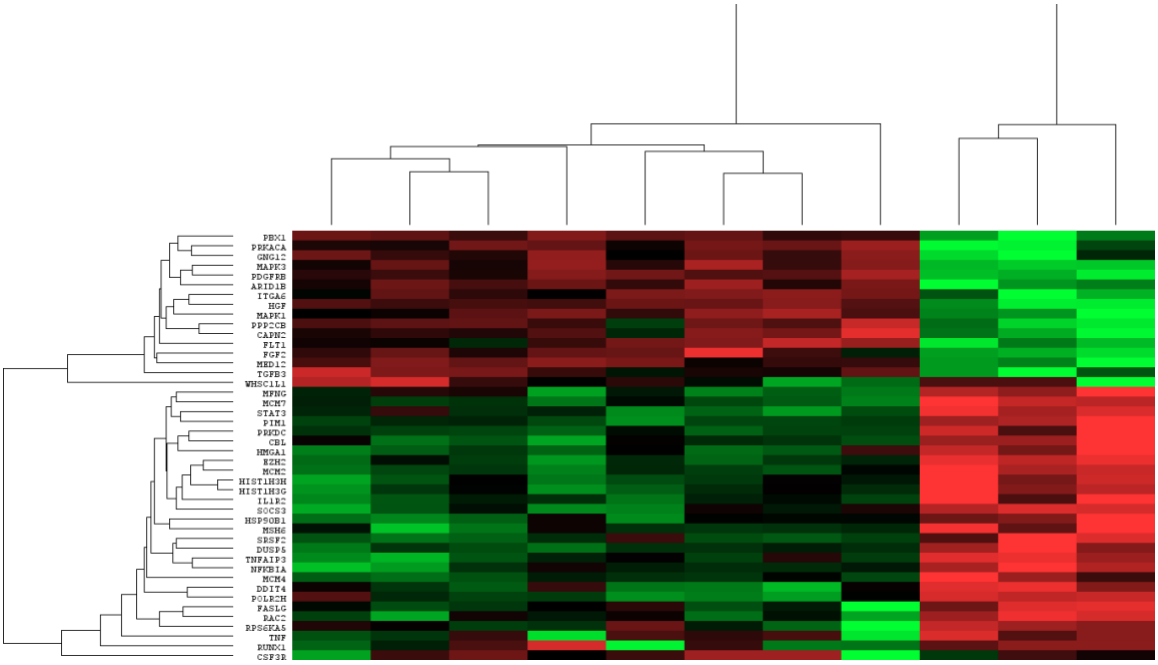


Figure 6-2. Heatmap of differential gene expression in BIA-ALCL vs. healthy controls. Note the hierarchal clustering of tumor specimens (BIA-ALCL) and healthy control tissue (benign breast implant capsules). Red denotes increase gene expression while green signifies decreased gene expression.

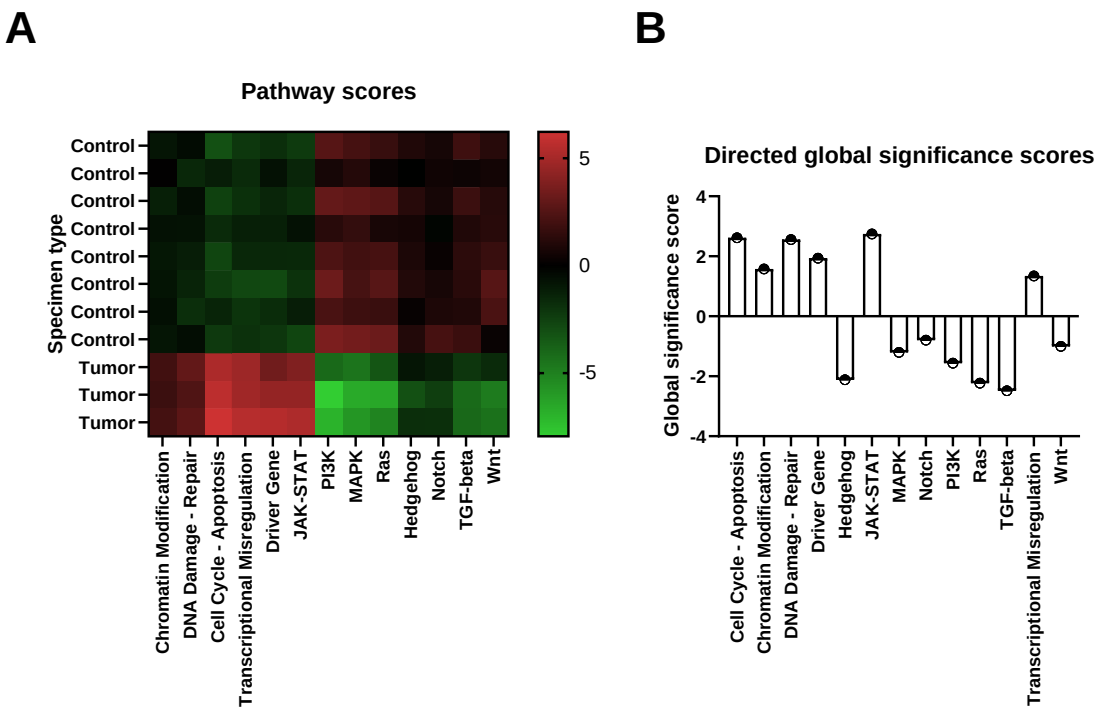


Figure 6-3. A) Heatmap of pathway scores. B) Directed global significance scores.

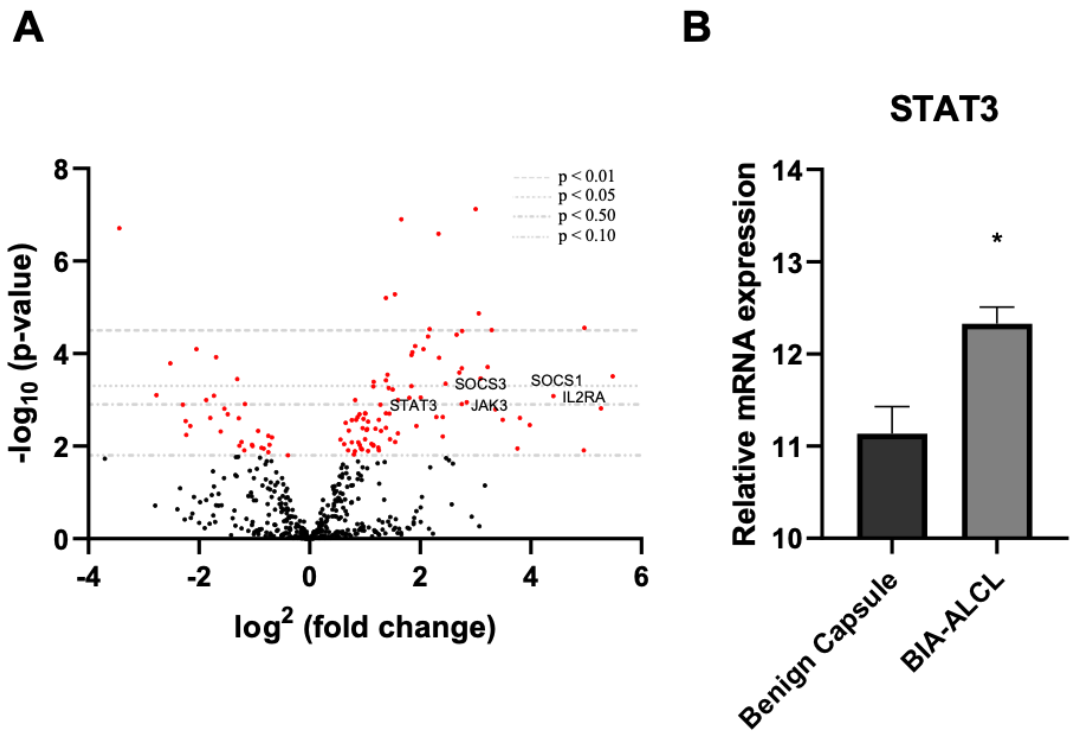


Figure 6-4. A) **Volcano plot of differential gene expression for the JAK-STAT3 pathway. B) Relative mRNA expression of STAT3.**
Horizontal lines denote various false discovery rate thresholds. *$p < 0.05$.

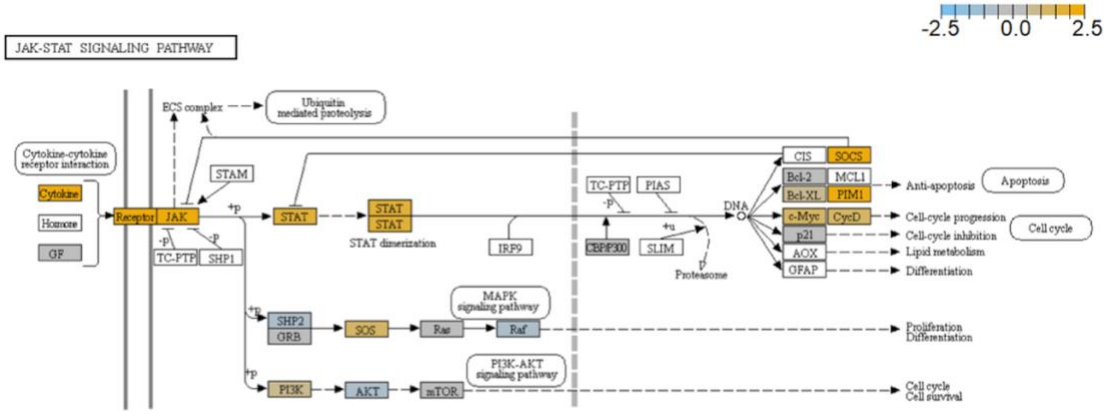


Figure 6-5. JAK-STAT signaling pathway.
Overview of KEGG pathway diagram of differentially expressed JAK-STAT3 signaling pathway genes in BIA-ALCL. Orange denotes increased expression while blue signifies decreased expression.

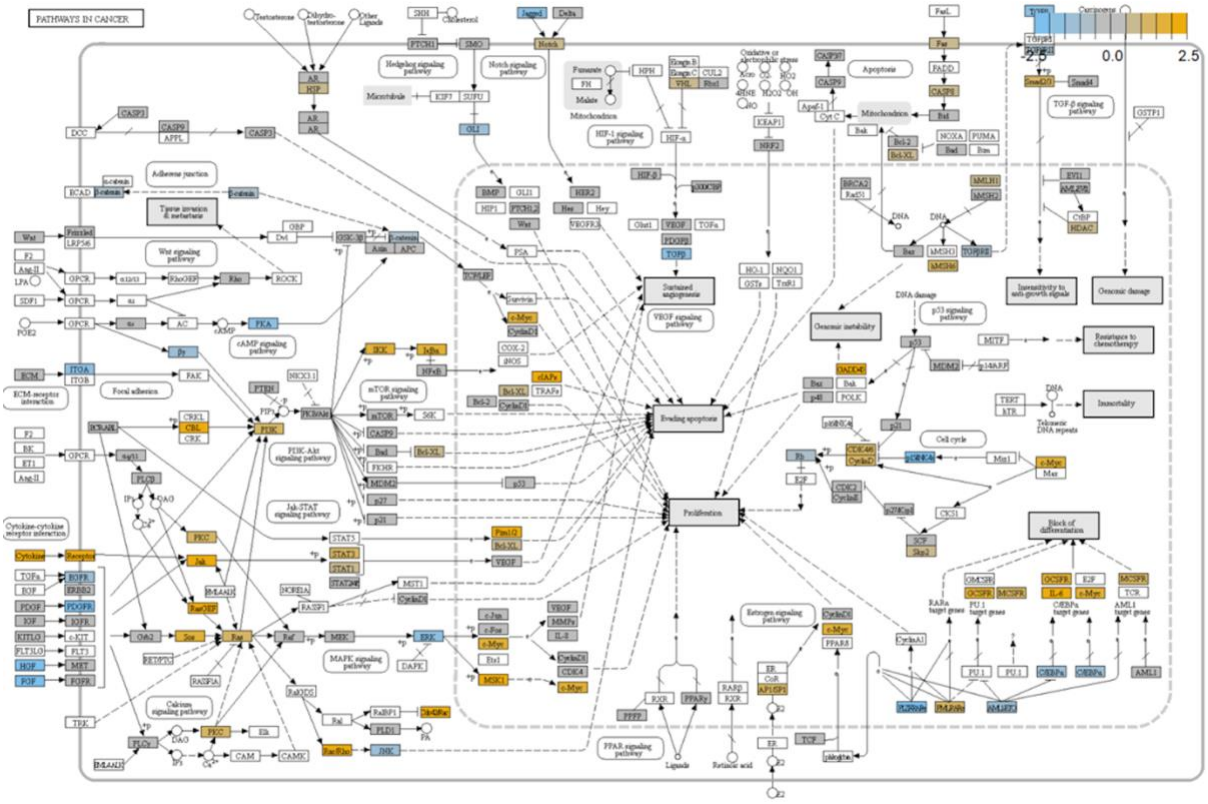


Figure 6-6. KEGG pathway showing global gene expression and interaction between 13 cancer-associated pathways in BIA-ALCL.
Orange indicates increased gene expression and blue indicates decreased gene expression.

6.1.9 pSTAT3 is aberrantly activated in BIA-ALCL

Work in other types of ALK- ALCL has shown that activating mutations in the JAK-STAT pathway leads to increased nuclear localization of pSTAT3$_{Y705;56}$ however, this has not been investigated in BIA-ALCL. Having established significant genetic alterations in JAK-STAT pathway contributors in BIA-ALCL patient samples in previous experiments, we set out to determine the activation status of *STAT3* at the protein level using IHC analysis. Representative pSTAT3 images with paired H&E images are shown in **Figure 6-7A**. Cell counts performed on 5-10 high-magnification images per sample revealed that BIA-ALCL samples had a significantly higher average of pSTAT3+ cells per HPF (68.65±31.57) than benign capsular tissues (23.93±16.93; $p < 0.031$) (**Figure 6-7B**).

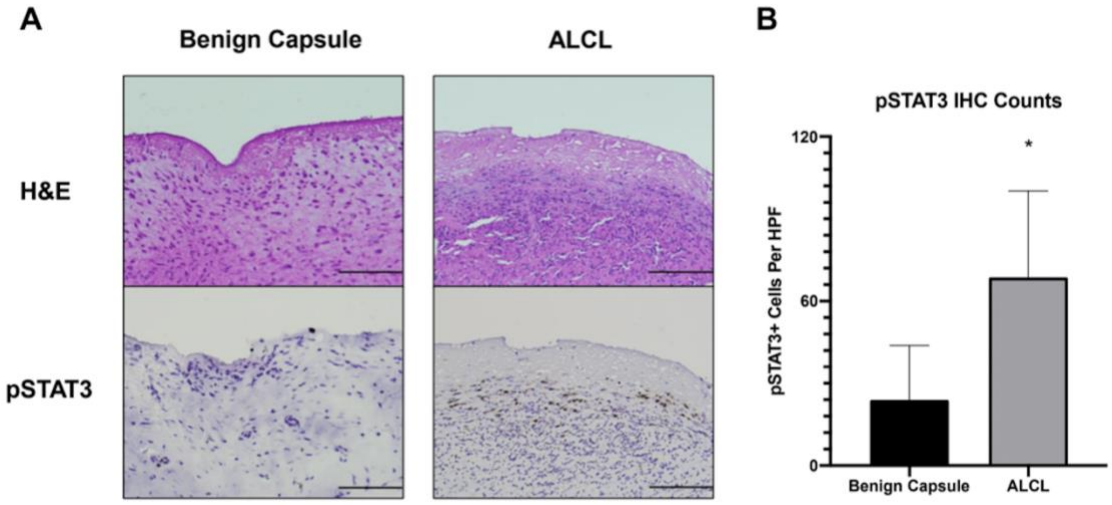


Figure 6-7. pSTAT3 Signaling in BIA-ALCL samples.
A) H&E and pSTAT3 immunohistochemical staining performed on benign capsular tissue and tumor samples isolated from patients with biopsy-proven BIA-ALCL. B) pSTAT3+ cell counts per HPF performed on independent benign capsules (n = 4) and BIA-ALCL

Discussion

Recent work has established BIA-ALCL as a highly treatable specific type of ALK- ALCL that is associated with textured-surface breast implants.[25,30] Early investigation implicated activating genetic mutations as the central drivers in BIA-ALCL tumorigenesis and progression. However, the evidence to substantiate these claims was lacking—specifically regarding the translation of known genetic mutations to oncogenic pathway activation.[131] This current study sought to identify the molecular drivers of BIA-ALCL using hybridization-based transcriptional profiling to identify actionable genetic mutations leading to oncogenic pathway activation—aiding in the diagnosis and treatment of the disease. With the knowledge that other types of ALK- ALCL rely on aberrant JAK-STAT pathway activation, we hypothesized that BIA-ALCL samples would harbor genetic mutations within this pathway that would lead to an increase in nuclear localization of pSTAT3. Consistent with our hypothesis, we found that BIA-ALCL tumors exhibit widespread JAK-STAT3 pathway upregulation, including a 2.26-fold-change in *STAT3* expression. Constitutive pathway activation directly correlated with increased pSTAT3 nuclear localization in BIA-ALCL tissues by IHC, demonstrating that increased *STAT3* gene expression results in protein phosphorylation and downstream events that stimulate proto-oncogenes (*MYC*, *CCND1*, and *PIM1*).

Data from this study revealed several key characteristics of BIA-ALCL that underpin a disease pathogenesis that is unique and distinct from other types of lymphoma. Despite activating mutations in JAK-STAT pathway constituents occurring in the minority (~20%) of T-cell lymphomas, gain-of-function mutations traditionally lead to enhanced growth capabilities of affected cells.[132] Using Nanostring technology, our

current study identified a decrease in growth factor (*PDGF*, *FGF2*) and growth factor-receptor (*PDGFR*) related gene expression in BIA-ALCL samples. Further, GSS and pathway analysis scoring revealed decreased activation in pathways involved in growth and differentiation, including PI3K, Wnt, and MAPK. Within hematologic malignancies, overexpression of PI3K or Wnt signaling has been linked to a poorer prognosis [133] and treatment resistance. BIA-ALCL remains very treatable, albeit through complete surgical excision, indolent disease process, which may explain the downregulation of PI3K rather than indicating a functional change in cellular growth capacity or the nutrient availability within the tumor microenvironment. Tumor cells undergo oncogenesis in multiple ways—namely through rapid upregulation of growth pathways superseding regulatory measures that would otherwise limit cellular expansion or through the careful avoidance of apoptotic and immune clearance protocols for defective or mutated cells.[134] GSS and pathway analysis performed in this study reveal that BIA-ALCL cells most likely avoid clearance, as the highest scores obtained in this study were in pathways involved in DNA damage repair, transcriptional dysregulation, and cell cycle control-apoptosis. Importantly, mutations in the p53 protein family—a strong activator of apoptotic paradigms—have been linked to BIA-ALCL cases.[79] Taken together, the data from this study support the notion that BIA-ALCL tumor cells have modest cellular growth activation and achieve lymphomatosis through the alteration of cell-cycle checkpoints and avoidance of apoptosis paradigms.

Our sequencing data corroborate other BIA-ALCL studies by Blombery et al.,[51,52] Di Napoli et al.,[53] and Oishi et al.,[16] who independently implicated JAK-STAT3 pathway activation in disease pathogenesis. Importantly, Blombery and colleagues were the first to

link gain-of-function mutations in STAT3 p.S614R and JAK1 somatic variant (G1097V) with the disease.[52] In a follow-up study, this group identified the first somatic *STAT3* mutation conserved between 7 of 11 independent BIA-ALCL samples studied using targeted-next generation sequencing (NGS), implicating shared JAK-STAT activation in disease progression. Similarly, Oishi and colleagues interrogated 15 unique BIA-ALCL tumor specimens by NGS.[16] In addition to the known oncogenic variants, this group identified a *STAT3* p.S616R variant, while simultaneously discovering two instances of novel STAT3 Y640F gain-of-function mutations, as well as a JAK1 missense variant (G1097D). Both the S616R and Y640F amino acid substitutions affect the SH2 domain, which is known to activate *STAT3* constitutively, suggesting that these gain-of-function mutations could be definitively linked to nuclear localization of activated protein. Following up on this idea, Letourneau et al. linked dual activating JAK1 (G1079V), STAT3 (p.S614R) mutations, and high pSTAT3 expression in a single case of BIA-ALCL.[77] Our study builds on the current literature by providing direct evidence that activating mutations in the JAK-STAT pathway lead to pSTAT3 nuclear localization. Specifically, we found a 2.26-fold increase in *STAT3* expression in BIA-ALCL tumor specimens relative to controls ($p < 0.014$), and these data were strengthened by the 3-fold increase in nuclear localization of pSTAT3 protein ($p < 0.03$). Our work suggests that JAK-STAT activation is pervasive across independent BIA-ALCL samples and is a distinct feature separating disease tissue from benign capsular controls.

The JAK-STAT pathway has multiple distinct levels of activation, and elevated pSTAT3 nuclear localization could be an indication of a chronic inflammatory milieu within the breast pocket rather than a true pathognomonic finding in ALCL. To

investigate changes within the T-cell population specifically, Di Napoli et al. interrogated the molecular signatures of microdissected BIA-ALCL tumor specimens (n = 6) as compared with normal T-cells and other peripheral T-cell lymphomas.[17] Gene set enrichment analyses revealed *STAT3* activation when comparing transcripts from BIA-ALCL tumor specimens to healthy T-cells, suggesting that *STAT3*-related signaling mechanisms are inherent to the BIA-ALCL disease process, not necessarily to all T-cell lymphomas. In line with this thinking, Lechner and colleagues demonstrated that BIA-ALCL tumor cell lines (TLBR-1) showed increased activation of *STAT3* and that exposure to a *STAT3*-specific inhibitor resulted in tumor cell death;[83] molecular testing of TLBR 2 - 4 later showed similar results.[82] Collectively, these data suggest a possible avenue of lymphomagenesis that may progress from chronic inflammation to true oncogenesis through the JAK-STAT3 pathway.[11] This theory introduces multiple levels of potential intervention to ablate JAK-STAT signaling and prevent transformation to BIA-ALCL or limit the progression of the disease to a higher stage.

Localized BIA-ALCL typically responds to en bloc resection, which includes removal of the implant with complete capsulectomy and obtaining clear margins. However, in advanced disease, which can include lymph node involvement or distant metastasis, per NCCN guidelines, patients should receive adjuvant chemotherapy, or radiation.[32] Currently approved recommendations include CHOP therapy and dose-adjusted EPOCH. Nevertheless, ALK- ALCL cases convey 5-year survival rates around 49%, and BIA-ALCL, which exhibits 5-year survival rates around 75%, are more likely to follow a relapsing-remitting course than patients with ALK+ ALCL.[135,136] As such, our data showing pervasive JAK-STAT activation in BIA-ALCL might inform treatment

strategies for advanced disease to improve long-term outcomes. Specifically, the presence of JAK-STAT inhibitors represents an untapped area of potential research for affected patients. Work by Chen and colleagues revealed that ALK- ALCL cells, *in vitro*, depend on *JAK1* and *STAT3* for survival.[88] Further, this group demonstrated that pSTAT3+ ALK- ALCL cells *in vitro* and in a xenograft model of ALCL were sensitive to JAK inhibitors, including ruxolitinib. Taken together, with the pervasive JAK-STAT activation seen in the current study, JAK inhibitors represent an attractive area of future research in patients with advanced disease refractory to standard adjuvant therapy recommendations.

6.1.10 Limitations

While this pilot study revealed several key characteristics of BIA-ALCL, some limitations should be considered when interpreting the results. While statistically significant differences in gene expression were identified, it is important to note that our study may have been somewhat limited by statistical power due to the small number of tumor specimens available to interrogate. While this is an inherent limitation of studying a rare disease, these gene expression profiles may not be representative of all BIA-ALCL tumors, particularly when considering differences across pathologic stage. Nevertheless, the fact that we did achieve statistical significance in our pathway (JAK-STAT) and genes of interest (e.g., *STAT3*) demonstrates that the present study was adequately powered to detect those differences. Transcripts that did not show differential expression profiles may be a result of limited statistical power that could result in a type II error, or it may simply be indicative of a lack of gene involvement. Nevertheless, it is possible that gene expression patterns and downstream effects may show differences in a larger study.

Conclusions

Oncogenic signaling in BIA-ALCL is highly complex and exhibits widespread JAK-STAT pathway involvement as well as other cancer-associated pathways. Differential expression of the JAK-STAT3 pathway may provide a mechanism for malignant transformation of lymphocytes residing within benign breast implant capsular tissue. These data provide novel insights into the biological basis of BIA-ALCL and highlight the potential role of *STAT3* as a potential biomarker, as well as providing a novel therapeutic target. Future work should seek to determine the role of *STAT3* as a diagnostic tool and prognostic indicator and to identify whether or not expression correlates with disease severity (e.g., TNM staging). Large scale, functional analyses are needed to determine further the central role genetics may play in BIA-ALCL tumorigenesis and progression.

CHAPTER 7. CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

BIA-ALCL is an emerging cancer on the immune system associated with textured surface breast implants. Although the knowledge of the disease has evolved rapidly in recent years, specifically regarding diagnosis and treatment, the evidence supporting current clinical recommendations has not been critically appraised. Widespread variations in epidemiological reporting have only complicated the ability to mitigate risks associated with textured devices. Adding to the complexity of the disease, the molecular mechanisms responsible for BIA-ALCL tumorigenesis have yet to be elucidated. This chapter provides a brief summary of the findings of this dissertation and establishes research priorities for current and future research endeavors.

Current evidence aligns with consensus guidelines and treatment recommendations

Recent treatment advances have been highlighted by complete surgical resection and device removal as the standard of care in the majority of cases. Other advancements include the reservation of adjuvant therapy for cases with advanced disease or those refractory to oncologic resection, as well as reconstructive techniques following complete resolution. Despite the limited number of high-quality studies related to the diagnosis and treatment of BIA-ALCL, this study demonstrated that current evidence supports clinical recommendations and aligns with National Comprehensive Cancer Network consensus guidelines. It also provides a comprehensive clinical update on the epidemiology and pathophysiology of the disease, in addition to the advances in diagnosis and treatment as just described. Given the number of women with breast implants who are at risk for developing the disease, this study reinforced current clinical guidelines while bringing a

heightened awareness of this emerging disease to clinicians from diverse specialties including breast surgeons, surgical oncologists, plastic surgeons, and pathologists, as well as general practitioners that encounter patients with textured breast implants in their daily practice.

Smooth surface devices are oncologically safe; the risk of BIA-ALCL is not well defined

This is the first study to review the epidemiology of BIA-ALCL systematically. Not surprisingly, this study demonstrated substantial gaps in the current knowledge regarding risk profiles and BIA-ALCL tumorigenesis for currently available textured-surface breast devices in the U.S. market. With the removal of Allergan textured surface devices from the U.S. market, the risk of BIA-ALCL for currently available textured devices is not well-defined. Given these findings, an accurate risk of BIA-ALCL cannot. As such, surgeons are strongly advised against utilizing textured devices regardless of whether it is for cosmetic or reconstructive purposes until more accurate risk profiles can be determined.

As previously mentioned, certain governmental regulatory agencies (e.g., U.S. FDA) maintain that there is a possible association between smooth surface devices and BIA-ALCL. Refuting those claims, this study also illustrated the oncologic safety of smooth surface devices. Despite this finding, larger prospective studies with head-to-head comparisons between smooth surface and textured breast devices are needed before definitive conclusions should be drawn.

JAK-STAT3 pathway upregulation is a key molecular feature of BIA-ALCL tumors

Previous molecular studies have sought to determine the molecular events responsible for oncogenic transformation. However, the biologic mechanisms remain poorly understood. Hybridization-based transcriptional profiling was used to compare molecular signatures in BIA-ALCL tumors to healthy controls in order to investigate the molecular mechanisms of the disease. This represents one of the first studies undertaken that attempts to better understand the pathogenesis of this rare but emerging disease. Of particular interest was the observation that BIA-ALCL tumors exhibit pervasive JAK-STAT3 upregulation that results in downstream events that promote tumorigenesis through the alteration of cell-cycle checkpoints and avoidance of apoptosis paradigms. Based on these findings, a novel mechanism was proposed whereby an over-active immune system facilitates the malignant transformation of peri-implant lymphocytes via the JAK-STAT3 pathway. These data highlight the JAK-STAT pathway as a novel therapeutic target for patients with advanced disease while providing avenues for future investigation that might lead to an increased understanding of the mechanisms of lymphomagenesis in BIA-ALCL and potentially other types of ALK- ALCL.

Future directions

In-vivo model of BIA-ALCL

An *in vivo* model of BIA-ALCL is needed to further elucidate the molecular mechanisms not only responsible for tumorigenesis but also disease progression. Our group has previously suggested that CRISPR-Cas9 technology may be used to engineer a murine model of pathogenesis under a variety of conditions.[19] Perhaps more feasible would be the development of a xenograft model using established tumor cell lines to further interrogate mechanisms of pathogenesis and to assess the efficacy of targeted therapies (e.g., “JAKanibs”). Although, the reluctance among investigators to deposit

tumor cell lines in tumor repositories complicates such an endeavor. As mentioned in this dissertation, previous cell lines have since been discontinued by ATCC. Another interesting avenue of research would be the use of an IL-10 knockout in order to further investigate the role of the chronic inflammation and to assess possible differences that may exist in how smooth surface and textured devices influence the inflammatory milieu.

Macrophage phenotype and particulate matter digestion

Macrophage phenotype is known to influence the tumor microenvironment. Specifically, differentiation to an M2 or alternatively activated phenotype exhibits anti-inflammatory, pro-tumor effects.[137] Further investigation should seek to determine the predominant macrophage phenotype in BIA-ALCL and if polarization to tumor-associated macrophages plays a role in shaping the tumor microenvironment to promote progression and metastasis. Conceptually, this could be achieved by using a macrophage polarization model co-cultured with BIA-ALCL tumor cells. Although this is an oversimplification of the cellular and molecular events under experimental conditions, such an investigation may inform future avenues of research that could potentially reprogram M2 macrophages to an M1 phenotype which could allow for targeted therapies, possibly alleviating the necessity of complete surgical resection or the need for adjuvant therapy under certain conditions.

As discussed in chapter three, macrophage digestion of foreign particles, possibly from the surface of textured devices, may lead to frustrated phagocytosis resulting in a chronically over-activated immune system that predisposes to errors in DNA replication and driver gene mutations (e.g., STAT3).

Correlation of STAT3 expression with clinical outcomes

As shown in this dissertation, BIA-ALCL tumors overexpress *STAT3*. More extensive studies are needed to determine the extent of *STAT3* expression in BIA-ALCL. Specifically, future work should seek to correlate *STAT3* expression across tumor stage and determine if expression correlates with clinical outcomes, which may highlight the role of *STAT3* as a potential biomarker and prognostic indicator.

Implant surface characteristics

Perhaps the most important distinguishing feature of this disease is the marked distinction between cases based on implant surface characteristics and, more specifically, texturization. The lack of an association between smooth-surface breast devices and BIA-ALCL, as demonstrated in this dissertation, unequivocally establishes the role of texturization in the pathogenesis of the disease. As such, concerted research efforts to better define differences in the material properties between smooth and textured breast devices and how potential differences can influence a host-specific response leading to tumorigenesis are warranted.

APPENDIX: SOUTHEASTERN SOCIETY OF PLASTIC AND RECONSTRUCTIVE SURGEONS RESEARCH GRANT

Purpose of Project: Genetic susceptibility is thought to play a major role in the pathogenesis of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). Previous genetic studies using next-generation sequencing methods have identified oncogenic mutations in the JAK/STAT3 pathway among several others. However, both studies were limited by sample size and a lack of controls, making it difficult to draw larger conclusions about the role of genetics in the pathogenesis of BIA-ALCL. Furthermore, the manner in which these genes and others are expressed and how they affect the tumor microenvironment in BIA-ALCL have yet to be elucidated. Thus, the molecular mechanisms responsible for the tumorigenesis in BIA-ALCL remain undefined, which remains a critical barrier to advancing our scientific understanding of this disease. Gene discovery by DNA microarray has led to the identification of novel genes in many cancers as well as major breakthroughs in tumor molecular biology. This study aims to utilize the systematic application of transcriptome-wide microarray analysis in banked BIA-ALCL tumor specimens and healthy control tissue in order to define the molecular mechanisms of BIA-ALCL. Defining the molecular mechanisms of BIA-ALCL is essential for guiding future research efforts that ultimately seek to improve patient safety.

Background: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is an emerging and potentially lethal cancer of the immune system that is associated with textured-surface breast implants .To date, over 500 cases have been reported worldwide, and 16 deaths have occurred as a result of receiving this type of breast implant.

Conversely, no cases of BIA-ALCL have been reported in patients with smooth-surface breast implants. BIA-ALCL has been shown to occur in women undergoing breast augmentation or post-mastectomy breast reconstruction at similar frequencies. The number of reported cases continues to rise, with the incidence increasing 15% over the last year alone. As such, BIA-ALCL poses a significant public health risk to women undergoing breast augmentation or implant-based breast reconstruction. Genetic susceptibility is thought to play a major role in the pathogenesis of this emerging cancer. Several hypotheses attempting to explain the pathogenesis of BIA-ALCL have been postulated. The current consensus is that BIA-ALCL occurs as a result of a chronic inflammatory state that leads to unregulated T-cell proliferation in a genetically susceptible individual. The exact cause of the chronic inflammation, including allergic inflammation, remains a highly debated topic, and the molecular mechanisms by which chronic inflammation leads to aberrant T-cell clonal expansion in BIA-ALCL have yet to be elucidated. Arguably, only genetic predisposition in the setting of chronic inflammation could account for the relatively low number of reported cases. Previous genetic studies of BIA-ALCL have been limited by small sample size and a lack of adequate controls. Previous genetic studies using next-generation sequencing methods have identified oncogenic mutations in the JAK/STAT3 pathway among several others. However, both studies were limited by sample size and a lack of adequate controls, making it difficult to draw larger conclusion about the role of genetics in the pathogenesis of BIA-ALCL. As such, the molecular mechanisms that lead to tumorigenesis in BIA-ALCL remain undefined which remains a critical barrier to advancing our scientific understanding of this disease. Gene discovery by transcriptome-wide microarray has led

to the identification of novel genes in many cancers as well as major breakthroughs in tumor molecular biology. This study aims to utilize the systematic application of transcriptome-wide microarray to measure differential gene expression in banked BIA-ALCL tumor specimens and healthy control tissue. The use of a transcriptome-wide microarray to define the molecular mechanisms of BIA-ALCL in this study is innovative, as is the use of a healthy control tissue for genetic comparisons. Defining the molecular mechanisms responsible for the pathogenesis of BIA-ALCL is critical for guiding the direction of future BIA-ALCL research and improving patient safety. For example, uncovering differentially expressed clusters of genes tumors will assist in determining the molecular networks that control the progression of the pathogenesis of BIA-ALCL. Additionally, knowing how gene expression affects tumorigenesis in BIA-ALCL will allow for the identification of oncogenic mutations in the future through targeted rather than shotgun approaches to genetic sequencing. Lastly, derivation of such data could have profound implications, which could ultimately lead to the development of clinical gene arrays for pre-operative risk stratification that could identify high-risk women undergoing breast augmentation or implant-based breast reconstruction.

Methods: This pilot study follows a case-control study design Identification, recruitment and enrollment of eligible participants: Healthy control group (controls) Inclusion criteria: (a) Female patients; (b) age >22 years; (c) presenting for breast implant exchange with textured or smooth surface breast implants will be screened for study inclusion. The control group will be identified during routine pre-operative clinic visits for breast implant exchange at the University of Kentucky (UK), MD Anderson Cancer Center, and the Mayo Clinic (Jacksonville). Patients will not be compensated. Drs. Vasconez,

Clemens, Rinker, and DeCoster will be responsible for enrolling and obtaining written informed consent from participants at their respective institutions. We expect to enroll approximately eight participants in our control group (n=8 (smooth surface=4, textured surface=4)). We do not anticipate problems with obtaining control samples given the volume of implant exchange performed annually at each institution. Approximately 10mg of breast implant capsule tissue will be collected at the time of surgery. Tissue will be frozen and stored at -80C. The Biospecimen Procurement and Translational Pathology Shared Resource Facility (BPTP SRF) at UK will assist with obtaining informed consent, tissue collection, storage, and preparation of all specimens identified and received at UK. All tissue will be de-identified. Identification, recruitment, and enrollment of eligible participants: BIA-ALCL group (cases) FFPE/Frozen BIA-ALCL specimens (n=4) have already been collected and are currently available at a biorepository that is directed by Dr. Clemens and located at MD Anderson. All tissue will be de-identified. All cases have been reviewed by a board-certified, fellowship-trained hematopathologist in order to verify the accuracy of the BIA-ALCL diagnosis. In consultation with the Office of Research Integrity at UK, the determination has been made that IRB approval is not required as long as the tissue is de-identified and is for research purposes only.

RNA extraction and microarray analysis RNA will be extracted from tissues in FFPE/ frozen states using Qiagen RNeasy minikit (50) and analyzed with a 2100 Bioanalyzer (Agilent) to ensure integrity. RNA (300 ng) from 3 independent samples per group will be used for complementary DNA (cDNA) synthesis and labeling, using a GeneChip Whole Transcript cDNA Synthesis and Amplification Kit and a GeneChip WT Terminal Labeling Kit (Affymetrix). Labeled cDNA samples will be hybridized using a RES HT

ARRAY WT Protocol on an Affymetrix HTA array plus PICO processing and will be scanned at the Microarray Shared Resource Facility at the University of Kentucky. Intensity scans from 3 independent GeneChips/groups will be subjected to gene expression analysis using Partek Genomic Suite, version 7.18. Variations among the samples in each group will be examined by principal components analysis and subjected to hierarchical and partition clustering with the Partek Genomic Suite.

Functional gene network analysis The gene expression data derived from microarray analysis will be subjected to Ingenuity Pathways Analysis (IPA; Ingenuity Systems) to generate functional molecular networks. A fold change cutoff of 2.0 will be established to identify and assign molecules to the Ingenuity Knowledge Base. Gene expression changes will be considered in the context of physical, transcriptional, or enzymatic interactions of the gene/gene products and then grouped according to interacting gene networks. Expression of selected genes from cluster analysis will be confirmed by Realtime polymerase chain reaction (PCR).

Statistical analysis Significance of differences in microarray data among cases and controls will be analyzed with Fisher's exact and ANOVA tests as appropriate using Partek Genomic suite. ANOVA with Tukey's honest significant difference post hoc test will be applied using SPSS version 25 and will be used to determine the significance levels of real-time PCR data. Subgroup analysis will also be performed to assess for gene expression differences between implant surface types and to assess for temporal differences in gene expression.

Budget:

Personnel:

-Henry Vasconez, MD, 1% effort $4,019

-Tim Butterfield, PhD, 1% effort $1,237

-Betsy Fink, BS, 2% effort $1,418

$314

$900

Supplies:

-Qiagen RNeasy MiniKit (50) Total RNA Isolation Kit

-Qualitative real-time PCR kit for microarray validation

-Tissue Microarray slides (12 @ $25/slide) $300

Other:

-Shipping of tumor and control specimens from collaboration sites $300

-Tissue banking of BIA-ALCL and control specimens (12 samples @ $35/sample) $420

-RNA Isolation (12 samples @ $15/sample) $180

-Test Tissue Microarray $10

-RES HT ARRAY/PICO WT PROTOCOL, Affymetrix HTA array plus Pico WT processing

(12 specimens @ $457.39/sample) $5,489

Total: $14,587

Collaboration:

Henry Vasconez, M.D. Principal Investigator (1.0% effort) Dr. Vasconez is the William S. Farish Endowed Chair in Plastic Surgery and is Professor of Plastic Surgery at the University of Kentucky College of Medicine. He will assume overall responsibility for the project. He will facilitate the accrual of study-eligible women from which control specimens can be obtained at the University of Kentucky. Additionally, Dr. Vasconez will be responsible for oversight of the receipt and transfer of specimens from MD Anderson Cancer Center to the Genomics Core facility at the University of Kentucky Markey Cancer Center. Dr. Vasconez will contribute his expertise to the analysis of data and preparation of manuscripts related to the research.

Tim Butterfield, Ph.D. Co-Principal Investigator (1.0% effort) Dr. Butterfield is an Associate Professor of Rehabilitation Science and Physiology at the University of Kentucky. He has an expertise in chronic inflammatory-related disease states and has published several times in the area of Transcriptome-wide microarray. Dr. Butterfield will also contribute his expertise to the analysis of data and preparation of manuscripts related to the research.

Other Significant Contributors:

Ryan DeCoster, M.D. Dr. DeCoster is a post-doctoral research fellow within the Division of Plastic Surgery at the University of Kentucky. Dr. DeCoster is currently pursuing a research fellowship in plastic surgery while obtaining a Ph.D. in Clinical and Translational Science. The focus of his dissertation is on the molecular mechanisms of BIA-ALCL. Dr. DeCoster is currently funded under a National Institutes of Health (NIH)/National Cancer Institute (NCI) T32 training grant. Dr. DeCoster will contribute his expertise with data and preparation of manuscripts related to the research.

Mark Clemens, M.D. Dr. Clemens is an Associate Professor of Plastic Surgery at the University of Texas MD Anderson Cancer Center. Dr. Clemens is considered by many as the world's leading expert in BIA-ALCL and has published extensively on the topic. Dr. Clemens serves as the American Society of Plastic Surgeons (ASPS) liaison to the Food and Drug Administration for BIA-ALCL and chairs a subcommittee for the ASPS overseeing national research and education efforts for this cancer. He currently runs the BIA-ALCL tissue biorepository at MD Anderson. He will facilitate the accrual of study-eligible women from which control specimens can be obtained at MD Anderson Cancer Center. Additionally, Dr. Clemens will be responsible for overseeing the preparation and

shipment of BIA-ALCL and control specimens from MD Anderson Cancer Center to the University of Kentucky. Dr. Clemens will contribute his expertise with data and preparation of manuscripts related to the research.

Brian Rinker, M.D. Dr. Rinker is Professor of Plastic Surgery at the University of Kentucky College of Medicine. He has accepted a position as the Chief of Plastic Surgery at the Mayo Clinic (Jacksonville). Dr. Rinker will facilitate the accrual of study-eligible women from which control specimens can be obtained at the Mayo Clinic (Jacksonville). Lastly, Dr. Rinker will contribute his expertise with data and preparation of manuscripts related to the research.

Betsy Fink, BS, Research Associate (2.0% effort) Mrs. Fink has 25+ years' experience with clinical research study coordination on funded studies, including the National Institutes of Health. Her role in this study will include assistance with obtaining informed consent as well as tissue collection and serving as a liaison to the BPTP SRF and MicroArray Core. Mrs. Fink is proficient with IRB preparation, maintenance, and documentation for the Division of Plastic Surgery at the University of Kentucky and will provide support to this project in those areas as well.

Facilities:

Biospecimen Procurement and Translational Pathology Shared Resource Facility (BPTP SRF) The NCI-designated Markey Cancer Center BPTP SRF is located on the same campus as the PI and within short walking distance. The BPTP SRF collects, processes, annotates, stores, and distributes biospecimens to support translational research in cancer and other diseases. BPTP SRF support includes obtaining Institutional

Review Board (IRB) certification, collecting Informed Consents or the acquisition, and the processing of targeted biospecimens in accordance with specific research protocols.

MicroArray Core Facility The MicroArray Core Facility at the University of Kentucky is located a short walking distance from the office of the PI. He has access by appointment on a fee-for-service basis. The MircoArray Core provides comprehensive state-of-the-art microarray services and resources for the analysis of gene expression, including Affymetrix GeneChip Technology, preparation of RNA samples, experimental design, and bioinformatics support for genomic data reduction and analysis. Fee-based services include assessment of RNA quality and concentration with an Agilent 2100 Bioanalyzer and a NanoDrop 2000 spectrophotometer, as well as RNA labeling, chip hybridization, scanning and data collection. Available instrumentation includes an Affymetrix GCS 3000 7G scanner, GeneChip Fluidics Station 450, GeneChip Hybridization Oven 640 and a Statistical and Bioinformatics Software Computer Workstation. Services also include statistical analysis of microarray data and experimental design consultation (Arnold Stromberg, Ph.D.).

Other Financial Awards and Conflicts of Interest:

Dr. DeCoster is currently funded by an NIH/NCI(T32CA16003) T32: *Oncology Research Training for Surgeon-Scientists* training grant.

Dr. Vasconez is the William S. Farish Endowed Chair in Plastic Surgery. Dr. Clemens receives funding from the Plastic Surgery Foundation.

Dr. Butterfield is currently funded by an NIH RO1 (1R01AT009268-01A1). There are no conflicts of interest to report with any of the study personnel.

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VITA

Ryan C. DeCoster, M.D.

EDUCATION

Graduate

2017 - Present **Ph.D. candidate**, Clinical and Translational Science
University of Kentucky College of Medicine
Lexington, Kentucky

Professional

2017 **M.D.,** with distinction
University of Kentucky College of Medicine
Lexington, Kentucky

Undergraduate

2013 **B.S.,** Topical Major-Biophysics, magna cum laude with departmental honors.
University of Kentucky College of Arts and Sciences
Lexington, Kentucky

PROFESSIONAL EXPERIENCE

2018 – Present NIH/NCI T32 Post-Doctoral Fellowship (Tumor Biology)
PI: B. Mark Evers, M.D., F.A.C.S.
Lucille P. Markey Cancer Center
University of Kentucky
Lexington, Kentucky
Mentor: B. Mark Evers, M.D., F.A.C.S.

2017-2018 Post-Doctoral Fellowship
Division of Plastic and Reconstructive Surgery
University of Kentucky College of Medicine
Lexington, Kentucky

MILITARY EXPERIENCE

Dates of Military Service: July 2004-May 2010
Branch: United States Navy
Highest Rank Achieved: Hospital Corpsman 2nd Class-HM2 (FMF), E-5
Discharge Type: Honorable

2009-2010	Education and Training Petty Officer Battalion Aid Station, Headquarters Co. 2nd Battalion, 6th Marines Camp Lejeune, North Carolina
2008-2009	Senior Line Company Corpsman Echo Co, Battalion Landing Team 2/6 26th Marine Expeditionary Unit Camp Lejeune, North Carolina
2007-2008	Senior Line Corpsman 1st platoon, Echo Co 2nd Battalion, 6th Marines Camp Lejeune, North Carolina
2006	Hospital Corpsman Detention Hospital, Joint Medical Group Joint Task Force Guantanamo Bay, Cuba
2006	Hospital Corpsman Task Force Gold Joint Task Force Guantanamo Bay, Cuba
2005	Emergency Medical Technician, Casualty Receiving Department USNS Comfort T-AH20 Joint Task Force Hurricane Katrina
2004-2006	Hospital Corpsman, Department of Internal Medicine and Pediatrics Naval Hospital, Beaufort South Carolina

COMMITTEE RESPONSIBILITIES

University of Kentucky

1) 2019-pres Trainee Advisory Council, Markey Cancer Center
2) 2019-pres Trainees in Research Advisory Committee, College of Medicine

HONORS AND AWARDS

2019 National Institutes of Health, National Cancer Institute Ruth L Kirschstein, M.D. National Research Service Award (T32CA160003): *Oncology Research Training for Surgeon-Scientists* PI: B. Mark Evers, M.D.

2019 Markey Cancer Center Trainee Travel Award ($500)
Lucille P. Markey Cancer Center
University of Kentucky

2018 Undergraduate Research Enhancement Award ($500)
College of Health Sciences
University of Kentucky

2018 National Institutes of Health, National Cancer Institute Ruth L Kirschstein, M.D. National Research Service Award (T32CA160003): *Oncology Research Training for Surgeon-Scientists* PI: B. Mark Evers, M.D.

2016 Society for Vascular Surgery Medical Student Travel Scholarship ($750)

2013 Research Travel Grant ($400)
Office of Undergraduate Research
University of Kentucky

2012 Summer Scholar Appointment
Center for Muscle Biology
University of Kentucky

2010 French Fourragere
Echo Co., 2nd Battalion, 6th Marines
Camp Lejeune, North Carolina

2009 Navy and Marine Corps Achievement Medal
Echo Co., Battalion Landing Team 2/6
26th Marine Expeditionary Unit

Camp Lejeune, North Carolina

2008 Sea Service Deployment Ribbon (3rd award)
Echo Co., Battalion Landing Team 2/6
26th Marine Expeditionary Unit

2008 Marine Corps Rifle Expert

2008 Navy Good Conduct Medal

2008 Letter of Appreciation, Commanding Officer 2nd Battalion, 6th Marines
Second Marine Division Association 67th Anniversary
Camp Lejeune, North Carolina

2007 Marine Corps Meritorious Mast
Echo Co, 2nd Battalion, 6th Marines
Camp Baharia, Iraq

2007 Combat Action Ribbon
Echo Co, 2nd Battalion, 6th Marines
Fallujah, Iraq

2007 Iraqi Campaign Service Medal with Eagle, Globe and Anchor Device
Echo Co, 2nd Battalion, 6th Marines
Al Anbar Province, Iraq

2007 Sea Service Deployment Ribbon (2nd award)
Echo Co, 2nd Battalion, 6th Marines
Al Anbar, Iraq

2006 Blue Jacket Sailor of the Year
Naval Hospital Beaufort, South Carolina

2006 Navy Pistol Sharpshooter

2006 Joint Service Achievement Medal
Detention Hospital, Joint Medical Group
Joint Task Force Guantanamo Bay, Cuba

2006 Joint Service Achievement Medal
Task Force Gold
Joint Task Force Guantanamo Bay, Cuba

2006 Global War on Terrorism Expeditionary Medal

2006 Sea Service Deployment Ribbon (1st award)

Joint Task Force Guantanamo Bay, Cuba

2005 Humanitarian Service Medal
Joint Task Force Hurricane Katrina
USNS Comfort T-AH20
New Orleans, Louisiana

2005 Letter of Commendation
Commanding Officer, USNS Comfort T-AH20
Joint Task Force Hurricane Katrina
New Orleans, Louisiana

2004 National Defense Service Medal

2004 Global War on Terrorism Service Medal

2004 Meritorious Promotion
Naval Recruit Training Command
Great Lakes, Illinois

MEMBERSHIP IN SCIENTIFIC SOCIETIES

2016-2017 Special Operations Medical Association
2016-present American College of Surgeons, *resident member*
2015-present Lexington Medical Society
2013-present American Medical Association, *resident member*
2013-present Kentucky Medical Association
2010-present University of Kentucky Alumni Association

RESEARCH PRODUCTIVITY

Editorial Consultant/Journal Reviewer

2020 – pres *Plastic and Reconstructive Surgery Global Open* (Experimental section)

2019 – pres *Plastic and Reconstructive Surgery* (Experimental section)

2019 – pres *Annals of Plastic Surgery* (Editorial Board: Peripheral Nerve Surgery and Research, Reconstructive, and Research sections)

Publications

1. Burns, JC, **DeCoster, RC**, Dugan, AJ, Davenport, D, Vasconez, HC (2020) Trends in the surgical management of lower extremity Gustilo IIIB/IIIC injuries. *Plastic and Reconstructive Surgery.* [in press]

2. **DeCoster, RC**, Clemens, MW, Di Napoli, A, Lynch, EB, Bonaroti, AR, Rinker, BD, Butterfield, TA, Vasconez, HC, Clemens, MW (2020) Cellular and molecular mechanisms of breast implant-associated anaplastic large cell lymphoma. *Plastic and Reconstructive Surgery.* [accepted]

3. Vyas, KS, **DeCoster, RC**, Burns, JC, Dugan, AJ, Rodgers, LT, Shrout, MA, Mercer, JP, Coquillard, C, Baratta, MD, Rinker, BD, Vasconez, HC (2020) Autologous fat grafting does not increase risk of oncologic recurrence in the reconstructed breast. *Annals of Plastic Surgery.* [Epub ahead of print]

4. **DeCoster, RC**, Rinker, BD, Butterfield, TA (2020) The role of muscle-derived stem cell-enriched scaffolds for treating volumetric muscle defects. *Plastic and Reconstructive Surgery.* 145 (1):202e-203e.

5. **DeCoster, RC**, Rinker, BD, Butterfield, TA, Vasconez, HC (2019) Oncogenic drivers of breast implant-associated anaplastic large cell lymphoma. *Plastic and Reconstructive Surgery*. 145(1):195e-196e.

6. **DeCoster, RC**, Bautista, RF, Evers, BM (2019) Comment on training the surgeon-scientist in today's healthcare environment. *Annals of Surgery.* 270(6): e124-e125.

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13. Covey, SE**, DeCoster, RC**, Wallace, CC, Moore, EM, Vasconez, HC (2019) Dermal substitutes in the setting of flap delay: A reconstructive technique to enhance flap viability. *The American Surgeon.* 85(5): e235-e237.

14. Coquillard, C, Boustany, A, **DeCoster, RC**, Vasconez, HC (2019) Muir-Torre syndrome presenting as a sebaceous carcinoma of the nasal ala. *The American Surgeon.* 85(3): e115-e117.

15. Shrout, M., **DeCoster, R,** Wermeling, R, Vasconez, HC (2019) Risk factors for squamous cell carcinoma: A case for red pigment in tattoos. *The American Surgeon.* 85(2): e77-e78.

Abstracts, peer-reviewed

1. **DeCoster, RC**, Lynch, EB, Bonaroti, AR, Vasconez, HC, Clemens, MW (2020) Current Risk of Breast Implant-Associated Anaplastic large Cell Lymphoma: A Systematic Review of Epidemiological Studies. Southeastern Society of Plastic and Reconstructive Surgeons 63rd Annual Scientific Meeting. Virtual meeting due to COVID-19. [accepted for podium presentation]
2.
3. **DeCoster, RC**, Clemens, MW, Lin, KY, Miranda, R, Medeiros, J, Rinker, BD, Butterfield, TA, Vasconez, HC (2019) Transcriptome-wide Microarray to Determine Molecular Mechanisms of Breast Implant-Associated Anaplastic Large Cell Lymphoma. Southeastern Society of Plastic and Reconstructive Surgeons 62nd Annual Scientific Meeting, Naples, FL. [accepted for podium presentation]

4. **DeCoster, RC**, Bautista, RF, Rinker, BD, Butterfield, TA, Vasconez, HC (2019) The Role of Oncogenic JAK/STAT3 Pathway Mutations in the Pathogenesis of Breast Implant Associated-Anaplastic Large Cell Lymphoma. Southeastern Society of Plastic and Reconstructive Surgeons 62nd Annual Scientific Meeting (June 8-12th, 2019), Naples, FL. [accepted for podium presentation]

5. Burns, JC, **DeCoster, RC**, Vyas, KS, Mercer, JP, Vasconez, HC (2019) Oncologic Safety of Autologous Fat Grafting to the Reconstructed Breast. Southeastern Society of Plastic and Reconstructive Surgeons 62nd Annual Scientific Meeting (June 8-12th, 2019), Naples, FL. [accepted for podium presentation; Glancy competition]

6. Wetzel, M, **DeCoster, RC**, Stout, MA, Burns, JC, Shrout, MA, Dugan, AJ, Webster, JM, Vasconez, HC (2018), Appalachian Status is a Negative Predictor of Breast Reconstruction Following Breast Cancer Resection. Appalachian Translational Regional Network 8th Annual Summit, Lexington, KY. [accepted for podium presentation]

7. Burns, JC, **DeCoster, RC,** Davenport, DL, Vasconez, HC (2018) An Analysis of Trends in Lower Extremity Trauma Management: Is Lower Extremity Reconstruction a Thing of the Past? American Association of Plastic Surgeons 97th Annual Meeting. Seattle, WA. [accepted for podium presentation]

Abstracts, non-peer reviewed

1. Burns, JC, **DeCoster, RC**, Stout, MA, Shrout, MA, Webster, JM, Vasconez, HC (2018), Appalachian Status is a Negative Predictor of Breast Reconstruction Following Breast Cancer Resection, Kentucky Society of Plastic Surgeons Annual Meeting, Louisville, Kentucky (podium presentation).

GRANT ACTIVITY

Funded

Extramural funding

Plastic Surgery Foundation Pilot Research Grant DeCoster (PI)
07/01/2019 - 06/30/2020
The Biomechanical Effects of Deferoxamine on Irradiated Soft Tissue
Amount: $10,000

SESPRS Research Grant Award Butterfield/Vasconez (Co-PIs)
07/01/2018 – 06/30/2019
Transcriptome-Wide Microarray to Determine Molecular Mechanisms in Breast Implant-Associated Anaplastic Large Cell Lymphoma
Amount: $15,000
Role: Post-Doctoral Fellow

Intramural funding

UK UGR Summer Research and Creativity Fellowship Grant
05/03/2012 - 08/15/2012
Fiber Type Transition in Eccentric Exercised Normal vs. Stretch-Activated Channel Blocked New Zealand Rabbits
Amount: $2,000
Mentor: Tim Butterfield, Ph.D.