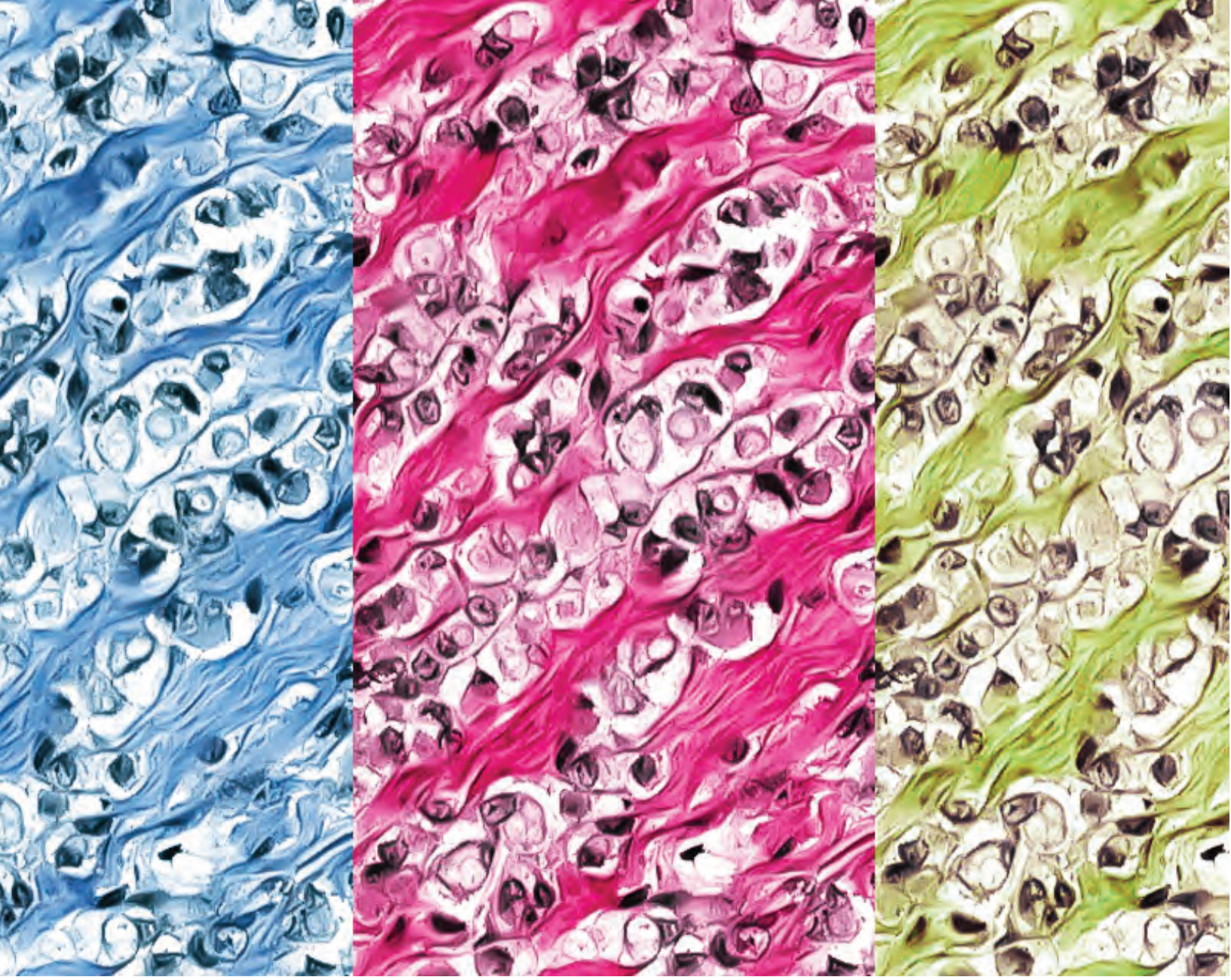


ILC

SYMPOSIUM 2023

SEPTEMBER 28-30TH, 2023
THE ASSEMBLY, PITTSBURGH, PA

#ILCSymposium

WELCOME

Welcome to the 2023 International Invasive Lobular Carcinoma (ILC) symposium in Pittsburgh, the City of Bridges. The ILC symposium is made possible through the work of the local and international organizing committees, the patient advocates of GlobMob, and collaboration between UPMC Hillman Cancer Center, UPMC Magee-Womens Hospital, Magee-Womens Research Institute and the University of Pittsburgh. We are grateful to many sponsors and supporters of the Symposium.

The ILC Symposium 2023 focuses on various topics of importance and relevance to research scientists, clinicians, and advocates who are interested in ILC research and clinical trials. We hope that participation in this symposium will help foster collaborations leading to advancements in ILC prevention, diagnosis, and treatment.

We look forward to your participation in this meeting. Thank you for what you do to contribute to improve the lives of patients with ILC.

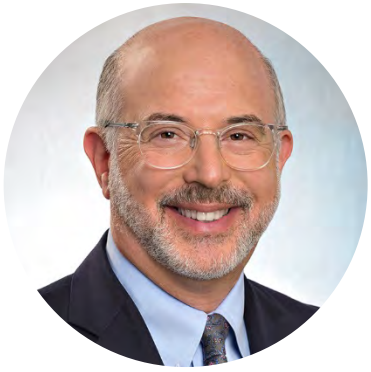
Steffi Oesterreich, PhD, on behalf of the ILC Symposium Organizing Committee

TABLE OF CONTENT

KEYNOTE TALKS

Cathrin Brisken, MD, PhD

Cathrin Brisken, MD, PhD, is an internationally renowned Associate Professor at EPFL, specializing in endocrine control of breast development and cancer. She holds MD and PhD degrees from Georg August University, with postdoctoral experience at Whitehead Institute, Harvard, and ISREC. Her research centers on estrogen and progesterone signaling in breast carcinogenesis, aiming to enhance prevention and treatment. Dr. Brisken's lab pioneers in vivo approaches to understand hormonal impacts on mammary glands and employs humanized mouse models for studying hormone actions. She's a member of IBCSG and co-founded the International Cancer Prevention Institute.



Stuart Schnitt, MD

Stuart J. Schnitt, M.D. is a renowned breast pathology expert, currently serving as Chief of Breast Oncologic Pathology at Dana-Farber/Brigham and Women's Cancer Center. He is a prolific author with over 350 publications, focusing on breast diseases, and is the author of the widely used breast pathology textbook "Biopsy Interpretation of the Breast." He has also contributed to the "World Health Organization Classification of Tumors of the Breast." Dr. Schnitt's distinguished career includes serving as President of the United States and Canadian Academy of Pathology, receiving numerous awards, and mentoring numerous breast pathology fellows. His research has significantly advanced our understanding of benign breast diseases, breast cancer risk, and stromal-epithelial interactions in breast tumor progression.

SPECIAL LECTURE

Yossi Yarden, PhD

Dr. Yosef Yarden's research focuses on growth factor roles in tumor progression and developing anti-cancer drugs. He holds a BSc from Hebrew University and a PhD in Immunology from Weizmann Institute. He trained at Genentech and Whitehead Institute. Currently, he is the Harold & Zelda Goldenberg Chair in Molecular Cell Biology and Director of the Dwek Institute for Cancer Therapy Research at Weizmann Institute. His research centers on drug-resistant non-small-cell lung cancer (NSCLC) with EGFR mutations. Traditional treatments have limitations due to evolving drug resistance. Dr. Yarden proposes an upfront treatment combining antibodies and TKIs to block drug evasion routes, potentially extending lung cancer patient survival.

INTERNATIONAL ORGANIZING COMMITTEE

Steffi Oesterreich, PhD
Chair
Professor, Univ of Pittsburgh, Co-Director, Womens Cancer Resarch Center, Co-Leader Cancer Biology Program, UPMC HCC and MWRI

Patrick Derksen, PhD
Professor of Experimental & Preclinical Oncology, University Medical Center Utrecht

Rachel C. Jankowitz, MD
Associate Professor of Clinical Medicine, University of Pennsylvania, Director of the Rena Rowan Breast Center at Abramson Cancer Center

Rita Mukhtar, MD
Associate Professor of Surgery, University of California San Francisco, Principal Investigator of the Lobular Breast Cancer Research Program

Anne Vincent-Salomon, MD, PhD
Department Head of Pathology and Pole Coordinator of Pathology-Genomics and Immunology, Institut Curie

Christine Desmedt, PhD
Assistant Professor of Oncology, KU Leuven, Head of the Laboratory for Translational Breast Cancer Research

LOCAL ORGANIZING COMMITTEE

Adrian V. Lee, PhD
Director, Institute for Precision Medicine, Univ. of Pittsburgh and UPMC; Professor of Pharmacology & Chemical Biology and Human Genetics, Univ. of Pittsburgh, Women's Cancer Research Center, UPMC Hillman Cancer Center; Magee-Womens Research Institute & Foundation

Priscilla McAuliffe, M.D. PhD
Assistant Professor of Surgery, University of Pittsburgh, UPMC Magee Womens Hospital, UPMC Hillman Cancer Center

Jagmohan Hooda, PhD, MBA
Symposium Director
Senior Research Scientist, University of Pittsburgh, UPMC Hillman Cancer Center

Daniel D. Brown, PhD
Website and Media Manager
Senior Research Scientist, Institute for Precision Medicine, University of Pittsburgh/UPMC Hillman Cancer Center
Laughing Mantis Studio

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Eli Coutch
Logistics Coordinator
University of Pittsburgh, UPMC Hillman Cancer Center

GLOBMOB ORGANIZING COMMITTEE

Lori Petitti
Co-chair
Independent ILC advocate (also: Breast Cancer Care & Research Fund BCCRF)

Claire Turner
Co-chair
Lobular Breast Cancer UK (LBCUK)

Siobhan Freeney
Lobular Ireland

Myiesha Gibson
Independent advocate

Julia Katherine Levine
Independent ILC advocate (also: Metastatic Lobular Advocate, ILC Symposium FB page, LBCA Lead Research Advocate)

Judy Hallinen
Breast Cancer Research Advocacy Network (bcRAN)

Christine Hodgdon
Guiding Researchers & Advocates to Scientific Partnerships (GRASP)

Laurie Hutcheson
Lobular Breast Cancer Alliance Inc.

Yen Lam
Independent advocate (also: BCRF research campaign)

Christine McKay, MSW
Canadian Breast Cancer Network (CBCN) Lobular Breast Cancer Ambassador

Susan MacDonald
Independent ILC advocate (also: Cleveland Clinic Lobular Fund, and LBCA Research Advocate)

Diane Mapes
Fred Hutchinson Cancer Center,
Seattle, WA, USA;
Independent ILC advocate (Two ILC Science & Support FB groups/ILC Symp FB group w/Julia Katherine)

Julia Maues
Guiding Researchers & Advocates to Scientific Partnerships (GRASP)

Flora Migyanka
Independent Advocate, ILC and Founder, Dynami Foundation – (LBCA)

Rian Terveer
European Lobular Breast Cancer Consortium (ELBCC)

Stephanie Walker
Individual MBCI advocate, MBC Alliance-BECOME project, LBBC

Ma'isah Wise
Independent advocate

GlobMob

Thursday, September 28th, 2023

INTERNATIONAL INVASIVE LOBULAR BREAST CANCER (ILC) SYMPOSIUM 2023 PITTSBURGH, PA – SEPTEMBER 28-30, 2023

7:00 AM – 8:00 AM	**REGISTRATION, AND BREAKFAST**
8:00 AM – 8:05 AM	**WELCOME AND OVERVIEW FOR ILC WORKSHOP** ***Moderators:*** Emilia Diego, MD (UPMC Magee Womens Hospital and UPMC Hillman Cancer Center, Pittsburgh, PA) Lori Petitti (Independent ILC Advocate) Clair Turner (Lobular Breast Cancer, UK)
8:05 AM – 8:50 AM	**Part 1: Overview, Basic Science, and Biology of ILC and Clinical Consideration of Primary/Early Stage ILC** **Lead**: *Steffi Oesterreich (UPMC Hillman Cancer Center, Pittsburgh, PA)*
8:55 AM – 9:45 AM	**Part 2: Metastatic ILC, Current Treatment Strategies and Imaging** **Lead**: *Julia Levine (Independent ILC advocate)*
9:45 AM – 10:00 AM	**COFFEE BREAK AND COLLABORATION**
10:05 AM – 10:45 AM	**Part 3: Progress in ILC Advocacy** **Lead**: *Lourie Hutcheson (Lobular Breast Cancer Alliance)*
10:50 AM – 11:35 AM	**Part 4: Communications and Social Media: Communication Tips, Telling your story and more** **Lead**: *Diane Mapes (Fred Hutchinson Cancer Center, Seattle, WA; Independent ILC advocate)*
11:35 AM – 12:30 PM	**LUNCH, COLLABORATION, VISIT POSTERS AND SPONSOR TABLES**
12:30 PM – 12:35 PM	**Introduction to International Invasive Lobular Breast Cancer Symposium 2023** **Moderators:** *Robert Ferris, MD, PhD (UPMC Hillman Cancer Center, Pittsburgh, PA)* *Steffi Oesterreich, Ph.D (UPMC Hillman Cancer Center, Pittsburgh, PA)*
12:35 PM – 12:45 PM	**Introduction by:** *Flora Migyanka (Breast Cancer Advocate, Dynami)* *Anne Vincent-Salomon, MD, PhD (Institut Curie, Paris, France)*
12:45 PM – 1:30 PM	**Keynote talk** **Invasive Lobular Carcinoma: Where Have We Been and Where Are We Going?** *Stuart Schnitt, MD (Dana-Farber Cancer Institute, Boston, MA) - The Leigh Pate Memorial Lectureship on Lobular Breast Cancer*
1:30 PM	**SESSION 1: Pathology, Diagnosis, ILC Variants and Lobular Neoplasia** **Session chair:** *Rohit Bhargava, MD (UPMC Magee-Womens Hospital, Pittsburgh, PA)*
1:35 PM – 1:55 PM	**Homogenization of Pathological Diagnosis of ILC** *Maxim De Schepper, MD, PhD (KU Leuven, Belgium)*
1:55 PM – 2:15 PM	**Lobular Carcinoma In Situ – Current Concepts and Challenges** *Tari King, MD (Dana-Farber Cancer Institute, Boston, MA)*

2:15 PM – 2:30 PM	**Oral presentation selected from Abstract submissions** **E-cadherin inactivation shapes tumor microenvironment specificities in ILC** *Lounes Djerroudi, MD (Institut Curie, Paris)*
2:30 PM – 2:45 PM	**Oral presentation selected from Abstract submissions** **Spatial profiling of mixed invasive ductal-lobular carcinoma reveals intrinsic molecular subtype and oncogenic signaling heterogeneity** *Osama Shah, Ph.D (UPMC Hillman Cancer Center, Pittsburgh, PA)*
2:45 PM – 3:05 PM	**COFFEE BREAK AND COLLABORATION**
3:05 PM	**SESSION 2: E-cadherin and the ILC Tumor Microenvironment** **Session chair**: *Rebecca Riggins, PhD (Georgetown University, Washington, DC)*
3:10 PM – 3:30 PM	**E-cadherin to F-actin Linkage Reveals Candidate Tumor Suppressors in Lobular Breast *Cancer*** *Patrick Derksen, PhD (UMC Utrecht, Netherlands)*
3:30 PM – 3:50 PM	**Lobular Carcinoma In Situ – Current Concepts and Challenges** Val Brunton, PhD (University of Edinburgh Scotland)
3:50 PM	**SPECIAL SESSION: Signaling by Adhesion and Growth Factor Receptors. Their Role in ILC Biology and Treatment** **Session Chair:** *Patrick Derksen, PhD (UMC Utrecht, Netherlands)*
3:55 PM – 4:25 PM	**SPECIAL LECTURE** **Regulation of Metastasis and Dormancy by Nucleo-cytoplasmic Transport and Redox Potential** *Yossi Yarden, PhD (Dwek Institute for Cancer Therapy Research, Rehovot, Israel)*
4:25 PM – 4:45 PM	**Targeting Survival Cues to Treat Lobular Breast Cancer** *Thijs Koorman, PhD (UMC Utrecht, Netherlands)*
4:45 PM – 5:05 PM	**Therapeutic Targeting of HER2 Drive Mutations in ILC** *Ariella Hanker, PhD (University of Texas Southwestern, Dallas, TX)*
5:05 PM – 5:25 PM	**A Unique Chromatin State Drives Therapeutic Resistance in ILC** *Rinath Jeselsohn, MD (Dana-Farber Cancer Institute, Boston, MA)*
5:25 PM – 7:00 PM	**POSTER SESSION – Odd number presenters (wine and cheese served)**
7:00 PM	**DINNER ON YOUR OWN** ** **Special Event:** Dinner and Entertainment at Gateway Clipper Fleet. **Requires separate registration for the event.** Please check registration details on the symposium website under social activities.

Friday, September 29th, 2023

7:00 AM – 7:30 AM	**Yoga (optional). Additional details on the symposium website under social activities.**
7:30 AM – 8:30 AM	**BREAKFAST**
8:30 AM – 8:35 AM	**Instruction by:** *Leonie Young, PhD, RCSI (Dublin, Ireland)*
8:35 AM – 9:10 AM	**Keynote talk** **A new look at lobular carcinoma development: through the teat** *Cathrin Brisken, MD, PhD (Swiss Federal Institute of Technology Lausanne, Switzerland, The Institute of Cancer Research, London, UK)*
9:15 AM	**SESSION 3: Modeling ILC** **Session chair:** *Sean Egan, PhD (University of Toronto, Canada)*
9:20 AM – 9:40 AM	**Understanding ILC models** *Adrian Lee, PhD (UPMC Hillman Cancer Center, Institute for Precision Medicine. Pittsburgh, PA)*
9:40 AM – 10:00 AM	**Exploring New Targets: Molecular Profiling of Metastatic Patient-Derived Models** *Damir Vareslija, PhD (Royal College of Surgeons, Dublin, Ireland)*
10:00 AM – 10:15 AM	**Oral presentation selected from Abstract submissions** **Characterization of Resistance to CDK4/6 Inhibitors and Endocrine Therapy in Invasive Lobular Breast Cancer Cell Lines** *Christina L. Gersch, BS, BSN (University of Michigan, Michigan)*
10:15 AM – 10:35 AM	**COFFEE BREAK AND COLLABORATION**
10:35 AM	**How Researchers, Clinicians and Patient Advocates Can Accelerate Lobular Breast Cancer Research – In Memory of Leigh Pate**
10:40 AM – 10:50 AM	Leigh Pate: How it all started *Steffi Oesterreich, PhD (UPMC Hillman Cancer Center, Pittsburgh, PA)*
10:50 AM – 10:55 AM	**The Genesis of the Lobular Breast Cancer Alliance** *Lori Petitti (Independent ILC Advocate)*
10:55 AM – 11:00 AM	**Leigh Pate: A short life, a long view** *Diane Mapes (Independent ILC Advocate)*
11:00 AM – 11:05 AM	**How one person sparked a movement** *Flora Migyanka (Independent Advocate/Founder, Dynami Foundation)*
11:05 AM – 11:10 AM	**Sowing the seeds of Change' - Leigh's Legacy** *Siobhan Freeney (Lobular Ireland)*
11:10 AM – 11:15 AM	**Leaning In with Lobular** *Claire Turner (Lobular Breast Cancer UK)*
11:15 AM – 11:25 AM	**Building Bridges in Research Outreach and Lobular Cancer Biology** *Matt Sikora, PhD (University of Colorado Denver, Anschutz Medical Campus, Denver, CO)*
11:25 AM – 11:35 AM	**BCRF ILC Legacy Project – Lobular Breast Cancer Biobank** *Jagmohan Hooda, PhD, MBA (UPMC Hillman Cancer Center, Pittsburgh, PA)*
11:35 AM – 1:00 PM	LUNCH AND POSTER SESSION – Even number presenters

1:00 PM – 1:40 PM **Panel Discussion – Diversity, Equity, Inclusion, Justice (DEIJ) Aspects in ILC Research, Treatment and Advocacy**
Session chair: *Stephanie Walker, MBCI Advocate, MBC Alliance-Become Project*
Advisor: *Julia Maues (Breast Cancer Advocate, GRASP)*
Panel Members:
Kathryn Demanelis, PhD (UPMC Hillman Cancer Center, Pittsburgh, PA),
Claire Turner (Lobular Breast Cancer UK),
Yen Lam, Myiesha Gibson, and Ma'isah Wise (all independent advocates)

1:45 PM **SESSION 4: Unique Imaging Modalities in ILC**
Session chair: *Vincent Vandecaveye, MD, PhD (KU Leuven, Belgium)*

1:50 PM – 2:10 PM **Challenges and Potential Solutions for Imaging of Invasive Lobular Carcinoma**
Matthew Covington, MD (University of Utah, Salt Lake City)

2:10 PM – 2:30 PM **Imaging and ILC, Advances and Opportunities**
Hannah Linden, MD, FACP (Fred Hutch Cancer Center, University of Washington, Seattle)

2:30 PM – 2:50 PM **Estrogen Receptor (ER)-targeted PET: Clinical Applications and Interpretation**
Gary Ulaner, MD (Hoag Family Cancer Institute, University of Southern California, Irvine)

2:50 PM – 3:10 PM **Whole Body Diffusion-Weighted MRI in Lobular Breast Cancer: Development and Clinical Applications for Staging and Response Assessment**
Vincent Vandecaveye, MD, PhD (KU Leuven, Belgium)

3:10 PM – 3:30 PM **COFFEE BREAK AND COLLABORATION**

3:30 PM **SESSION 5: Genetics of ILC –Use of clinical specimens and data**
Session chair: *Elinor Sawyer, PhD (Kings College, London, UK)*

3:35 PM – 3:55 PM **Hereditary lobular breast cancer syndrome associated with germline CDH1 variants**
Giovanni Corso, MD, PhD (European Institute of Oncology, University of Milan, Italy)

3:55 PM – 4:15 PM **Identification of Targetable Vulnerabilities in ILC Using Comprehensive Genomics Profiling**
Ethan Sokol, PhD (Foundation Medicine, Boston, MA)

4:15 PM – 4:35 PM **IDA – How an Individualized Digital Aid for ILC Can Assist Patients and Research**
Maria Margarete Karsten, MD, PhD (Charité-Universitatsmedizin Berlin, Germany)

4:35 PM **SESSION 6: Challenges in Treatment of ILC**
Session Chair: *Vikram Gorantla, MD (UPMC Hillman Cancer Center, Pittsburgh, PA)*

4:40 PM – 5:00 PM **When the Breast Cancer Researcher Becomes the Patient with ILC**
Susanne Fuqua, PhD (Baylor College of Medicine, Houston)

5:00 PM – 5:20 PM **Differential Genomic and Transcriptomic Analysis of Invasive Lobular Carcinoma Versus Invasive Ductal Carcinoma**
Jason Mouabbi, MD (University of Texas, MD Anderson Cancer Center, Houston)

Friday, September 29th, 2023

5:20 PM – 5:40 PM

Distinct features of ILC vs IDC in four NSABP randomized trials of adjuvant chemotherapy

Julia Foldi, MD, PhD (UPMC Hillman Cancer Center, Pittsburgh, PA)

5:40 PM – 6:00 PM

Exercise is Medicine in the Setting of Oncology

Kathryn Schmitz, PhD, MPH (UPMC Hillman Cancer Center, Pittsburgh, PA)

6:10 PM

DINNER ON YOUR OWN

** Special Event: Baseball game night for attendees. **Requires separate registration for the event.** Please check registration details on the symposium website under social activities.

Saturday, September 30th, 2023

7:30 AM – 8:50 AM	**Round Table Clinical and Scientific Discussions Over Breakfast**
8:55 AM	**SESSION 7: Local Treatment of ILC** **Session chair:** *Bhuvaneswari Ramaswamy, MD (The Ohio State, Columbus, OH)*
9:00 AM – 9:20 AM	**Surgical management of ILC: challenges and opportunities** *Rita Mukhtar, MD (UCSF, San Francisco, CA)*
9:20 AM – 9:40 AM	**Surgical management of the axilla in lobular cancer** *Priscilla McAuliffe, MD, PhD (UPMC Magee-Womens Hospital and UPMC Hillman Cancer Center, Pittsburgh, PA)*
9:40 AM	**SESSION 8: Treatment Resistance and Metastases** **Session chair:** *Fresia Pareja, MD, PhD (Memorial Sloan Kettering Cancer Center, NYC, NY)*
9:45 AM – 10:05 AM	**Liquid biopsy in ILC: What can we learn about clinical and molecular evolution?** *Massimo Cristofanilli, MD (Sandra Edward Meyer Cancer Center, Weill Cornell, NYC, NY)*
10:05 AM – 10:25 AM	**ILC-focused biomarkers of progression and prognosis** *Peter Simpson, PhD (University of Queensland, Australia)*
10:25 AM – 10:45 AM	**Metastatic Spread in Patients with Mixed ILC/NST: Results from Post-Mortem Tissue Donation Programs** *Karen Van Baelen, MD (KU Leuven, Belgium)*
10:45 AM – 11:10 AM	**COFFEE BREAK AND COLLABORATION**
11:10 AM – 12:40 PM	**Case Reports** **Session Chairs:** *Nancy Davidson, MD (Fred Hutch Cancer Center, Seattle, WA)* **Presenters:** *Rachel Jankowitz, MD (University of Pennsylvania, Philadelphia)* *Kit Lu, MD (UPMC, Harrisburg, PA)* *Elinor Sawyer, PhD (Kings College London, UK)* *Dhaval Mehta, MD (UPMC, Monroeville, PA)* *Chuck Geyer, MD (NSABP, Pittsburgh, PA)*
12:40 PM – 1:10 PM	**Discussions of Research Priorities and Potential Collaborations** **Session chair:** *Steffi Oesterreich PhD (UPMC Hillman Cancer Center, Pittsburgh, PA)* **Panel members:** *Christine Desmedt, PhD (KU Leuven, Belgium)* *Jason Mouabbi, MD (UT MD Anderson Cancer Center)* *Patrick Derksen, PhD (UMC Utrecht, Netherlands)* *Rita Mukhtar MD (UCSF, San Francisco, CA)*
1:10 PM	**CLOSING REMARKS and BOXED LUNCH To-Go**

Posters are sorted and listed by the study category below;

1) Clinical characteristics
2) Diagnosis
3) Treatments strategies
4) Prognosis
5) Metastasis
6) Biomarker
7) TME
8) Genetics
9) Epigenetics
10) Mulitiomics

- Poster Setup: Please ensure that all posters are mounted on the designated poster boards no later than Thursday at lunchtime. If preferred, you may also set up your posters on Thursday morning during breakfast.

- Thursday Evening Poster Session, 5:25 PM to 7:00 PM, (**Odd Number Posters**): For posters with odd numbers, the presenting author should be present at their poster during the poster session on Thursday evening from 6:00 PM to 7:00 PM.

- Friday Poster Session, 11:35 AM to 1:00 PM, (**Even Number Posters**): For posters with even numbers, the presenting authors should be available at their posters during the second poster session on Friday from 12:00 PM to 1:00 PM.

- Poster Evaluation: A panel of judges from the poster judging committee will evaluate all posters. The top three posters will be selected and announced during the closing session of the symposium on Saturday. These selected posters will also receive awards for their outstanding presentations.

Poster Number	PrimaryAuthor	Title	Category
1	Gitte Zels	Overview of patients with invasive lobular breast carcinoma included in the post-mortem tissue donation program, UPTIDER	Clinical Characteristics
2	Qian Zhao	Clinical Characteristics and Outcomes of Invasive Lobular Carcinoma	Clinical Characteristics
3	Jasmine Timbres	Risk factors pre-disposing to LCIS and ILC	Clinical Characteristics
4	Josephine Van Cauwenberge	Reporting on invasive lobular carcinoma in clinical drug trials and trials investigating gene expression profiles and molecular screening programs – a systematic review	Clinical Characteristics
5	Siobhan Freeney	Enhancing Knowledge and Research in Europe for Invasive Lobular Breast Cancer: Unique Challenges across European Countries	Clinical Characteristics

Poster Number	PrimaryAuthor	Title	Category
6	Helen Coulthard	“they… kept saying there's nothing there: British Women’s accounts of delayed lobular breast cancer diagnosis	Diagnosis
7	Laurie Hutcheson	Individuals with Invasive Lobular Carcinoma (ILC) Raise their Voices about ILC and Surgery	Diagnosis
8	Luca Nicosia	Contrast-Enhanced Mammography (CEM) compared to Breast Magnetic Resonance (MRI) in the management of breast lobular neoplasia: a new scenario in facing the diagnosis of an insidious disease	Diagnosis
9	Manisha Bahl	Pre-Operative MRI for Screening Digital Breast Tomosynthesis-Detected Invasive Lobular Carcinoma: Who Benefits Most?	Diagnosis
10	Annapurna Gupta	Downregulation of Arginosuccinate synthase 1 confers Tamoxifen Resistance in Invasive Lobular Breast Cancer	Treatment Strategies
11	Bernadette Heemskerk-Gerritsen	The effect of (neo)adjuvant chemotherapy on long-term survival outcomes in Invasive Lobular Breast Cancer Patients treated with endocrine therapy: a retrospective cohort study	Treatment Strategies
12	Steffi Oesterreich	Personalized circulating tumor DNA (ctDNA) testing for detection of progression and treatment response monitoring in patients with metastatic invasive lobular carcinoma (mILC)	Treatment Strategies
13	Christina Gersch	Characterization of Resistance to CDK4/6 Inhibitors and Endocrine Therapy in Invasive Lobular Breast Cancers Cell Lines	Treatment Strategies
14	Jie Bin Liu	Predicting Response to HER2 Tyrosine Kinase Inhibitors and Antibody Drug Conjugates in ERBB2 Mutant ILC Using CRISPR/Cas9 Knock-in Cell lines and Patient-derived Organoids	Treatment Strategies
15	Jordan Swartz	Efficacy of PARP inhibitor Talazoparib on ER+ ILC breast cancer models	Treatment Strategies
16	Melinda Sanders	High prevalence of HER2-low among ILC with residual disease following neoadjuvant therapy provides therapeutic opportunities with HER2-antibody-drug conjugates	Treatment Strategies
17	William Yang	E-cadherin loss imparts mitotic vulnerabilities rendering breast cancer cells synthetic lethal to crizotinib and up-regulation of Src signalling reverses this effect	Treatment Strategies
18	Xilal Y. Rima	Coupling bioengineered microsystems with high-resolution microscopy to investigate dormancy and drug resistance in invasive lobular carcinoma	Treatment Strategies
19	Zhangyi Luo	Inhibition of iRhom by CD44-targeting Nanocarrier for Improved Cancer Immunochemotherapy	Treatment Strategies

Poster Number	PrimaryAuthor	Title	Category
20	Francesca Magnoni	Comparison of long-term outcome between high risk lobular versus ductal breast cancer: a propensity score matched study	Prognosis
21	Rebecca Riggins	Glutamate metabolic enzymes associate with increased tumor size in black women with invasive lobular breast cancer: a single-institution study	Prognosis
22	Simran Sandhu	Racial Disparities in Survival in Lobular Breast Cancer	Prognosis
23	Baylee Porter	Glucocorticoid receptor activation inhibits tumor cell growth while increasing metastatic characteristics in models of invasive lobular breast cancer	Metastasis
24	Bernadette Heemskerk-Gerritsen	Localization of metastatic disease in patients with a history of lobular carcinoma of the breast	Metastasis
25	Esme Bullock	Cancer-associated fibroblast driven paracrine IL-6/STAT3 signalling promotes migration and dissemination of Invasive Lobular Carcinoma cells	Metastasis
26	Martin Blankfard	Development of a comprehensive cancer profiling assay utilizing circulating tumor cells in metastatic breast cancer	Metastasis
27	Andi Cani	Serial monitoring of circulating tumor cells and circulating tumor DNA in metastatic lobular breast cancer identifies intra-tumor heterogeneity and precision and immuno-oncology biomarkers of therapeutic importance	Biomarker
28	Chinasa A Ufondu	Assessing the Effect of E-cadherin loss on Estrogen Receptor activity in Human Mammary Epithelial Cell Models	Biomarker
29	Christina Addison	Increased Sox9 expression is associated with a more invasive ILC cell phenotype	Biomarker
30	Luyu Jia	Mutating E-cadherin in Rats to Model Lobular Breast Cancer	Biomarker
31	Madeleine Shackleford	WNT4 regulates cellular metabolism via intracellular activity at the mitochondria in invasive lobular carcinoma cells	Biomarker
32	Maggie Musick	The tumorigenic effects of E-cadherin loss in early ILC tumorigenesis	Biomarker
33	Ye Cao	Investigating RET as a Novel Therapeutic Target in Breast Cancer Brain Metastasis Using Patient-Derived Organoid	Biomarker
34	Lounes Djerroudi	E-cadherin inactivation shapes tumor microenvironment specificities in invasive lobular carcinoma	TME
35	Lynda Bennett	Understanding ILC biology through combined spatial transcriptomics and proteomics	TME
36	Matteo Serra	Spatially resolved analysis of tumor microenvironment revealed biologically driven subgroups with distinct clinical outcome in invasive lobular carcinoma	TME

Poster Number	PrimaryAuthor	Title	Category
37	Emmanuelle mouret-fourme	Clinical Characteristics of Lobular Breast Carcinoma in CDH1 genetic predisposition. Experience from the Institut Curie	Genetics
38	Fresia Pareja	Non-Lobular Invasive Breast Carcinomas with Bi-Allelic Pathogenic CDH1 Somatic Alterations: a Histologic, Immunophenotypic and Genomic Characterization	Genetics
39	Higinio Dopeso	Genetic and Epigenetic Basis of Breast Invasive Lobular Carcinomas Lacking CDH1 Genetic Alterations	Genetics
40	Rita Canas-Marques	Aberrant E-cadherin (E-cad) Expression in Lobular Carcinoma in Situ (LCIS): A Comprehensive Evaluation of N-terminal, Extracellular, and C-terminal E-cadherin Domains by	Genetics
41	Jing Yu	Lobular-like invasive mammary carcinoma: Is this a ductal cancer, lobular cancer, or a distinct entity?	Genetics
42	Sumayya Abdul Qadir	Developing and Characterizing a Model for Recurrent Invasive Lobular Breast Carcinoma with Nf1 and HER2/Neu Mutations	Genetics
43	Joseph Sottnik	Estrogen receptor interaction with Mediator of DNA Damage Checkpoint 1 (MDC1) mediates epigenomic remodeling and gene regulation in ILC cells	Epigenomics
44	Sanghoon Lee	Chromatin accessibility landscape and active transcription factors in primary human invasive lobular and ductal breast carcinomas	Epigenomics
45	Fangyuan Chen	Integrated analysis of tumor transcriptomics and immune landscape in primary lobular cancer – a case-series study	Multiomics
46	Fresia Pareja	Integrative Deep Learning and Genomics Approach Reveals Alternative CDH1 Inactivation Mechanisms	Multiomics
47	Osama Shiraz Shah	Multi-omic characterization of ILC and ILC-like cell lines as part of ILC cell line encyclopedia (ICLE) defines new models to study potential biomarkers and explore therapeutic opportunities	Multiomics
48	Sarah Nash	Transcriptomic and immune heterogeneity underpin the biology of pleomorphic invasive lobular breast cancer	Multiomics
49	Susrutha Puthanmadhom Narayanan	Transcriptomic analysis identifies enrichment of cAMP/PKA/CREB signaling in Invasive Lobular Breast Cancer	Multiomics
50	Osama Shiraz Shah	Spatial profiling of mixed invasive ductal-lobular carcinoma reveals intrinsic molecular subtype and oncogenic signaling heterogeneity	Multiomics

Abstracts of Poster and Trainee Presentation

(Ordered by the poster number listed in the table in the previous page)

Clinical Characteristics, Poster #1

Overview of patients with invasive lobular breast carcinoma included in the post-mortem tissue donation program, UPTIDER

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Background: Invasive lobular carcinoma (ILC) represents 15% of invasive breast cancer diagnoses and has distinct clinicopathological features in comparison to no-special type breast cancer. There is an unmet scientific and clinical need to better characterize metastatic ILC (met-ILC). Here, we present the clinical and pathological findings of patients with met-ILC included in our post-mortem tissue donation program UPTIDER (NCT04531696).

Methods: 6 patients with primary pure, estrogen receptor-positive (ER+) ILC underwent rapid autopsy through the UPTIDER program. Primary and post-mortem samples with at least 10% tumour cellularity of all patients underwent centralized pathological review for histology, stromal tumour infiltrating lymphocytes (sTIL) scores as well ER and progesterone receptor (PR) expression for 4 patients. Clinical data on age and disease progression were extracted from patient files.

Results: Three of the 6 patients with ER+ ILC showed disseminated disease at diagnosis. The others had an average distant recurrence free survival of 163 months (range: [55–358]). Median age at initial diagnosis was 52 years (range: [37-80]). The average time between metastatic spread and death was 44.8 months (range: [15–83]). In total, 175 metastatic samples were taken at autopsy with a median of 26 lesions per patient (range: [22–47]), retrieved from a median of 8 different sites (range: [4-16]). Both primary and metastatic

samples showed low sTIL scores with a mean of 0.66% (range: [0-1], n=5) and 1.67% (range: [0–13-33], n=145) respectively. ER expression was maintained in a median of 95% samples per patient (range: [71-100]), while PR expression was only preserved in 63% of the samples in one of 4 patients.

Conclusions: Post-mortem metastatic samples of primary ER+ ILC have very low sTIL levels and most lesions retain ER+ status. Next, we will join forces with University of Pittsburgh Rapid Autopsy Program to better characterize met-ILC using various omics techniques.

Lay Abstract:
Of all patients with breast cancer (BC), 15% get diagnosed with invasive lobular BC (ILC). During disease progression, distant tumours will lose or gain certain characteristics in comparison to the primary tumour, as well as between distant tumours. This is driven by alterations at a DNA level, the local tissue environment and treatment. This is referred to as tumour heterogeneity. This heterogeneity is responsible for therapeutic failure and disease progression. To study and tackle this, there is a scientific and clinical need to better characterize heterogeneity in metastatic BC. One way to achieve this is through post-mortem tissue donation programs, a procedure in which many tumour samples are collected after death for the purpose of translational research. Here, we present the findings of patients with metastatic ILC from included in our post-mortem tissue donation program, UPTIDER (NCT04531696).

Clinical Characteristics, Poster #2

Clinical Characteristics and Outcomes of Invasive Lobular Carcinoma

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Background: Infiltrating ductal carcinoma (IDC) is the most predominant type of breast cancer, followed by invasive lobular carcinoma (ILC), which accounts for roughly 5%-15%. ILC exhibits features associated with a good prognosis, such as low grading and positive hormone receptors. ILC is still a relatively complicated disease. We reviewed patients diagnosed with ILC in our center between 2008-2022 in order to gain a better understanding of this type and to help formulate clinical treatment strategies.
Methods: This retrospective study included 1,456 patients diagnosed with ILC at our center during the period 2008-2022, after excluding patients with incomplete clinical information as well as follow-up information. We analysed the clinicopathological characteristics and survival data of ILC and compared them with IDC in the same period.
Results: The median age was 53 years old, with 90.5% of patients in the 40-80 age group. 43.3% of ILC patients had multifocal tumors. 89.6% of patients were hormone receptor positive, while 63.1% of patients had no HER2 amplification. Mastectomy was performed in 72.6 % of the patients, while only 26.2 % underwent breast-conserving surgery. The median follow-up was 34.5 months. The prognosis of patients was comparable in both surgical approaches. ILC had a worse prognosis than IDC compared with IDC patients during the same period, which held true after excluding the effects of time period, staging, hormone receptor and HER2 status.
Conclusion: Patients with ILC received the same survival benefit from mastectomy versus breast-conserving surgery. ILC had a worse prognosis compared to IDC.

Lay Abstract:
Background: ILC exhibits features associated with a good prognosis, such as low grading and positive hormone receptors. However, ILC is still a relatively complicated disease.
Methods: This retrospective study included 1,456 patients diagnosed with ILC at our center during the period 2008-2022, after excluding patients with incomplete clinical information as well as follow-up information.
Results: Mastectomy was performed in 72.6 % of the patients, while only 26.2 % underwent breast-conserving surgery. The median follow-up was 34.5 months. The prognosis of patients was comparable in both surgical approaches. ILC had a worse prognosis than IDC compared with IDC patients during the same period, which held true after excluding the effects of time period, staging, hormone receptor and HER2 status.
Conclusion: Patients with ILC received the same survival benefit from mastectomy versus breast-conserving surgery. ILC has a worse prognosis compared to IDC.

Clinical Characteristics, Poster #3

Risk factors pre-disposing to LCIS and ILC

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Lobular Carcinoma In-Situ (LCIS) is a non-invasive breast lesion and is considered as a risk factor for breast cancer than a true precursor, as it increases the risk of breast cancer bilaterally. However, studies have shown that LCIS is clonally similar to invasive lobular carcinoma (ILC), supporting the argument that it is a pre-cursor lesion. This study aimed to identify shared and distinct risk factors between LCIS and ILC, as if LCIS is a precursor of ILC, the risk factors are expected to be similar.
The GLACIER case-control study conducted in the UK between 2007-2012 focused on ILC, LCIS, and LCIS and invasive concurrent cases. Data on reproductive history and hormone usage were collected, and logistic regression analysis was used to explore associations. Missing data were multiple imputed using chained equations. Results revealed common risk factors for LCIS and ILC. Breastfeeding had a protective effect on both LCIS (OR: 0.63, 95%CI: 0.46-0.86) and ILC (OR: 0.69, 0.57-0.84), while hormone replacement therapy (HRT) increased the risk for both LCIS (OR: 1.62, 95%CI: 1.21-2.17) and ILC (OR: 1.22, 95%CI: 1.01-1.48). Taking HRT for 10-years or more greatly increased the risk of both LCIS and ILC. Notably, combined estrogen and progestogen HRT increased ILC risk (OR: 1.91, 95%CI: 1.15-2.83), but not LCIS risk. Increasing age at first childbirth was linked to ILC, not LCIS. Comparing LCIS to ILC, the study suggests substantial overlap in risk factors for LCIS and ILC, supporting the notion of LCIS as a potential precursor to ILC. This information is crucial for informed decision-making, particularly for women on extended HRT, who should be aware of risks for both conditions, and could benefit from increased breast monitoring.

Lay abstract:
Lobular Carcinoma In-Situ (LCIS) is a breast condition that has not yet developed into invasive cancer, but increases the risk of developing invasive breast cancer. Research suggests LCIS might evolve into invasive lobular carcinoma (ILC), a type of breast cancer. This study examined similarities between LCIS and ILC risk factors in UK cases from 2007-2012. Findings indicate shared risk factors: breastfeeding lowers risk, while hormone replacement therapy (HRT) raises risk for both conditions, with long-term HRT increasing even risk further. Interestingly, combined estrogen and progestogen HRT increases ILC risk more, while late childbirth raises ILC risk, not LCIS risk. Similar risk factor patterns between LCIS and ILC support the idea of LCIS developing into ILC. This is vital for women, especially those on extended HRT, to understand risks and consider additional breast scans.

Clinical Characteristics, Poster #4

Reporting on invasive lobular carcinoma in clinical drug trials and trials investigating gene expression profiles and molecular screening programs – a systematic review

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Background: Clinical, histological and molecular differences between invasive lobular carcinoma (ILC) and breast cancer (BC) of no-special type support the idea of ILC as a separate entity. Unfortunately, evidence on treatment efficacy for ILC is often lacking. We aimed to map out the lack of documentation and representation of patients with ILC in clinical drug trials (CT), trials investigating gene expression profiles (GEPs) and molecular screening programs (MSPs).
Methods: Phase 3 and 4 CT for novel BC treatments were identified on Pubmed and clinicaltrials.gov by use of keywords linked to treatment strategies, GEPs and MSPs and 'breast cancer'. CT were included if a manuscript was available on the 15th of January 2023. Inclusion and exclusion criteria were reviewed to see if patients with ILC or non-measurable disease were excluded. Documentation of ILC was assessed and if reported, percentage of ILC, central pathology for ILC and ILC subgroup analyses were evaluated.
Results: In total, 81 CT were included (14 neoadjuvant, 11 adjuvant and 56 metastatic). Non-measurable disease was an exclusion criterium in 19.8% of the CT (14.3% neoadjuvant and 25% metastatic) and non-measurable disease with the exception of bone-only disease was excluded in 30.4% of the metastatic CT. Inclusion of patients with ILC was documented in 11/81 CT (13.6%: 35.7% neoadjuvant, 9.1% adjuvant,

8.9% metastatic). Inclusion rates varied between 2.0 and 16.3%. Only 2/11 CT had specific sub-analyses on ILC and no CT reported central pathology for ILC.
Conclusions: ILC is greatly overlooked in the majority of CT with poor representation, documentation and lack of specific sub-analyses. Eligibility criteria and definitions of treatment response do not reflect the unique biology and clinical course of ILC. It is critical that these gaps in inclusion and study of ILC are closed. ILC deserves much more attention from both clinical investigators and pharmaceutical industries.

Lay abstract
Although previous studies demonstrated that differences between lobular breast cancer (ILC) and breast cancer of no special subtype (NST) exist, they are often treated the same. It is unclear if treatments that were recently developed for breast cancer are as effective for ILC as for NST. We looked into all manuscripts published on these recently developed treatments to see if they reported how many patients with ILC were involved in the trials. The aim was to uncover the extent of the knowledge gap concerning ILC treatment. In total 81 publications were found. Only 11/81 (13.6%) reported how many patients with ILC they included. The percentage of patients with ILC included, varied from 2.0 to 16.3% and only 2 of the studies did specific sub-analyses for the patients with ILC. We conclude that ILC is greatly overlooked in clinical drug trials. Patients with ILC deserve much more attention in clinical trials.

Clinical Characteristics, Poster #5

Enhancing Knowledge and Research in Europe for Invasive Lobular Breast Cancer: Unique Challenges across European Countries

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Background
In 2019, Invasive Lobular Cancer (ILC) Patient Advocates attended the 3rd meeting of the European Lobular Breast Cancer Consortium (ELBCC), a scientific community focused on lobular research and treatment. European Lobular Breast Cancer Advocates (ELBCA) now have a network of ILC patient advocates across Europe.
ILC is classified by the World Health Organisation as the most common Special Type of Breast Cancer. In 2022 there were 576,300 diagnoses of breast cancer in Europe, 15% ~86,500 were ILC.
ILC is a histologically distinct breast cancer with diverse presentations including a tendency to metastasize to unique locations.
Current endocrine therapies may have different effectiveness for ILC making treatment challenging.
Current imaging tools are often unreliable with up to 30% of ILC tumours not identified on mammograms and detection is often delayed.

Unique Challenges
There are no standardised European diagnostic or treatment protocols for ILC. ELBCA addresses this with collaborative efforts between ILC medical professionals and researchers. It's a major challenge because each European country has a different health care system and economic policy structure.
It's also a challenge to streamline the translation of clinically accurate information to European patients in their own language. Not all European countries are members of the EU, there are a minimum of 24 official

European languages. Established European breast cancer patient organizations often don't distinguish between different histological subtypes.
Current European policy and administrative processes have hindered the formation of a not-for-profit Advocacy Organization. Without such an organization, fundraising and collaborative efforts with others is difficult.

Opportunities
ELBCA has made significant progress towards raising awareness and increasing knowledge of ILC across Europe. Our poster will expand on these accomplishments and detail our short and long term goals.

Lay Abstract
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Unique Challenges across European Countries
In 2019, Invasive Lobular Cancer (ILC) Patient Advocates attended the 3rd meeting of the European Lobular Breast Cancer Consortium (ELBCC), a scientific community focused on lobular research and treatment. European Lobular Breast Cancer Advocates (ELBCA) now have a network of ILC patient advocates across Europe.
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ILC is a histologically distinct breast cancer with diverse presentations including a tendency to metastasize to unique locations.
Current endocrine therapies may have different effectiveness for ILC making treatment challenging.
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There are no standardised European diagnostic or treatment protocols for ILC. ELBCA addresses this with collaborative efforts between ILC medical professionals and researchers. It's a major challenge because each European country has a different health care system and economic policy structure.
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Current European policy and administrative processes have hindered the formation of a not-for-profit Advocacy Organization. Without such an organization, fundraising and collaborative efforts with others is difficult.
Opportunities
ELBCA has made significant progress towards raising awareness and increasing knowledge of ILC across Europe. Our poster will expand on these accomplishments as well as the following goals:
Work with European and International ILC research and patient communities to better understand this unique histological subtype.
Elevate ILC research priorities and inform European policy to recognize ILC as a distinct disease.
Enhance education of ILC among primary HCP's and patients.
Establish a recognized European ILC Advocacy Organization and collaborate with established BC organizations.
Attend major BC research and clinical conferences. Lobby to achieve increased funding for ILC research and clinical trials

Diagnosis, Poster #6

“they… kept saying there's nothing there: British Women’s accounts of delayed lobular breast cancer diagnosis

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Background
Mammograms are the standard screening tool for breast cancer, yet 19% of lobular tumours have a false negative result, resulting in delayed diagnosis and treatment (1,2). There is no research on this experience, so the research question for this study was, ‘What is the lived experience of individuals whose lobular breast cancer (LBC) was missed on mammogram screening?’
Method
Twelve participants aged 51-64 years diagnosed with LBC who had all received a false negative mammogram in the UK were recruited from social media platforms. In-depth semi-structured interviews were conducted online and analysed using Interpretive Phenomenological Analysis (IPA) (5).
Results
Three interrelated themes are presented. Theme 1: Previous diagnostic delay and distress, represents the difficult experience of obtaining a diagnosis for lobular breast cancer after a false-negative result. Theme 2: Ongoing screening doubts and future fears relates to continuing problems following treatment, as many women were increasingly likely to experience fear of recurrence having lost ‘faith’ in mammograms and typically were not offered alternative screening methods. Theme 3: Developing knowledge, support and confidence evinces women talking positively about the support and information provided by breast cancer charities and online groups, which provided them with knowledge and skills to advocate for themselves and others.
Conclusions
The experience of false-negative mammograms has been ignored in the literature. False-negative results led to a difficult journey to diagnosis, loss of faith in mammography, heightened fear of recurrence and potentially anticipated future treatment delays. The findings suggest a need for self-advocacy promotion, greater knowledge and psychological support and improved training of healthcare professionals.

Lay Abstract
Objective: To examine the experience of women with lobular breast cancer who experienced a delayed diagnosis because their cancer was missed on mammograms.
Methodologies: In-depth interviews were carried out with 12 women with a lobular diagnosis that had been initially missed on mammograms. The data was analysed using IPA, which focuses on participants' experience, and the findings were presented as themes.
Results: The main themes were the distress of a missed mammogram and delayed diagnosis, the lack of faith in future screening, and the importance of charities and support groups to help people advocate for themselves.
What this means for patients: This is the first time that the experience of delayed diagnoses due to inaccurate screening has been researched. More work is needed on education about breast cancer screening for patients and health professionals, as well as extra psychological support when cancers are missed.

Diagnosis, Poster #7

Individuals with Invasive Lobular Carcinoma (ILC) Raise their Voices about ILC and Surgery

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The Lobular Breast Cancer Alliance (LBCA) is committed to raising awareness and promoting research for invasive lobular carcinoma (ILC). In June 2023, LBCA surveyed ILC patients treated with surgery to identify and share patient experiences and challenges related to breast surgery. The survey asked about pre-operative imaging, surgical decisions, margins, and included open-ended questions. Results demonstrated that patients had concerns about the extent of disease and risk of recurrence resulting from surgical decisions. Survey analysis will be presented at SABCS 2023. 1031/1482 respondents provided comments and permission to share. We were incredibly moved by the volume of individuals who commented, and the variety and pathos of their remarks. We felt compelled to honor and give voice to these respondents.

Results
Responses reviewed fell under one or more of six categories: ILC-specific information is needed (N=274), Breast density and ILC diagnosis (N=36), Challenges with surgical decision making (N=266), Better imaging/detection needed (N=224), ILC-specific treatments needed (N=132), Follow-up and recurrence (N=63).

Sample of Responses
“My team provided very little info about ILC and I felt very much on my own.”
“I had 2 mammograms saying I was fine. …There should be more extensive testing when you have dense breast tissue. I was lucky.”
“I wish I would have understood that risk of positive margins can be higher so I would have been better mentally prepared for the need for multiple surgeries.”
“I was never told by my team of any need or recommendation to obtain regular scans after an ILC diagnosis…then I was stage 4... I can’t help but wonder if my recurrence would have been found before spreading so far.”
“Now concerned about places ILC may metastasize further that may not be picked up on scans.”
Conclusion
It is extremely critical that ILC receives the research attention it needs.

Lay Abstract:
The Lobular Breast Cancer Alliance (LBCA) is committed to raising awareness and promoting research for invasive lobular carcinoma (ILC). In June 2023, LBCA conducted a survey of individuals with ILC about their experience with surgery. In addition to responding about surgical experience, respondents had the opportunity to provide an open-ended response related to their experience with ILC and surgery. These responses were reviewed and categorized by the authors. Because of the depth and variety of these responses, the authors wanted to honor these comments by presenting them in this abstract and poster. Patients continue to convey the critical need for more ILC research to identify ILC specific treatments and better methods of detection and the need for ILC information for people affected by this disease and caregivers.

Diagnosis, Poster #8

Contrast-Enhanced Mammography (CEM) compared to Breast Magnetic Resonance (MRI) in the management of breast lobular neoplasia: a new scenario in facing the diagnosis of an insidious disease

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Purpose: to compare the diagnostic performance (detection, assessment of correct disease extent and multifocality/centricity) of Contrast-Enhanced Mammography (CEM) Versus Breast Magnetic Resonance (MRI) in the study of lobular neoplasms.
Methods: we retrospectively selected all the patients who underwent surgery for a lobular breast neoplasm, either an in situ or an invasive tumor, and had undergone both breast CEM and MRI examinations during the pre-surgical planning. Wilcoxon Signed Rank test was performed to assess the differences between size measurements using the different methods and the post-surgical pathological measurements, considered the gold standard. The agreement in identifying multifocality/multicentricity among the different methods and the pathology was assessed using the Kappa statistics.
Results: we selected 19 patients, of which one presented a bilateral neoplasm. Then, the images of these 19 patients were analyzed, for a total of 52 malignant breast lesions. We found no significant differences between the post-surgical pathological size of the lesions and the calculated size with CEM and MRI (p-value of the difference respectively 0.71 and 0.47). In all 20 cases, neoplasm detection was possible both with CEM and MRI. CEM and MRI showed an excellent ability to identify multifocal and multicentric cases (K statistic equal to 0.93 for both the procedures), while K statistic was 0.11 and 0.59 for FFDM and US, respectively.
Conclusion: The findings of this study suggest that CEM is a reliable imaging technique in the preoperative setting of patients with lobular neoplasm, with comparable results to breast MRI.

Lay Abstract:
Due to an insidious proliferative pattern with a lack of desmoplastic and fibrotic reactions, lobular carcinoma remains clinically and radiologically elusive in many cases. Detecting lobular carcinoma with conventional imaging techniques, such as Full Field Digital Mammography or Ultrasound, represents a real radiological challenge. Indeed, it is well known that breast Magnetic Resonance Imaging (MRI) improves the management of patients diagnosed with breast lobular neoplasia. Contrast Enhanced Mammography (CEM) is a relatively new method showing huge potential in the study of breast malignancies, although its role applied specifically to lobular neoplasms has been little investigated. In our work, we sought to compare the performance of CEM to Magnetic Resonance in detecting, assessing lesion extent, and identifying multifocality and pluricentric lobular neoplasms. Based on our preliminary results, we think CEM could be proposed as a replacement or complement to breast MRI in managing patients with breast lobular neoplasia.

Treatment Strategies, Poster #10

Downregulation of Arginosuccinate synthase 1 confers Tamoxifen Resistance in Invasive Lobular Breast Cancer

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Invasive Lobular Carcinoma (ILC) is an estrogen receptor-positive, unique subtype of breast cancer. ILC patients have worse long-term disease-free and overall survival primarily due to the development of resistance to antiestrogens like tamoxifen (TAM). Our goal is to determine the drivers of tamoxifen resistance and target therapy to improve the efficacy of anti-estrogen therapy.
We have developed tamoxifen-resistant (TAMR) ILC cell lines, MDA-MB-134-VI-TAMR and SUM44PE-TAMR, through prolonged exposure to TAM. The IC50 for TAM is ≥2-fold higher for the TAMR vs. parental cells. Metabolomics and RNA-seq analyses of parental and TAMR cells revealed differential expression of multiple genes and pathways in TAMR cells. Combined analysis of the data revealed dysregulation of Alanine, Arginine, and Glutamate (AAG) metabolism, Purine metabolism, and Arginine and Proline metabolism in TAMR cells. Of the 15 common dysregulated genes between these pathways, downregulation of ASS1 was associated with poor disease-free survival in TAM-treated patients [PMID:18821012]. qPCR and western-blot analysis revealed downregulation (~50%, $p<0.001$) of ASS1 in TAMR cells. Methylation specific PCR revealed CpG island methylation of the ASS1 promoter in TAMR cells. ASS1 expression was upregulated upon 5-aza-2'-deoxycytidine (dAZA) treatment. Pre-treatment of TAMR cells with dAZA reduced IC50-TAM from 14.34μM to 8.36μM (SUM44PE) and from 16.0μM to 8.7μM (MDA-MB-134-VI). ASS1 utilizes aspartate for arginine synthesis, while Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), regulated by p70S6K, directs it to pyrimidine biosynthesis. Phospho-p70S6K and phospho-CAD levels are elevated in TAMR cells. Inhibition of dihydroorotate dehydrogenase (DHODH), an enzyme downstream of CAD involved in pyrimidine biosynthesis, by Farudostat significantly hampers TAMR cell proliferation on exposure to TAM.
Our study, for the first time, demonstrates ASS1 downregulation in TAMR-ILC cells and potential mechanisms. Restoring ASS1 expression by demethylating agent or inhibiting pyrimidine biosynthesis by targeting DHODH reduced TAMR cell growth, offering potential therapeutic strategies to treat ILC patients.

Lay abstract

Invasive Lobular Carcinoma (ILC) is a unique subtype of breast cancer that is understudied, facing many challenges including late detection, higher stage at diagnosis and late recurrence. Even though ILC are often dependent on estrogen for their growth, they develop resistance that results in recurrence. The greatest challenge is that patients with this subtype of breast cancer are treated as invasive ductal cancers of the breast. Our lab is focusing primarily on ILC, and we have developed Tamoxifen-resistant ILC cells to study how to improve sensitivity to tolerable antiestrogen therapies. We have identified ASS1 as one metabolic pathway that is silenced in these cells and have preliminary data that shows that reactivating this pathway can improve responses to tamoxifen. We are proposing further work to understand mechanisms and opportunities to improve outcomes in these unfortunate patients.

Treatment Strategies, Poster #11

The effect of (neo)adjuvant chemotherapy on long-term survival outcomes in Invasive Lobular Breast Cancer Patients treated with endocrine therapy: a retrospective cohort study

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Background
Despite histological and molecular differences between invasive lobular carcinoma (ILC) and carcinoma of no special type (NST), in the Dutch guidelines no distinction is made regarding the use of chemotherapy. However, ILC may be less sensitive to chemotherapy than NST. Studies on the long-term outcome of chemotherapy in ILC patients, though, are scarce and show inconclusive results.

Methods
We selected 520 women from the Erasmus Breast Cancer Cohort with estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-) ILC treated with endocrine therapy and with an indication for chemotherapy. We compared patients actually treated with chemotherapy (n=379) with those without chemotherapy (n=141). Multivariable Cox proportional hazards models were used to estimate the association of chemotherapy on recurrence-free survival (RFS), breast cancer-specific survival (BCSS), and overall survival (OS). Additionally, we used the Inverse Probability of Treatment Weighting (IPTW) method with tumor size, lymph node involvement and tumor grade included in the treatment model, to obtain the average treatment effects (ATE) of chemotherapy on RFS, BCSS and OS.

Results
Patients in the chemotherapy group were younger (51 vs 61 years, p<0.001), had a higher T-status (T3+ 33% versus 14%, p<0.001) and more positive lymph node involvement (80% versus 49%, p<0.001) than patients without chemotherapy. Chemotherapy treatment was not associated with better RFS (HR 1.20, 95%CI 0.63-2.31), BCSS (HR 1.24, 95%CI 0.60-2.58), or OS (HR 0.97, 95%CI 0.56-1.66). This was confirmed by the results from the IPTW method, with non-significant ATEs of chemotherapy on RFS (1.74, 95%CI -0.54-4.02), BCSS (0.84, 95%CI -1.21-2.89), and OS (-0.99, 95%CI -2.70-0.72).

Conclusion
Chemotherapy is not associated with improved RFS, BCSS or OS for ER+/HER2- ILC patients treated with endocrine therapy and with an indication for chemotherapy.

Lay abstract

Invasive lobular breast cancer (ILC) may be less sensitive to chemotherapy than carcinoma of no special type (NST). We aimed to investigate whether adding chemotherapy upon endocrine therapy lowers the risks of disease recurrence and of dying from breast cancer, and additionally improves overall survival for ILC patients. We selected 520 women from our institutional breast cancer registry with a history of hormone-sensitive ILC who were treated with endocrine therapy and who had an indication for chemotherapy according to the national guidelines. We compared patients actually treated with chemotherapy (n=379) with those who received no chemotherapy (n=141). We used two different statistical approaches to estimate the added value of chemotherapy. We observed no differences in the risk of recurrence of breast cancer and dying from it, nor a difference in overall survival between patients with and without chemotherapy, indicating no added value of chemotherapy.

Treatment Strategies, Poster #12

Personalized circulating tumor DNA (ctDNA) testing for detection of progression and treatment response monitoring in patients with metastatic invasive lobular carcinoma (mILC)

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mILC presents unique clinical challenges and can be difficult to monitor radiographically. More accurate biomarkers are needed for real-time assessment of response to treatment. This real-world study demonstrates the feasibility of longitudinal ctDNA testing for treatment response monitoring in patients with mILC. Longitudinal plasma samples (n=219) from 60 patients with mILC (median age at baseline: 62.9 years; range: 32.2-79.7) treated between 7/22/21 and 6/15/23 were analyzed. ctDNA was detected and quantified using a personalized, tumor-informed assay (Signatera™). Receptor status included: HR+ (80%), HER2+ (10%), TNBC (2%), and not available (8%). CDH1 (75%) and PIK3CA (42%) were the top two mutated genes; p.Q23* was the most common CDH1 variant (5%). Of the 60 patients, 37 (62%) had detectable ctDNA at all timepoints tested while on treatment. Imaging/biopsy results after baseline were available for 32/37 patients, 18 (56%) of whom had detectable ctDNA within 3 months of imaging. Of these, 16 (88%) patients had progressive (N=10) or stable (N=6) disease. The remaining two patients had no evidence of disease (NED) prior to the first ctDNA testing, but later had significant ctDNA increase indicating molecular recurrence with imaging pending. Of the 60 patients, 14 (23.3%) had undetectable ctDNA at all timepoints tested. Of these, 6 (42%) had imaging results within 3 months of ctDNA testing and no patient showed evidence of disease progression. Of the remaining 9/60 patients, 3 achieved sustained ctDNA clearance after therapy initiation, 2 converted from undetectable to detectable ctDNA, and 4 had transient clearance/low ctDNA levels (0.038-1.007 mean tumor molecules/mL) while on treatment and NED. In this study, ctDNA detection correlated well with clinical status determined by conventional monitoring tools in mILC patients. Personalized, longitudinal ctDNA testing may have utility in detecting progression and monitoring treatment response in patients with mILC.

Lay Abstract:
Detection of metastatic invasive lobular carcinoma (mILC) and its progression can be challenging using conventional imaging techniques. In this study, we evaluated the feasibility of circulating tumor DNA (ctDNA), a minimally invasive and blood-based biomarker, for treatment response monitoring in a real-world cohort of 60 patients with mILC. A personalized, tumor-informed assay (SignateraTM) was used for the detection and quantification of ctDNA in serial plasma samples. We observed that all ctDNA-positive patients with imaging results available had progressive/stable disease, whereas none of the ctDNA-negative patients with imaging results available had evidence of progressive disease. These results indicate a correlation between ctDNA detection and clinical disease status as well as the potential utility of serial ctDNA monitoring for real-time assessment of response to treatment in patients with mILC.

Treatment Strategies, Poster #13

Characterization of Resistance to CDK4/6 Inhibitors and Endocrine Therapy in Invasive Lobular Breast Cancers Cell Lines

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Background: Approximately 10% of breast cancer types are classified as invasive lobular carcinoma (ILC) and are estrogen receptor (ER) – positive (+) with a loss of E-cadherin and exhibit diffuse growth patterns making it harder to detect than invasive ductal carcinoma (IDC). Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors are the newest addition to the standard of care for patients with metastatic, ER+ breast cancer, which includes patients with ER+ ILC. Herein, we uncover mechanisms of resistance to CDK4/6 inhibitors and endocrine therapy and identify strategies to overcome resistance.
Methods: To compare ILC to IDC we generated cell lines resistant to CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) and endocrine therapies (tamoxifen, fulvestrant, and hormone-free) in 7 cell lines: MDA-MB-134, SUM44PE, MCF-7, T47D, MDA-MB-361, BT474, and ZR-75-1. Cells were selected for resistance to the CDK4/6 inhibitors and antiestrogens then RNA-Seq was performed using NovaSeq-6000 platform (Illumina) to identify pathways associated with resistance.
Results: The ILC and IDC models of CDK4/6 inhibitor resistance exhibited hallmark signature pathways involved in cell signaling: E2F targets, G2M checkpoint, JAK/STAT signaling, and estrogen response. The ILC cell line, SUM44PE, treated with Palbociclib exhibited an upregulation of the hallmark epithelial mesenchymal transition (EMT) markers: CDH2 (N-Cadherin), VIM (vimentin), FN1 (fibronectin). The ILC cell line, MDA-MB-134, treated with either palbociclib or endocrine therapy conditions resulted in an upregulation of androgen receptor (AR). MDA-MB-134 cells deprived of estrogens or maintained in the presence of antiestrogens upregulated the ERBB2/HER2 pathway. Both ILC and IDC cell lines showed varying patterns of multidrug resistance (MDR) proteins.
Conclusions: Human breast cancer cell line models of ILC and IDC resistant to CDK4/6 inhibitors or endocrine therapies were characterized using RNA-Seq. We confirmed previously reported hallmark pathways as well as discovered novel pathways of resistance in ILC cells including: EMT pathways, AR, HER2, and MDR genes.

Lay Abstract:
Background: Approximately 10% of breast cancer types are classified as invasive lobular carcinoma (ILC) and are estrogen receptor (ER) – positive (+) with a loss of E-cadherin and are harder to detect than invasive ductal carcinoma (IDC). Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors have become a new standard of care for patients with ER+ breast cancer. Herein, we uncover mechanisms of resistance and identify strategies to overcome them.
Methods: To compare ILC to IDC we generated cell lines resistant to CDK4/6 inhibitors and endocrine therapies and performed RNA-Seq with functional analysis.
Results: The ILC and IDC models of CDK4/6 inhibitor resistance exhibited hallmark signature pathways involved in cell signaling. The ILC cell lines exhibited differences in epithelial mesenchymal transition (EMT) markers, androgen receptor (AR), ERBB2/HER2 pathway, and multidrug resistance (MDR) proteins.
Conclusions: We confirmed previously reported hallmark pathways as well as discovered novel pathways of resistance in ILC cells

Treatment Strategies, Poster #14

Predicting Response to HER2 Tyrosine Kinase Inhibitors and Antibody Drug Conjugates in ERBB2 Mutant ILC Using CRISPR/Cas9 Knock-in Cell lines and Patient-derived Organoids

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Background: Activating mutations in ERBB2 (HER2) are enriched >4-fold in invasive lobular carcinoma (ILC) with a rate of up to 19% in metastatic ILC. ILC is a histologic subtype of breast cancer characterized by loss of E-cadherin (CDH1), suggesting a potential interaction between loss of CDH1 and mutations in ERBB2. Recent trials have demonstrated promising single agent efficacy using the irreversible pan-HER tyrosine kinase inhibitor (TKI), neratinib, in patients with metastatic ERBB2 mutant ILC. However, further studies on combination therapies with other anticancer agents are needed to increase response rate and progression free survival for these patients. HER2-targeted antibody drug conjugates (ADC), particularly trastuzumab deruxtecan (T-DXd), have shown great promise in HER2-low metastatic breast cancer. Yet, their efficacy as a single agent or with HER2 TKIs in HER2-low and -mutant ILC is unknown and warrants investigation.

Methods: Past studies analyzing ERBB2 mutations used overexpression of ERBB2 mutant cDNA, but this approach does not faithfully recapitulate the human disease. To model ERBB2 missense mutations as found in human breast cancers, we used CRISPR-based prime editing to generate a panel of isogenic ILC cell lines and patient-derived organoids (PDO) harboring ERBB2 wild-type (WT) or ERBB2 mutations (S310F or V777L). Both of these ERBB2 mutations have been previously characterized and are known to be activating mutations. We then used them to test neratinib and other TKIs with ADCs, including T-DXd and trastuzumab emtansine (T-DM1).

Results: We successfully introduced single copy, heterozygous activating ERBB2 mutations (S310F or V777L) into two ERBB2-nonamplified metastatic ILC cell lines (MDA-MB-134 and SUM44PE) and one ERBB2-nonamplified metastatic ILC PDO (IPM-BO-053). Positive clones carrying the mutations were verified by Sanger sequencing and droplet digital PCR and subsequently pooled together. We further demonstrated that these mutations hyperactivated HER2 and downstream signaling pathways. ILC cell lines harboring these mutations showed enhanced sensitivity to both HER2 TKIs and ADCs. In contrary, ERBB2 mutations did not alter responses of IPM-BO-053 PDOs to HER2 TKIs but significantly increased responses to ADCs.

Interestingly, we also observed accelerated HER2 protein degradation upon heregulin stimulation in ERBB2 mutant ILC PDOs, suggesting activating ERBB2 mutations may enhance ADC/HER2 complex internalization, degradation, and release of payloads. Lastly, we explored drug synergy between HER2 TKIs and ADCs in isogenic IPM-BO-053 PDOs and found that combination of T-DXd and neratinib or afatinib showed synergy (Combination Index <1).
Conclusions: Although the reason for the discrepancies in drug response between ILC cell lines and PDOs is not clear, we hypothesize that response in 3D PDOs might be more faithfully representing response seen in patients. We will generate additional ILC PDOs with knock-in ERBB2 mutations to validate our findings. Irreversible HER2 TKIs, such as neratinib and afatinib, showed synergy with T-DXd in ERBB2 mutant ILC PDOs. This holds important therapeutic implications in light of current treatment options for ILC. In future experiments, we will test if neratinib or afatinib increases endocytic uptake of T-DXd using a fluorescently labeled endocytosis tracker. Further, an in-depth molecular characterization of our isogenic cell lines and PDOs models is ongoing to gain mechanistic insights into how activating ERBB2 mutations increase HER2 internalization and degradation. Our in vitro studies provide a strong foundation for in vivo testing of neratinib or afatinib with T-DXd for ERBB2 mutant ILC.

Lay Abstract
Activating ERBB2 (HER2) mutations are enriched in invasive lobular carcinoma (ILC) of the breast and thus potential targets for HER2 therapies. My project aims to characterize the functional roles of recurrent ERBB2 mutations in ILC using innovative preclinical models and to provide rationale for specific combination targeted therapy for patients with ERBB2 mutant ILC, in order to enhance anti-tumor activity and/or delay drug resistance.

Treatment Strategies, Poster #15

Efficacy of PARP inhibitor Talazoparib on ER+ ILC breast cancer models

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Background/Purpose: Anti-estrogens are a cornerstone of treatment for invasive lobular carcinoma (ILC) as nearly all ILC express estrogen receptor α (ER). However, anti-estrogen resistance remains a pervasive clinical problem. We previously linked ER activity and anti-estrogen resistance in ILC to ILC-specific activity of MDC1 (mediator of DNA damage checkpoint 1) as an ER co-regulator. We subsequently found that in ILC cells, canonical functions of MDC1 in DNA repair may be compromised, manifesting as dysfunctional DNA double-strand break repair. Based on this, we hypothesized that ILC cells may be sensitive to PARP inhibitors as a mechanism to exploit this DNA repair dysfunction.
Experimental Design: ILC cell lines MDA-MB-134VI (MM134) and SUM44PE (44PE), and IDC cell lines MCF7 and T47D, were treated with FDA-approved PARP inhibitor talazoparib and/or anti-estrogens tamoxifen or fulvestrant. Proliferation and long-term survival was assessed by dsDNA quantification. Parallel studies examined talazoparib response in long-term estrogen-deprived (LTED) anti-estrogen resistant variants of MM134 and 44PE.
Results: ILC cells were sensitive to single-agent talazoparib (IC50 = 38nM and 13nM in MM134 and 44PE, respectively) and showed compromised long-term viability after drug wash-out. In contrast, T47D IDC cells were talazoparib-resistant (IC50 = 140nM); MCF7 showed initial sensitivity (IC50 = 20nM) but proliferated normally after drug wash-out. Combining anti-estrogens with talazoparib did not reduce ILC cell sensitivity to talazoparib, suggesting PARP inhibitor sensitivity in these cells is not explicitly linked to proliferation. LTED variants of 44PE and MM134 were sensitive and resistant to talazoparib, respectively, associated with differential activity of ER and MDC1 in these models.
Conclusions: ILC cells are sensitive to PARP inhibition with talazoparib, which may be associated with an unappreciated DNA repair deficiency. Developing biomarkers of ER, MDC1, and DNA repair activity in ILC is critical toward understanding PARP inhibitor sensitivity in primary versus anti-estrogen resistant ILC.

Lay Abstract

Despite being typically cast as a “low risk” breast cancer, Invasive lobular carcinoma (ILC) is linked to poor long-term outcomes and resistance to anti-estrogens, which is complicated by a lack of ILC-tailored treatments. Our research identified a potential treatment vulnerability in ILC cells, related to the ability of cells to repair damage to their DNA. We found ILC cells have a defect in their DNA repair capacity, which can be exploited using FDA-approved “PARP inhibitors” like talazoparib. We tested whether ILC cells are sensitive to talazoparib, compared to invasive ductal carcinoma (IDC) cells. ILC cells were more sensitive to talazoparib than IDC cells, and less able to survive treatment. However, some anti-estrogen resistant ILC cells were PARP inhibitor-resistant, related to changes in DNA repair. Our work identifies PARP inhibitors as a potential treatment for ILC, and will be explored in tumor models with paired biomarker studies for DNA repair activity.

Treatment Strategies, Poster #16

High prevalence of HER2-low among ILC with residual disease following neoadjuvant therapy provides therapeutic opportunities with HER2-antibody-drug conjugates

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Background: Invasive lobular carcinoma (ILC) accounts for 5-15% of breast cancer (BC). Most ILCs are strongly hormone receptor positive. Adjuvant endocrine therapy (ET) is the usual treatment. The use of chemotherapy alone or in combination with ET is controversial and does not improve survival outcomes for most patients with ILC compared to ET alone. However, neo-adjuvant ET (NET) may be warranted to downstage some ILC permitting breast conservation. HER2-Low BC spectrum demonstrates variable HER2 expression and HR status. HER2-Low BC are defined by an immunohistochemical score of 1+ or 2+ with negative FISH. The prevalence and implications of HER2-Low in ILC are poorly characterized. This study investigates rates of HER2-Low among patients with ILC treated with neoadjuvant therapy (NAT) and correlates HER2-Low with clinicopathologic features predictive of outcome.
Methods: Patients with ILC treated from 2018 to 2022 at our institution were identified from a breast cancer database. HER2-Low status was correlated with tumor characteristics and treatment data.
Results: Between 2018 and 2022, 196 women with ILC were treated at our institution. Median age at diagnosis was 63 years (25–90). The majority of ILC were HR+/HER2- (93%), intermediate to low grade (87%), and HER2-Low (52%). Among the HR+/HER2- patients, 11 received neoadjuvant chemotherapy and 26 received NET. Patients receiving NAT presented with pT2/pT3 (85%), multifocal tumors (76%) and positive nodes (65%). Twenty-one (56%) were HER2-Low. No difference in clinicopathologic features was observed between HER2-0 and HER2-Low. Among HR+/HER2- patients receiving NAT, none achieved pCR and 89% were RCB II/III. Among NAT patients with RCB II/III, 55% were HER2-Low and among NET patients with RCB II/III, 68% were HER2-Low.
Conclusion: HER2-low status is common in this ILC cohort, including patients who received NAT. High prevalence of HER2-low among ILC with residual disease following NAT provides therapeutic opportunities with HER2-antibody-drug conjugates.

LAY ABSTRACT

The goal of this study is to investigate the prevalence of HER2-low expression in patients with invasive lobular carcinoma (ILC) treated at our institution, including patients with ILC treated with presurgical (neoadjuvant) therapies. Patients with invasive lobular carcinoma who are selected for neoadjuvant chemo- or endocrine therapy have primary tumor characteristics associated with more aggressive disease. HER2-low breast cancer (BC) is a newly defined subset of HER2-negative BC with a HER2 immunohistochemical score of 1+ or 2+ and lacking HER2 gene amplification. Recent clinical trials have demonstrated significant

clinical benefit from novel HER2 antibody-drug conjugates to treat HER2-Low BC. We found 52% of patients with ILC at our institution are categorized as HER2-Low and 55% of patients with large volume of residual tumor after neoadjuvant treatment were HER2-Low. High prevalence of HER2-low among ILC, especially in patients with residual disease following NET, provides therapeutic opportunities with HER2-antibody-drug conjugates.

Treatment Strategies, Poster #17

E-cadherin loss imparts mitotic vulnerabilities rendering breast cancer cells synthetic lethal to crizotinib and up-regulation of Src signalling reverses this effect

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Loss of E-cadherin, a vital cell adhesion molecule, is commonly observed in cancers and is the pathognomonic alteration in lobular breast cancer. Beyond its established roles in cell adhesion, E-cadherin is also implicated in mitotic processes such as spindle orientation and centrosome clustering. Our previous research has demonstrated that the clinical ROS1 inhibitor crizotinib exhibits synthetic lethality with E-cadherin loss. We conducted genetic perturbation screens of E-cadherin defective breast cancer cells exposed to crizotinib, which revealed an increased dependency on genes regulating the spindle assembly checkpoint and kinetochore function. Live cell imaging showed that crizotinib disrupted chromosome alignment and segregation, leading to delayed mitotic progression, mitotic slippage, multinucleation and eventual mitotic catastrophe. Furthermore, our findings suggest that the negative regulator of Src-family kinases, CSK, controls these processes and its inactivation results in crizotinib resistance. Preliminary data indicates that the effect of CSK on crizotinib resistance may be mediated through Aurora-A kinase activation and restoration of centrosome maturation. Additionally, loss of CSK appears to confer increased polyploidy tolerance following crizotinib exposure. Altogether, our results highlight the critical role of E-cadherin in mitosis and offer potential new avenues for targeting E-cadherin defective breast cancers.

Lay Abstract

The E-cadherin molecule is often missing in lobular breast cancer cells. While its main job is to help cells stick together, it also plays a role in cell division. Previous work in our lab have found that a drug called crizotinib can be effective against cancers that do not have E-cadherin. Our current work has discovered that crizotinib causes problems in cell division, resulting in mistakes that can ultimately cause the cell to die. We identified a molecule called CSK that regulates this process and when CSK is turned off, the cancer cells become resistant to crizotinib. We also find that CSK may be involved in a pathway that controls the formation of important structures during cell division. This research could lead to new treatments that targets these vulnerabilities in lobular breast cancer.

Treatment Strategies, Poster #18

Coupling bioengineered microsystems with high-resolution microscopy to investigate dormancy and drug resistance in invasive lobular carcinoma

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Rationale: Invasive lobular carcinoma (ILC) tumors are often low-grade, hormone-receptor-positive (HR+), HER2-negative, and E-cadherin-negative. ILC patients face low rates of long-term disease-free, overall survival, and more tumor cells in circulation compared to invasive ductal carcinoma (IDC), indicating drug resistance and dormancy as possible metastatic mechanisms. Despite their differences, ILC and IDC are treated similarly in the clinic with HR+ dormancy investigations focusing on IDC. Therefore, investigating dormancy and drug-resistance biomarkers specific to ILC is a necessity. The extracellular matrix (ECM) has profound implications on dormancy, motivating bioengineered models to isolate cellular interactions compatible with high-resolution microscopy.
Methods: We developed tamoxifen-resistant (TAMR) ILC cell lines with extended treatments of low-dose tamoxifen in MDA-MB-134-VI and SUM44-PE cell lines. Light-induced molecular adsorption, in situ photopolymerization, and droplet-based microfluidics were utilized to investigate ECM-cell and cell-cell interactions. Dual treatment of hypoxia and mitogen deprivation was used to test for dormancy propensity. Immunofluorescence was performed with live-cell epifluorescence, confocal microscopy, and total internal reflection fluorescence microscopy.
Results: We recapitulated the morphological and molecular characteristics of IDC and ILC with the bioengineered microsystems, revealing the necessity of intercellular E-cadherin to form complex three-dimensional structures. As such, IDC formed complex structures, including the formation of acini, whereas ILC cells formed discohesive structures, providing a physiologically relevant model to investigate ILC dormancy. The ILC cells cultured in the microsystems upregulated p27Kip1 across the microsystems under hypoxic and mitogenic stress. Furthermore, p27Kip1+ cells downregulated Ki67 and exhibited smaller nuclei, indicating quiescent cells.
Conclusion: The bioengineered recapitulated the distinct differences between IDC and ILC and the inability of ILC cells to form complex structures mediated by E-cadherin. Furthermore, p27Kip1 is distinctly upregulated under dormancy-inducing conditions and absent in controls. Therefore, the microsystems provide a facile model for cell-ECM and cell-cell interactions to investigate ILC dormancy.

Lay Abstract

ILC patients often experience relapse after long timeframes, likely due to the tumor cells acquiring resistance to therapeutics and the arrest of cellular growth in distant sites, termed dormancy. Although delayed recurrence occurs in HR+ cancers, this clinical challenge is more common in ILC. Therefore, we aim to investigate dormancy in ILC by understanding the molecules mediating its induction through a variety of bioengineered techniques to simulate the tumor microenvironment and molecular biology to identify proteins differentially expressed in dormant cells. The work's success will aid in understanding late relapse in ILC patients and promote further research in identifying biomarkers of dormancy and strategies to target these cells to improve outcomes.

Treatment Strategies, Poster #19

Inhibition of iRhom by CD44-targeting Nanocarrier for Improved Cancer Immunochemotherapy

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Abstract:
The multifaceted chemo-immune resistance is the principal barrier to achieving cure in breast cancer patients. Identifying a target that is critically involved in chemo-immune-resistance represents an attractive strategy to improve cancer treatment. IRhom1 (RHBDF1) plays a role in cancer cell proliferation and its expression is negatively correlated with immune cell infiltration. Here we showed that iRhom1 decreased chemotherapy sensitivity by regulating the MAPK14-pHSP27 axis. In addition, iRhom1 inhibited the cytotoxic T-cell response by reducing the stability of ERAP1 protein and the ERAP1-mediated antigen processing and presentation. To facilitate the therapeutic translation of these novel findings, we developed a biodegradable nanocarrier that was effective in codelivery of iRhom1 pre-siRNA (pre-siiRhom) and chemotherapeutic drugs. Importantly, this nanocarrier was highly effective in tumor targeting and penetration through both EPR and CD44-mediated transcytosis in tumor endothelial cells as well as tumor cells. Inhibition of iRhom1 further facilitated tumor targeting and uptake through inhibition of CD44 cleavage. Co-delivery of pre-siiRhom and doxorubicin led to significantly enhanced antitumor efficacy and activated tumor immune microenvironment in breast cancer models. Targeting iRhom1 together with chemotherapy represents a novel strategy to overcome chemo-immune resistance in breast cancer treatment.

Lay Abstract:
IRhom1 is an oncoprotein that has been shown to be overexpressed in breast cancer and enhance tumor cell proliferation. We report here that iRhom1 is also critically involved in resistance to chemotherapy and immunotherapy. We have also developed a new nanoparticle-based drug carrier that is highly effective in codelivery of iRhom1 siRNA (an oligonucleotide-based therapeutic that inhibit the synthesis of iRhom1) and doxorubicin, leading to significantly improved antitumor activity in a murine breast cancer model.

Prognosis, Poster #20

Comparison of long-term outcome between high risk lobular versus ductal breast cancer: a propensity score matched study

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BACKGROUND
After recent MonarchE trial approval of abemaciclib for adjuvant treatment of hormone receptor-positive, Her2-negative, node-positive breast cancer (BC), the frequency of BC patients potentially eligible for abemaciclib is unclear. There are conflicting data regarding the biological behavior and long-term outcomes between invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). With this study we would like to retrospectively evaluate the real-world data and long-term outcome of ILC compared to IDC selected with high-risk features, according to MonarchE trial selection criteria.

METHODS
15,071 patients operated at the European Institute of Oncology for a first primary, non-metastatic, hormone receptor-positive, HER2-negative BC from 2000 to 2008 were selected. 11,981 (79.5%) patients presented an IDC, 1524 (10.1%) an ILC. Applying the same eligibility criteria from the MonarchE study, we identified two high-risk groups.

RESULTS
A total of 2,872 (21.3%) patients were selected as high-risk, including 361/1,524 ILC (23.7%) and 2,511/11,981 IDC (21%). 322 high-risk ILC were matched with similar high-risk IDC. The median follow-up was 13.2 years for survival. In the matched set, the cumulative incidence of 10-years axillary lymph node and contralateral BC recurrences were higher in ILC vs IDC.
Disease free survival (DFS) and overall survival (OS) were not statistically significantly different between the two histotype groups. At multivariate analysis, age < 35 years, pT2-3, axillary involvement with more than 10 positive axillary nodes were found to be predictors of unfavorable DFS and OS in the overall high-risk population.

CONCLUSIONS
Findings from this study demonstrated rates of concordant long-term outcome status by histologic subtype, suggesting an equivalent clinical management and therapeutic strategy for these two different histological entities with such high-risk features. Our real-world data demonstrated an approximately 21% rate of high-risk patients, potentially eligible for adjuvant abemaciclib treatment, suggesting that ILC patients might benefit most from this therapy.

Lay Abstract
A recent important study called MonarchE trial confirmed the ability in interfering with the growth and spread of breast cancer by a drug, abemaciclib, administered after surgery in combination with hormonal therapy for the treatment of breast cancer at higher risk of coming back (recurrence) to other parts of the body. Our study was conducted on a selected group of 2,872 patients with breast cancer with this higher risk, extracted from a large population of 15,071 patients operated for breast cancer at the European Institute of Oncology in Milan (Italy) between 2000 and 2008. It reported a higher rate of recurrence in the axillary lymph nodes and the contralateral breast in patients with invasive lobular carcinoma compared with those with invasive ductal carcinoma. These results would suggest that abemaciclib might be of greater benefit for women with lobular breast cancer than for invasive ductal breast cancer.

Prognosis, Poster #21

Glutamate metabolic enzymes associate with increased tumor size in black women with invasive lobular breast cancer: a single-institution study

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Background and Objective: Clinical and preclinical evidence suggests that Invasive Lobular Carcinoma (ILC) may be less dependent on glucose metabolism than Invasive Ductal Carcinoma (IDC), instead relying more on lipid and/or amino acid metabolism. We previously reported that metabotropic glutamate receptor expression is increased in endocrine therapy-resistant cell line models of ILC. We hypothesized that increased expression of glutamate transporters and regulatory enzymes associate with poor prognostic features in ILC.
Methods and Analysis: We used multispectral immunofluorescence staining to measure four functionally interrelated proteins (CD98, GLUD1/2, GPX4, and SLC7A11) in two racially diverse cohorts of 72 and 50 women with ILC and IDC, respectively, who had surgery at MedStar Georgetown University Hospital with a median follow-up time of 8.4 years. Primary tumor samples were assessed by two-fold redundant tissue microarray (TMA). SLC7A11 and CD98 form a heterodimer that exports glutamate from, and imports cystine into, the cell. GLUD1/2 are two isoenzymes that convert glutamate into α-ketoglutarate to replenish the TCA cycle, or recycle ammonia to support amino acid synthesis. GPX4 is an enzyme that protects cells from lipid peroxidation-induced death.
Results: High expression of CD98 was significantly associated with poor overall survival in women with ILC, but not IDC. The percentage of ILC and IDC tumor cells stained positive for GLUD1/2 and GPX4 was significantly higher than for CD98 or SLC7A11, so further analyses focused on these enzymes. In multivariate analyses, GPX4, GLUD1/2, and estrogen receptor (ER) expression were associated with tumor size in ILC, but not IDC. This relationship was markedly stronger in Black women with ILC. In univariate analyses, GLUD1/2 and GPX4 expression were each associated with tumor size in Black women with ILC, but not in the entire cohort or in IDC.
Conclusion: GLUD1/2 and GPX4 expression may have prognostic value in ILC, especially among Black women.

LAY ABSTRACT

Published clinical and laboratory studies have suggested that invasive lobular breast cancer (ILC) may be more dependent on proteins than sugars to obtain the energy they need to grow and spread. We measured the expression of four markers that contribute to how tumor cells take up and metabolize one particular amino acid (the building block of proteins) called glutamate. In a racially diverse cohort from our medical center, we found that two of these markers that regulate glutamate were highly abundant in large tumors in women with ILC, but not IDC. The link between tumor size and marker expression was strongest in Black women. Black women are significantly underrepresented in studies of ILC, and it is our goal to actively increase representation and identify social and genetic ancestry-associated factors associated with ILC outcomes.

Prognosis, Poster #22

Racial Disparities in Survival in Lobular Breast Cancer

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Purpose: The aim of our study was to characterize differences in incidence and survival, and identify predictors of survival, among women of varying race/ethnicities with lobular breast cancer.
Methods: Using the SEER database, we performed a retrospective cohort study of women diagnosed with ILC between 1998 and 2019. We collected demographic data on race, age at diagnosis, year of diagnosis, marital status, and income. Clinical data included grade, size, laterality, clinical stage, T and N stage, ER/PR/HER2 receptor status, surgery type, chemotherapy, radiation, and cause of death. Differences between racial groups were assessed using Chi-square tests or one-way ANOVA. Breast cancer-specific survival was compared between the racial groups using the Kaplan-Meier method. To identify predictors of survival, Cox-proportional hazard models were constructed. Statistical analyses were performed using SAS® and P values <0.05 were considered significant.
Results: 22,656 women with invasive lobular breast cancer were identified, including 19,081 White, 1400 Black, 1476 Asian, and 699 women of Other race. Black women presented more high-grade, advanced clinical stage, N2 stage, ER-positive disease, and had a lower rate of unilateral mastectomy than White women and women of other race. Asian women were comparatively younger, had more ER-/PR- disease, and received chemotherapy (Table 1). The five-year BCSS was worse in Black women (P<0.0008). The five-year BCSS rate in Black, White, Asian, and women of other race were 94.7%, 97.1%, 97.5%, 96.8%, respectively. Predictors of worse survival include Black race (HR1.36, P=0.0018) and ER-/PR- receptor subtype (HR2.19, P=<0.0001) (Table 2). Radiation receipt was associated with improved survival (HR0.84, P= 0.004) while receiving chemotherapy did not affect survival. Additionally, women in the age groups 30-39 (HR1.363, P=0.046), 60-69 (HR1.147,P=0.052), and 70-79 (HR1.477, P=<0.0001) had worse survival compared to women in the 50-59 age group.
Conclusion: There are differences in clinical presentation of invasive lobular breast cancer according to race. Black women had more advanced disease, while Asian women were younger. In women with ILC, overall survival for Black women at the 5-year time point was significantly lower compared to other racial groups. Our data provides insight into the complex interactions of race, clinical characteristics, and survival outcomes in lobular breast cancer.

Lay Abstract:
Purpose: Our study aimed to uncover why survival rates in lobular breast cancer differ among racial groups.
Methods: Using the SEER database, we examined data from 22,656 women diagnosed with invasive breast cancer from 1998-2109. We looked at demographic factors and clinical factors such as race, age, cancer characteristics, treatment, and more. We compared these factors between different racial groups using statistical tests and plotted survival rates. Our analysis helped to construct models to understand how these factors impact survival.
Results: Among the patients, Black women had higher rates of advanced cancer stages. Comparatively, Asian women were younger, diagnosed later and had higher rates of receiving chemotherapy. Notably, Black patients had the lowest 5-year survival rates. Radiation was associated with improved survival and interestingly, chemotherapy showed no significant impact.
Conclusion: Our data provides important insights into the complex interactions of race, clinical characteristics, and survival outcomes in lobular breast cancer.

Note: There are two large tables submitted in the original word file which is not copied in this master file. Table names are below.
Table 1: Descriptive Demographic and Clinical Data
Table 2: COX proportional hazard model

Metastasis, Poster #23

Glucocorticoid receptor activation inhibits tumor cell growth while increasing metastatic characteristics in models of invasive lobular breast cancer

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Estrogen receptor (ER)-positive invasive lobular breast cancer (ILC) is the second most common histological subtype of breast cancer. The unique genotypic and phenotypic characteristics of ILC include e-cadherin loss that is associated with “single file” tumor architecture in the breast and an unusual metastatic pattern characterized by discontinuous tumor cell foci invading serosal surfaces the peritoneal lining and abdominal organs. While ER is a known driver of ILC relapse and anti-estrogen treatment can reduce early disease progression to metastasis, very little is known about glucocorticoid receptor (GR) contribution to ILC’s distinctive biology. Our group has previously shown that GR-regulated cell cycle gene expression in ER+ infiltrating ductal carcinoma is associated with decreased tumor cell proliferation. In this study we examined isogenic GR+ and GR- ILC models SUM44 (ectopic GR) and MM134 (endogenous GR) and found that GR activation resulted in reduced activity of the “Estrogen-mediated S phase entry” gene expression pathway and associated decreased ILC cell proliferation following GR activation. Interestingly, our GR-mediated gene expression data also suggested that GR activation contributes to increased ILC integrin expression, potentially accounting for increased GR+ tumor cell-ECM adherence to serosal surfaces. GR expression also is associated with more efficient mesothelial cell clearance while a GR+ versus GR- xenograft MIND model revealed both decreased primary mammary gland tumor growth and increased distal metastasis to bone. In conclusion, GR activity in ILC appears to reduce ILC cell proliferation gene expression pathways associated with G1/S phase entry while increasing gene expression pathways associated with the metastatic cascade. In future studies, selective GR modulation and its potential role in inhibiting ILC metastasis will be evaluated.

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Lay Abstract:
Invasive lobular breast carcinoma (ILC) is a highly prevalent histological subtype of breast cancer. Our understanding of metastatic spread poses an unmet clinical challenge. The stress steroid hormone known as cortisol (ie glucocorticoids) contributes to ILC biology through interaction with the hormone receptor glucocorticoid receptor (GR). The aim of this study is to understand how previous findings of glucocorticoid regulation and GR activity in infiltrating ductal carcinoma contribute to similar processes observed in ILC such as cellular growth and cellular migration (ie metastasis). Hormonal homeostasis driven by the body’s stress response is an integral part of ILC tumor biology which we hypothesize that activation above baseline may promote enhanced invasion and metastatic spread. Thus, understanding the contributions of glucocorticoids to these processes will significantly improve current therapeutic intervention strategies.

Metastasis, Poster #24

Localization of metastatic disease in patients with a history of lobular carcinoma of the breast

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Introduction: Representing 10-15% of all breast cancer (BC) cases, invasive lobular carcinoma (ILC) is the second most common histological type of BC after invasive carcinoma of no special type (NST). Metastatic behavior and long-term survival seems to be different for ILC and NST although the results of previous studies are inconsistent. Therefore, this study aims to investigate whether there is a difference between ILC and NST in risk and pattern of loco-regional recurrence (LRR) and distant metastasis (DM), and in recurrence-free survival (RFS), distant disease-free survival (DDFS), overall survival (OS) and breast cancer-specific survival (BCSS).
Methods: For this matched retrospective cohort study we used data of patients from the Erasmus BC Cohort. ILC patients were matched to NST patients on age at diagnosis, year of birth, stage and ER-status. Differences in metastatic sites were determined using Chi-squared tests. The association between histological type and RFS, DDFS, OS and BCSS was estimated using Cox proportional hazard models.
Results: We included 584 ILC patients and 1434 NST patients. ILC patients were more likely to have DM to abdominal organs, while lung and lymph node metastases were more common in NST patients. Further, the median time to disease recurrence was longer in ILC patients. Multivariable analyses showed no differences between ILC and NST in RFS (HR 1.01, 95% CI 0.82-1.25), DDFS (HR 0.99, 95% CI 0.80-1.23), and BCSS (HR 1.01, 95% CI 0.80-1.27). ILC patients had a higher risk to die due to any cause (HR 1.20, 95% CI 1.00-1.44).
Conclusion: Histological BC type was not a prognostic factor regarding the risk of disease recurrence and dying from BC. However, a different metastatic pattern was observed, with more abdominal metastases and longer time to recurrence in ILC patients. These differences should be taken into consideration during decision-making processes.

Lay abstract

The aim of this study was to investigate whether after the initial diagnosis of breast cancer, recurrence and spreading of the disease differs between patients with invasive lobular cancer (ILC) type and invasive cancer of no-special type (NST), and whether this influences the risk of dying from breast cancer. We compared 584 ILC patients with 1434 NST patients, who were comparable regarding age, year of birth, stage and hormone sensitivity. We observed no differences in the risk of recurrence of breast cancer and dying from it between ILC patients and NST patients. However, ILC patients were more likely to have spreading to abdominal organs, while spreading to lung and lymph nodes was more common in NST patients. Further, the time to disease recurrence was longer in ILC patients.

Metastasis, Poster #25

Cancer-associated fibroblast driven paracrine IL-6/STAT3 signalling promotes migration and dissemination of Invasive Lobular Carcinoma cells

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Cancer associated fibroblasts (CAFs) contribute to many aspects of breast cancer tumorigenesis. However, their role in invasive lobular carcinoma (ILC), the second most common subtype of breast cancer, has not previously been extensively investigated. Although ILC tumours present many positive prognostic indicators, this does not translate to better outcomes compared to the most common subtype of breast cancer, invasive ductal carcinoma (IDC). ILC and IDC patients are treated using the same therapies, despite the myriad clinical, molecular and histological differences. No ILC-specific treatments are currently available. As CAFs are the most abundant non-transformed cell type in the ILC microenvironment, our work focuses on investigating paracrine signalling from CAFs to ILC tumour cells and identifying any ILC-enriched potential therapeutic targets.
Conditioned media from primary ILC patient-derived CAFs was used to stimulated SUM44PE cells. This led to activation of the MAPK pathway and phosphorylation of STAT3, a pro-tumorigenic transcription factor. IL-6 was identified as the CAF-secreted factor driving STAT3 activation. An RNAseq experiment allowed us to define a 42-gene IL-6 dependent signature (IL6GS). *IL6*, pSTAT3 and the IL6GS score were all significantly more highly expressed in ILC than IDC in the TCGA dataset.
IL-6 stimulation caused an elongated mesenchymal-like cell shape in SUM44PE cells and significantly increased their migration. A CRISPR screen of IL6GS genes identified *MUCL1* as driving the IL-6 dependent increase in migration, a novel STAT3 target that is more highly expressed in ILC. In a zebrafish embryo xenograft assay, IL-6 treatment led to significantly more SUM44PE cells being disseminated throughout the embryos and also promoted increased angiogenesis.
Altogether, this work identifies the CAF driven IL-6/STAT3 pathway as enriched in ILC and promoting increased tumour cell migration and dissemination *in vitro* and in the zebrafish assay. This suggests that this may be a novel therapeutic target for treatment of ILC.

Lay Summary
Lobular breast cancer is the second most common subtype of breast cancer and is defined by loss of E-cadherin, a protein which helps cells to stick together. Because of this, the tumours grow in a single-file line and so cancer cells come into contact with lots of non-cancerous 'healthy' cells. The most abundant type of these 'healthy' cells in Lobular tumours are cancer-associated fibroblasts (CAFs). CAFs have been shown to promote progression of other types of breast cancer but their role has not been investigated in Lobular breast cancer.

We have demonstrated that CAFs isolated from Lobular breast cancer patients secrete a protein called IL-6 which switches on tumour-promoting pathways and genes in Lobular breast cancer cells. IL-6 and the genes it switches on are very highly activated in lobular tumours compared to the most common subtype, Ductal breast cancer. IL-6 stimulation of Lobular cancer cells increased their movement and causes them to elongate – features that cause cancer cells to spread through a patient. We demonstrated that a gene called *MUCL1* drives this increase in migration. When Lobular cancer cells were injected into a zebrafish embryo, IL-6 stimulation also increased the spread of the cells throughout the embryo. This suggests that targeting IL-6 may be a good treatment option to reduce the spread of Lobular breast cancer in patients.

Metastasis, Poster #26

Development of a comprehensive cancer profiling assay utilizing circulating tumor cells in metastatic breast cancer

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Background: Liquid biopsy evaluation of circulating tumor cells (CTC) has provided valuable clinical information for patients with metastatic breast cancer, specifically where a tissue biopsy is not feasible. Combining biomarkers associated with circulating epithelial cells with single-cell whole-genome sequencing to assess copy number variations (genomic instability - GI), we differentiate rare cells from true CTCs. Additionally, we can determine the presence or absence of regions that contain gene amplifications (e.g., ERBB2). Here we report on CTC analysis using both protein biomarkers and single cell genomics.
Methods: Relevant cell line controls of epithelial origin and known expression levels of ER or HER2 were spiked into healthy donor whole blood at varying concentrations. All nucleated cells were deposited on glass slides at high density. Immunofluorescence staining of slides was performed to identify the presence and localization of nuclei (via DAPI), pan-CK, HER2, ER, CD45 and CD31. Cells classified as CTCs were further characterized for expression of ER and HER2 protein. CTC candidates were imaged and isolated for whole genome amplification. Single-cell sequencing data was analyzed for copy number to detect GI as well as amplification at the ERBB2 locus.
Results: The limit of CTC detection was 1 per 6M WBC. The sensitivity and specificity for HER2 detection was determined to be 94% and 97%, respectively. ER assay sensitivity and specificity were 91% and 100%, respectively. Overall accuracy was 95% for HER2 and 94% for ER detection. Overall precision across all relevant variables produced %CVs <10% for HER2 and ER. Gene amplification was detected with 85% sensitivity and 94% specificity.
Conclusion: These results reflect the validation of a novel clinical assay combining CTC detection with single-cell GI and amplification of the ERBB2 locus.

Lay version
Liquid biopsy evaluation of circulating tumor cells (CTC) has provided valuable clinical information for patients with metastatic breast cancer, specifically where a tissue biopsy is not feasible. Here we report a blood-based assay that can both identify breast cancer CTCs and using single cell genomics determine the level of genomic instability as well as ERBB2 (HER2) gene amplification status of the patient.

Biomarker, Poster #27

Serial monitoring of circulating tumor cells and circulating tumor DNA in metastatic lobular breast cancer identifies intra-tumor heterogeneity and precision and immuno-oncology biomarkers of therapeutic importance

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Despite treatment advances, metastatic lobular breast cancer (mLBC) remains incurable mainly due to tumor heterogeneity and evolution leading to exhaustion of treatment options. However, longitudinal molecular monitoring of the evolving disease is not routinely performed and is unfeasible or ambiguous with tissue biopsies or ctDNA. Circulating tumor cells (CTCs) are common in LBC, and we hypothesized that single-cell CTC genomic profiling allows detection and monitoring of the clinically-relevant biomarkers, their heterogeneity and evolution. We analyzed 120 individual CTC, 26 ctDNA, 15 white blood cells and 24 tissue samples, from 15 CTC-positive mLBC patients. CTC were enriched with CellSearch® and isolated as single cells with the DEPArray™ system. CTC, WBC, and ctDNA underwent targeted scNGS at 733x depth covering ~500 genes and 1.1Mb of genomic space to detect mutations, copy number alterations, tumor mutation burden (TMB) and microsatellite instability (MSI) with 99.1% of single cells and 95.2% of ctDNA informative samples.

Our CTC-based precision medicine reporting platform, MI-CTCSeq, detected targetable CTC alterations in 10 of 15 patients (67%). 14 of 22 alterations (64%) in 8 of 11 patients (73%) harbored actionable alterations not shared between all three analyte types (tissue, CTC and ctDNA), including undetectable mutations in each of the three. 13 patients (87%) displayed inter-CTC genomic heterogeneity of driver mutations. 1 of 4 (25%) patients with CTC available in >1 timepoint displayed fluctuations in their CTC subclonal makeup between timepoints. Data from this patient's 6 tissue, 5 ctDNA samples, and 28 individual CTC over 7 timepoints revealed in unprecedented detail heterogeneity and evolution in response to endocrine, chemo and immunotherapy pressures. Our novel detection of single-cell TMB and MSI showed highly concordant (R 0.81) CTC and tissue TMB and intra patient, inter-CTC TMB and MSI heterogeneity. These data support the non-invasive biomarker detection and monitoring by liquid biopsy in mLBC.

Lay Abstract

Metastatic lobular breast cancer is incurable due to the tumor molecular evolution that leads to therapy failure. However, monitoring of tumor molecular evolution is not performed in the clinic since tissue biopsies are invasive and costly whereas free-floating tumor DNA in blood is in limited amounts. Given that circulating tumor cells (cell that break off from a tumor and end up in blood) are plentiful in metastatic lobular breast cancer, and that tumor cells in a patient are not all identical, isolation and analysis of a number of individual circulating tumor cells represents a unique opportunity to monitor tumor evolution for treatment purposes.

In this study we analyzed over 180 samples from 15 metastatic lobular patients, including 120 circulating tumor cells and several tissue and free-floating DNA samples to detect cancer driving mutations and cancer immunotherapy biomarkers using a new method developed by us. This analysis revealed extensive heterogeneity of cancer driving mutations (presence of cells with or without the mutation). Mutations were often not present in all three matched samples from individual patients (circulating tumor cells, tissue, and free-floating tumor DNA). We also show that monitoring by circulating tumor cells reveals tumor evolution in response to endocrine and immunotherapy in unprecedented detail. Further, our new method to detect immunotherapy biomarkers in individual circulating tumor cells reveals good, but imperfect concordance with tissue biomarkers, indicating further heterogeneity among single tumor cells within individual patients. Altogether, our data suggest that single-cell profiling in metastatic lobular breast cancer represents a unique opportunity to monitor tumor heterogeneity and evolution with the potential to inform therapy and extend survival.

Biomarker, Poster #28

Assessing the Effect of E-cadherin loss on Estrogen Receptor activity in Human Mammary Epithelial Cell Models

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Abstract
Estrogen receptor (ER) expression and E-cadherin (CDH1) loss are the two most common molecular characteristics of invasive lobular carcinoma (ILC), but the interaction between E-cadherin loss and ER signaling during ILC tumorigenesis is not well understood. Further, estrogen-only hormone replacement therapy increases the risk of ILC, but not the more common invasive ductal carcinoma (IDC), highlighting a potentially unique role for ER in ILC initiation and progression. Our goal is to identify mediators that drive estrogen-induced tumorigenesis using the mouse mammary intraductal (MIND) model, which better recapitulates the progression of ILC and response to estrogen in vivo. We aim to identify how loss of E-cadherin in normal human mammary epithelial cells (HMECs) may lead to reprogramming of ER and promote the development of ILC. We have developed a CRISPR/Cas9 approach for knocking out CDH1 in primary HMECs. We injected control and knockout cells into #3 and #4 mammary glands of adult female NSG mice, with estrogen supplemented in their drinking water, and assessed for engraftment using bioluminescence imaging (BLI). BLI suggests enhanced engraftment of CDH1 knockout cells compared to CDH1 wild-type HMECs. Importantly, immunofluorescence of tissue one-month post-MIND injections showed engraftment of human cells positive for cytokeratin-5. Since primary HMECs showed minimal ERα expression by flow cytometry (only 2-5% ER+ cells), we also overexpressed ESR1 using lentiviral constructs in primary HMECs. We will study estrogen response in the CDH1-knockout versus control HMECs in vitro and in vivo to examine whether loss of E-cadherin contributes to ILC-specific estrogen activity. We predict that E-cadherin loss will accelerate estrogen-driven tumorigenesis by reprograming ER DNA binding through the dysregulation of pathways and transcription factors linked to E-cadherin. Understanding the unique interplay between E-cadherin loss and ER in ILC could lead to new therapeutic strategies for the prevention and treatment of ILC.

Lay Abstract
ILC are uniquely estrogen-driven among breast cancers, as nearly all ILC are estrogen receptor α (ER)-positive and increased breast cancer risk after hormone-replacement therapy is most strongly linked to ILC. Furthermore, clinical, epidemiological, and laboratory studies suggest that ER function is unique in ILC cells. Since ~95% of ILC tumors show E-cadherin loss, we aim to study how this loss affects ER using human mammary epithelial cells (HMECs) derived from donor tissue. We developed HMECs with decreased E-cadherin expression and injected them into the mammary glands of female mice. We then monitored the mice to see if the human cells survived and were incorporated into the mouse mammary gland. Our goal is to mimic the ILC phenotype both in human cells and mice to study how ER impacts the initiation and progression of the disease. This study could lead to new therapeutic approaches for preventing and treating ILC.

Biomarker, Poster #29

Increased Sox9 expression is associated with a more invasive ILC cell phenotype.

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Invasive Lobular Breast Cancer (ILC) is often diagnosed at more advanced stages, with higher nodal involvement and ~30% of ILC patients being diagnosed with multiple concurrent metastatic sites. ILC also has higher recurrent rates post-surgery and responds poorly to chemotherapy hence identification of more effective therapies to prevent or treat metastatic ILC is urgently needed. We recently isolated the more invasive VIVA1 cells from the representative ILC cell line MDA-MB-134VI. Orthotopic VIVA1 tumor cell administration in NSG mice, showed robust tumor growth in mammary ducts with spontaneous metastasis to the spleen, ovary, liver, adrenal gland and bone. RNAseq analysis showed Sox9 (SRY box transcription factor 9) was one of the most highly upregulated genes in VIVA1 cells. KEGG pathway analysis suggested significant alterations in a number of metabolic pathways including calcium signaling which had the highest number of altered genes. Interestingly, 7/16 genes in this KEGG pathway are predicted Sox9 targets. We thus hypothesize that Sox9 promotes ILC cell invasion and metastasis in part by altering metabolic signaling. We have confirmed that siRNA-mediated depletion of Sox9 results in impaired VIVA1 cell invasion in vitro, concomitant with reduced gene expression of the calcium signaling proteins CACNA1C (Calcium Voltage-Gated Channel Subunit Alpha1 C) and RYR1 (Ryanodine receptor 1). We also performed metabolomic analysis of Sox9 expressing or depleted VIVA1 cells and found significant alterations in metabolites associated with glycolysis and the pentose phosphate pathway. Our findings to date in our metastatic ILC model suggest that Sox9 may play an integral role in promoting metastatic growth of ILC in part via its ability to alter tumor cell metabolism. Given that ILC does not readily respond to chemotherapy, understanding the role of and targeting these metabolic vulnerabilities may lead to novel effective therapies for metastatic ILC.

Lay Abstract
Invasive lobular carcinoma (ILC) that has spread beyond the breast to other sites in the body (i.e. metastasized), does not respond well to current treatments used for metastatic breast cancers. To find new targets that might stop the growth of these metastatic tumors, we used a model of ILC that mimics metastatic spread from the breast. We found the Sox9 gene was increased in ILC cells with enhanced ability to spread. We confirmed that reducing levels of Sox9 blocked the ability of ILC cells to invade; a feature associated with their ability to spread. We further found that invasive ILC tumor cells have altered cellular metabolism, and if we inhibited expression of Sox9, this altered metabolism was decreased. Our findings suggest that therapeutically targeting Sox9 or critical nodes regulating the observed altered metabolism may be beneficial to treat metastatic ILC.

Biomarker, Poster #30

Mutating E-cadherin in Rats to Model Lobular Breast Cancer

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Invasive lobular cancer (ILC) is the most common special histological subtype of breast cancer, accounting for 8-14% of all breast cancer cases. 95% of ILCs are ER+ and treated with endocrine therapy. Optimal rodent models of ILC are needed to investigate ILC evolution and new treatment strategies. Mouse models of ILC have been made by mutating CDH1 (which encodes E-cadherin), a hallmark of ILC, but the resulting tumors have not been well characterized for ER and estrogen dependence. None of the hundreds of mouse models of breast cancer have been reported to exhibit estrogen dependence with the exception of perhaps STAT1 knockout mice. Rats, however, have mammary tissue more similar to humans, and easily generate ER+, estrogen-dependent mammary tumors. Our lab has successfully modelled ER+, estrogen-dependent ductal breast tumors in rats using intraductal injection of lentiviral oncogene or CRISPR/Cas9 genome editing, but a rat model of ILC is still lacking. In this study, we aim to model ILC in rats by intraductal injection of virus to CRISPR-edit the CDH1 locus. We will first examine the effect on rat mammary gland development, proliferation and apoptosis. Next, we will combine somatic deletion of CDH1 with activation of PIK3CA, the most commonly mutated protooncogene in human breast cancer. We will characterize the resulting tumors for histopathology, ER and PR expression, estrogen dependence, transcriptome and metastasis to distant organs, and compare the data with human ILCs. In summary, this study will develop a clinically relevant rat model of ER+ ILC, and will help understand ILC evolution and discover new treatment strategies.

Lay abstract:
Invasive lobular cancer (ILC) is a common subtype of breast cancer, accounting for 8-14% of all breast cancer cases. 95% of ILCs express estrogen receptor (ER) and are treated with hormone therapy. Clinically relevant models are needed to study the biology and develop therapies for ILC. Breast tumors in mice rarely express ER or respond to hormone therapy, and current mouse models of ILC have not been well characterized in these two aspects. Rats, however, have mammary tissue more similar to humans, and easily generate ER+, hormone-responsive mammary tumors. In this study, we aim to develop a clinically relevant rat model of ER+ ILC by manipulating the commonly mutated genes in rat mammary cells, and we will test whether the resulting tumors recapitulate the characteristics of human ILCs. This novel rat model will help understand ILC evolution and discover new treatment strategies.

Biomarker, Poster #31

WNT4 regulates cellular metabolism via intracellular activity at the mitochondria in invasive lobular carcinoma cells

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Wnt ligand WNT4 behaves in an atypical manner in invasive lobular carcinoma (ILC) and is a key ILC-specific estrogen receptor (ER) target. We previously showed that WNT4 engages with the PI3K-mTOR pathway via p70S6K and maintains downstream pathway activation, ultimately regulating cellular proliferation, metabolism, and survival. In ILC cells, we found WNT4 is critical in mitochondrial function and oxidative phosphorylation (OXPHOS), but the specific mechanisms are not yet understood. To explore the mechanistic role for WNT4 in ILC metabolism, we performed proximity-dependent biotinylation with mass spectrometry (BioID) to identify WNT4 signaling partners. BioID showed that unlike canonical Wnt protein WNT3A, WNT4 was localized to the mitochondria with putative partners STAT1, DHRS2, and mTOR. We explored WNT4 regulation of cellular metabolism further using global metabolomics comparing the effects of WNT4 knockdown inhibition or over-expression. We observed WNT4 signaling regulates OXPHOS; WNT4 knockdown suppressed OXPHOS as well as fatty acid and glutamate metabolism pathways, but not glycolytic activity. Taken together these data support that WNT4 influences cellular metabolism and mitochondrial function and plays a key role in unique metabolic phenotype of ILC. Determining the role of WNT4 within the mitochondria will allow us to better understand ILC metabolism and therefore how to better target WNT4 signaling pathways for potential treatment options.

Lay Abstract:
Proteins called ‘Wnt ligands’ are important for how tissues and organs in the body grow and develop, but we found that one Wnt ligand important in invasive lobular carcinoma (ILC) – WNT4 – has unusual functions in these cancer cells. In ILC, WNT4 is important for controlling how cancer cells metabolize fuels to support their growth and survival, and may direct ILC cells to preferentially use fats and amino acids instead of sugars. This parallels some observations on how ILC tumors use fuel. To learn about WNT4 in ILC metabolism, we used mass spectrometry to identify cellular proteins that WNT4 may partner with. These studies showed that, unexpectedly, WNT4 is physically located at the mitochondria, the sub-cellular units that produce energy from available fuels. This work will help us understand how ILC use fuel, and help identify treatment approaches targeted against the specific ways that ILC metabolize fats, proteins, and sugars.

Biomarker, Poster #32

The tumorigenic effects of E-cadherin loss in early ILC tumorigenesis

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E-cadherin loss, a hallmark of invasive lobular carcinoma (ILC), is an early and nearly universal event in ILC and associated precursor lesions (e.g. lobular carcinoma in situ, LCIS). However, ILC tumorigenesis, i.e. the progression from atypical lobular hyperplasia (ALH) to LCIS to ILC is not well understood, which confounds patient risk evaluation and treatment. Studies to date strongly support that E-cadherin has direct tumor suppressor roles that are pivotal to breast tumorigenesis, but mechanistic studies in human cells are limited. To model early ILC tumorigenesis, we use human mammary epithelial cells (HMECs), combining various modes of E-cadherin suppression with other candidate oncogenic hits, to determine how E-cadherin inhibition or loss remodels the HMEC genome to facilitate ILC tumorigenesis. In RNA-seq of three independent HMEC models (HMEC lines 122, 153, and 184, with CCND1 over-expression), we found that antibody inhibition of E-cadherin increases gene expression signatures associated with transformation and cancer phenotypes. For example, the hallmark signature of epithelial-mesenchymal transition (EMT) was activated in all three models (q=5.09E-8, 1.52E-18, 4.65E-13). Notably, the increased EMT signatures upon E-cadherin inhibition are accompanied by coordinate shifts in gene expression toward a more mesenchymal phenotype (e.g. increased SNAI2, VIM, FN1, ZEB1), and a basal or myoepithelial-like phenotype (e.g. decreased EPCAM / increased ITGA6). Initial experiments with E-cadherin knock down by siRNA have shown similar changes in EMT gene expression by qPCR. Parallel studies in CDH1-knockout HMEC models are underway to compare how E-cadherin suppression relates to complete loss-of-function observed in most clinical ILC. Taken together, E-cadherin suppression shifts pre-cancerous HMEC cells toward a more myoepithelial-like (i.e. dedifferentiated) gene expression phenotype, supporting E-cadherin loss remodeling the cellular transcriptome to facilitate ILC tumorigenesis.

Lay Abstract:
E-cadherin loss occurs in ~95% of invasive lobular carcinoma (ILC) and ~80% of lobular carcinoma in situ (LCIS), but our understanding of how E-cadherin loss promotes ILC initiation in human mammary cells is limited. To better understand the cellular processes that are affected by E-cadherin loss, we are investigating how the reduction in E-cadherin expression in healthy human mammary epithelial cells (HMECs) leads to a tumor-promoting state. So far, we have used HMECs derived from three donors and have seen similar shifts toward a tumor-promoting state. We have also observed differences between the HMECs which could help us determine what types of cells within the breast are more susceptible to becoming ILC. Further investigation of these similarities and differences could help us to find new biomarkers that can be used to improve current risk assessment and treatment strategies for LCIS and ILC.

Biomarker, Poster #33

Investigating RET as a Novel Therapeutic Target in Breast Cancer Brain Metastasis Using Patient-Derived Organoid

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Breast cancer brain metastasis (BrM) has become a major contributor to mortality from breast cancer. Current therapeutic strategies for breast cancer brain metastases are mostly limited to surgery, chemotherapy, and radiotherapy. There is an urgent need to better understand BrM and to define novel therapeutic targets.
We recently reported frequent overexpression of RET, a receptor tyrosine kinase, in BrM. The ligand for RET is glial cell-derived neurotrophic factor (GDNF), which is expressed at high levels in the brain. This drives our hypothesis that GDNF enhances breast cancer growth and colonization in brain through RET signaling pathway, which evaluates the potential of RET as a novel therapeutic target for breast cancer BrM patients.
We use three-dimensional (3D) patient-derived organoids (PDO) as a major model for the study of RET in BrM. We demonstrated that RET expression and signaling were increased in breast cancer BrM compared with primary tumors in clinical samples. We overexpressed RET in two primary PDOs (IPM-BO-056, IPM-BO-086) and one metastatic PDO (IPM-BO-053) using lentivirus. We determined that RET was activated by GDNF treatment, which was inhibited by Pralsetinib (BLU-667), a highly selective inhibitor of RET in RET-overexpressing PDOs. And preliminary growth assay data in IPM-BO-056 showed elevation of growth rate in RET overexpressing organoid after GDNF treatment, which could also be inhibited by Pralsetinib. In contrary, GDNF didn't promote growth in RET-EV organoids. RET downstream signalings AKT and ERK weren't stimulated by GDNF treatment, but the average level of phosphorylation was higher in RET-OE PDOs than RET-EV PDOs. One possible reason is that the overexpressed RET is super-active already and cannot be activated more. Meanwhile, we're also exploring other possible downstream signals of RET. In conclusion, our preliminary in vitro study provides initial clues on RET as a novel therapeutic target for breast cancer BrM patients.

Lay abstract

Breast cancer brain metastasis (BrM) has become a major contributor to mortality from breast cancer. But current therapeutic strategies are limited. There is an urgent need to better understand BrM and to define novel therapeutic targets. We recently reported frequent overexpression of RET in BrM. Its ligand GDNF is expressed at high levels in the brain. So we hypothesize that GDNF enhances breast cancer growth and colonization in brain through RET signaling pathway, which evaluates the potential of RET as a novel therapeutic target for breast cancer BrM patients. We use three-dimensional (3D) patient-derived organoids (PDO) as a major model for this study. We've successfully overexpressed RET in several PDOs. We determined that RET and downstream signalings were activated by GDNF treatment, which was inhibited by Pralsetinib, a highly selective inhibitor of RET. In vitro growth assay determined that GDNF promoted cell proliferation in RET overexpressing PDOs. In conclusion our preliminary study provides initial clues on RET as a novel therapeutic target for breast cancer BrM patients.

TME, Poster #36

Spatially resolved analysis of tumor microenvironment revealed biologically driven subgroups with distinct clinical outcome in invasive lobular carcinoma

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Background: Invasive lobular carcinoma (ILC) is the second most common histological breast cancer subtype; however, little is known about its tumor microenvironment (TME). Here, we aimed to study ILC TME using spatial transcriptomics (ST).
Methods: We performed ST (Visium 10x Genomics) on frozen tumor samples from 41 primary hormone receptor positive (HR+), HER2-negative (HER2-) ILCs. Relative hematoxylin/eosin (H&E) slides were morphologically annotated (QuPath software). After cross-samples integration, ST spots were clustered using hierarchical clustering. The information coming from the spatial organisation of the morphological annotation and the gene expression-based clustering was integrated and used as input for a patient level classification (using intNMFalgorithm). METABRIC (HR+, HER2- ILC samples, n = 122) was used as external validation cohort.
Results: Four groups showing different biological characteristics were identified. Differences in terms of morphology (annotation) and pathway enrichment analysis based on marker genes (GSEA, MSigDb) were observed between groups. This information allowed us to annotate our groups as: proliferative (P, n = 12, enriched in tumor cells and proliferation-related pathways), normal-stroma enriched (NSE, n = 10, enriched in fibroblasts and carcinoma in situ), metabolic (M, n = 9, enriched in metabolic-related pathways) and metabolic-immune enriched (MIE, n = 10, enriched in adipose tissue, metabolic and immune related pathways). Of note, a significative higher presence of macrophages M2 was found in MIE group (from xCell). Using group-specific gene signatures, we were able to reproduce the same 4 groups in the METABRIC. Of note, we observed significative differences in RFS in METABRIC ($p = 0.03$), with NSE presenting better and P and M worse disease outcome.
Conclusions: We identified 4 biologically driven HR+, HER2- ILC subgroups describing tumor microenvironment heterogeneity. Of note, two of the three groups associated to worse outcome were related to metabolism and not related to proliferation, highlighting the importance of metabolism in the biology of ILC. Further validation is warranted.

Lay abstract

Tumor microenvironment (TME) is the set of normal cells, vessels and molecules that surround and feed a tumor cell. Little is known about the TME of invasive lobular carcinoma (ILC). In this work, we aimed to study TME heterogeneity in ILC. To do so, we performed spatial transcriptomics (ST) on 41 frozen tumor samples obtained from patients with estrogen receptor-positive, HER2-negative ILC. ST is a technique that allows us to sequence the RNA of a slice of tissue by keeping the spatial information of the RNA expression. In brief, small spots (containing from 10 to 50 cells) can capture the RNA of the slice of tissue that has been attached on the ST slide. The captured RNA is then sequenced and, thanks to the spatial information of the spots, mapped back to the original tissue slice. Hematoxylin/eosin sections relative to each sample were morphologically annotated by assigning a cell type (label) to each cell and structure present in the slide. We performed clustering analysis in each sample (at the spot level), identifying groups of spots (across all our patients) sharing common characteristics from their gene expression point of view.

The information coming from the spot level clustering analysis and the morphological annotation was merged and used as input for a clustering analysis at the patient level. We identified four groups of patients inside our cohort. The proliferative group (P) presented a grater quantity of tumor cells and an enrichment in proliferation-related pathways, the normal and stroma-enriched (NSE) presented higher quantity of fibroblast and carcinoma in situ, the metabolic group (M) showed an enrichment in metabolic-related pathways, while the metabolic-immune enriched (MIE) presented a higher quantity of adipose tissue, macrophages M2 and an enrichment in metabolic and immune pathways. We validated our findings in an external cohort (METABRIC), and we observed differences in disease outcome between groups (with NSE showing better and M and P worse outcome for relapse free survival).

Of note, two of the three groups associated to worse disease outcome (M and MIE) were related to metabolism and not to proliferation, showing an important implication of metabolism in the biology of ILC.

Genetics, Poster #37

Clinical Characteristics of Lobular Breast Carcinoma in CDH1 genetic predisposition. Experience from the Institut Curie.

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Hereditary diffuse gastric cancer and hereditary lobular breast cancer may be linked to monoallelic germline pathogenic variants (PV) of the CDH1 gene. CDH1 genetic testing is recommended in patients fulfilling either family or individual criteria. More recently, the inclusion of the CDH1 gene in the HBOC panel has identified families whose history was less suggestive than before. In this context, risks estimates, genomic characteristics underlying gastric and breast tumorigenesis are still poorly characterized and need to be consolidated. Between 2008 and July 2023, we identified 38 carriers of PV of CDH1 gene (31 women and 7 men). 15 women had at least one breast cancer, 2 had a breast and gastric cancer, 6 had gastric cancer alone, 15 were free of cancer (median age 38).
The carriers come from 23 different families . While N=25/38 (66%) of carriers had both gastric and breast cancer in their family, N=10/38 (26%) had only breast cancer and N=3/38 (8%) had only gastric cancer.
All breast cancers were diagnosed before the genetic test was carried out, apart from 2 contralateral cancers diagnosed after the genetic test. The mean age at diagnosis was 49.7 years. 4 out of 17 patients were metastatic at diagnosis. Among the 24 breast cancers, 18 were invasive lobular carcinoma (ILC). Among ILC, 61% were of grade 2, more than 50% were HR +, and 16% were HER2-positive.
We will report at the congress the comparison of clinico-pathological characteristics and prognosis of these CDH1 carriers patient vs those of ILC patients tested negative for CDH1 genetic predisposition.

Lay abstract
Hereditary diffuse gastric cancer and hereditary lobular breast cancer may be linked to a rare genetic predisposition due to germline pathogenic variants (PV) of the CDH1 gene.
We will review the criteria that should lead us to consider this type of hereditary predisposition on the basis of personal characteristics and/or family history.
It is important to identify the rare families affected by this type of hereditary predisposition, in order to adapt surveillance and management. Finally, we will present the experience of the Institut Curie, and the characteristics of lobular cancers identified in this genetic context (18 cases identified in 17 patients) and we will compare lobular cancers in this genetic context with lobular cancers without an identified mutation.

Genetics, Poster #38

Non-Lobular Invasive Breast Carcinomas with Bi-Allelic Pathogenic CDH1 Somatic Alterations: a Histologic, Immunophenotypic and Genomic Characterization

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Inactivation of CDH1, which encodes for E-cadherin, is the hallmark alteration of breast invasive lobular carcinoma (ILC). Albeit vanishingly rare, bi-allelic CDH1 alterations may be found in non-lobular breast cancers (NL-BCs). Here, we sought to determine the clinicopathologic and genetic features of NL-BCs harboring CDH1 bi-allelic genetic alterations. We re-analyzed the targeted sequencing data from 5,842 breast cancers (BCs) previously subjected to paired tumor-normal targeted sequencing utilizing an FDA-authorized multigene panel to identify BCs with bi-allelic CDH1 inactivating mutations lacking lobular histological and immunohistochemical features. We compared the genetic makeup of NL-BCs with CDH1 bi-allelic genetic alterations with that of CDH1-altered ILCs and invasive ductal carcinomas of no special type (IDC-NSTs), matched by clinicopathologic features. Out of 5,842 BCs, we identified only 7 (0.11%) cases lacking lobular features and harboring bi-allelic CDH1 alterations, including CDH1 inactivating mutations associated to loss of heterozygosity of the wild-type allele (n=6) and homozygous deletion (n=1). NL-BC were mostly of histologic grade 2 (5/7; 71%) and ER-positive/HER2-negative (4/7; 57%) and were histologically heterogenous, including invasive ductal carcinomas with focal neuroendocrine (n=3) or apocrine (n=2) features, a mucinous carcinoma (n=1), and an IDC-NST (n=1). NL-BCs displayed absent (3/5) or aberrant (weak/heterogenous; 2/5) E-cadherin expression as detected by immunohistochemistry. TP53, PIK3CA, FGFR1 and NCOR1 were found to be recurrently altered in NL-BCs with CDH1 bi-allelic genetic alterations. As compared to IDC-NSTs, CDH1-mutant NL-BCs less frequently harbored mutations affecting GATA3 (0% vs 47%, p=0.03). No genomic differences with CDH1-mutant ILCs were observed. Taken together, our findings indicate that NL-BCs with CDH1 bi-allelic genetic alterations are vanishingly rare, predominantly correspond to IDCs with special histologic features, and display a genetic makeup similar to luminal B ER-positive BCs.

LAY ABSTRACT

In breast cancer (BC) genetic code errors resulting in abrogation of the two copies of the CDH1 gene are seen almost exclusively in invasive lobular carcinoma (ILC). Extremely rarely, however, CDH1 genetic code errors can be seen in other breast cancer subtypes. We analyzed the genetic data of over 5,000 cases to identify BCs other than ILC harboring alterations in CDH1. Our analysis resulted in the identification of only 7 non lobular BC with alterations in both copies of CDH1. These cases had a heterogenous appearance and genetically resembled luminal BCs. These findings indicate, that although vanishingly rare, CDH1-altered non-lobular BCs exist.

Conflicts of interest: J.S.R.-F. reports receiving personal/consultancy fees from Goldman Sachs, Bain Capital, REPARE Therapeutics, Saga Diagnostics, MultiplexDX and Paige.AI, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of AstraZeneca, Merck, Daiichi Sankyo, Roche Tissue Diagnostics and Personalis, outside the scope of this study. B.W. reports research funding from Repare Therapeutics, outside the scope of the submitted work. All other authors declare no financial or non-financial competing interests.

Genetics, Poster #39

Genetic and Epigenetic Basis of Breast Invasive Lobular Carcinomas Lacking CDH1 Genetic Alterations

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The hallmark molecular alteration of invasive lobular carcinoma (ILC) of the breast is bi-allelic inactivation of CDH1, which encodes for E-cadherin and results in the distinctive discohesive phenotype of this breast cancer histologic special type. A subset of ILCs lack CDH1 genetic or epigenetic inactivation, however, despite displaying a lobular phenotype and their molecular basis has yet to be determined. Through targeted sequencing data reanalysis of 364 primary ILCs previously subjected to paired tumor/normal targeted sequencing using the FDA-cleared MSK-IMPACT assay, we identified 25 ILCs lacking CDH1 bi-allelic genetic alterations. Promoter methylation assessment using revealed CDH1 gene promoter methylation in 10/16 (63%) cases interrogated. The majority (9/10) of these cases also displayed 16q loss and loss of E-cadherin expression by immunohistochemistry. Whole-genome sequencing (WGS) analysis of three cases lacking bi-allelic CDH1 genetic or epigenetic inactivation revealed pathogenic genetic alterations in other genes with pivotal roles in cell-cell adhesion, including a novel deleterious fusion gene inactivating CTNND1 (p120) and an AXIN2 loss-of-function mutation. AXIN2 knock out (KO) using CRISPR-Cas9 genome editing techniques in estrogen receptor-positive MCF-7 cells resulted in the acquisition of lobular-like features, such as increased migratory properties and anoikis resistance. Our findings indicate that CDH1 promoter methylation is frequent in ILCs lacking CDH1 bi-allelic genetic alterations, and that inactivating genetic alterations affecting other cell-cell adhesion genes, including CTNND1 or AXIN2, may underpin a subset of ILCs. Taken together, these findings provide support to the notion that ILCs constitute a convergent phenotype.

Lay abstract

Invasive lobular carcinoma (ILC) is a type of breast cancer with tumor cells that are not connected to each other, mostly due to alterations in a specific gene called CDH1 (E-cadherin). This gene plays important roles in cell-cell adhesion. Some ILCs have the typical lobular characteristics but lack CDH1 genetic inactivation and the cause for their appearance under the microscope is not yet known. Here we aimed to discover new mechanisms causing ILC in addition to CDH1 alterations. By investigating the genetic profiles of 364 ILCs we identified 25 cases lacking inactivation in both copies of the CDH1 gene. We observed that CDH1 gene was frequently (65%) turned off by an epigenetic modification in its regulatory region. We also identified alterations in other genes involved in intercellular connection, such as CTNND1 or AXIN2. Our findings show that ILCs lacking alterations in the CDH1 gene have inactivation of other genes involved in cell adhesion.

Conflict of interest: J.S.R.-F. reports receiving personal/consultancy fees from Goldman Sachs, Bain Capital, REPARE Therapeutics, Saga Diagnostics, MultiplexDX and Paige.AI, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of AstraZeneca, Merck, Daiichi Sankyo, Roche Tissue Diagnostics and Personalis, outside the scope of this study. B.W. reports research funding from Repare Therapeutics, outside the scope of the submitted work. All other authors declare no financial or non-financial competing interests.

Genetics, Poster #40

Aberrant E-cadherin (E-cad) Expression in Lobular Carcinoma in Situ (LCIS): A Comprehensive Evaluation of N-terminal, Extracellular, and C-terminal E-cadherin Domains by

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While ~15% of invasive lobular carcinomas show aberrant E-cad expression, the frequency of aberrant E-cad expression in LCIS is poorly defined. Furthermore, among LCIS cases with aberrant E-cad expression, the E-cad molecule domains that are aberrantly expressed and the relationship to the expression of other components of the cadherin-catenin complex and to LCIS subtype have not been previously analyzed. We studied 50 cases of LCIS (22 classic A, 10 classic B, 9 florid, 9 pleomorphic) by immunohistochemistry to identify the frequency and patterns of expression of cadherin-catenin complex components including the E-cad N-terminal (N), extracellular (ECD), and C-terminal (C) domains, p120 catenin, and beta-catenin. Quantitative real-time PCR (RT-qPCR) was performed to measure expression levels of CDH1 in aberrant cases and compared to that in cases of E-cad negative LCIS and DCIS. Loss of membrane expression of all 3 E-cad domains was seen in 34 cases (68%) whereas aberrant expression of one or more E-cad domains was seen in 16 (32%) including 3/22 classic type A, 3/10 classic type B, 5/9 florid and 5/9 pleomorphic LCIS. The frequency of aberrant expression of E-cad domains was N+ECD+C=5; N+ECD=5;C+ECD=2; C only=2; ECD only=1; N only=1. Aberrant E-cad expression was most often partial, fragmented membrane staining. Among cases with aberrant E-cad expression, aberrant expression of p120 catenin, beta-catenin, or both was seen in 4 cases, 3 cases and 5 cases, respectively. Aberrant E-cad LCIS cases had low CDH1 expression levels by RT-qPCR, similar to E-cadherin negative LCIS and different from DCIS cases ($p=0.02$). Our results demonstrate for the first time that aberrant E-cad expression is seen in all LCIS subtypes and may be due to expression of various E-cad domains, singly and in combination. This, in turn, likely reflects different mechanisms of E-cad alterations in LCIS, the underlying nature of which merits further study.

Lay Abstract:

Lobular carcinoma in situ (LCIS) is considered both a marker of increased breast cancer risk as well as a lesion that itself can directly progress to invasive lobular breast cancer. The cells that make up LCIS are confined to the small breast ducts (called lobules) but they do not stick together very well the way normal cells do. This is due to the loss of expression of a molecule on the surface of the LCIS cells called E-cadherin. We found that while most LCIS cases show total loss of expression of E-cadherin, about one third show abnormal (aberrant) expression of various parts of this molecule. Understanding why this happens could be important for further understanding the biology of LCIS and, in turn, may lead to a better understanding of what makes some cases of LCIS progress to invasive lobular carcinoma.

Genetics, Poster #41

Lobular-like invasive mammary carcinoma: Is this a ductal cancer, lobular cancer, or a distinct entity?

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Biallelic inactivation of the CDH1 gene, i.e. CDH1 mutation coupled with loss of heterozygosity of the other allele is considered a hallmark of lobular carcinoma which results in typical discohesive growth pattern of the tumor cells. These molecular events result in loss of E-cadherin protein or aberrant reactivity (i.e. lack of circumferential membranous staining) by immunohistochemistry in lobular carcinoma. This study describes an unusual type of carcinoma with low- to intermediate-grade nuclei with discohesive, diffusely infiltrative cells but showing retained circumferential membranous immunoreactivity for both E-cadherin and p120. We termed these “lobular-like invasive mammary carcinomas” (LiMCa). We analyzed the clinical-pathologic features of 166 LiMCa and compared them with 104 classical invasive lobular carcinomas (ILCs) and 100 grade 1 and 2 invasive (ductal) carcinomas of no special type (IDCs). An exploratory, hypothesis generating analysis of the genomic features of 14 randomly selected LiMCa and classical ILCs (7 from each category) was performed utilizing an FDA-authorized targeted capture sequencing assay (MSK-IMPACT). Despite histomorphologic similarities to classical ILC, the discohesion in LiMCa was independent of E-cadherin/p120 immunophenotypic alteration. Correspondingly, 5 out of 7 LiMCa were CDH1 wild-type, whereas all ILCs analyzed harbored CDH1 loss of function mutations coupled with loss of heterozygosity of the CDH1 wild-type allele. However, 4 of the 6 evaluable LiMCa were positive for CDH1 promoter methylation. Tumor size of LiMCa were often underestimated on imaging and they showed frequent positive margins on first resection similar to ILC. Survival outcomes for LiMCa, ILC, and IDC in this cohort were associated with prognostics scores of Magee Equation 2 (ME2) in addition to traditional prognostic factors in multivariable models. CDH1 promoter methylation may partially explain the typical morphology seen in LiMCa; however, further studies are warranted to better define the molecular basis of the discohesive cellular morphology in LiMCa.

Lay Abstract:
We examined the clinical and pathologic features of an unusual group of breast cancers that we termed as lobular-like invasive mammary carcinoma (LiMCa) and compared these with 100 invasive ductal carcinoma (IDC) and 104 invasive lobular carcinoma (ILC). We defined LiMCa as a low to intermediate grade invasive breast carcinoma with single cell infiltrative growth pattern (i.e. “lobular-like”) but with retained circumferential membranous expression for cell adhesion proteins, E-cadherin and p120 (a property of ductal cancers). Based on exploratory molecular analysis of select cases, we found that LiMCa generally lack the typical CDH1 gene mutations seen in lobular carcinomas but a proportion of them demonstrate CDH1 gene promoter methylation. LiMCa demonstrate clinical-pathologic features that are intermediate between invasive ductal carcinoma and invasive lobular carcinoma. Tumor prognosis is related to pathologic tumor and nodal stage as well as multivariable prognostic score (Magee Equation).

Genetics, Poster #43

Estrogen receptor interaction with Mediator of DNA Damage Checkpoint 1 (MDC1) mediates epigenomic remodeling and gene regulation in ILC cells

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Estrogen receptor α (ER) has unique regulatory activities in invasive lobular carcinoma (ILC) cells associated with endocrine response and anti-estrogen resistance, which we linked to ILC-specific interaction between ER and Mediator of DNA Damage Checkpoint 1 (MDC1). MDC1 is a cornerstone of DNA damage repair yet in ILC cells has a novel, critical role in ER-mediated gene regulation. Defining this putative ILC-specific ER co-regulator function of MDC1 may identify the mechanisms underpinning ILC-specific functions of ER. We profiled the ER and MDC1 cistrome in ILC cells and found that at ER-regulated genes, ER binds distal enhancers, while MDC1 binds promoters, suggesting that MDC1 may facilitate the action of ER-bound enhancers at target gene promoters. To understand how MDC1 may regulate enhancer/promoter regulation, we performed MDC1 immuno-precipitation mass spectrometry (IP-MS) in ILC vs invasive ductal carcinoma (IDC) cell lines to profile partners that mediate ER-MDC1 activity. In total, we identified 2,221 MDC1-interacting proteins. IDC cell lines present preferential MDC1 association with DNA repair proteins. In ILC cell lines, MDC1 additionally associated with a cohort of epigenomic regulators. We identified 234 epigenomic partners associated with MDC1 with 66 of these proteins also interacting with ER. Importantly, treatment of MM134 with ER-antagonist fulvestrant remodeled MDC1 associated proteins to mirror that seen in IDC cells. To identify MDC1 critical epigenomic partners, we are undertaking an siRNA screen of 228 putative partners from IP-MS for their role in ER-driven phenotypes. We further explored how MDC1 regulates the epigenome and profiled chromatin accessibility by MNase-seq after MDC1 or ER knockdown, which suggests that MDC1 plays distinct roles in chromatin remodeling in ILC cells. Taken together, ER interaction with MDC1 may build gene regulatory complexes at ILC-specific ER target genes that facilitates chromatin remodeling, gene regulatory, and ultimately endocrine response and resistance in ILC.

Lay abstract
Mediator of DNA damage checkpoint 1 (MDC1) is a protein which regulates how cells repair DNA damage. However, in invasive lobular carcinoma (ILC), MDC1 is “hijacked” by the estrogen receptor (ER), creating new ILC-specific functions for MDC1 in controlling how genes are turned on or off. ER-MDC1 together may be important for how ILC cells respond to the hormone estrogen, and resist anti-estrogen treatments. To better understand the ILC-specific activity of ER-MDC1, we performed experiments to identify other cellular proteins that partner with ER-MDC1, and compared MDC1 partners in ILC cells versus other breast cancer cells. We found that in ILC cells, MDC1 partners are shifted away from DNA repair proteins (as in other cancer cells) and are instead utilized to control gene expression. This work can help define anti-estrogen response and resistance in ILC cells, and lead to new therapies targeting the specific functions of ER-MDC1 in ILC cells.

Epigenomics, Poster #44

Chromatin accessibility landscape and active transcription factors in primary human invasive lobular and ductal breast carcinomas

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Background: Invasive lobular breast carcinoma (ILC), the second most prevalent histological subtype of breast cancer, exhibits unique molecular features compared with the more common invasive ductal carcinoma (IDC). While genomic and transcriptomic features of ILC and IDC have been characterized, genome-wide chromatin accessibility pattern differences between ILC and IDC remain largely unexplored.
Methods: Here, we characterized tumor-intrinsic chromatin accessibility differences between ILC and IDC using primary tumors from The Cancer Genome Atlas (TCGA) breast cancer assay for transposase-accessible chromatin with sequencing (ATAC-seq) dataset.
Results: We identified distinct patterns of genome-wide chromatin accessibility in ILC and IDC. Inferred patient-spe- cific transcription factor (TF) motif activities revealed regulatory differences between and within ILC and IDC tumors. EGR1, RUNX3, TP63, STAT6, SOX family, and TEAD family TFs were higher in ILC, while ATF4, PBX3, SPDEF, PITX family, and FOX family TFs were higher in IDC.
Conclusions: This study reveals the distinct epigenomic features of ILC and IDC and the active TFs driving cancer progression that may provide valuable information on patient prognosis.
Keywords: Invasive lobular breast carcinoma, Invasive ductal breast carcinoma, Differential chromatin accessibility landscape, EGR, SOX, TEAD, FOX family transcription factors, Transcriptional regulation

Lay Abstract

Invasive lobular carcinoma (ILC), also known as infiltrating lobular carcinoma, begins in the milk-producing glands (lobules) of the breast. Because it is an invasive type of cancer, ILC can spread beyond its original tumor site. ILC has worse prognosis in patients, and exhibits unique clinical features, such as patient age, tumor grade, and size, compared with more common invasive ductal carcinoma (IDC). We characterized the open space and the degree to which nuclear macromolecules are able to physically contact DNA to turn on gene expression in ILC and IDC. This study reveals the distinct features of turning genes on and off between ILC and IDC and the active transcription factors, which are proteins that regulate the expression of genes that can drive cancer progression. Our study will provide valuable information on patient therapeutic outcomes or recurrence.

Epigenomics, Poster #45

Integrated analysis of tumor transcriptomics and immune landscape in primary lobular cancer – a case-series study

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Immunotherapy is extensively explored in oncology. In breast cancer, pembrolizumab has been approved in TNBC, while results for ER+ subtype were less favorable. Invasive lobular breast cancer (ILC) consists of 10-15% of BC. Despite being ER+ in 80-90% cases, certain ILC subtype showed immune enrichment[1, 2], with clinical benefit rate of 26% in GELATO trial for metastatic ILC[3]. However, immune landscape of ILC and causal factors remain largely under-studied.

We here performed integrated analysis of RNA-seq and multispectral IHC (mIHC) from a series of 13 treatment naïve ER+ primary ILCs[4]. Pairwise analysis of immune cell density showed higher correlation between CD4+ with CD8+ T cells, and macrophages with Foxp3+ Tregs, with CD4 T cells as 'correlation hubs'. Unsupervised clustering of immune cell type density generates 5 subclusters, showing cluster 3 with high CD8 and CD4 T, and cluster1 with high Treg. Based on subclusters, patients were classified into three groups (immune-active / suppressive / neutral), and single-gene logistic classifiers were built from RNA-seq to predict patient classes, identifying 257 genes with accuracy of 1 (class predictors). Pairwise correlation of each gene and immune cell type generates 545 genes (correlation predictors), and intersection of both predictors leads to 49 genes, mostly with negative correlation with stromal macrophage infiltration. Expression of the 49-gene signature showed better prognosis in ER+ patients (METABRIC[5] and SCAN-B[6]), which was more prominent in ILC.

In summary, we performed integrated analysis of tumor transcriptomics and immune landscape in a series of 13 primary ILC cases. Patients showed distinct immune infiltration in cell type density and activation status, which can be predicted via expression of specific genes in tumor-rich regions. Of note, expression of negative predictors of macrophage infiltration relates to better prognosis, which indicates tumor associated macrophage (TAM) being one dominant negative factor in ILC microenvironment.

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Lay Abstract

This study focused on invasive lobular breast cancer (ILC), to unravel association of tumor with the immune system. By analyzing gene expression and immune cell presence in 13 cases of ILC, we identified tight collaborations between specific immune cells. Grouping patients based on immune patterns, we devised a gene-based method to forecast immune behavior. Remarkably, genes linked to lower levels of unfavorable immune cells (macrophage) were associated with better patient outcomes, particularly in ILC cases. These findings underscore the intricate interplay between immune responses and transcriptomic factors in ILC. The results hold potential for refining therapeutic strategies and enhancing our comprehension of immune-tumor in ILC.

Multiomics, Poster #46

Integrative Deep Learning and Genomics Approach Reveals Alternative CDH1 Inactivation Mechanisms

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Breast cancer (BC) is heterogenous and includes histologic subtypes with distinctive phenotypes and pathognomonic genetic alterations, constituting genotypic-phenotypic correlations. Invasive lobular carcinoma (ILC), the most common special BC histologic subtype, is characterized by a distinctive discohesive phenotype, caused by CDH1 bi-allelic inactivation, most frequently in the form of CDH1 inactivating mutations with loss-of-heterozygosity (LOH) of the wild-type allele. Here we sought to identify convergent molecular mechanisms in ILCs lacking these alterations by integrating deep learning (DL) methods and genomics. We employed a DL-algorithm, using pathology whole-slide images (WSIs) as input, previously trained to detect CDH1 bi-allelic mutations in BC. The algorithm was applied to WSIs of 1,057 BCs previously subjected to paired tumor-normal targeted sequencing using the FDA-approved MSK-IMPACT assay. Cases lacking CDH1 bi-allelic mutations by targeted sequencing but predicted to be mutant by the DL-model were investigated through reanalysis of targeted sequencing data, CDH1 promoter methylation assessment and/or whole genome sequencing analysis. Our analyses identified 34 cases lacking CDH1 bi-allelic mutations by targeted sequencing that were predicted by the DL-model to be mutant. We observed prevalent CDH1 gene promoter methylation (n=18) in these cases. Reanalysis of targeted sequencing/WGS revealed inactivating genetic mechanisms other than bi-allelic mutations in a subset of cases, including CDH1 homozygous deletion (n=3), intragenic deletion with LOH (n=1), a novel deleterious fusion gene affecting CDH1, resulting in deletion of its 5'UTR and exons 1 and 2 (n=1), associated with LOH, and likely pathogenic non-coding CDH1 alterations (n=2), associated with LOH. Taken together, we identified epigenetic or alternative genetic (coding and non-coding) mechanisms of CDH1 inactivation in 74% (25/34) of cases. Phenotypes caused by convergent molecular mechanisms affecting a single gene/pathway are detectable by DL methods applied to H&Es. The integration of genomics and DL can result in identification of novel biological mechanism underlying histologic entities.

LAY ABSTRACT

Breast invasive lobular carcinoma (ILC) has a characteristic discohesive appearance, caused predominantly by errors in the genetic code (mutations) of CDH1, a gene in charge of intercellular connections. Here we applied advanced computational methods applied to pathology images integrated with genetic analysis to identify alternative mechanisms causing ILC. Our approach applied to >1000 breast cancers identified alternative mechanisms abrogating the gene CDH1 in 74% of cases lacking CDH1 mutations, including the loss of both copies of this gene, loss of key regions of CDH1, a novel large rearrangement in the genetic code of CDH1 and novel alterations in non-coding DNA regions of CDH1. This work demonstrates how the combination of artificial intelligence applied to pathology images and genetic analysis can reveal novel mechanisms of disease.

Conflict of interest:

YW, MB, JHB, JS, MCHL, RAG, AC, BR, JDK, JO, DSK and TJF receive salary and own stock from Paige. CK is a paid a consultant and equity holder at Paige. BW reports research support from Repare Therapeutics, outside the scope of the current study. SC receives grant support (to MSK) from Daiichi-Sankyo and AstraZeneca, financial interests in Totus Medicine and Odyssey Biosciences, and consulting fees from Lilly, Novartis, Paige.ai, AstraZeneca, SAGA, Boxer Capital, & Prelude Therapeutics. J.S.R.F reports receiving personal/consultancy fees from Goldman Sachs, Bain Capital, REPARE Therapeutics, Saga Diagnostics and Paige.AI, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of Astrazeneca, Merck, Daiichi Sankyo, Roche Tissue Diagnostics and Personalis, outside the scope of this study. . B.W. reports research funding from Repare Therapeutics, outside the scope of the submitted work. All other authors declare no financial or non-financial competing interests.

Multiomics, Poster #47

Multi-omic characterization of ILC and ILC-like cell lines as part of ILC cell line encyclopedia (ICLE) defines new models to study potential biomarkers and explore therapeutic opportunities

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Invasive lobular carcinoma (ILC), the most common histological "special type", accounting for ~10-15% of all BC diagnoses, is characterized by unique features such as E-cadherin loss/deficiency, lower grade, hormone receptor positivity, larger diffuse tumors, and specific metastatic patterns. Despite ILC being acknowledged as a disease with distinct biology that necessitates specialized and precision medicine treatments, discovering new treatments has been hindered due to the scarcity of well-characterized cell line models. To address this, we generated the ILC Cell Line Encyclopedia (ICLE), providing a comprehensive multi-omic characterization of ILC and ILC-like cell lines (n=17). Using consensus multi-omic subtyping, we confirmed the luminal status of previously established ILC cell lines and uncovered additional ILC/ILC-like cell lines with luminal features suitable for modeling ILC disease. Furthermore, most of these luminal ILC/ILC-like cell lines also showed RNA and DNA copy number similarity to ILC patient tumors. Similarly, ILC/ILC-like cell lines also retained molecular alterations at similar frequency to both primary and metastatic ILC tumors. Importantly, ILC/ILC-like cell lines recapitulated the CDH1 alteration landscape of ILC patient tumors including enrichment of truncating mutations in and biallelic inactivation of the CDH1 gene. Using whole-genome optical mapping, we uncovered novel genomic-rearrangements including structural rearrangements in CDH1, functional gene fusions, and characterized breast cancer specific patterns of chromothripsis in chromosomes 8, 11 and 17. In addition, using integrative DNAm and RNA analysis, we identified epigenetic activation of TFAP2B – a transcription factor that is preferentially expressed in lobular disease. Finally, we analyzed publicly available RNAi loss of function breast cancer cell line datasets and revealed numerous putative vulnerabilities cytoskeletal components, focal adhesion and PI3K/AKT pathway. The top candidate based on druggability score was PKN3, protein-kinase C-related kinase 3, which also showed high expression in ILC vs NST patient tumors.

In summary, we addressed the lack of suitable models to study E-cadherin deficient breast cancers by first collecting both established and putative ILC models, then characterizing them comprehensively to show their molecular similarity to patient tumors along with uncovering their novel multi-omic features as well as highlighting putative novel druggable vulnerabilities.

Lay Abstract:
Invasive lobular carcinoma (ILC) is a type of breast cancer that makes up about 10-15% of all cases. It is characterized by E-cadherin loss/deficiency and shows distinct clinical and biological features. However, studying it has been tough due to limited model systems. To help, we created the ILC Cell Line Encyclopedia, detailing the features of 17 ILC-related cell lines. This study confirmed certain characteristics of known ILC lines and discovered others that mimic ILC. Many of these lines also showed genetic patterns seen in ILC patients, including specific changes in the CDH1 gene. We found new genetic changes and also identified the mechanism regulating, TFAP2B, a putative biomarker of lobular disease. We also identified various druggable gene targets and pathways in these cell lines. In short, we've gathered and studied ILC/ILC-like cell line models, showing they resemble patient tumors and revealing potential new treatment targets.

Multiomics, Poster #48

Transcriptomic and immune heterogeneity underpin the biology of pleomorphic invasive lobular breast cancer

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Background: Invasive Lobular Carcinoma (ILC) encompasses several histological subtypes, with pleomorphic ILC representing 15% of cases and being associated with aggressive disease. These patients have limited treatment options, representing a clinically unmet need. Previous studies have identified ILC gene-expression subtypes, but their prognostic utility is unknown. Moreover, recent studies have highlighted the importance of the immune microenvironment in governing the biology of ILC, however its role in pleomorphic ILC is unknown. Thus, a comprehensive characterisation of aggressive ILCs, notably pleomorphic disease is warranted.
Methods: RNA and targeted DNA sequencing were undertaken in an exclusively pleomorphic ILC cohort (n = 47). Findings were validated in METABRIC and TCGA studies. Stromal tumour infiltrating lymphocytes were histologically quantified in a cohort of non-pleomorphic (n = 100) and pleomorphic ILCs (n = 63). The spatial distribution of immune subpopulations were quantified in a subset of pleomorphic ILCs with high TIL counts (n = 20) using NanoString Digital Spatial profiling technology.
Results: We identify a prognostic gene expression signature consisting of 62 genes which were significantly associated with survival in the pleomorphic ILC cohort; and was independently validated in the METABRIC cohort. Through sequencing, we show that TP53, FAT1 mutations and HER2 alterations were enriched in pleomorphic ILC and FGFR1 alterations associated with worse outcomes. However PIK3CA mutations were more common in non-pleomorphic ILC. Pleomorphic ILCs are more immunogenic than non-pleomorphic cases with higher TILs. Whilst gross quantification of TILs was not associated with clinical outcome, further characterisation of immune subpopulations through digital spatial profiling demonstrated significantly higher numbers of CD68+ cells (macrophages) in poor prognosis pleomorphic ILCs.
Conclusion: Overall the study reveals novel insights into the transcriptomic, genomic and immune landscape in pleomorphic ILC identifying drivers of clinically aggressive disease. We also provide new insights into the relevance of macrophages in pleomorphic ILC.

Lay Abstract:
Invasive lobular carcinoma (ILC) is the second most common type of breast cancer accounting for 10-15% of all breast cancers. In classic ILC (the most common type), the tumour cells are small and look alike. However, a rarer ILC subtype exists known as pleomorphic ILC accounting for 15% of all ILC. Here tumour cells show much greater variability from one cell to the next, and the nuclei which are the cell structures containing DNA, are much larger compared to classic ILC. Pleomorphic ILCs are associated with more aggressive disease and they are poorly understood. In particular there is a lack of knowledge about the genes which are expressed in these tumours and the role of different cell types such as immune cells that are present in and around the tumour play a role in the biology of the cancer.

Methods: Here we use a technique called RNA sequencing to study the genes which are being expressed in a cohort of 47 pleomorphic ILCs and assess how these are related to patient survival. We also study the number of immune cells in pleomorphic vs non-pleomorphic ILCs under the microscope. We also use a new technique called 'Digital Spatial Profiling' which can look at many changes in one experiment to provide greater detail on the types of immune cells present in a group of pleomorphic ILCs (n = 20).

Results: We find that a specific combination of genes can predict whether a patient will die after being diagnosed with pleomorphic ILC, which we further validate in other larger sets of ILCs. We show that pleomorphic ILC contains higher numbers of immune cells than non-pleomorphic ILC. We also find that high levels of a specific type of immune cell, macrophages, is associated with poor outcome.

Conclusion: Overall the study reveals new insights into the genes expressed and immune cells present in pleomorphic ILC, a rare ILC subtype and how these can predict the survival of women diagnosed with ILC.

Multiomics, Poster #49

Transcriptomic analysis identifies enrichment of cAMP/PKA/CREB signaling in Invasive Lobular Breast Cancer

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OBJECTIVE: Invasive lobular breast cancer (ILC) has unique clinicopathological and molecular hallmarks that differentiate it from the more common invasive carcinoma - no special type (NST). Despite these differences, ILC and NST are treated as a single entity, targeted therapies are lacking in ILC. We aimed to identify novel molecular alterations in ILC that could be exploited for therapeutic purposes.
METHODS: Differential gene expression and Geneset Enrichment and Variation analyses were performed on RNA-seq data from three large public breast cancer databases – the Sweden Cancerome Analysis Network-Breast (SCAN-B; luminal A ILC N=263, NST N=1162), The Cancer Genome Atlas (TCGA; luminal A ILC N=157, NST N=307) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; luminal A ILC N=65, NST N=533). Pathways enriched in overlapping differentially expressed genes from these datasets were clustered using Jaccard index to identify novel pathways related to ILC. The cAMP/PKA/CREB signaling was studied in ILC, ILC-like and NST cell lines and patient-derived organoids (PDOs) using Forskolin, an activator of the pathway.
RESULTS: There was a consistent pattern of up-regulation of cAMP/PKA/CREB related signaling in ILC compared to NST in SCAN-B, TCGA and METABRIC. Treatment with forskolin resulted in a greater increase in phospho-CREB in ILC cell lines and organoids than NST. CRISPR deletion of CDH1 in NST cell lines did not alter response of cells to forskolin as measured by phospho-CREB. Forskolin treatment caused growth inhibition in ILC and NST, with ILC cell lines being more sensitive to forskolin-mediated growth inhibition.
CONCLUSION: cAMP/PKA/CREB signaling is higher in ILC than NST, this was consistent across three separate datasets and was validated in cell lines and organoids. Loss of CDH1 is not sufficient to mediate this phenotype. Future studies should investigate the mechanisms for differential cAMP/PKA/CREB signaling in ILC and investigate the potential for therapeutic targeting in ILC.

Lay abstract

Invasive lobular breast cancer (ILC) is the most common special type of breast cancer and has unique clinical features, and response to treatment as compared to the no-special type (NST) breast cancer. However, the treatment of ILC is similar to NST and there is a need for drugs that specifically treat patients with ILC. Our goal was to study the gene expression of ILC and NST tumors to identify mechanisms that could be manipulated for treatment of ILC. Based on our results, a novel pathway involving the molecule cyclic AMP (cAMP) and related proteins (Protein-kinase A PKA, cyclic AMP response element binding protein CREB) appears to be more active in patients with ILC as well as in ILC tumor tissues and cells grown in the lab. A drug that activates this pathway, Forskolin, was able to inhibit cancer cell growth more profoundly in ILC than NST. We believe that cAMP/PKA/CREB pathway represents a potential mechanism that could be exploited for the treatment of ILC.

Multiomics, Poster #50

Spatial profiling of mixed invasive ductal-lobular carcinoma reveals intrinsic molecular subtype and oncogenic signaling heterogeneity

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Mixed invasive ductal and lobular carcinoma (mDLC) is a rare subtype of breast cancer displaying both E-cadherin proficient ductal and E-cadherin deficient lobular tumor regions within the same tumor. It remains unclear whether these tumor regions with distinct E-cadherin status and morphology also have distinct biology and prognosis. Give the rarity and complexity of mDLC diagnosis, we started by curating a well-annotated mDLC cohort and highlighted its histomorphic and E-cadherin heterogeneity and based on these features propose three mDLC subclasses namely intermixed, collision type and lobular-like IDC (LLIDC). Using state-of-the-art spatially resolved and single-cell line methodologies, we dissected the molecular heterogeneity of collision type mDLC tumors and revealed clinically significant biological distinctions between ductal vs lobular tumor regions. All lobular tumor regions showed CDH1 inactivation, either via genetic or epigenetic alterations. Moreover, tumor regions across mDLC cases had distinct clinically actionable molecular alterations in various components of ER signaling, Notch signaling and DNA repair response pathway presenting distinct druggable vulnerabilities. Notably, both senescence and ER signaling signatures were enriched in lobular, while MYC signaling signatures were enriched in ductal tumor regions. These findings raise several important questions on whether predominantly ductal vs lobular mDLC disease would respond differentially to standard of care endocrine and chemotherapies and should they be treated distinctly. Furthermore, profiling of a clinical ER+ mDLC case revealed mixture of ER-/TNBC ductal and ER+/luminal lobular tumors. This case also had ER- metastasis indicating individual tumor regions may have distinct prognosis and one may pose higher risk of recurrence than the other, another important finding, indicating the significance of histopathological and molecular profiling of individual tumor regions within mDLC to guide clinical decision making. Altogether, we curated a mDLC cohort and used spatially resolved.

multi-omic profiling to study intra-tumoral histological heterogeneity within mDLC and revealed that E-cadherin proficient ductal and E-cadherin deficient lobular regions have clinically significant biological differences including distinct oncogenic signatures, clinically actionable alterations and intrinsic subtype features. This biological heterogeneity within mDLC may cause prognostic uncertainty and therapy choice dilemma. Thus histopathological/molecular profiling of individual tumor regions within mDLC would lead to more precise clinical decision making and benefit patients with this disease.

Lay Abstract:
A rare breast cancer called mixed invasive ductal-lobular carcinoma (mDLC) shows histologic and E-cadherin heterogeneity. mDLC shows mixture of E-cadherin proficient ductal and E-cadherin deficient lobular tumor regions. It's uncertain if these individual tumor regions behave differently or lead to different outcomes. After studying histologic and E-cadherin features of mDLC, we saw three main patterns of these tumors. Then, using advanced spatial and single-cell profiling techniques, we discovered key biological differences between the ductal and lobular tumor regions including distinct molecular subtypes, oncogenic signatures and clinically druggable mutations. These differences can be indicative of distinct risk posed by individual tumor regions. For instance, one study case showed that one tumor component maybe be more aggressive than the other and pose higher risk of recurrence. This highlights the importance of closely examining each tumor region within mDLC tumors to guide treatment decisions. In essence, understanding the unique characteristics within mDLC tumors can help doctors make more accurate treatment decisions and predictions for patients.

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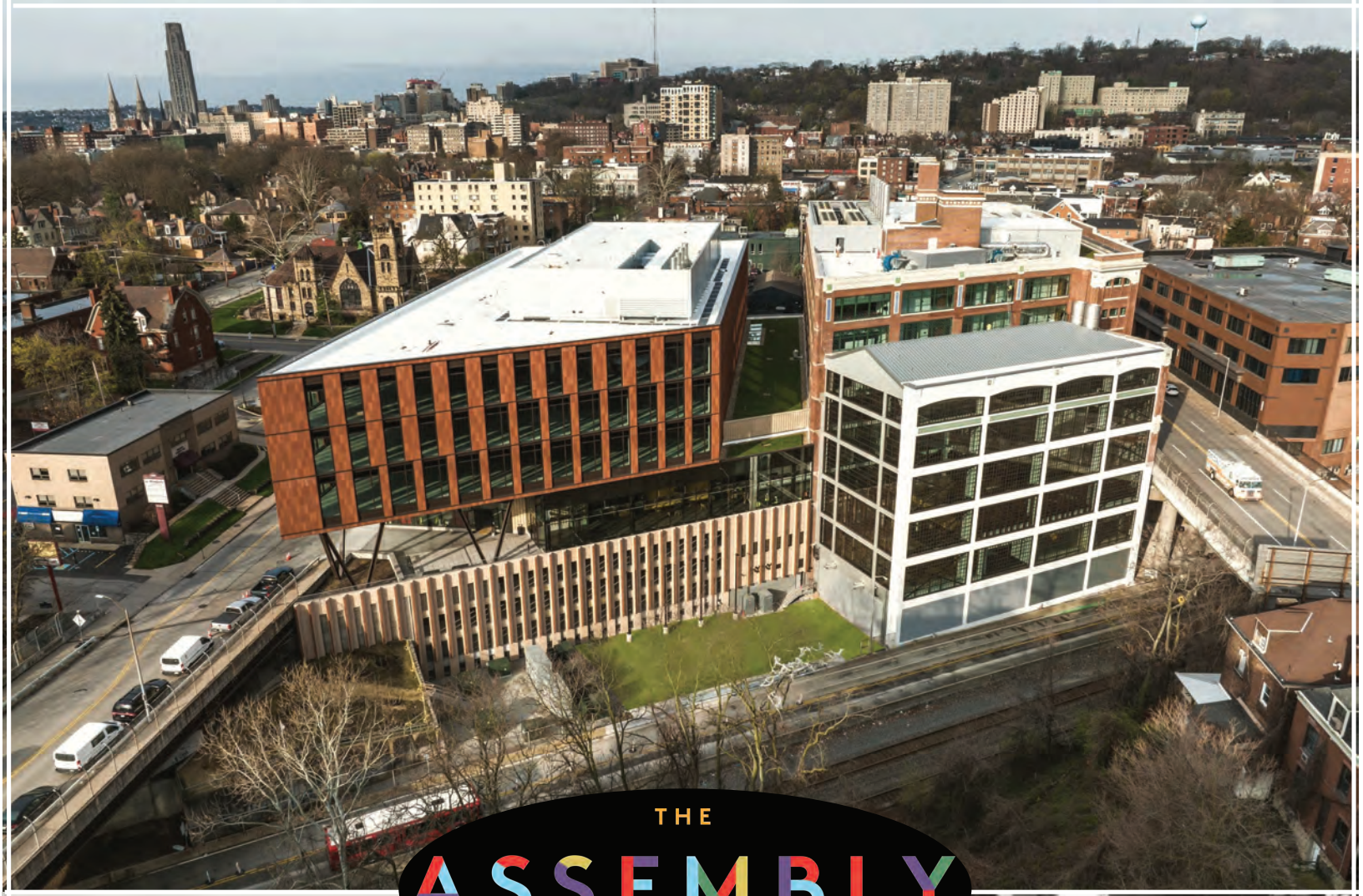

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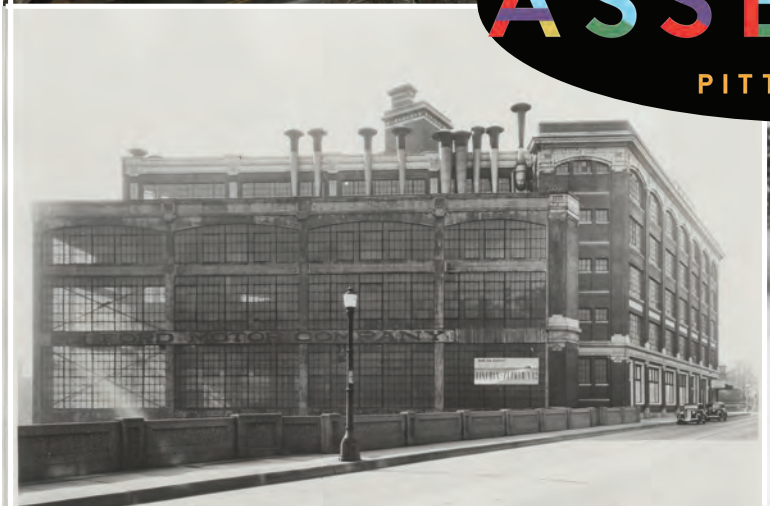

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