Breast Cancer SPORE Advocate Workshop

November 2, 11-12, 2020

Program Book

MAYO CLINIC
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Breast Cancer SPORE Advocate Workshop
Hosted by Mayo Clinic
Agenda
November 2020

Monday, November 2nd

Join meeting
Meeting Link For November 2nd Only
Meeting number (access code): 172 547 8504
Meeting password: BreastSPORE2020@
Times listed are Central Time

9:00 – 9:35	**Welcome, Introductions** *Matthew P. Goetz, M.D. and Cynthia Chauhan*
9:35 – 10:30	**Round table advocate introductions**
10:30 – 12:00	**Communications Workshop** **Amy Hauenstein, PhD.**

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Wednesday, November 11th

Join meeting
Meeting Link For November 11th Only
Meeting number (access code): 172 418 7187
Meeting password: BreastSPORE2020@
Times listed are Central Time

9:00	**Welcome, Introductions** *Matthew P. Goetz, M.D. and Cynthia Chauhan*
9:00 – 9:45	**New Drug Treatments for Endocrine Resistant Breast Cancer** *Matthew P. Goetz, M.D.* Co-Director, Mayo Clinic Women's Cancer Program Director, Mayo Clinic Breast Cancer SPORE
9:45 – 10:30	**New Approaches to TNBC** *Jennifer Pietenpol, Ph.D.* Director, Vanderbilt-Ingram Cancer Center Principal Investigator, Vanderbilt-Ingram Cancer Center Breast SPORE
10:30 – 10:45	**Break**
10:45 – 11:30	**New Approaches for HER2+ Breast Cancer** *Eric Winer, M.D.* Chief, Division of Women's Cancers, Dana-Farber Cancer Institute Principal Investigator, Dana-Farber/Harvard Cancer Center SPORE in Breast Cancer
11:30 – 12:15	**What is a biomarker? What makes a good biomarker?** *Andrea Richardson, M.D., Ph.D.* Director of Pathology, Community Practice, Johns Hopkins
12:15 – 12:30	**Break**
12:30 – 1:30	**Breast Cancer Vaccines for HER2+ and TNBC** *Keith Knutson, Ph.D.* Co-Director, Mayo Clinic Cancer Immunology and Immunotherapy Program
1:30 – 2:00	**Communication Review** **Amy Hauenstein, PhD.**

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Thursday, November 12th

Join meeting
Meeting Link For November 12th Only
Meeting number (access code): 172 213 8327
Meeting password: BreastSPORE2020@
Times listed are Central Time

11:30	**Welcome, Introductions** *Matthew P. Goetz, M.D. and Cynthia Chauhan*
11:30 – 12:00	**SPORES: Translational Science, NCI Perspective** *JoyAnn Phillips Rohan, Ph.D.* Program Director, Translational Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute
12:00 – 12:45	**New insights into the Genetic Basis for TNBC Susceptibility** *Fergus Couch, Ph.D.* Chair, Division of Experimental Pathology and Laboratory Medicine, Department of Laboratory Medicine and Pathology
12:45 – 1:00	**Break**
1:00 – 3:00	**Translational Research – Communicating with Scientists and Patients Workshop** **Amy Hauenstein, PhD.**
3:00 – 3:30	**Workshop wrap-up session**
4:00 – 5:00	**Breast SPORE Scientific Symposium** ***Join Here*** ***Immune cells in Breast Cancer: is it a big deal or not at all?*** *Roberto Salgado, M.D., Ph.D.* Scientific Collaborator of the Immuno-Task Force of the Breast International Group (BIG) Honorary Research Associate at the Division of Research at the Peter Mac Callum Cancer, Melbourne, Australia Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium Chair of International Immuno-Oncology Biomarkers Working Group

Institutional Directory

Baylor College of Medicine

Jane Marmion
6224 San Felipe St
Houston, Texas 77057-2810
(713) 785-3636
j77008@comcast.net

Julia Maues
4610 Ellicott St NW
Washington, D.C. 20016
(202) 380-7477
maues.ju@gmail.com

Eva May
108 Turquoise Creek Drive
Cary, North Carolina 27513
(919) 462-8071
eva.connector@gmail.com

Stephanie Miller
10907 Albury Drive
Houston, Texas 77096
(832) 729-5859
stephanie@stephaniedmiller.com

Josh Newby
1 Baylor Plaza
Houston, Texas 77007
(618) 580-0908
joshua.newby@bcm.edu

Susan Rafte
2228 Dunstan Rd.
Houston, Texas 77005
(832) 524-8505
susanrafte@me.com

Dana-Farber CI

Margaret Carvan
65 Clewley Road
Medford, Massachusetts 02155
(617) 694-1421
mcarvans57@aol.com

Elizabeth Frank
80 Park Street 75
Brookline, Massachusetts 02446
(617) 872-5873
lsalmonfrank@gmail.com

Barbara LeStage
360 Spring Street
Wrentham, Massachusetts 02093
(508) 954-3960
blestage@verizon.net

Nancy Poorvu
117 Woodridge Rd
Wayland, Massachusetts 01778
(617) 592-7897
nlp@poorvu.com

Duke Clinical Research Institute

Coleen Crespo
5832 Solitude Way
Durham, North Carolina 27713
919-824-3946
coleen.crespo@duke.edu

Learning Designs, LLC

Amy Hauenstein
amy@learningdesignsllc.com
Twitter: @amyhauenstein

Institutional Directory

Mayo Clinic, Rochester

Lori Denison
200 First Street SW
Rochester, Minnesota 55901-4834
(507) 251-2431
ldenison@mayo.edu

Barbara Goodnature
2922 17th Ave NW
Rochester, Minnesota 55901
(507) 358-8517
goodnatureb@hotmail.com

Carol Phillips
2475 Crimson Ridge Cir NW
Rochester, Minnesota 55901
(507) 358-5368
phillips.carol1@mayo.edu

Susan Quella
711 15th Ave NE
Rochester, Minnesota 55906
(507) 319-7227
susanrn71@gmail.com

Elda Railey
2709 Cunningham CT
Opelika, Alabama 36801
(214) 683-9937
erailey@researchadvocacy.org

Mary Lou Smith
718 South Wright Street
Naperville, Illinois 60540
(630) 420-2958
mlsmith@researchadvocacy.org

NIH/National Cancer Institute

JoyAnn Rohan
Program Director
9609 Medical Center Drive
3W206
Rockville, Maryland 20850
(240) 276-5150
joyann.rohan@nih.gov

Toby Hecht
Assistant Director,
Translational Research Program
Deputy Director,
Division of Cancer Treatment and Diagnosis
9609 Medical Center Drive
Rockville, Maryland 20850
(240) 276-5683
hechtt@mail.nih.gov

University of North Carolina, Chapel Hill

Vernal Branch
119 Landings Dr., #403
Mooresville, North Carolina 28117
(804) 551-1815
verbra@msn.com

Christine Foster
UNC Chapel Hill
450 West Drive
Chapel Hill, North Carolina 27719
(919) 536-2505
cefoster@med.unc.edu

Laura Jensen
3000 Galloway Ridge Rd., Apt B106
Pittsboro, North Carolina 27312
(919) 265-7507
ltjensen7@gmail.com

Institutional Directory

Marian Johnson-Thompson
111 Wenonah Way
Durham, North Carolina 27713-2461
(919) 544-3394
mjohnsonthompson@aol.com

Patty Spears
8605 Caolingian Court
Raleigh, North Carolina 27615
(919) 656-5401
paspears@med.unc.edu

Missy VanLokeren
704 Wayne Drive
Raleigh, North Carolina 27608
(603) 440-9535
missyvanlokeren@gmail.com

Vanderbilt

Lynne Cargen
9441 Ashford Place
Brentwood, Tennessee 37027
(615) 479-3186
lbcargen@gmail.com

Janet Piper
113 West Ridge Drive
Hendersonville, Tennessee 37075
(615) 429-1721
janet.piper@comcast.net

Institutional Directory

Planning Committee

Matthew Goetz, M.D.
Co-Director, Mayo Clinic Women's Cancer Program
Director, Mayo Clinic Breast Cancer SPORE
Goetz.Matthew@mayo.edu

Cynthia Chauhan
Chair, Mayo Clinic Breast SPORE Patient Advisory Committee
cynthiachauhan@aol.com

Joyann Rohan
joyann.rohan@nih.gov

Bobbi Jebens
Jebens.Bobbi@mayo.edu

Organizer

Tamara Walton
tamara.walton@nih.gov

* The directory is a list of attendees of the Breast Cancer SPORE Workshop and shall not be distributed beyond the explicit permission of Dr. Goetz or Cynthia Chauhan.

Alphabetical Directory

Vernal Branch
University of North Carolina, Chapel Hill
119 Landings Dr., #403
Mooresville, North Carolina 28117
Phone: (804) 551-1815
verbra@msn.com

Lynne Cargen
Vanderbilt
9441 Ashford Place
Brentwood, Tennessee 37027
Phone: (615) 479-3186
lbcargen@gmail.com

Margaret Carvan
Dana-Farber CI
65 Clewley Road
Medford, Massachusetts 02155
Phone: (617) 694-1421
mcarvans57@aol.com

Coleen Crespo
Duke Clinical Research Institute
5832 Solitude Way
Durham, North Carolina 27713
Phone: (919) 824-3946
coleen.crespo@duke.edu

Lori Denison
Mayo Clinic, Rochester
200 First Street SW
Rochester, Minnesota 55901-4834
Phone: (507) 251-2431
ldenison@mayo.edu

Christine Foster
University of North Carolina, Chapel Hill
450 West Drive
Chapel Hill, North Carolina 27719
Phone: (919) 536-2505
cefoster@med.unc.edu

Elizabeth Frank
Dana-Farber CI
80 Park Street 75
Brookline, Massachusetts 02446
Phone: (617) 872-5873
lsalmonfrank@gmail.com

Barbara Goodnature
Mayo Clinic, Rochester
2922 17th Ave NW
Rochester, Minnesota 55901
Phone: (507) 358-8517
goodnatureb@hotmail.com

Amy Hauenstein
Learning Designs, LLC
amy@learningdesignsllc.com
Twitter: @amyhauenstein

Toby Hecht
NIH/National Cancer Institute
Associate Director,
Translational Research Program
Associate Director, DCTD
9609 Medical Center Drive
Rockville, Maryland 20850
Phone: (240) 276-5683
hechtt@mail.nih.gov

Alphabetical Directory

Laura Jensen
University of North Carolina, Chapel Hill
3000 Galloway Ridge Rd., Apt B106
Pittsboro, North Carolina 27312
Phone: (919) 265-7507
ltjensen7@gmail.com

Marian Johnson-Thompson
University of North Carolina, Chapel Hill
111 Wenonah Way
Durham, North Carolina 27713-2461
Phone: (919) 544-3394
mjohnsonthompson@aol.com

Barbara LeStage
Dana-Farber CI
360 Spring Street
Wrentham, Massachusetts 02093
Phone: (508) 954-3960
blestage@verizon.net

Jane Marmion
Baylor College of Medicine
6224 San Felipe St
Houston, Texas 77057-2810
Phone: (713) 785-3636
j77008@comcast.net

Julia Maues
Baylor College of Medicine
4610 Ellicott St NW
Washington, District of Columbia 20016
Phone: (202) 380-7477
maues.ju@gmail.com

Eva May
Baylor College of Medicine
108 Turquoise Creek Drive
Cary, North Carolina 27513
Phone: (919) 462-8071
eva.connector@gmail.com

Stephanie Miller
Baylor College of Medicine
10907 Albury Drive
Houston, Texas 77096
Phone: (832) 729-5859
stephanie@stephaniedmiller.com

Josh Newby
Baylor College of Medicine
1 Baylor Plaza
Houston, Texas 77007
Phone: (618) 580-0908
joshua.newby@bcm.edu

Carol Phillips
Mayo Clinic, Rochester
2475 Crimson Ridge Cir NW
Rochester, Minnesota 55901
Phone: (507) 358-5368
phillips.carol1@mayo.edu

Janet Piper
Vanderbilt
113 West Ridge Drive
Hendersonville, Tennessee 37075
Phone: (615) 429-1721
janet.piper@comcast.net

Alphabetical Directory

Nancy Poorvu
Dana-Farber CI
117 Woodridge Rd
Wayland, Massachusetts 01778
Phone: (617) 592-7897
nlp@poorvu.com

Susan Quella
Mayo Clinic, Rochester
711 15th Ave NE
Rochester, Minnesota 55906
Phone: (507) 319-7227
susanrn71@gmail.com

Susan Rafte
Baylor College of Medicine
2228 Dunstan Rd.
Houston, Texas 77005
Phone: (832) 524-8505
susanrafte@me.com

Elda Railey
Mayo Clinic, Rochester
2709 Cunningham CT
Opelika, Alabama 36801
Phone: (214) 683-9937
erailey@researchadvocacy.org

JoyAnn Rohan
NIH/National Cancer Institute
Program Director
9609 Medical Center Drive, 3W206
Rockville, Maryland 20850
Phone: (240) 276-5150
joyann.rohan@nih.gov

Mary Lou Smith
Mayo Clinic, Rochester
718 South Wright Street
Naperville, Illinois 60540
Phone: (630) 420-2958
mlsmith@researchadvocacy.org

Patty Spears
University of North Carolina, Chapel Hill
8605 Caolingian Court
Raleigh, North Carolina 27615
(919) 656-5401
paspears@med.unc.edu

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University of North Carolina, Chapel Hill
704 Wayne Drive
Raleigh, North Carolina 27608
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missyvanlokeren@gmail.com

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Breast Cancer SPORE Advocate Workshop

Day 1 - Slides

November 2, 2020

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Skill Builders

A COMMUNICATIONS COURSE FOR RESEARCH ADVOCATES®

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Communications Skill Builders

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Session 1: Preparing for an online conference

Digital Audience Participation

Strategies

Audience Analysis + Preparing for the Q&A

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Digital Audience Participation Best Practices

- Electronic Etiquette
- Platform Familiarity
- Understanding Expectations & Participation Options
- Prepare

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Communication Strategy Preview

Information is not knowledge.

Two Major Challenges When Facing Information Overload

Communication Strategy Preview

- **Filtering** knowledge **for** <u>**use**</u>
- Effectively **sharing** knowledge **for** others to <u>**use**</u>

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“It's not information overload. It's filter failure.”

Clay Shirky

Illustration from The Economist

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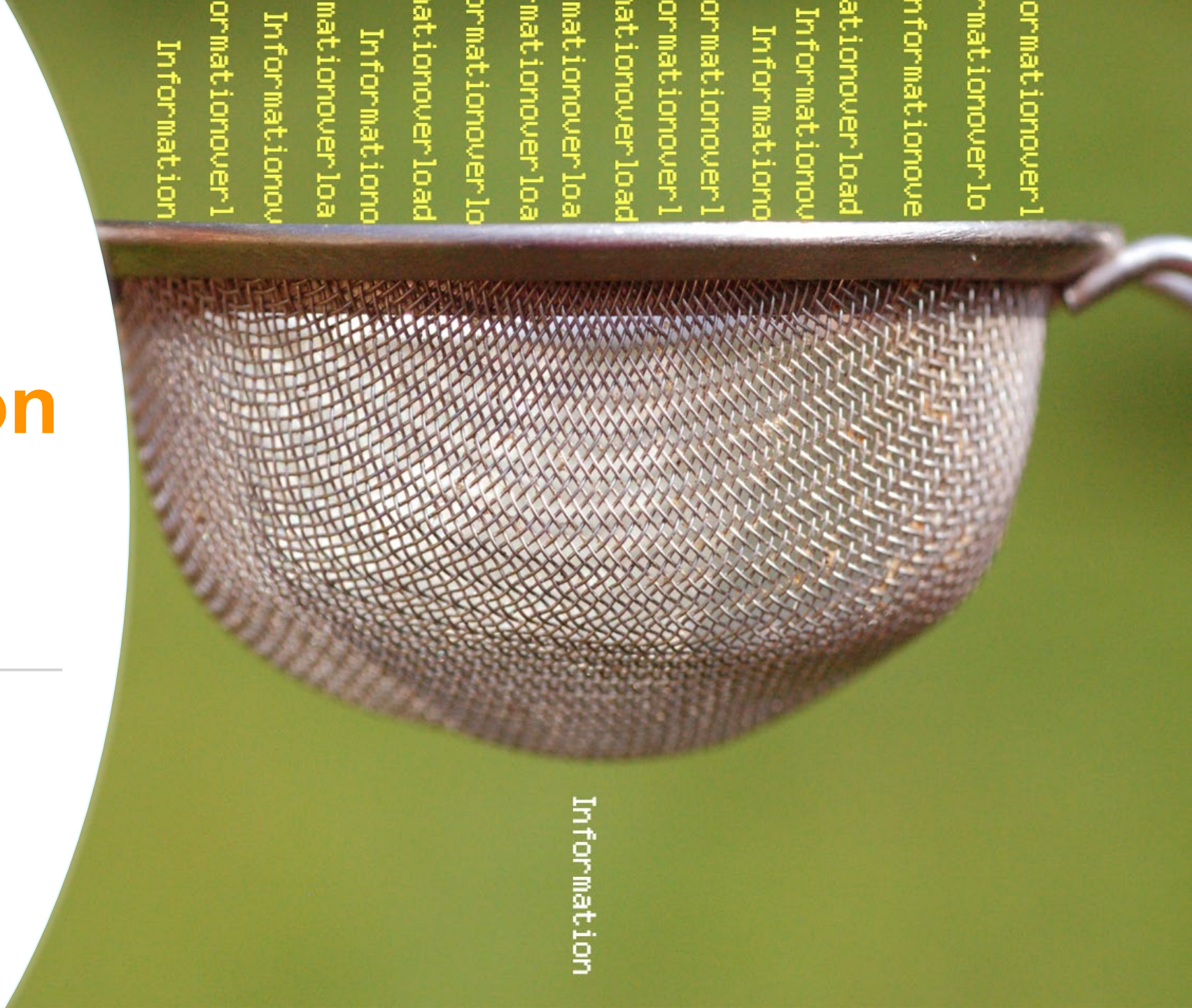
Communication Strategy Preview



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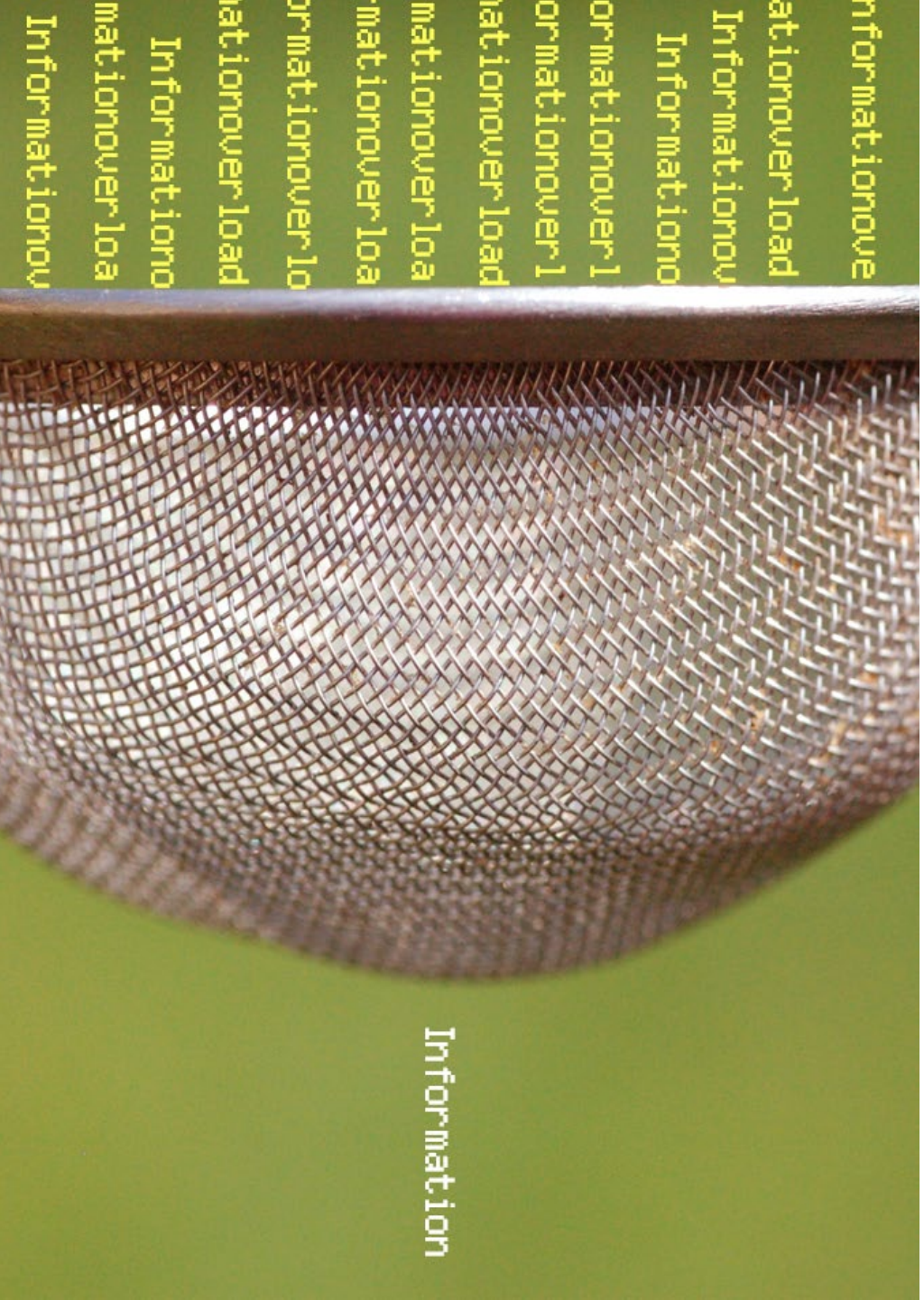
Critical Essence Model

- Takes information and organizes it into meaningful categories that have wide appeal.
- It understands that the audience has limited capacity to remember information and even less to translate information into knowledge for their own use.



Critical Essence Model

- Take all your notes from the conference
- Organize them under 3 headings (maximum)
- Align priority knowledge to specific audiences
- Strategically choose communication channels to delivery knowledge to audiences

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Group Dialogue

Who are your audiences?

What do they know they want to know?

What should they want to know?

What do they not know – that they should know?

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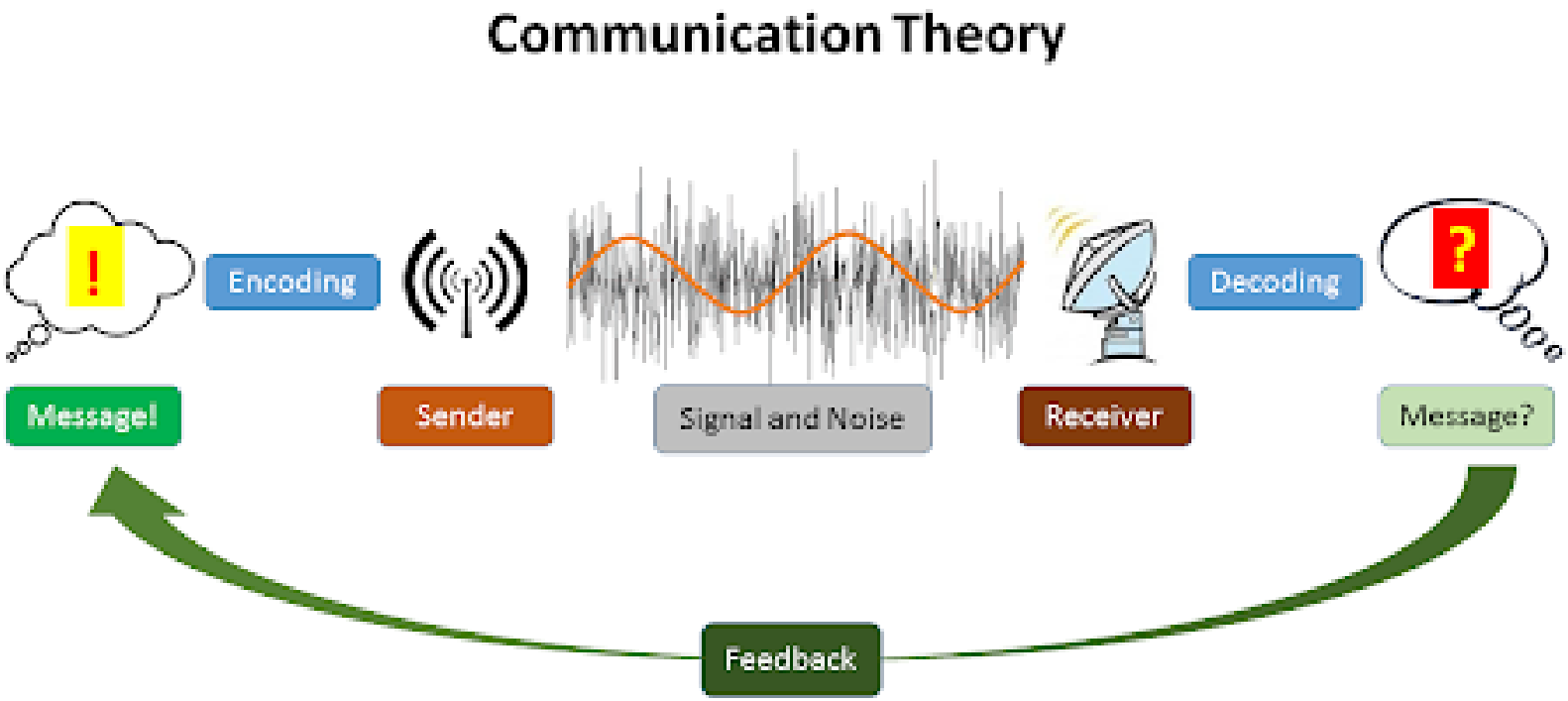
Communication Strategy Preview



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Critical Essence Model

- A strong communication framework is aspirational and designed to inspire stakeholders to take an action.
- It educates community members and tells the compelling story of how this knowledge can advance their vision, purpose, and goals.

Note-taking Strategy

DATE:

SPEAKER:	EXPERTISE:
	AFFILIATIONS:
TALK TITLE:	CONTACT:

QUESTIONS/KEY WORDS:	NOTES/ANSWERS/DEFINITIONS/EXAMPLES

CRITICAL ESSENCE SUMMARY:

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Information Organizing Strategy

We'll meet for 30 minutes to discuss organizing the notes you've taken.

11 Nov.

12 Nov.

We will work to create communication for knowledge sharing.

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Audience + Speaker Analysis

What can you find out in advance about the speaker that you and your audience(s) care about?

What questions can you anticipate that the speaker will answer? Put those in your question-column of your notes.

Use the notes column when those questions get answered and cross them out in the question-column to keep track.

If possible, ask any remaining questions or new ones that arise. Be careful to listen to others questions so as not to duplicate.

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Q&A Preparation

Prioritize your questions and ask the most pressing question that remains (again – be sure it hasn't been asked/answered previously)

Use the format you're most confident in – either unmute and ask verbally or use the chat function

Ask in as succinct a way as possible and be prepared to capture the answer

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DATE:

SPEAKER:	**EXPERTISE:**
	AFFILIATIONS:
TALK TITLE:	**CONTACT:**

QUESTIONS/KEY WORDS:	**NOTES/ANSWERS/DEFINITIONS/EXAMPLES**

CRITICAL ESSENCE SUMMARY:

Breast Cancer SPORE Advocate Workshop

Day 2 - Slides

November 11, 2020

New Drug Treatments for Endocrine Resistant Breast Cancer

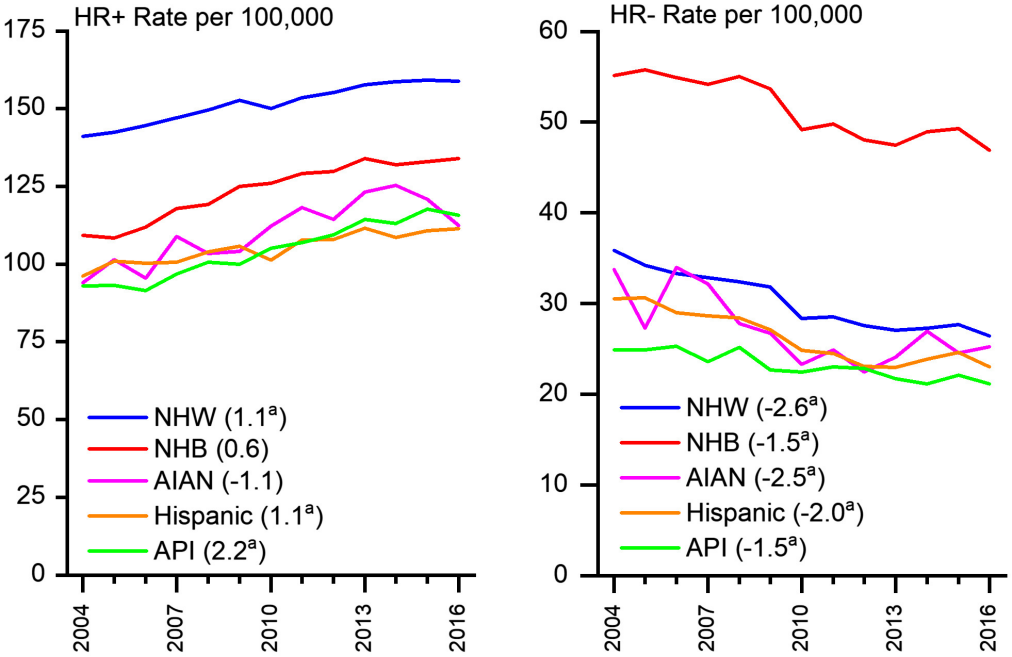
Matthew P. Goetz
Professor of Oncology and Pharmacology
Mayo Clinic
Rochester, MN

Outline

- Review the incidence and natural history of estrogen receptor (ER) positive breast cancer
- Define the impact of adjuvant hormonal therapies on the survival of women with ER+ breast cancer
- Discuss the emerging treatments for endocrine resistant breast cancer

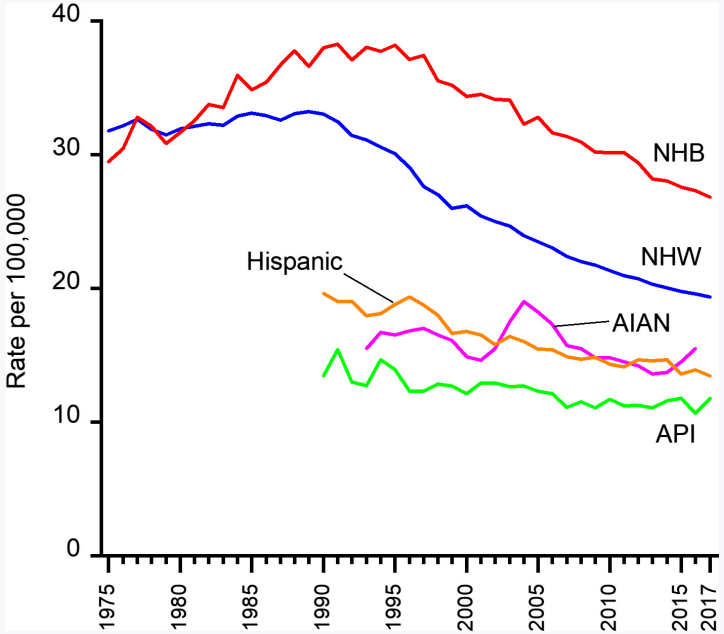
Age and Subtype Specific Incidence of breast cancer

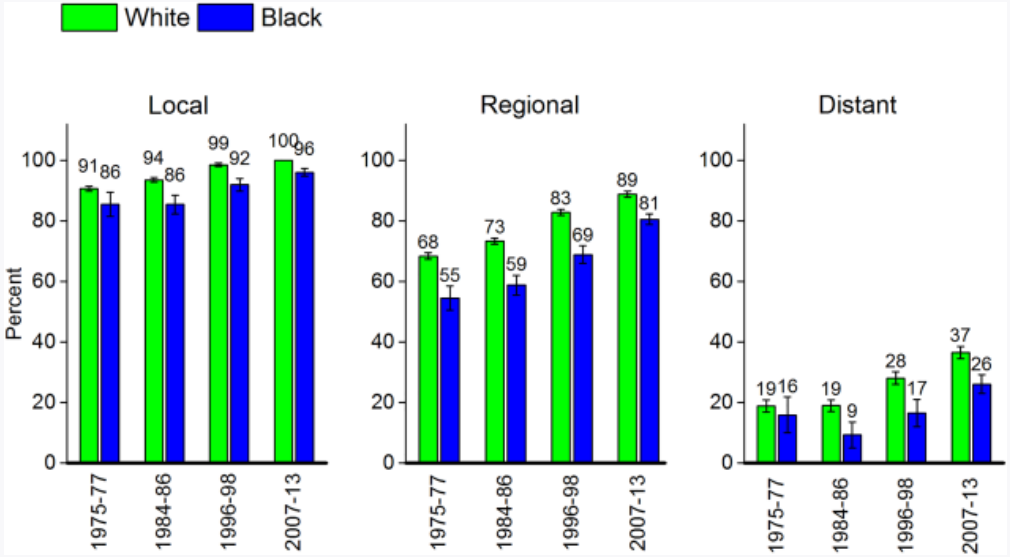
- **Globally, 2 million women estimated to develop breast cancer[1]**
- **In the U.S, 271,000 new cases of breast cancer--two-thirds are ER+[2]**
- **In the U.S., incidence of ER+ cancer still rising[3]**



1. Bray F, et al. Global Cancer Statistics 2018
2. Siegal et al. CA: A Cancer Journal for Clinicians 2019
3. DeSantis et al. CA: A Cancer Journal for Clinicians 2019

U.S Breast Cancer Mortality: Impact of Time and Stage





From 1989 to 2015, U.S. breast cancer death rates decreased by 39%, with 322,600 averted breast cancer deaths

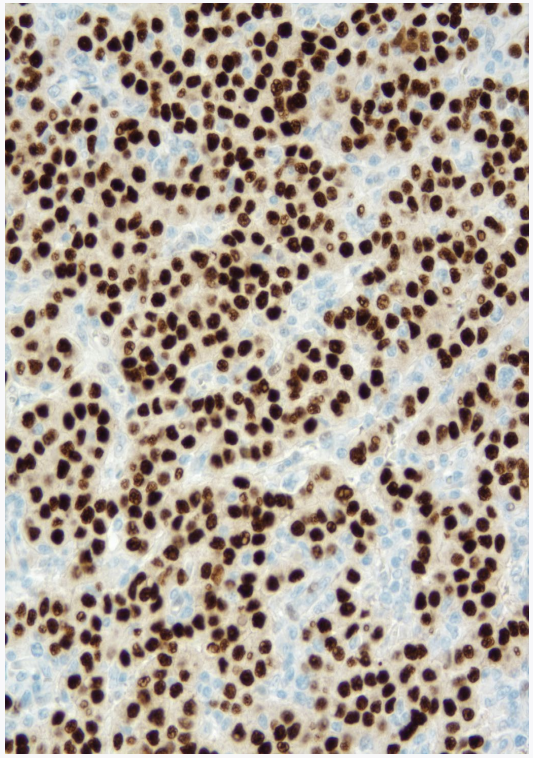
1. DeSantis et al. CA: A Cancer Journal for Clinicians 2019

Summary

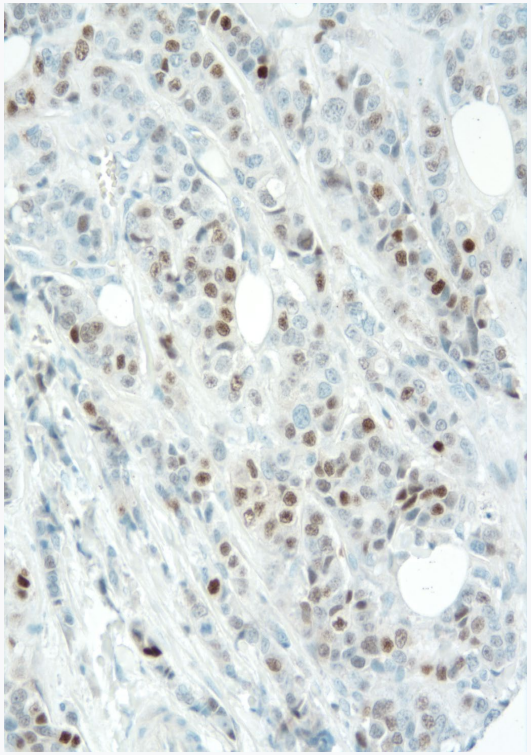
- The majority of breast cancers express the ER.
- In the U.S., the incidence of ER+ (but not ER-) breast cancer is slowly rising
- Substantial gains in mortality have been observed over time in women with both localized and metastatic breast cancer

IHC Detection of ER Expression in Breast Cancer

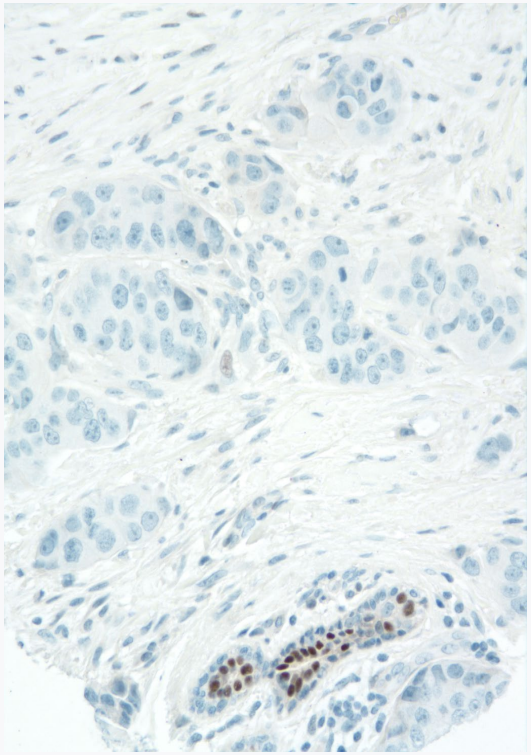
Strong



Weak



Negative

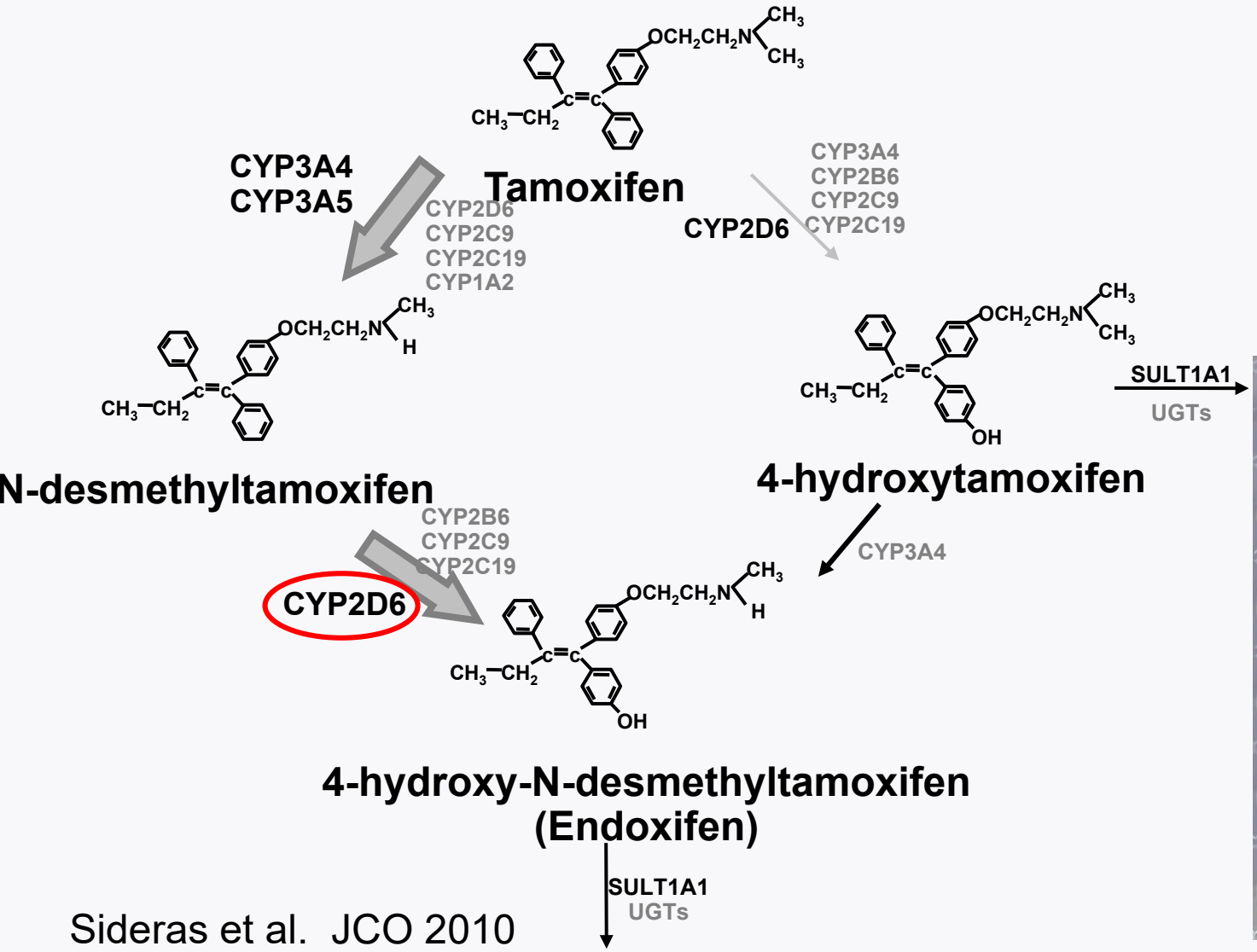


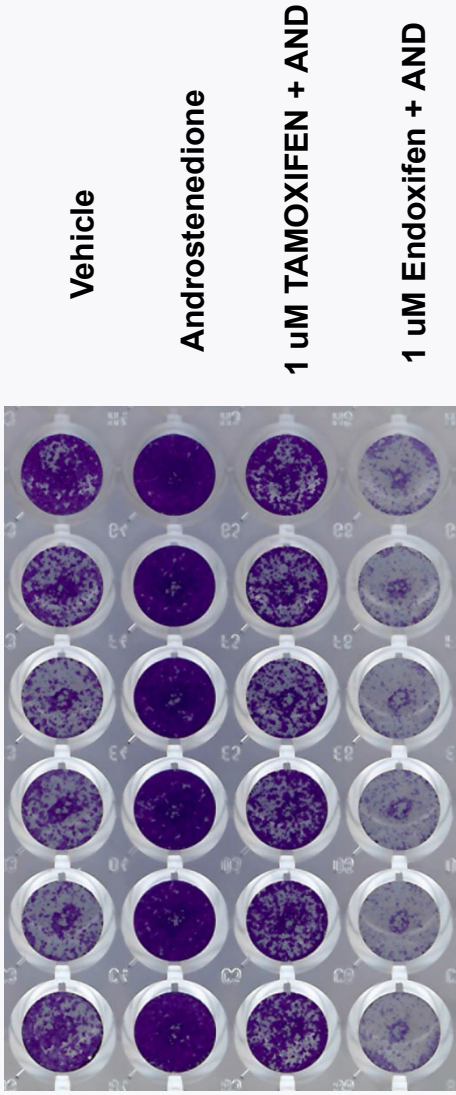
Breast Cancer
Endocrine Therapy

Therapeutic Strategies

- **Block or Degrade ER –**
  - Tamoxifen (SERM) (pre and postmenopausal)
  - Fulvestrant (Degrade ER) (postmenopausal)
- **Decrease estrogen synthesis**
  - ovarian estrogen production—LHRH agonists (Goserelin) or oophorectomy (Premenopausal)
  - Aromatase inhibitors (postmenopausal)

Tamoxifen Metabolism: Inhibition of Estrogen-mediated growth



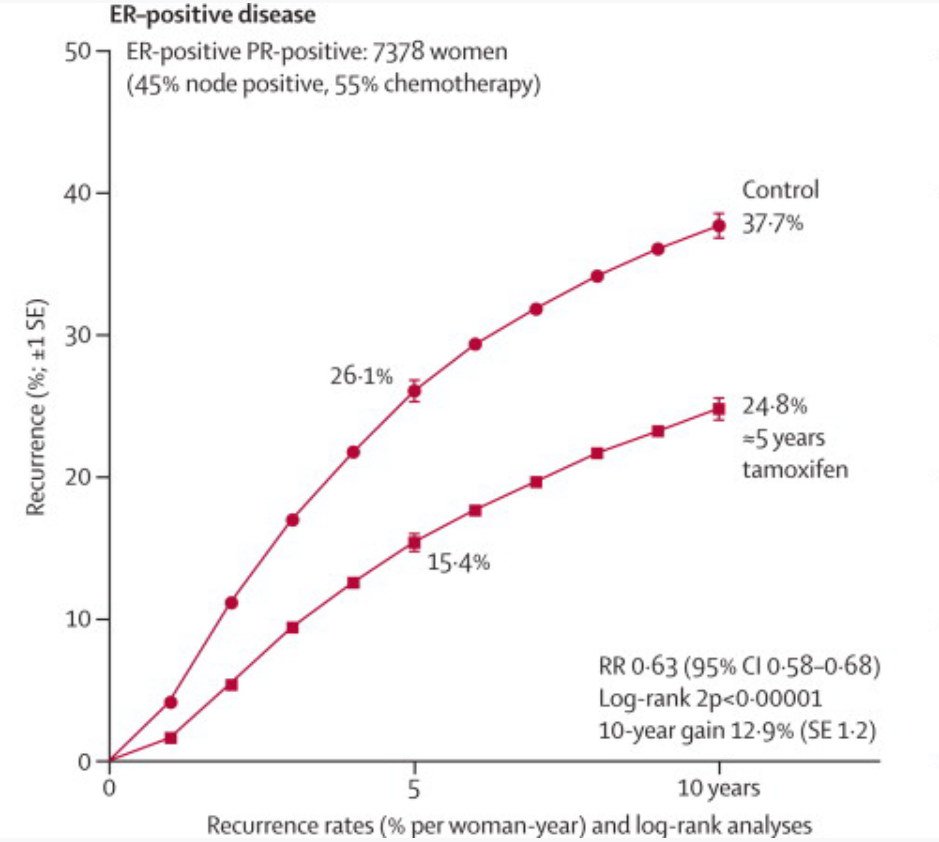


Sideras et al. JCO 2010

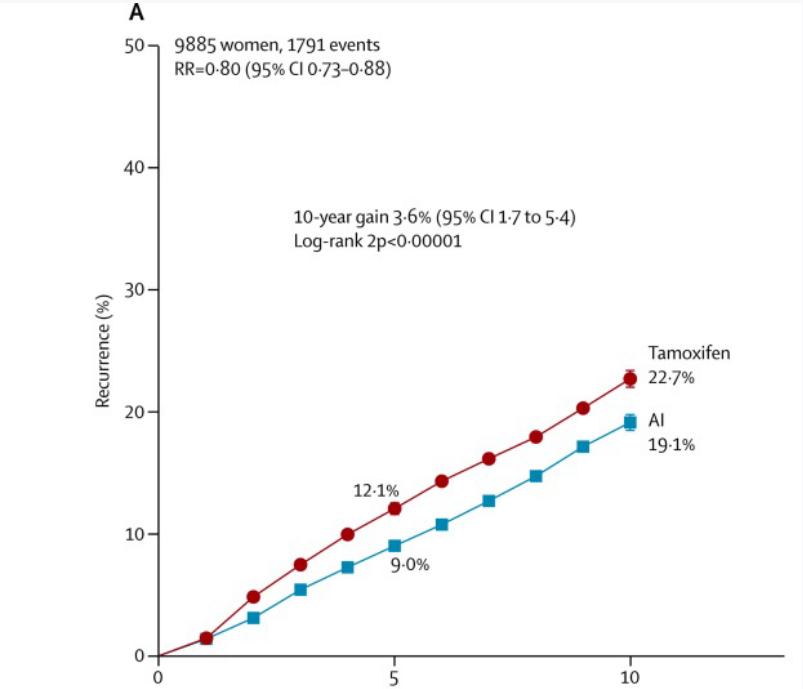
Jayaraman et al. Breast Can Res 2020

Tamoxifen and Aromatase Inhibitors: Adjuvant Treatment of Postmenopausal ER+ Breast Cancer

Tamoxifen vs Control



AI's vs Tamoxifen



Recurrence rate/year (%), events/woman-years and log-rank statistics

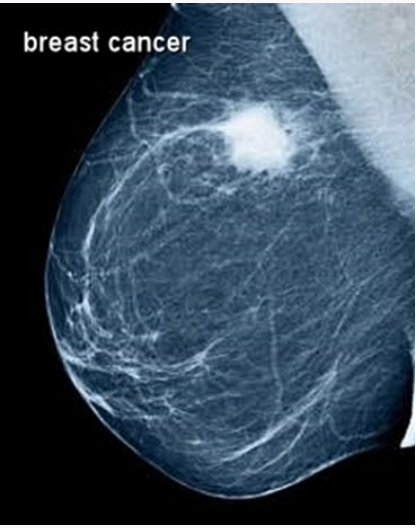
Allocation	Years 0–1	Years 2–4	Years 5–9	Year 10+
AI	1·62 (157/9691)	2·14 (285/13336)	2·33 (365/15648)	3·23 (20/619)
Tamoxifen	2·41 (230/9542)	2·62 (338/12906)	2·48 (372/14985)	4·54 (24/529)
Rate ratio (95% CI)	0·64 (0·52–0·78)	0·80 (0·68–0·93)	0·92 (0·79–1·06)	0·72 (0·39–1·30)
from (O-E)/V	−41·1/92·8	−34·1/149·0	−15·5/177·2	−3·6/10·7

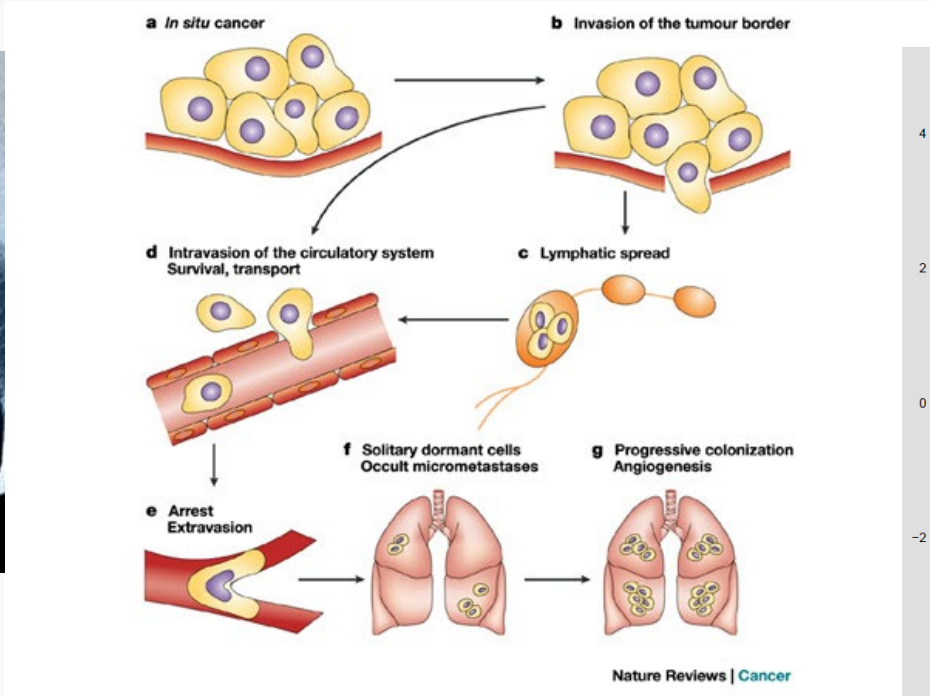
Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Lancet 2011 and 2015

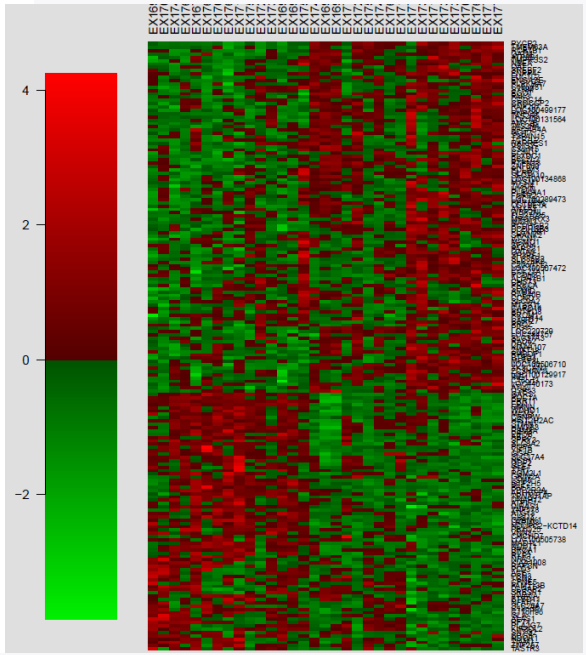
Summary

- Four decades of work focused on understanding the biology of ER breast cancer
  - Accurate identification of ER
  - Substantial benefit of adjuvant hormonal therapy in ER+ but not ER- breast ca
  - Improvements in adjuvant hormonal therapy (AI vs tamoxifen) have improved outcomes in the adjuvant setting
- Major goal: Identify resistance targets/pathways to prevent early and late resistance to hormonal therapy.

Heterogeneity of Breast Cancer

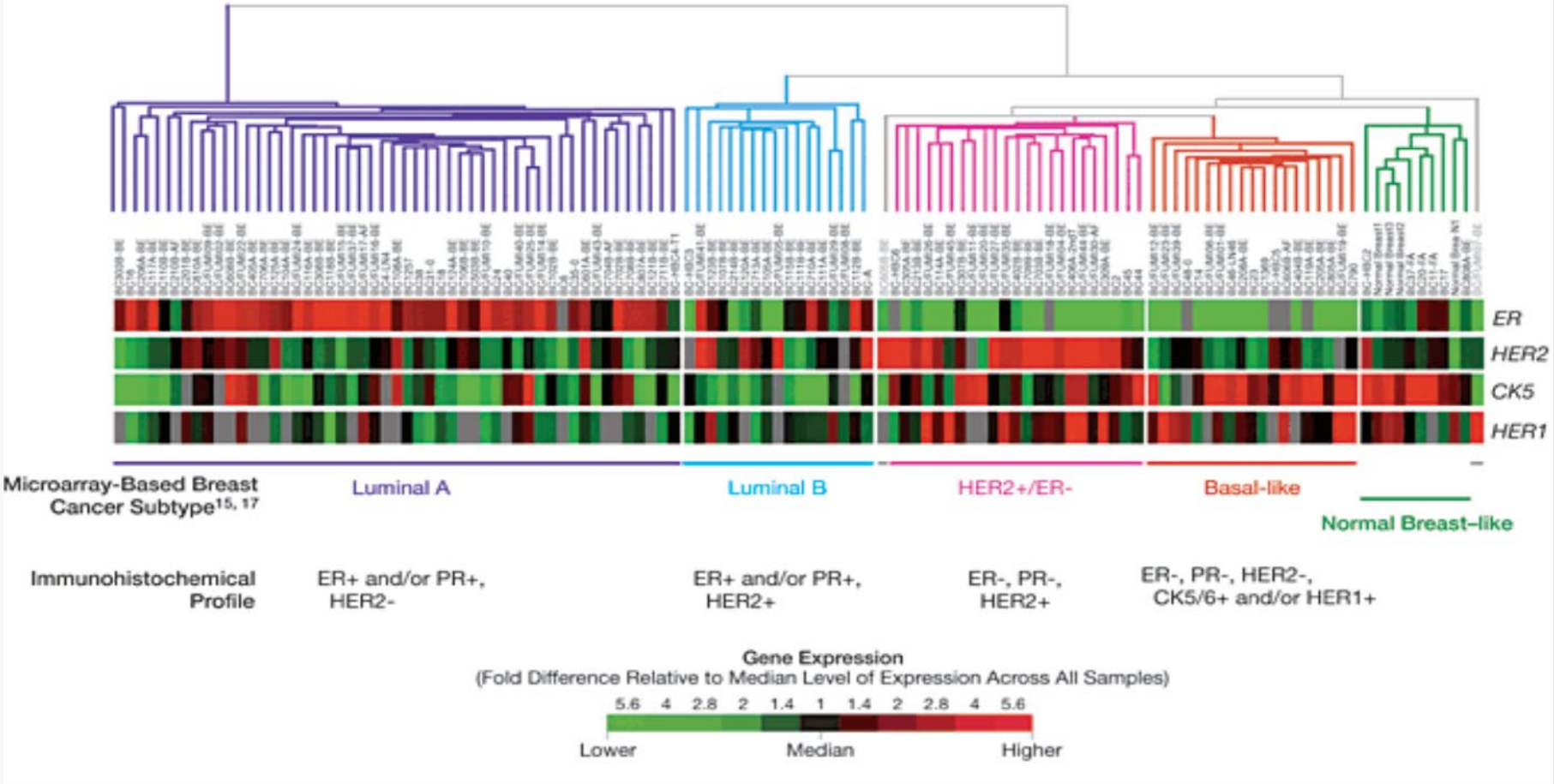






Sorlie et a al PNAS 2003

Breast Cancer Molecular Subtypes



TCGA. Nature. 2012.

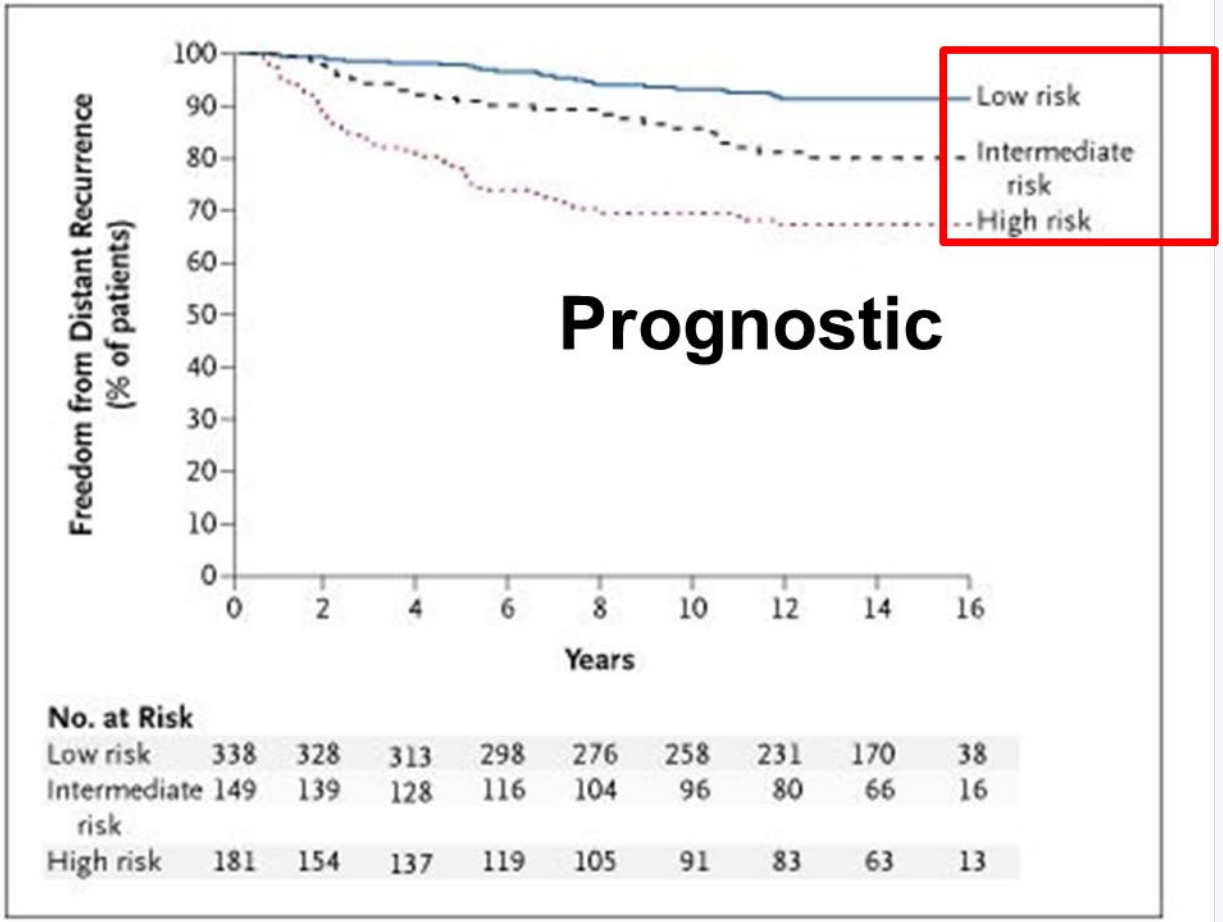
Endocrine Resistance

- **Primary Endocrine Resistance**
  - Recurrence within the first 2 years of adjuvant endocrine therapy while on endocrine therapy
  - Progression within first 6 months of initiating first-line endocrine therapy for the treatment of metastatic breast cancer (while on endocrine therapy)

Primary Endocrine Resistance

- **Summary of Genes/Pathways associated with primary endocrine resistance**
  - Low or absent ER
  - ER+/HER2+ (luminal HER2) (effects abrogated in the setting of trastuzumab)
  - Luminal B (heterogeneous)
    - Activation of Growth Factor Pathways (e.g. EGFR1)
    - Activation of proliferation genes
  - Commercially available gene expression profiles encompass many of these genes/pathways

21 Gene Recurrence Score: Distant Recurrence in NSABP B14



No. at Risk									
Low risk	338	328	313	298	276	258	231	170	38
Intermediate risk	149	139	128	116	104	96	80	66	16
High risk	181	154	137	119	105	91	83	63	13

The difference among the three recurrence score (RS) groups is significant (P<0.001)

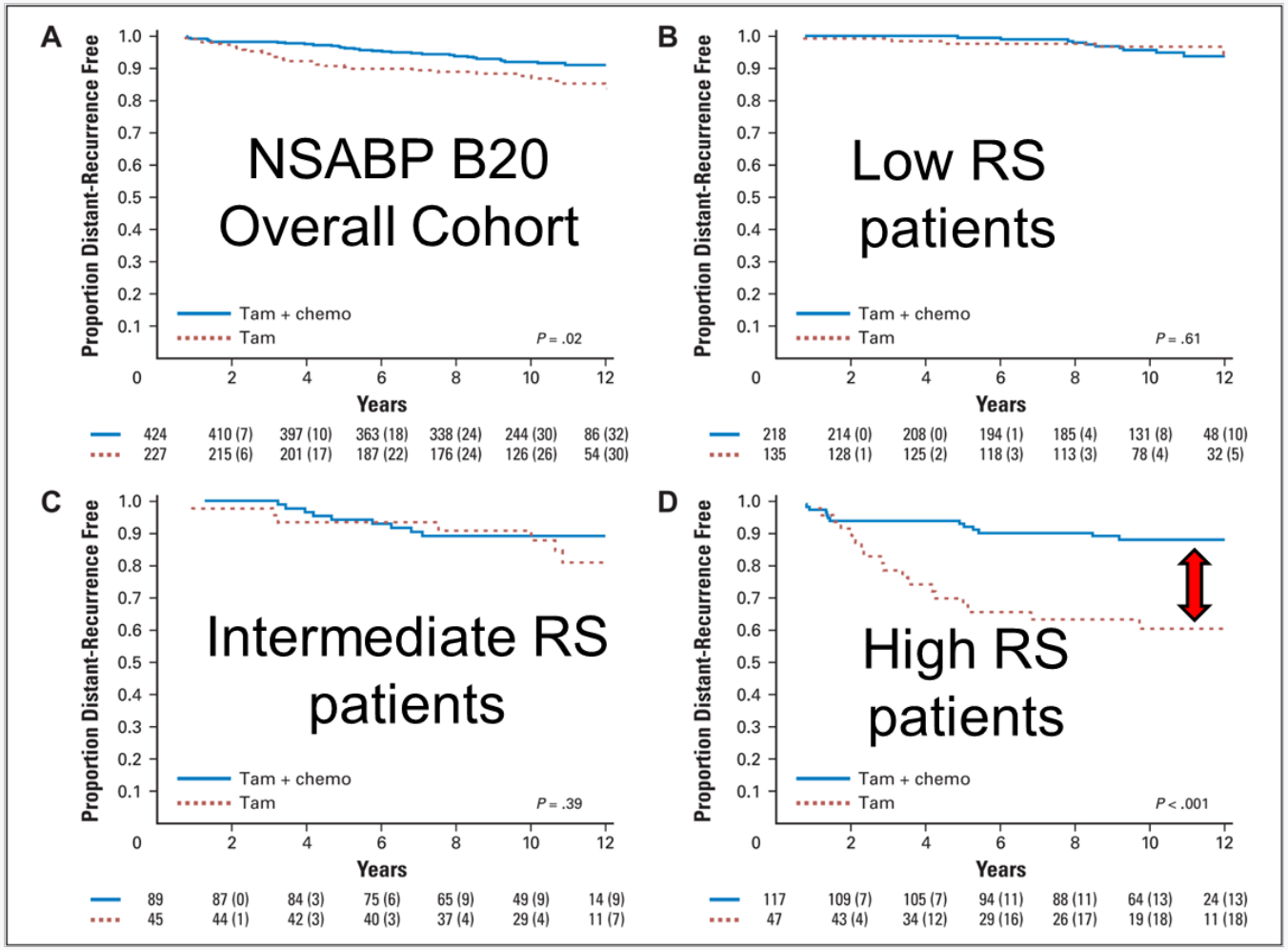
Paik et al. N Engl J Med 2004.

Chemotherapy and Early Recurrence

Can multi-gene panels predict drug response?

Most multi-gene panels are heavily weighted towards “proliferation” genes and thus “high risk” patients may gain the greatest benefit from systemic chemotherapy

Recurrence Score: Benefit of chemotherapy Restricted to High-Risk Group



Paik et al. JCO, 2006; 24: 3726

Prospective Validation of 21 gene Recurrence Score (TAILORx)

Eastern Cooperative Oncology Group

PACCT-1
Revised 6/06 – Addendum #1

SCHEMA

ER- Positive and/or PR-Positive Breast Cancer
Axillary Node-Negative
Candidate for Adjuvant Cytotoxic Therapy
In Addition to Hormonal Therapy

Pre-Registration

Rev. 6/06 — Submit tumor specimen for ONCOTYPE DX Assay[1]

Rev. 6/06
Rev. 6/06 — Registration[5]/Randomization[5]

Secondary Study Group – 1
Recurrence Score < 11(~29% of Population)
Patients = Registered

Primary Study Group
Recurrence Score 11-25
(~44% of Population)
Patients = Randomized

Secondary Study Group - 2
Recurrence Score > 25
(~27% of Population)
Patients = Registered

Rev. 6/06

Stratify
- Tumor Size: ≤2.0 cm vs ≥ 2.1 cm
- Post-menopausal vs. Pre- or Peri-menopausal[4]
- Planned chemotherapy: Taxane-containing (i.e. paclitaxel, docetaxel) vs. Non-taxane-containing
- Planned radiation therapy: whole breast, no boost planned vs. whole breast, boost planned vs. partial breast irradiation planned vs. no planned radiation therapy (for patients who had a mastectomy)

Rev. 6/06 — Arm A
Hormonal Therapy[2]

Arm B
Hormonal Therapy[2]

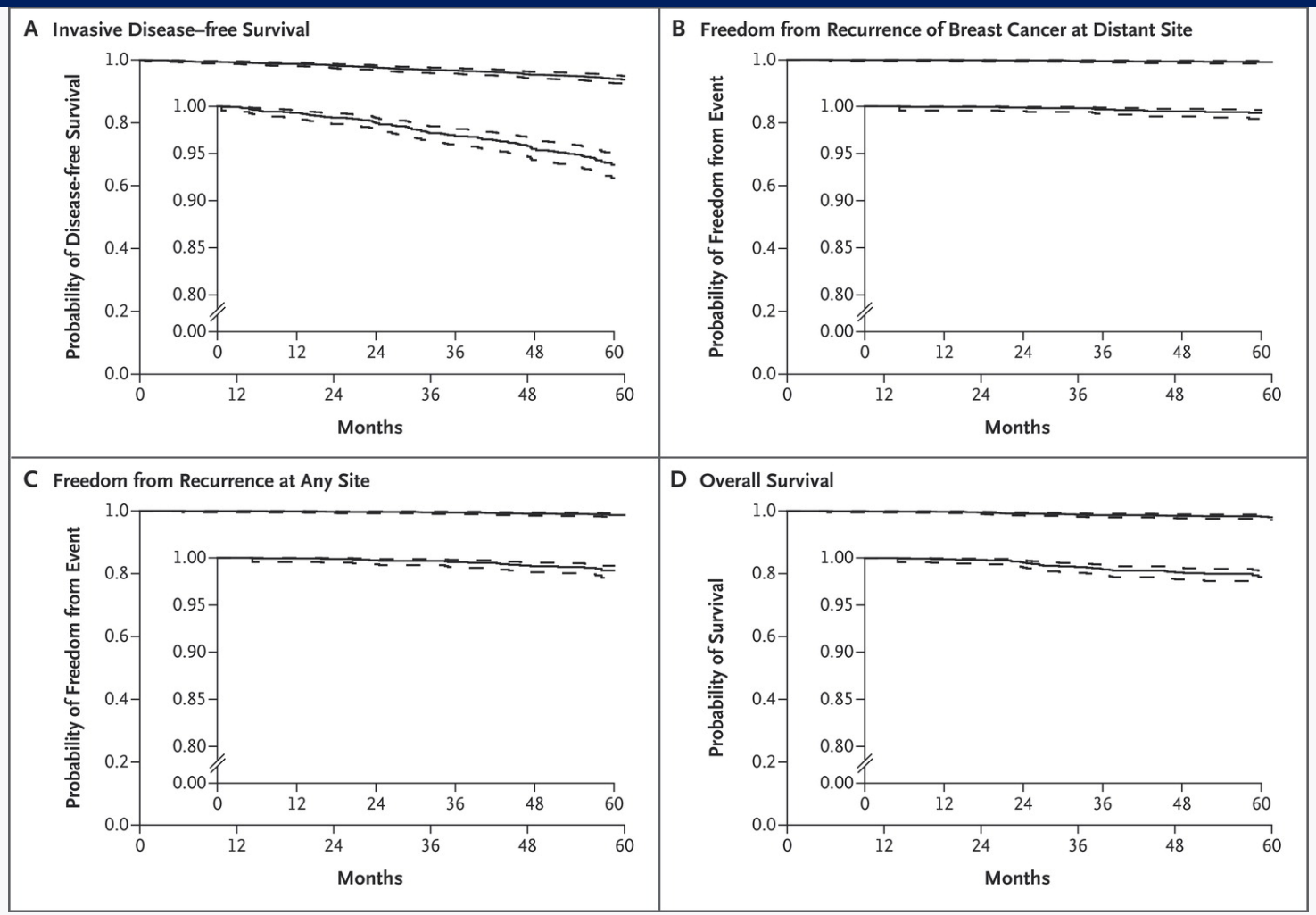
Arm C
Chemotherapy Plus Hormonal Therapy[3]

Arm D
Chemotherapy Plus Hormonal Therapy[3,5]

Accrual Goal = 10,046 Patients
Patients who have had breast conservation surgery will also be treated with radiotherapy.
Refer to Section 5.2 for RT guidelines

Clinical Trial ID: NCT00310180

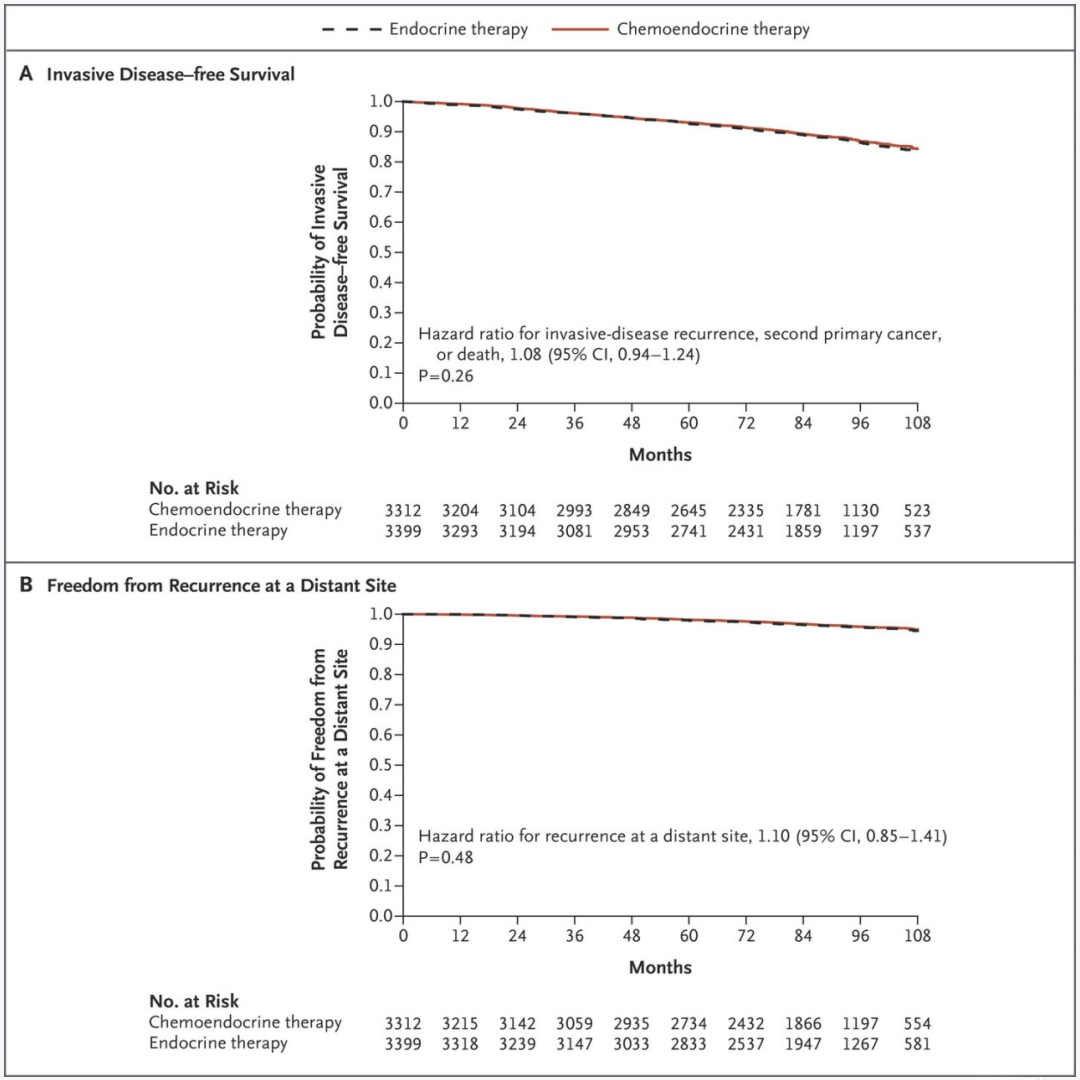
Kaplan–Meier Estimates of Key Endpoints 1626 patients with a recurrence score of 0 to 10.



Sparano JA et al. N Engl J Med 2015;373:2005-2014.

The NEW ENGLAND JOURNAL of MEDICINE

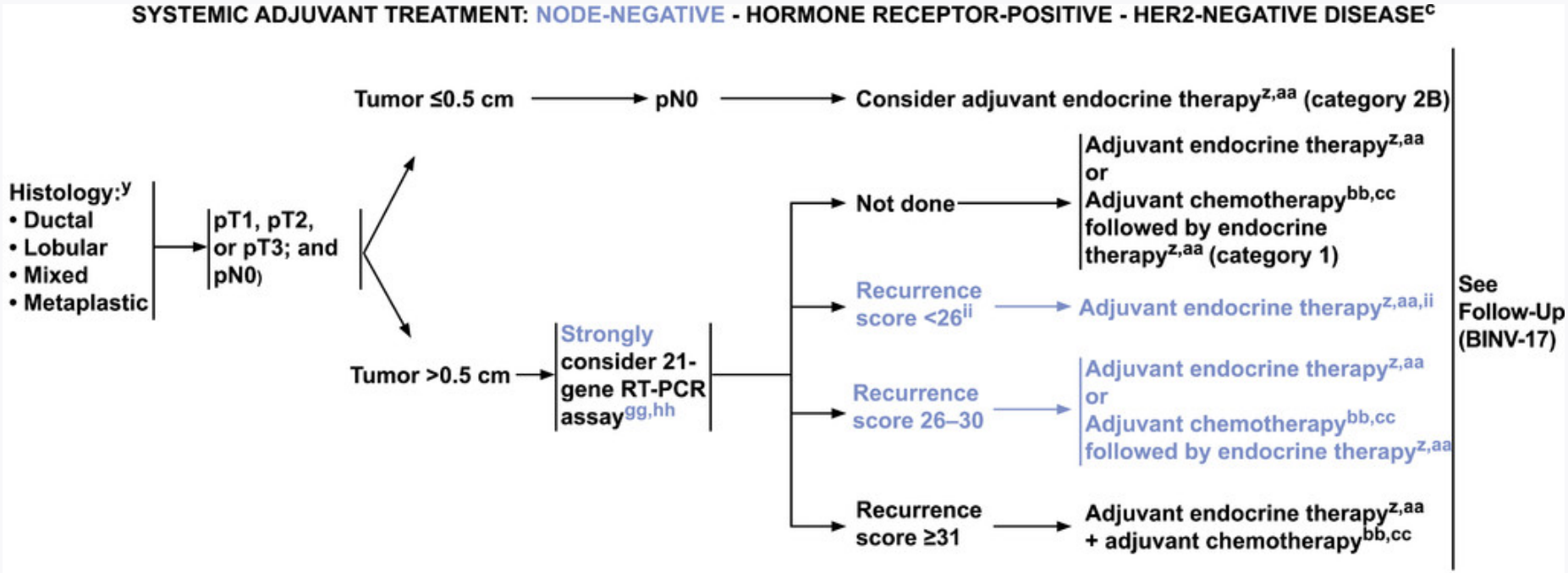
No Benefit of Chemotherapy for Patients with a Recurrence Score of 11 to 25



JA Sparano et al. N Engl J Med 2018;379:111-121.

The NEW ENGLAND JOURNAL of MEDICINE

NCCN Guidelines



[c]See Principles of HER2 Testing (BINV-A).

[y]Mixed lobular and ductal carcinoma should be graded based on the ductal component and treated based on this grading. For metaplastic carcinoma, the prognostic value of the histologic grading is uncertain. However, when a specific histologic subtype of metaplastic carcinoma is present and accounts for more than 10% of the tumor, the subtype is an independent prognostic variable.

[z]Consider adjuvant bisphosphonate therapy in postmenopausal (natural or induced) patients receiving adjuvant therapy.

[aa]Evidence supports that the magnitude of benefit from surgical or radiation ovarian ablation in premenopausal women with hormone receptor-positive breast cancer is similar to that achieved with CMF alone. See Adjuvant Endocrine Therapy (BINV-J).

[bb]Chemotherapy and endocrine therapy used as adjuvant therapy should be given sequentially with endocrine therapy following chemotherapy. Available data suggest that sequential or concurrent endocrine therapy with radiation therapy is acceptable. See Adjuvant Endocrine Therapy (BINV-J) and Preoperative/Adjuvant Therapy Regimens (BINV-K).

[cc]There are limited data to make chemotherapy recommendations for those >70 y of age. See NCCN Clinical Practice Guidelines for Older Adult Oncology.

[gg]Other prognostic multigene assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy. See Multigene Assays for Consideration of Addition of Adjuvant Systemic Chemotherapy to Adjuvant Endocrine Therapy (BINV-M).

[hh]Patients with T1b tumors with low grade histology should be treated with endocrine monotherapy as the TAILORx trial did not include patients with such tumors.

[ii]Consider the use of adjuvant chemotherapy in women 50 years of age or younger with a recurrence score of 16-25 based on an exploratory analysis from the TAILORx study demonstrating lower distant recurrences in women 50 years of age or younger randomized to chemotherapy.

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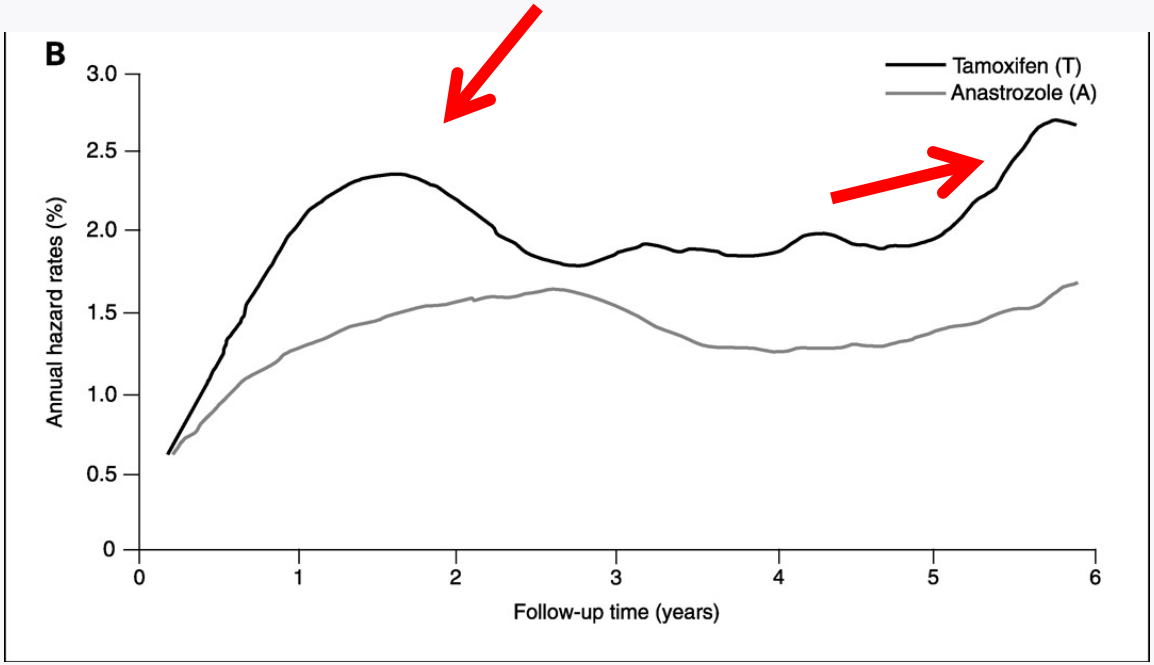
Goetz et al JNCCN 2019

Natural History of ER Positive Breast Cancer: Two Peaks for Recurrence over Time



- **First peak (primary endocrine resistance) addressed with improvements in endocrine therapy, selective use of chemotherapy and new targeted therapies (trastuzumab)**
- **Second Peak: (secondary resistance). What is the natural history?**

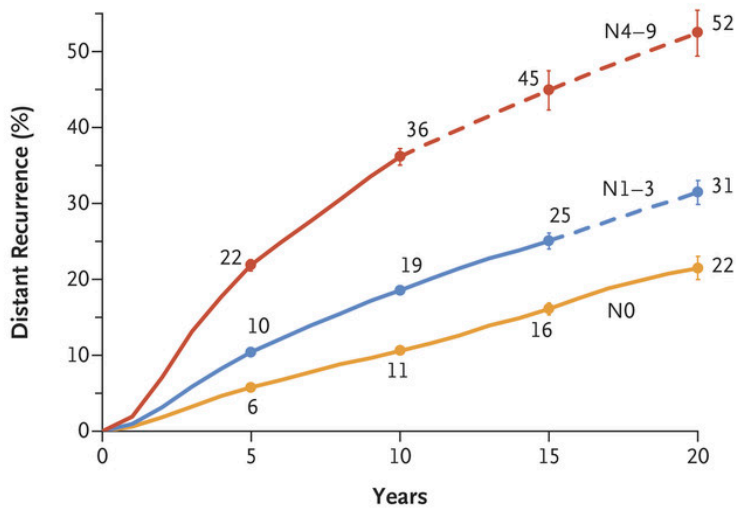
Buzdar et al. Clin Canc Res 2006

Endocrine Resistance

- **Secondary Clinical Resistance (Late Recurrence)**
  - Multi-modality therapies reduce “early” recurrences and prolong overall survival
  - What are accepted strategies to prevent late recurrences?

20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years

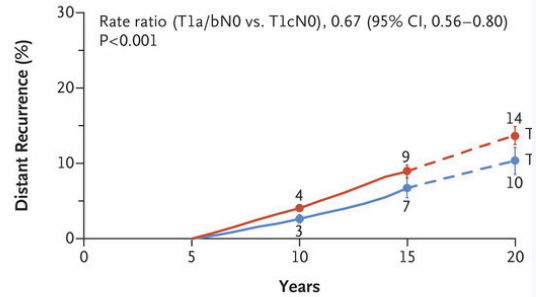
A Risk of Distant Recurrence



No. at Risk					
N4–9	12,333	8,116	2165	259	52
N1–3	31,936	23,576	7250	949	183
N0	29,925	24,081	8571	1982	414

No. of Events — annual rate (%)				
N4–9	2568 (4.8)	969 (4.0)	121 (3.1)	13 (2.2)
N1–3	3126 (2.2)	1421 (1.9)	241 (1.7)	39 (1.8)
N0	1646 (1.2)	835 (1.1)	272 (1.3)	68 (1.4)

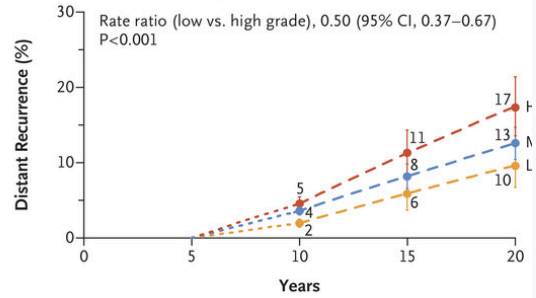
A Risk of Distant Recurrence, According to Tumor Size



No. at Risk				
T1cN0	13,875	5967	1641	309
T1a/bN0	5,527	2053	704	131

No. of Events — annual rate (%)			
T1cN0	413 (0.8)	171 (1.1)	46 (1.
T1a/bN0	96 (0.5)	47 (0.8)	12 (0.

C Risk of Distant Recurrence, According to Tumor Grade



No. at Risk				
High grade	3054	1010	188	2
Moderate grade	7363	2761	474	6
Low grade	3524	1258	239	6

No. of Events — annual rate (%)			
High grade	92 (0.9)	32 (1.3)	6 (2.6
Moderate grade	186 (0.7)	60 (1.0)	6 (1.1
Low grade	49 (0.4)	23 (0.8)	2 (0.6

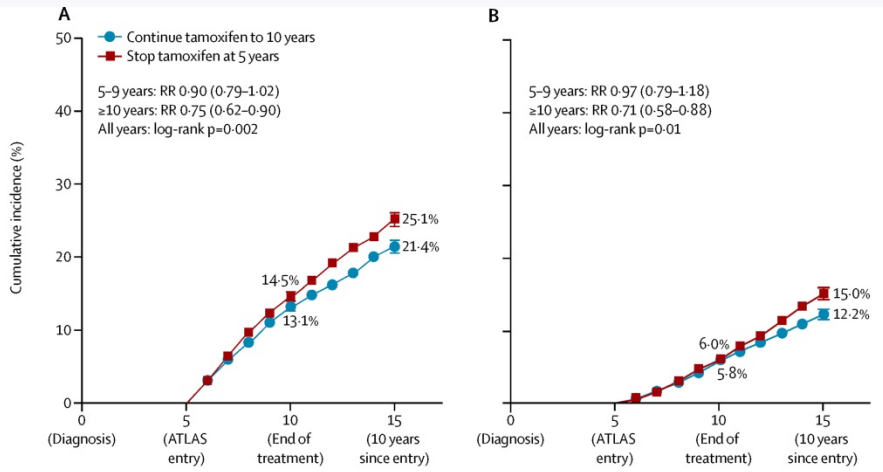
Pan et al. NEJM 2017

Summary

- The natural history of ER+ breast cancer
  - Late distant recurrences occur up to 20 years after the initial diagnosis for women receiving 5 years of endocrine therapy
  - Major factor driving the risk of late recurrence is tumor burden (tumor size, nodal status) at the time of initial diagnosis. However, some late recurrences occur for smallest tumors
- Can extending endocrine therapy reduce late recurrence?

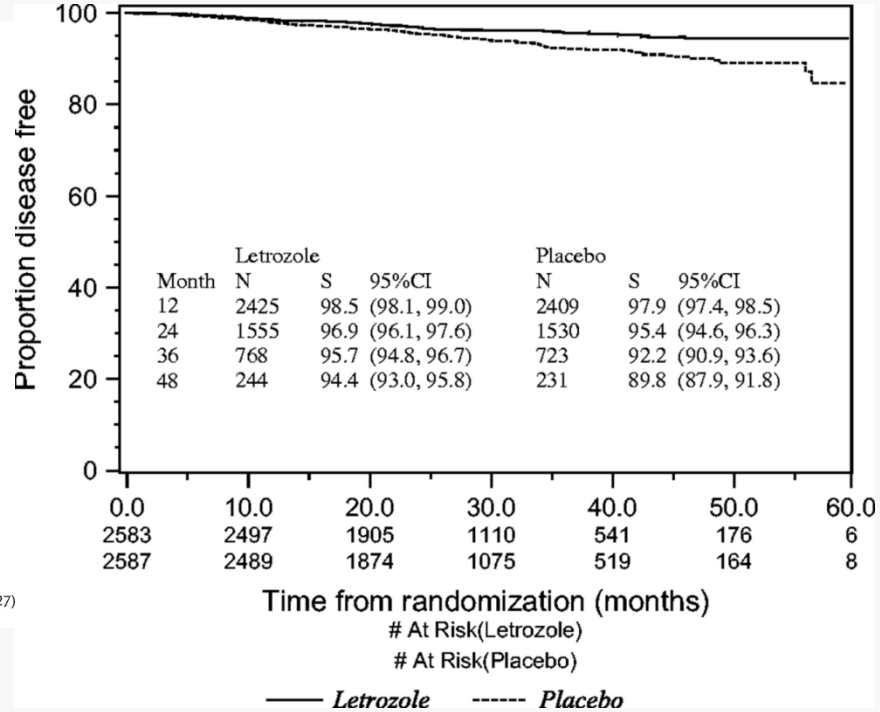
Extending Therapy Beyond 5 Years: 10 years of Total Endocrine Therapy

10 vs 5 yrs of Tamoxifen



	5–9 years	10–14 years	≥15 years	5–9 years	10–14 years	≥15 years
Continue tamoxifen to 10 years	2·83% (428/15115)	1·96% (165/8439)	2·54% (24/945)	1·17% (SE 0·09)	1·38% (SE 0·12)	1·64% (SE 0·39)
Stop tamoxifen at 5 years	3·16% (471/14889)	2·66% (214/8038)	3·03% (26/859)	1·21% (SE 0·09)	2·01% (SE 0·15)	2·29% (SE 0·47)
Rate ratio, from (O–E)/V	0·90 (SE 0·06)	0·74 (SE 0·09)	0·85 (SE 0·26)	0·97 (SE 0·10)	0·70 (SE 0·10)	0·79 (SE 0·27)
Log-rank O–E and variance V	−24·8/224·7	−29·1/94·7	−2·1/12·5	−3·2/94·0	−27·2/77·5	−2·5/10·6

Letrozole after 5 yrs of Tamoxifen



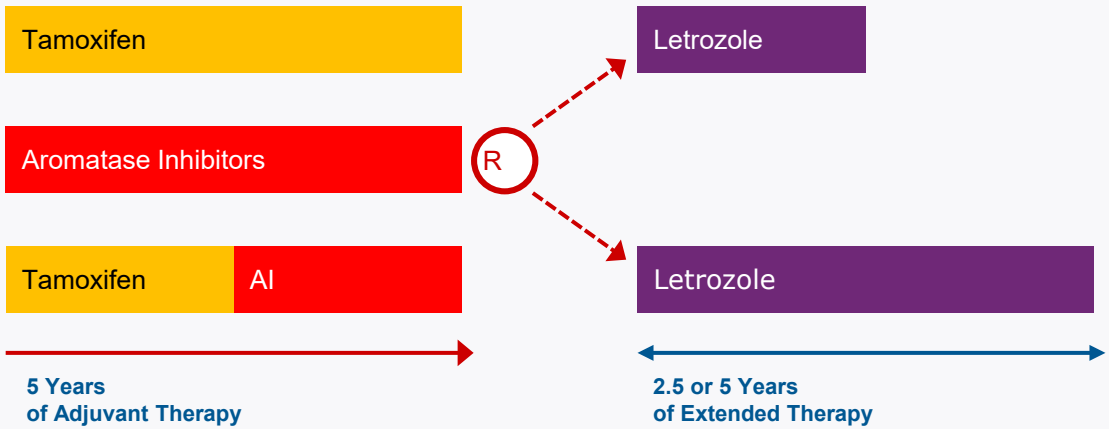
Davies et al. Lancet 2013
Goss P, Ingle JN et al. NEJM 2003 and JNCI 2005.

Strategies to Reduce Late Recurrence

- **Extended Adjuvant Endocrine Therapy:**
  - Tamoxifen for 10 years[1]
  - Tamoxifen for 5 years followed by AI for 5 years[2]
  - AI therapy for 7.5-10 years[3]
- **Benefit of 2nd five years of therapy "modest" compared to 5 years of therapy**
- **Research ongoing to**
  - Identify patients at high risk for late recurrence (Breast Cancer Index) (H/I)
  - Develop new therapeutic strategies

1. Davies et al. Lancet 2013
2. Goss PE and Ingle, JN et al. NEJM 2003
3. Goss PE and Ingle, JN et al. NEJM 2016

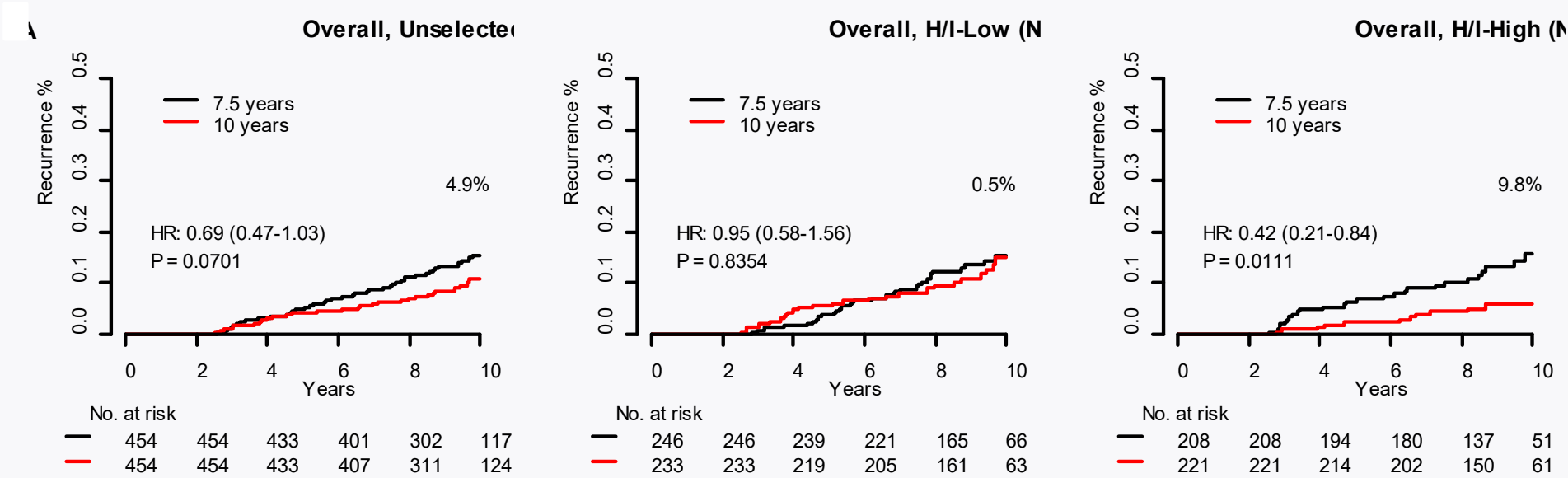
Investigation on the Duration of Extended Letrozole (IDEAL)



- Study explored whether a shorter extension of AI therapy is sufficient vs a full additional 5 years
- 88% of patients received either AI only (29%) or sequence of tamoxifen + AI (59%) in the first 5 years
- 73% of patients had LN+ disease
- HR 0.92 (0.74-1.16) for 5 vs 2.5 years (may change to 0.88 for blinded after events in the first 2.25y)
- Similar to studies such as ABCSG16A, results suggested that shorter duration of AI therapy might be as effective as full 10 years

1. Blok E J, et al. JNCI 2018; 110(1): djx134

Benefit from an Additional 2.5y vs 5y of Extended Endocrine Therapy is Dependent on Classification by BCI (H/I): Overall Cohort



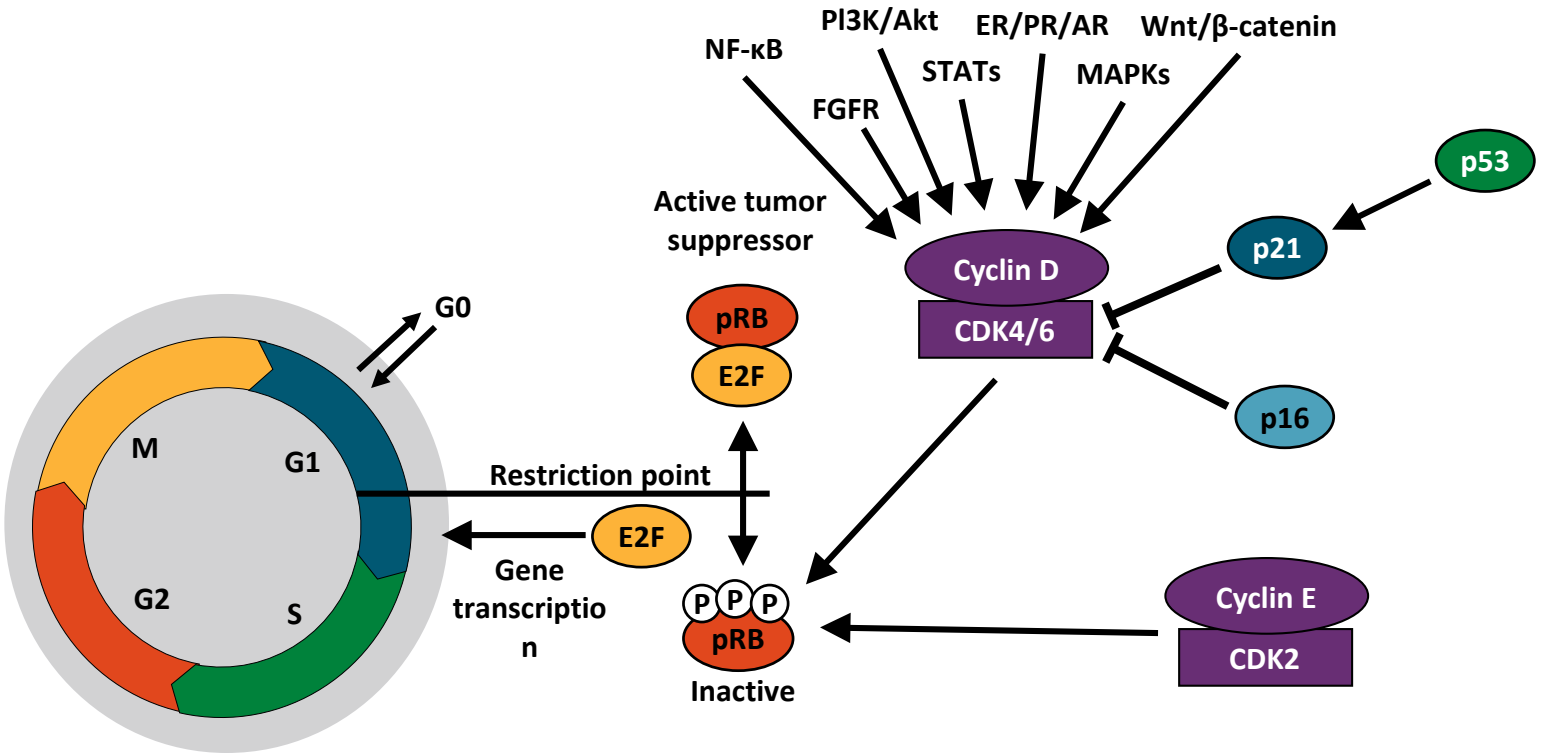
- Significant stratification response across endocrine driven (high H/I) and low endocrine low across the overall cohort
- Overall cohort included both N0 (27%) and N+ (73%) patients
- No significant interaction between: H/I status, nodal status, and benefit

1 . Noordhoek I et. Clin Canc Res 2020

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- **Cyclin-dependent kinases 4 and 6 (CDK 4/6 inhibitors)**
  - **Metastatic**
  - **Adjuvant**
- **PI3K/AKT/mTOR pathway**
- **The mutated ER: Targeting the ER for Degradation (SERD's)**
  - *ESR1* gene mutations
- **Other important drugs/drug targets (AURKA, Alisertib; BCL-2)**

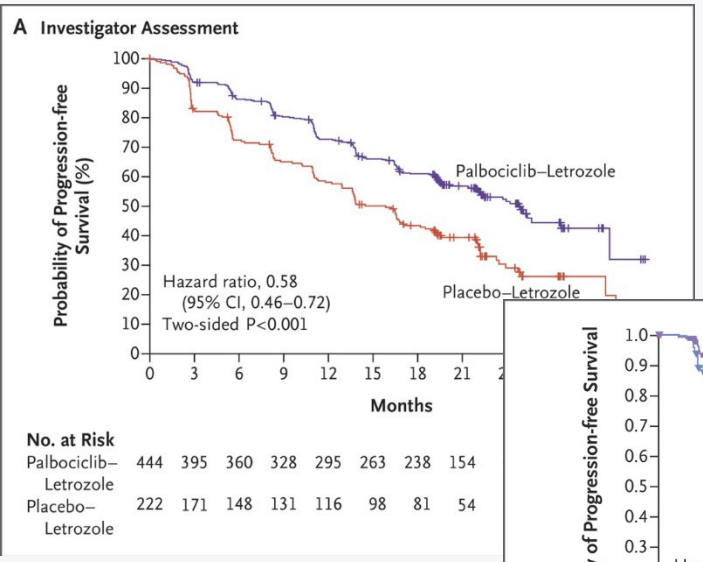
Cyclin D1 and CDK4/6 Regulate G1-S Checkpoint, Drive Cellular Proliferation Downstream of Signaling Pathways



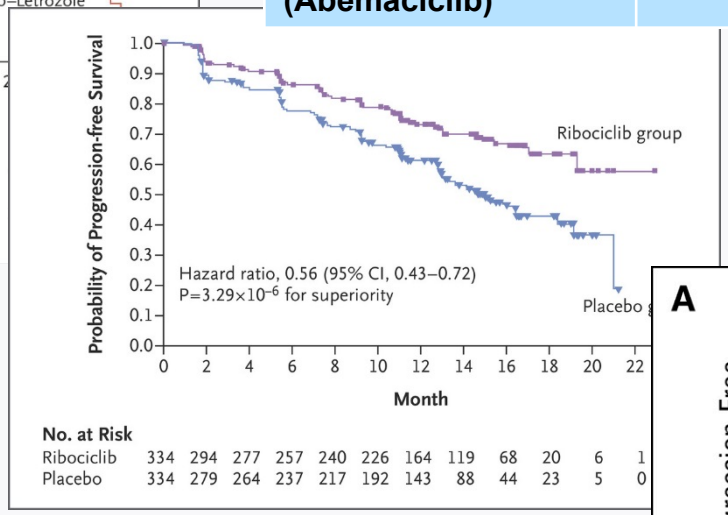
Lange. Endocr Relat Cancer. 2011;18:C19. Kundsen. Trends Cancer. 2017;3:39. Otto. Nat Rev Cancer. 2017;17:93. Corona. Drug Des Devel Ther. 2018.12:321. Tripathy. Clin Cancer Res. 2017;23:3251-3262.

First-line Metastatic ER+/HER2- Breast Cancer PALOMA-2, MONALEESA 2, and MONARCH 3

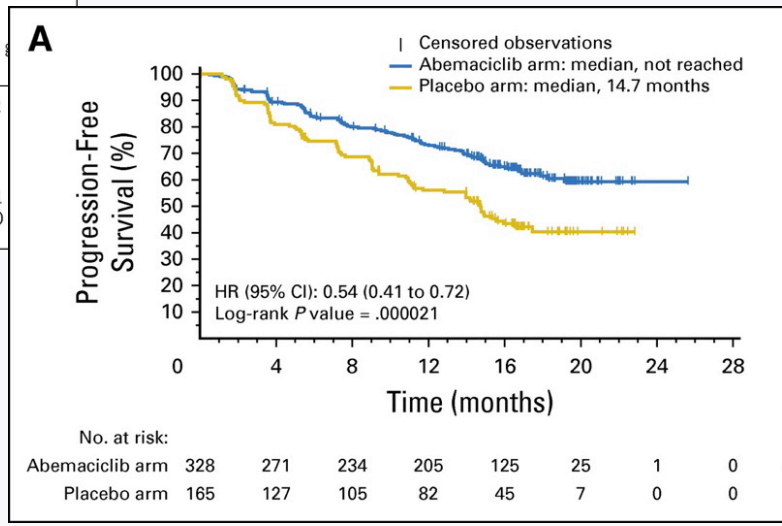
	HR	AI-Placebo	AI- CDK4/6i
PALOMA 2 (Palbociclib)	0.58	14.5 m	24.8 m
MONALEESA 2 (Ribociclib)	0.56	14.7 m	Not Reached
MONARCH 3 (Abemaciclib)	0.54	14.7 m	Not Reached



ORR: 55.3%



ORR: 52.7%



ORR: 59.2%

1) Finn et al. NEJM 2016
2) Hortobagyi et al. NEJM 2016
3) Goetz et al. JCO 2017

Survival Data from the 1st and 2nd line Settings

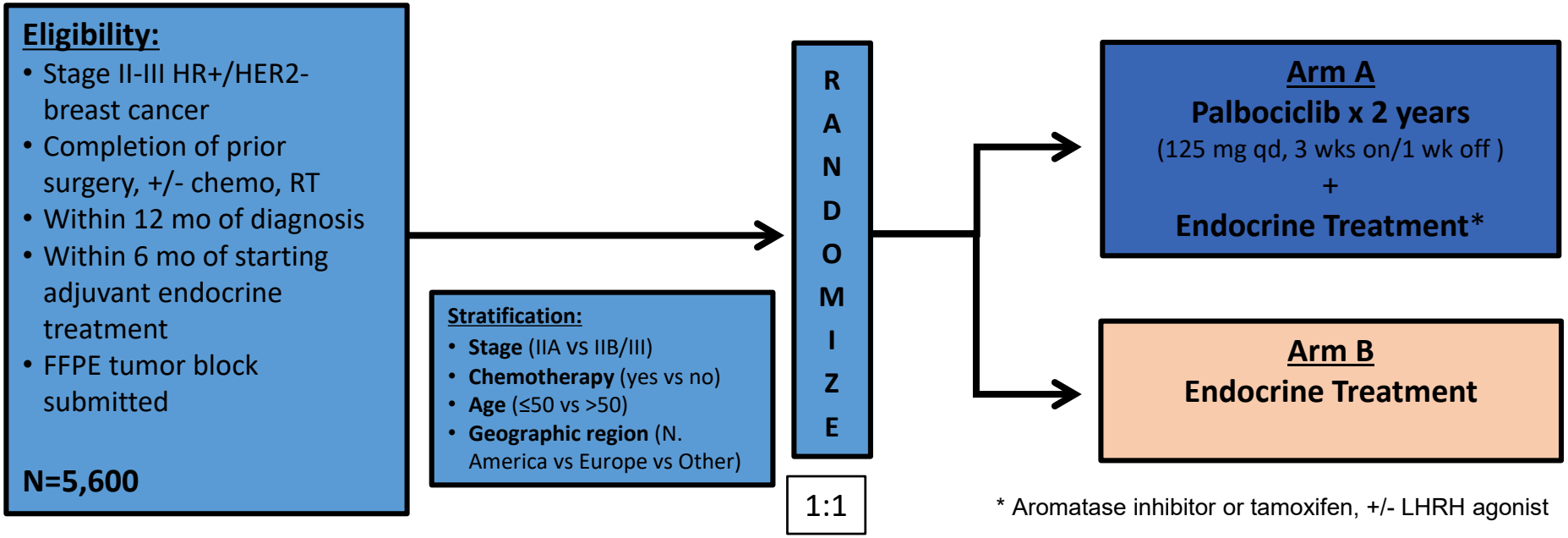
- **First line premenopausal setting (MONALEESA 7)**
  - **Ribociclib + ET prolonged OS compared with ET alone (HR 0.71; 95% CI, 0.54 to 0.95; P=0.009)**
- **Combined 1st/2nd line postmenopausal setting (MONALEESA 3)**
  - **Ribociclib + fulvestrant (F) prolonged OS vs F alone (HR 0.724; 95% CI, 0.57 to 0.92; P=0.004).**
- **2nd line Endocrine Resistant setting**
  - **Abemaciclib prolonged OS**
  - **Palbociclib (PALOMA 3): No overall OS Benefit**

Im et al. NEJM 2019
Slamon et al. NEJM 2020
Sledge et al. JAMA Oncology 2019

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- Cyclin-dependent kinases 4 and 6 (CDK 4/6 inhibitors)
  - Metastatic
  - Adjuvant
    - PALLAS: Palbociclib
    - monarchE: Abemaciclib

PALLAS: Phase III open-label study of palbociclib and adjuvant endocrine therapy



Primary Endpoint: invasive Disease-Free Survival (iDFS)

Mayer E et al, ESMO 2020

PALLAS: Patient Characteristics

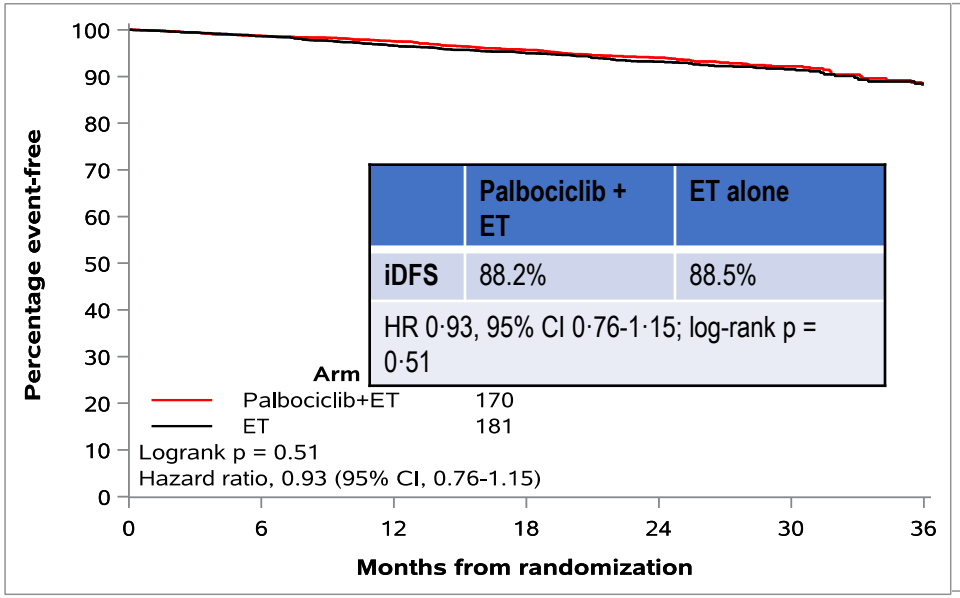
- Between 9/2015 and 11/2018, 5,760 patients were randomized and included in the ITT set.
- The majority had higher stage disease and had received prior chemotherapy.
- 58·7% had high clinical risk disease, described as:
  - ≥4 nodes involved (≥N2), or
  - 1-3 nodes with either T3/T4 and/or G3 disease

Variable	Palbociclib + ET (N=2,883)	ET (N=2,877)
Age (y) – median (range)	52 (25 – 90)	52 (22 – 85)
Stage		
IIA	504 (17·5%)	509 (17·7%)
IIB	968 (33·6%)	951 (33·1%)
III	1402 (48·6%)	1408 (48·9%)
T-Stage		
T0/T1/Tis/TX	557 (19·3%)	500 (17·4%)
T2	1603 (55·6%)	1636 (56·9%)
T3/T4	722 (25·0%)	741 (25·8%)
N-Stage		
N0	367 (12·7%)	383 (13·3%)
N1	1427 (49·5%)	1415 (49·2%)
N2	703 (24·4%)	709 (24·6%)
N3	385 (13·4%)	370 (12·9%)
Histologic Grade		
G1	300 (10·4%)	313 (10·9%)
G2	1622 (56·3%)	1658 (57·6%)
G3	836 (29·0%)	767 (26·7%)
Prior Chemotherapy	2384 (82·7%)	2370 (82·4%)
Initial Adjuvant Endocrine Therapy		
Aromatase inhibitor	1954 (67·8%)	1918 (66·7%)
Tamoxifen	923 (32·0%)	949 (33·0%)
Concurrent Adjuvant LHRH Agonist	532 (18·5%)	604 (21.1%)

Mayer E et al, ESMO 2020

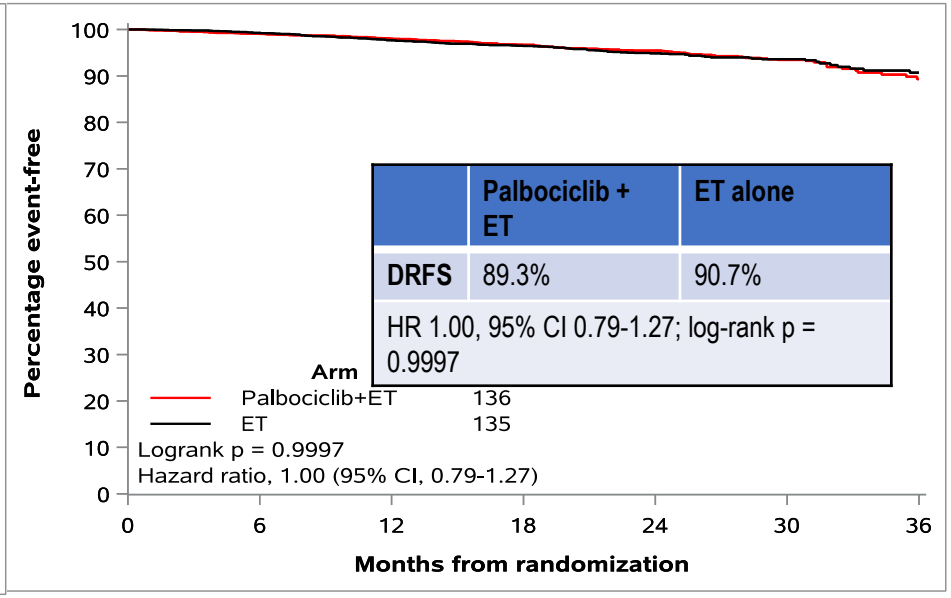
PALLAS: Primary Endpoint iDFS

iDFS



	Palbociclib + ET	ET alone
iDFS	88.2%	88.5%
HR 0·93, 95% CI 0·76-1·15; log-rank p = 0·51		

DRFS



	Palbociclib + ET	ET alone
DRFS	89.3%	90.7%
HR 1.00, 95% CI 0.79-1.27; log-rank p = 0.9997		

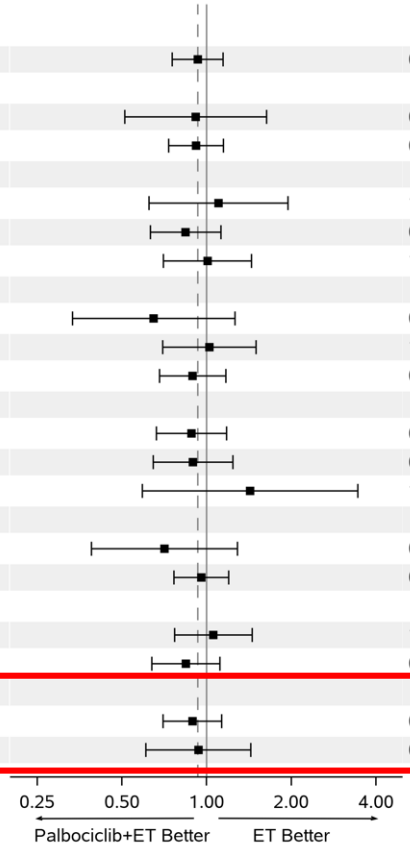
At a median follow-up of 23.7 months, no significant difference in either 3-year iDFS or DRFS was observed

Efficacy population: Intent-to-treat (ITT) principle, with patients withdrawing consent for all analysis excluded

Mayer E et al, ESMO 2020

PALLAS: Subgroup Analysis

Subgroup	Palbociclib+ET Events / N	ET Events / N	Hazard Ratio (95% CI)	P Value for Interaction
All patients	170 / 2883	181 / 2877	0.93 (0.75 - 1.14)	
Stage				0.99
IIA	21 / 504	25 / 509	0.91 (0.51 - 1.63)	
IIB/III	149 / 2370	156 / 2359	0.92 (0.73 - 1.15)	
T-Stage				0.60
T0/T1/Tis/TX	26 / 557	22 / 500	1.10 (0.62 - 1.94)	
T2	84 / 1603	101 / 1636	0.84 (0.63 - 1.12)	
T3/T4	60 / 722	58 / 741	1.01 (0.70 - 1.44)	
N-Stage				0.50
N0	14 / 367	23 / 383	0.65 (0.33 - 1.26)	
N1	53 / 1427	53 / 1415	1.02 (0.70 - 1.50)	
N2/N3	103 / 1088	105 / 1079	0.89 (0.68 - 1.17)	
Histologic grade				0.56
G1/G2	86 / 1922	100 / 1971	0.88 (0.66 - 1.18)	
G3	73 / 836	72 / 767	0.89 (0.65 - 1.24)	
GX	11 / 122	9 / 139	1.43 (0.59 - 3.44)	
Neo/adjuvant chemotherapy				0.36
No	18 / 498	27 / 507	0.71 (0.39 - 1.28)	
Yes	152 / 2384	154 / 2370	0.96 (0.77 - 1.20)	
Age group				0.29
≤50	79 / 1309	73 / 1304	1.06 (0.77 - 1.45)	
>50	91 / 1573	108 / 1573	0.84 (0.64 - 1.11)	
Clinical Risk				0.87
High risk	131 / 1710	136 / 1672	0.89 (0.70 - 1.13)	
Low risk	39 / 1172	45 / 1205	0.93 (0.61 - 1.43)	



Mayer E et al, ESMO 2020

VIRTUAL 2020 ESMO congress

• monarchE Study Design

monarchE EARLY BREAST CANCER

HR+, HER2-, high risk early breast cancer

High risk defined as:

- ≥4 positive axillary lymph nodes (ALN) OR
- 1-3 ALN and at least 1 of the below:
  - Tumor size ≥5 cm
  - Histologic grade 3
  - Centrally tested Ki67 ≥20%

N = 5637[a]

R 1:1

Stratified for:

- Prior chemotherapy
- Menopausal status
- Region

Abemaciclib (150mg twice daily for up to 2 years[b])
+ Standard of Care Endocrine Therapy
(5 to 10 years as clinically indicated)

Standard of Care Endocrine Therapy[b]
(5 to 10 years as clinically indicated)

Endocrine therapy of physician's choice

Other criteria:

- Women or men
- Pre-/ postmenopausal
- With or without prior adjuvant/neoadjuvant chemotherapy
- No distant metastases

Primary Objective: Invasive disease-free survival (STEEP criteria)
Key Secondary Objectives: Distant relapse-free survival, Overall survival, Safety, Patient reported outcomes, and Pharmacokinetics

[a] Recruitment from July 2017 to August 2019; [b] Treatment period = first 2 years on study treatment after randomization

VIRTUAL 2020 ESMO congress

monarchE EARLY BREAST CANCER

• Analysis Populations and Disposition

Median follow up at the interim analysis: ~15.5 months in each arm

- 12.5% of patients had completed the 2-year treatment period
- Over 70% of patients were still in 2-year treatment period

Abemaciclib + ET		ET Alone
N = 2808	ITT population	N = 2829
n = 2791	Received study treatment (Safety population)	n = 2800

VIRTUAL 2020 ESMO congress

monarchE EARLY BREAST CANCER

• High Risk Disease Characteristics

		Abemaciclib + ET N = 2808, n (%)	ET Alone N = 2829, n (%)
Number of positive lymph nodes	0	7 (0.2)	7 (0.2)
	1-3	1119 (39.9)	1143 (40.4)
	≥4 or more	1680 (59.8)	1679 (59.3)
Histological grade	Grade 1	209 (7.4)	215 (7.6)
	Grade 2	1373 (48.9)	1395 (49.3)
	Grade 3	1090 (38.8)	1066 (37.7)
Primary tumor size by pathology following definitive surgery	<2 cm	780 (27.8)	765 (27.0)
	2-5 cm	1369 (48.8)	1419 (50.2)
	≥5 cm	610 (21.7)	612 (21.6)
Central Ki-67	<20%	953 (33.9)	973 (34.4)
	≥20%	1262 (44.9)	1233 (43.6)
	Unavailable	593 (21.1)	623 (22.0)
Progesterone receptor status	Positive	2421 (86.2)	2453 (86.7)
	Negative	298 (10.6)	294 (10.4)

Note: where values do not add up to 100%, remaining data are missing, unavailable or could not be assessed

Additional high risk eligibility criteria for patients with 1-3 nodes	Abemaciclib + ET N = 2808, n (%)	ET Alone N = 2829, n (%)
Tumor size ≥5 cm (pathology) [a]	249 (8.9)	236 (8.3)
Tumor size ≥5 cm (imaging) [a, b]	152 (5.4)	158 (5.6)
Histologic grade 3 [a]	629 (22.4)	618 (21.8)
Central Ki-67 ≥20% only [c]	216 (7.7)	237 (8.4)

[a] Patients could be counted in more than one of the sub-categories under 1-3 positive lymph nodes; [b] Patients who received neoadjuvant chemotherapy may have been eligible based on imaging tumor size prior to receiving systemic therapy; [c] Patients not double counted; patients did not have tumor size ≥5 cm (either by pathology or imaging) or histologic grade 3

VIRTUAL 2020 ESMO congress

• Invasive Disease-Free Survival

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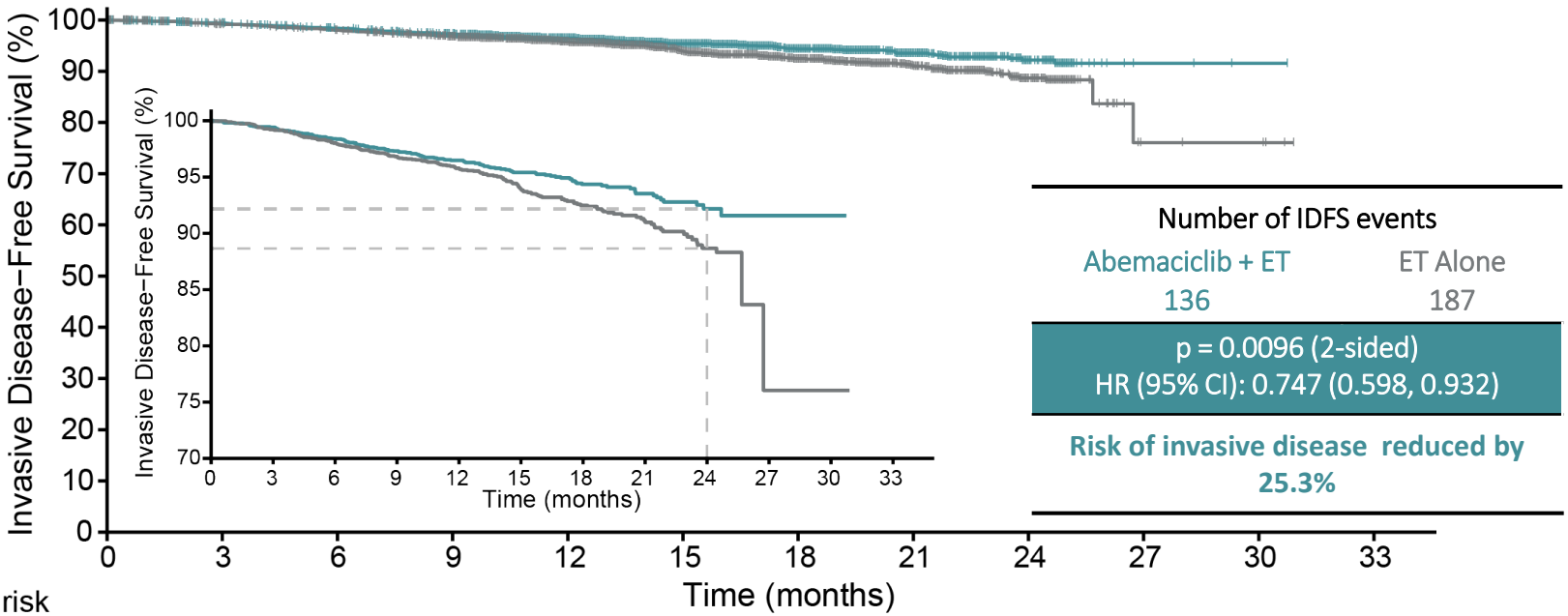


Number at risk

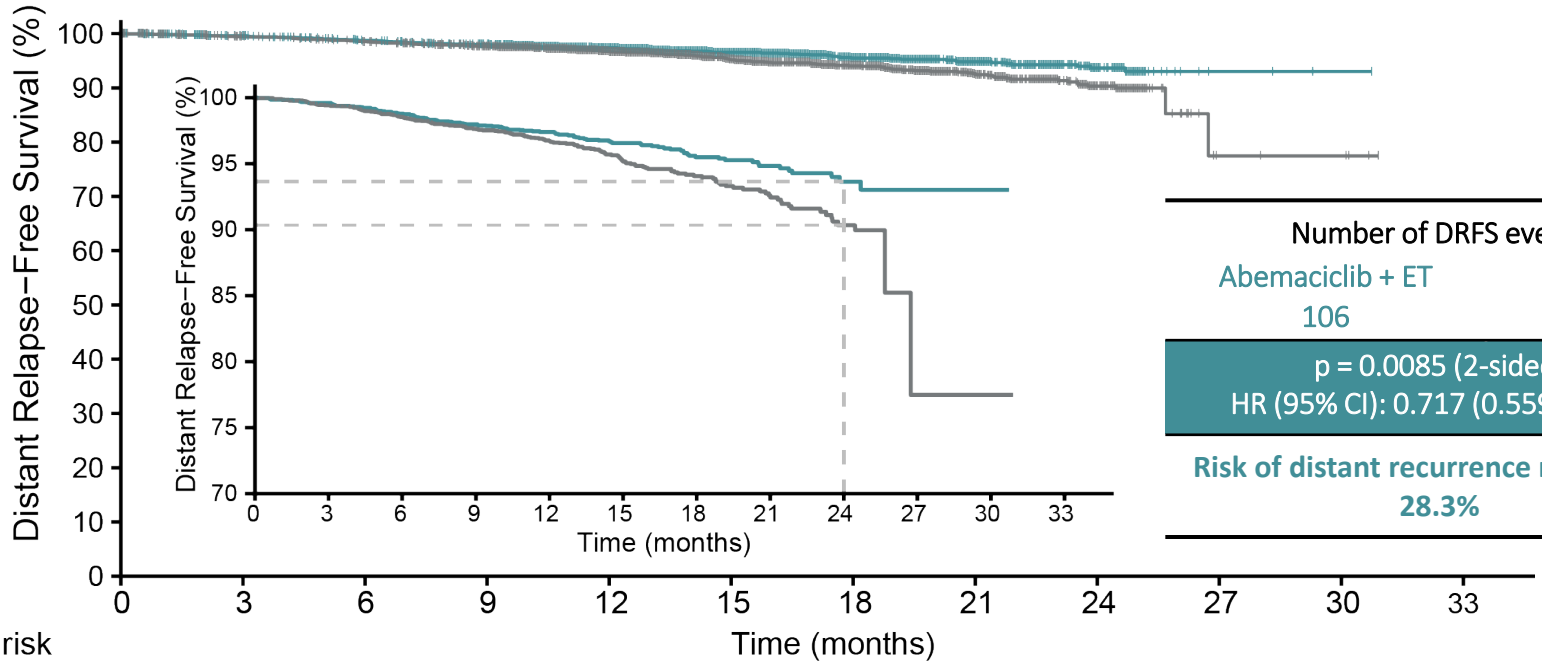
Abemaciclib + ET	2808	2676	2613	2543	1996	1371	918	566	245	3	1	0
ET Alone	2829	2699	2649	2562	2013	1405	932	586	262	7	6	0

Two-year IDFS rates were 92.2% (abemaciclib + ET arm) and 88.7% (ET arm) – 3.5% absolute difference

VIRTUAL 2020 ESMO congress

• Distant Relapse-Free Survival

monarchE EARLY BREAST CANCER



Number of DRFS events	
Abemaciclib + ET	ET Alone
106	152

p = 0.0085 (2-sided)
HR (95% CI): 0.717 (0.559, 0.920)

Risk of distant recurrence reduced by 28.3%

Number at risk	0	3	6	9	12	15	18	21	24	27	30	33
Abemaciclib + ET	2808	2680	2619	2555	2005	1378	925	573	247	3	1	0
ET Alone	2829	2704	2659	2576	2026	1417	941	590	263	7	6	0

Two-year DRFS rates were 93.6% (abemaciclib + ET arm) and 90.3% (ET arm) – 3.3% absolute difference

DRFS benefit consistent across all prespecified subgroups

Summary

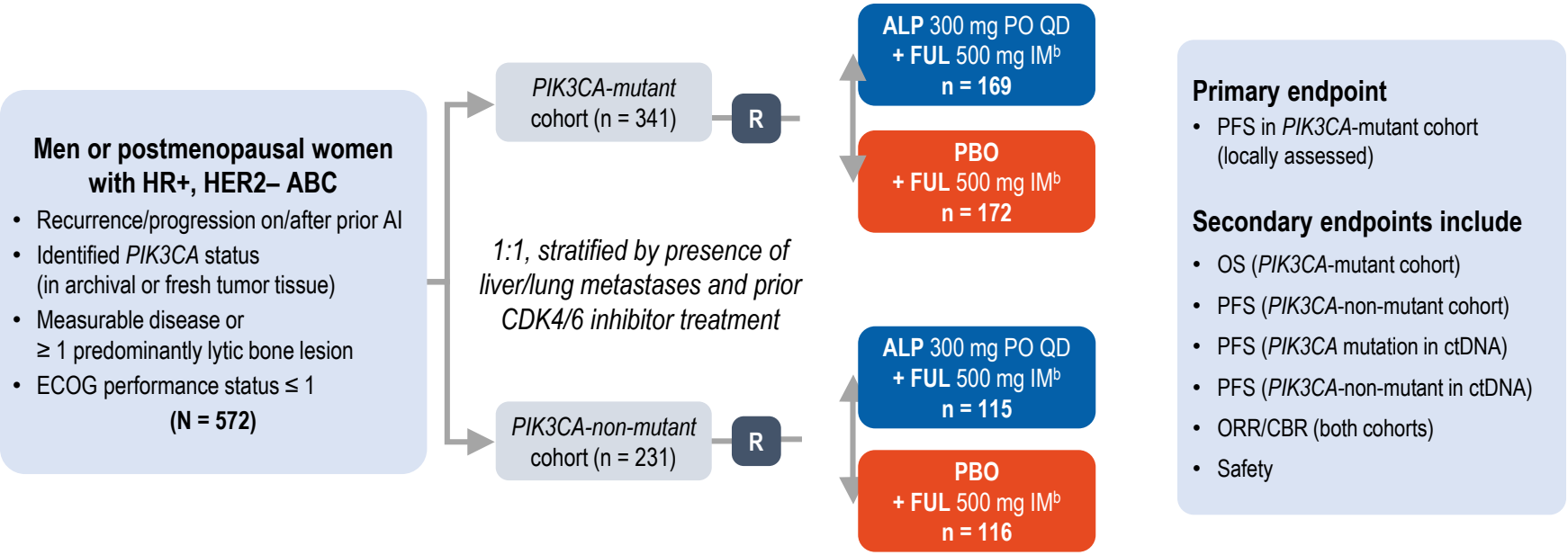
CDK 4/6 inhibitors have changed the landscape of ER+/HER2- BC

- **Important differences comparing CDK 4/6 inhibitors**
  - **Metastatic: Ribociclib and abemaciclib increased OS. No OS observed with palbociclib.**
  - **Adjuvant Abemaciclib but not palbociclib improved IDFS in the high risk adjuvant setting (Primary endocrine resistance).**
  - **Adjuvant Ribociclib (NATALEE): ongoing**
- **Additional follow-up is necessary to evaluate the sustainability of the early abemaciclib benefit**

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- Cyclin-dependent kinases 4 and 6 (CDK 4/6 inhibitors)
  - Metastatic
  - Adjuvant
- PI3K/AKT/mTOR pathway
- The mutated ER: Targeting the ER for Degradation (SERD's)
  - *ESR1* gene mutations

SOLAR-1: A Phase 3 Randomized, Double-Blind, Placebo-Controlled Trial (NCT02437318)[1]



- The primary endpoint included all randomized patients in the *PIK3CA*-mutant cohort; PFS was analyzed in the *PIK3CA*-non-mutant cohort as a proof of concept
- Safety was analyzed for all patients who received ≥ 1 dose of study treatment, in both cohorts

ABC, advanced breast cancer; AI, aromatase inhibitor; ALP, alpelisib; CBR, clinical benefit rate; ctDNA, circulating tumor DNA; ECOG, Eastern Cooperative Oncology Group; FUL, fulvestrant; HER2–, human epidermal growth factor receptor-2–negative; IM, intramuscular; ORR, overall response rate; OS, overall survival; PBO, placebo; PFS, progression-free survival; PO, oral; QD, once daily; R, randomization.

[a] More than 90% of patients had mutational status identified from archival tissue.

[b] Fulvestrant given on Day 1 and Day 15 of the first 28-day cycle, then Day 1 of subsequent 28-day cycles.

1. Andre F, et al. ESMO 2018. Abstract LBA3 [oral].

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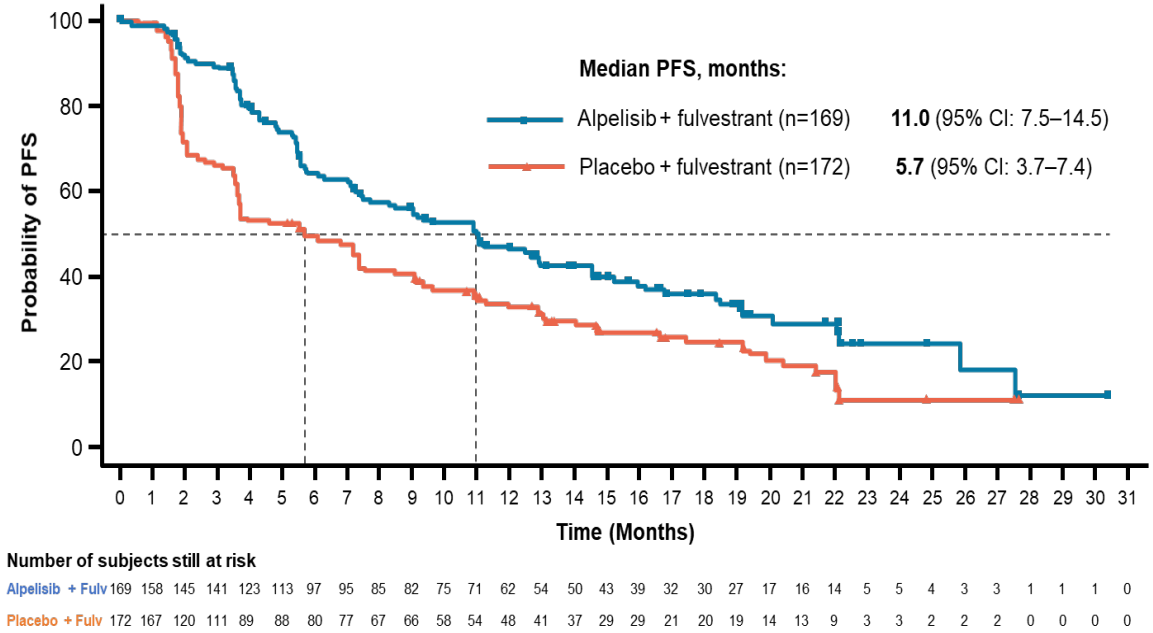
Primary Endpoint: Locally Assessed PFS in the *PIK3CA*-mutant Cohort[1,a]



Data cut-off: Jun 12, 2018	ALP + FUL (n = 169)	PBO + FUL (n = 172)
Number of PFS events, n (%)	103 (60.9)	129 (75.0)
Progression	99 (58.6)	120 (69.8)
Death	4 (2.4)	9 (5.2)
Censored	66 (39.1)	43 (25.0)
Median PFS (95% CI)	11.0 (7.5-14.5)	5.7 (3.7-7.4)
HR (95% CI)	0.65 (0.50-0.85)	
One-sided *P* value	0.00065	

CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.
[a] Mutation status determined from tissue biopsy.
1. Andre F, et al. ESMO 2018. Abstract LBA3 [oral].
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SOLAR-1: AEs

AEs ≥ 20% in Either Arm, n (%)	Alpelisib + FULV (n = 284)			Placebo + FULV (n = 287)		
	All	Grade 3	Grade 4	All	Grade 3	Grade 4
Any AE	282 (99.3)	183 (64.4)	33 (11.6)	264 (92.0)	87 (30.3)	15 (5.2)
Hyperglycemia	181 (63.7)	93 (32.7)	11 (3.9)	28 (9.8)	1 (0.3)	1 (0.3)
Diarrhea	164 (57.7)	19 (6.7)	0	45 (15.7)	1 (0.3)	0
Nausea	127 (44.7)	7 (2.5)	0	64 (22.3)	1 (0.3)	0
Decreased appetite	101 (35.6)	2 (0.7)	0	30 (10.5)	1 (0.3)	0
Rash*	101 (35.6)	28 (9.9)	0	17 (5.9)	1 (0.3)	0
Vomiting	77 (27.1)	2 (0.7)	0	28 (9.8)	1 (0.3)	0
Decreased weight	76 (26.8)	11 (3.9)	0	6 (2.1)	0	0
Stomatitis	70 (24.6)	7 (2.5)	0	18 (6.3)	0	0
Fatigue	69 (24.3)	10 (3.5)	0	49 (17.1)	3 (1.0)	0
Asthenia	58 (20.4)	5 (1.8)	0	37 (12.9)	0	0

- Alpelisib discontinued for hyperglycemia by 18 patients (6.3%), for rash by 9 patients (3.2%); no patients discontinued placebo due to either
- Maculopapular rash, all grade (grade 3): 14.1% (8.8%) with alpelisib vs 1.7% (0.3%) with placebo
- Safety similar in *PIK3CA*-mutant and *PIK3CA*-nonmutant cohorts

*Does not include maculopapular rash.

Slide credit: clinicaloptions.c

BYLieve: Alpelisib + Fulvestrant in *PIK3CA*m HR+/HER2- ABC After CDK4/6i + AI (Cohort A)

- International, open-label, multicohort, noncomparative phase II study

Men and pre/postmenopausal women with *PIK3CA*-mutant* HR+/HER2- advanced BC; immediate prior treatment CDK4/6i and ET, systemic CT, or ET; no prior PI3Ki; measurable disease or ≥ 1 predominantly lytic bone lesion; ECOG PS ≤ 2 (planned N = 340)

→ **Cohort A: Prior CDK4/6i + AI**
Alpelisib 300 mg PO QD +
Fulvestrant 500 mg IM†‡
(n = 127)

Current analysis

→ **Cohort B: Prior CDK4/6i +**
Alpelisib 300 mg PO QD +
Letrozole 2.5 mg PO QD‡
(minimum n = 112)

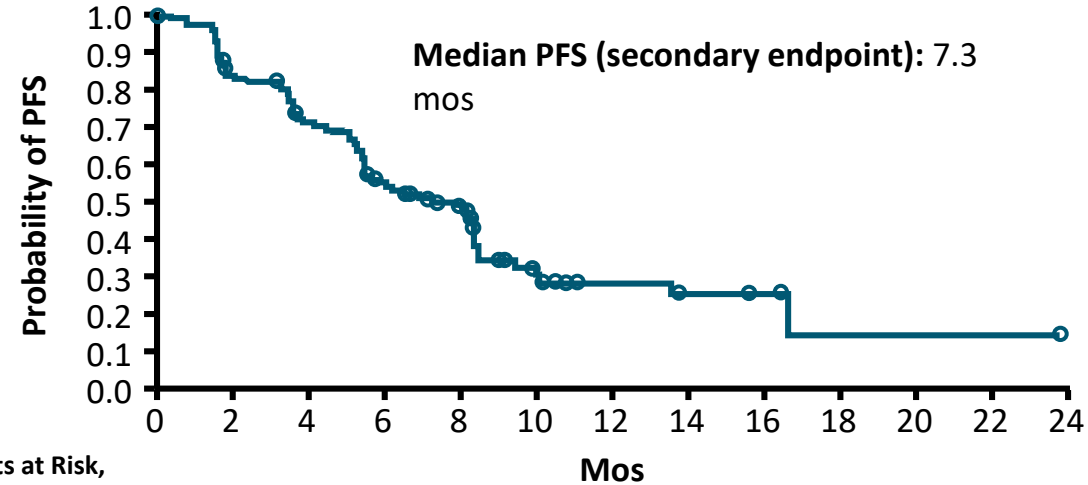
→ **Cohort C: Prior AI then CT or**
Alpelisib 300 mg PO QD +
Fulvestrant 500 mg IM†‡
(minimum n = 112)

*Centrally confirmed. †Fulvestrant given on Days 1 and 15 of cycle 1, Day 1 for subsequent cycles. ‡Men in letrozole cohort and premenopausal women received goserelin 3.6 mg SC Q28D or leuprolide 7.5 mg IM Q28D for adequate gonadal suppression.

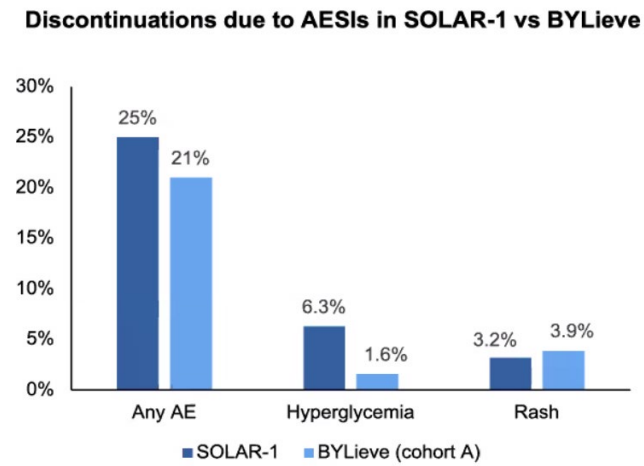
- Primary endpoint: proportion of each cohort alive without PD at 6 mos (RECIST v1.1)
  - Endpoint met if lower 95% CI > 30%
- Secondary endpoints (in each cohort): PFS, PFS2, ORR, CBR, DoR, OS, safety

Rugo. ASCO 2020. Abstr 1006. NCT03056755.

Alpelisib + Fulvestrant in *PIK3CA*m HR+/HER2- ABC After CDK4/6i + AI (Cohort A): Outcomes



Patients at Risk, n													
Prior CDK4/6i + AI (Cohort A)	121	95	77	54	40	15	8	5	4	1	1	1	0



- Primary endpoint met in Cohort A (95% CI lower bound > 30%): 50.4% (95% CI: 41.2% to 59.6%) alive without PD at 6 mos
  - In those with measurable disease at baseline (n = 100): ORR, 21.0%; CBR, 42.0%

Rugo. ASCO 2020. Abstr 1006.

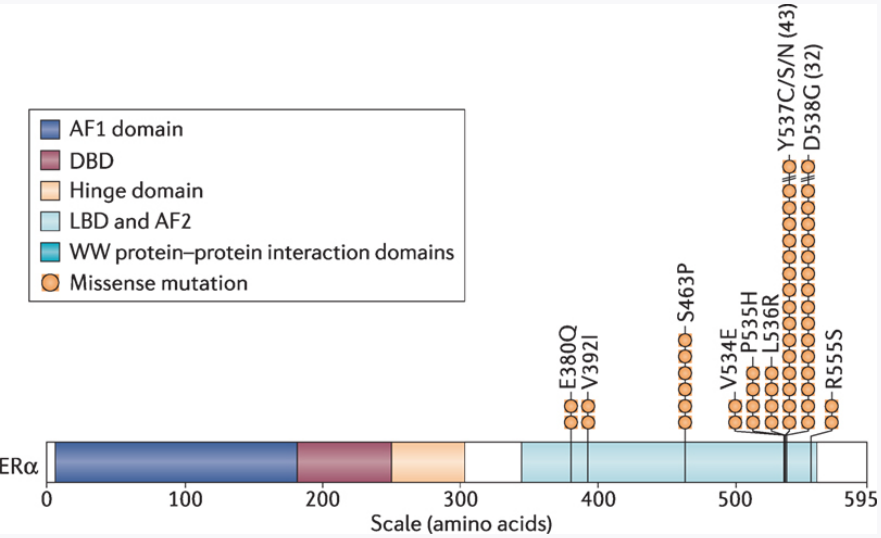
Conclusions

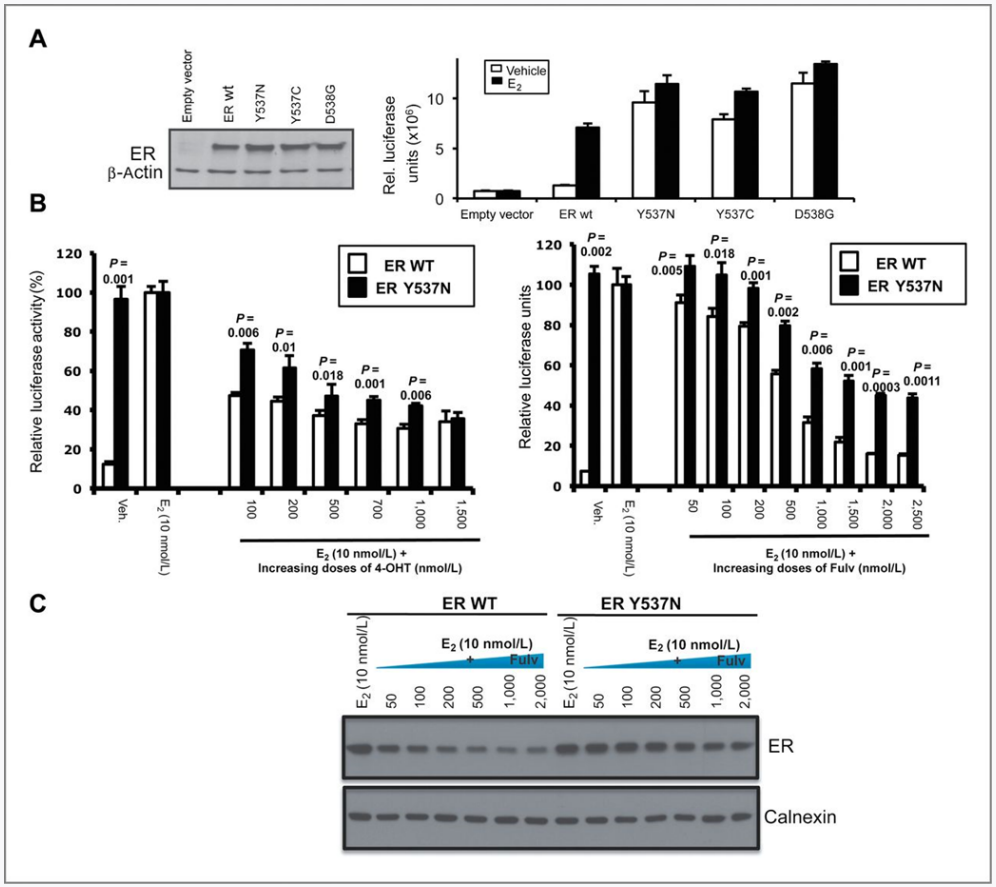
- Alpelisib: first PI3K inhibitor to result in
  - meaningful improvement in PFS in a predefined biomarker cohort (PIK3CA mutations)
- Benefit of alpelisib in PIK3CA mutation cohort appears to be similar whether PIK3CA is assessed from FFPE tumor tissue or by ctDNA
- Benefit of alpelisib after CDK 4/6 inhibitor
  - BYLieve (ASCO 2020): Pts with confirmed PIK3CA mutation: ORR was 20% (FUL) and 18% (LET)
- Concerns regarding the tolerability in general population

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- Cyclin-dependent kinases 4 and 6 (CDK 4/6 inhibitors)
  - Metastatic
  - Adjuvant
- PI3K/AKT/mTOR pathway
- **The mutated ER: Targeting the ER for Degradation (SERD's)**
  - *ESR1* gene mutations

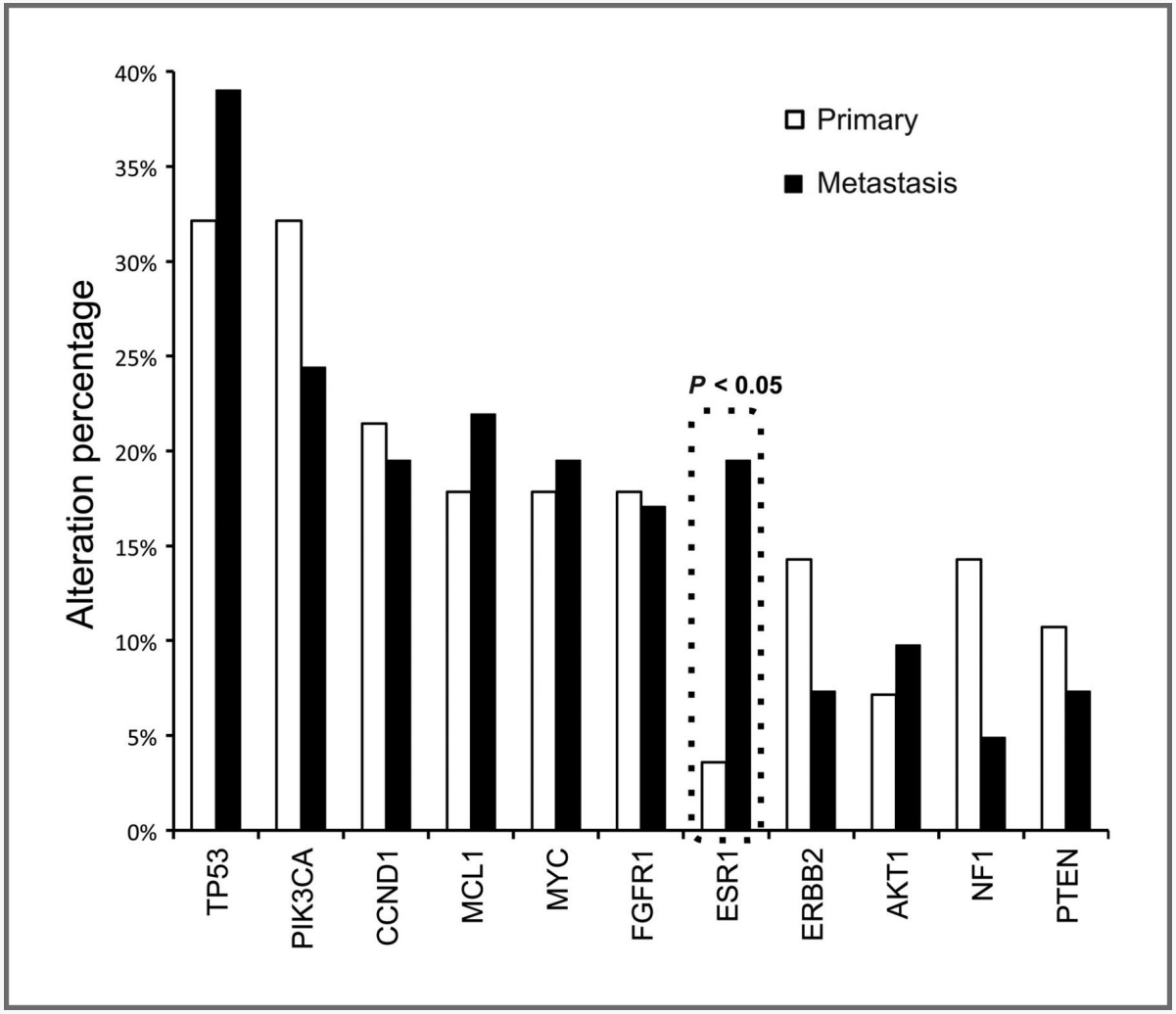
ESR1 mutations and effects on Estrogen Signaling





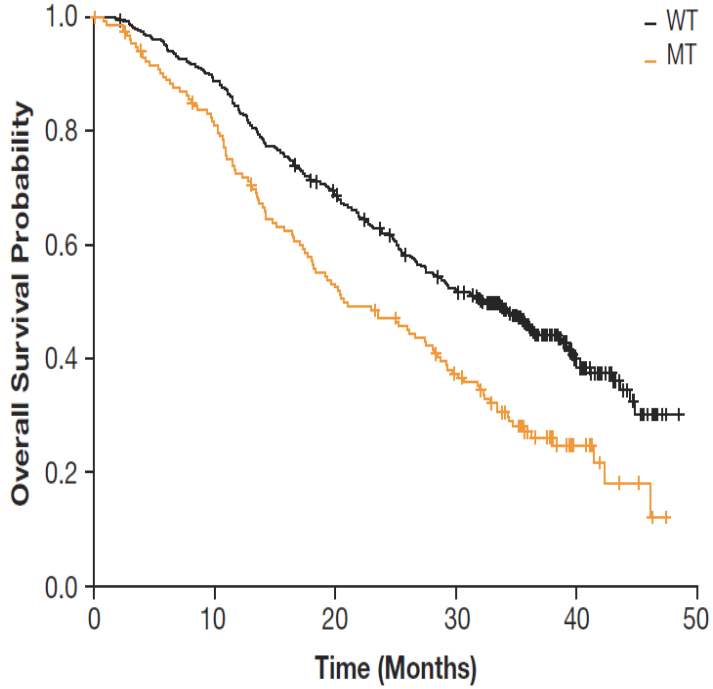
Jeselsohn R et al. Clin Cancer Res 2014;20:1757-1767

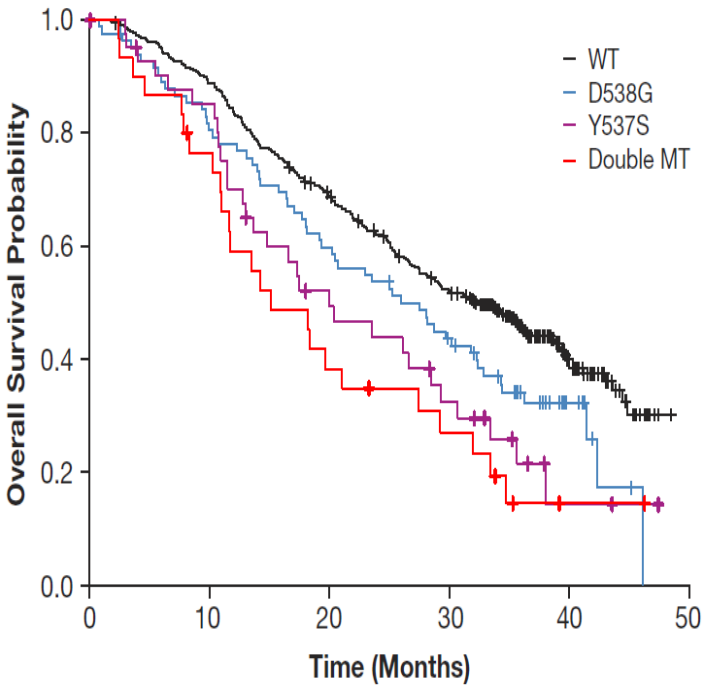
ESR1 mutations: Primary versus metastatic



Jeselsohn R et al. Clin Cancer Res 2014;20:1757-1767

Prognostic Effect of *ESR1* Mutation on OS





Mutations	N	Events	Median OS (95%CI) (months)	HR (95%CI)	P-value*
WT	385	217	32.1 (28.1-36.4)		
MT	156	112	20.7 (17.7 - 28.1)	1.40 (1.2 - 1.65)	0.000037
D538G	83	57	26.0 (19.2-32.4)	1.25 (1.02-1.54)	0.033
Y537S	42	30	20.0 (13.0-29.3)	2.31 (1.34-3.97)	0.0024
Double MT	30	24	15.2 (10.9-27.4)	1.77 (1.31-2.39)	0.00018

- Both D538G and Y537S mutations were poor prognostic factors associated with shorter OS
- In a multivariate analysis adjusting for sensitivity to prior hormonal therapy, visceral disease and ECOG status, the effect of ESR1 mutation (compared to wild-type) on OS remained significant

Chandarlapaty et al. JAMA Oncology 2016

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New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- **The mutated ER:** *ESR1* gene mutations
  - Better SERM's (Z-Endoxifen and Lasofoxifene)
  - Better oral SERD's

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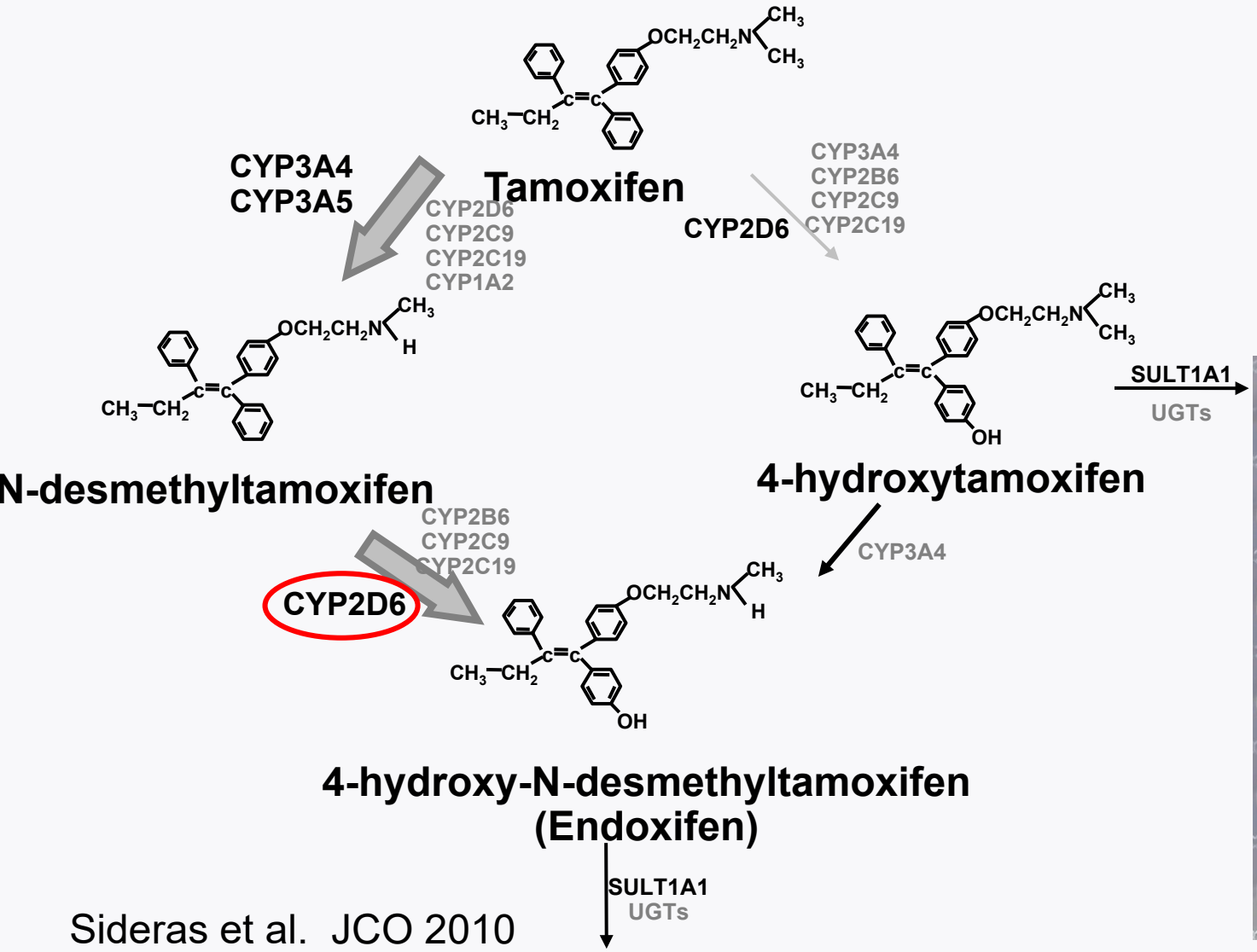
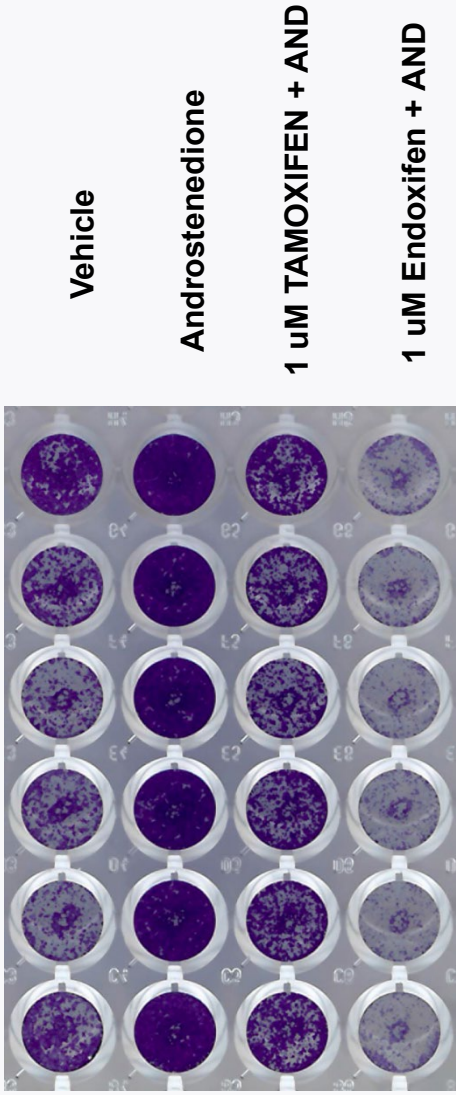
Tamoxifen Metabolism: Inhibition of Estrogen-mediated growth





Sideras et al. JCO 2010

Jayaraman et al. Breast Can Res 2020

Alliance A011203: Randomized Phase II Trial of Tamoxifen versus Z-endoxifen in Postmenopausal Women with Metastatic ER+, HER2- Breast Cancer

Matthew P. Goetz, Vera J Suman, Joel M. Reid, Mary Kuffel, Sarah A. Buhrow, Renee M. McGovern, John Black, Travis Dockter, Carrie Strand, William F. Symmans, Minetta C. Liu, John R. Hawse, Anna M. Storniolo, James H. Doroshow, Jerry M. Collins, Howard Streicher, Matthew M. Ames, James N. Ingle, Ann H. Partridge, Lisa A. Carey

A011203: A Randomized Phase II Trial of Tamoxifen and Z-endoxifen in Postmenopausal Women with Metastatic ER+, HER2- Breast Cancer (NCT02311933)

Key Eligibility Criteria

- Age ≥18 years
- Postmenopausal
- History of metastatic ER+/HER2- breast cancer and progression on a nonsteroidal AI
- Willingness to undergo a tumor biopsy

Biopsy confirms invasive breast cancer, ER+ (>10%, and HER2-

R 1:1

Z-Endoxifen 80 mg/day

Tamoxifen 20 mg/day → **Z-Endoxifen 80 mg/day)**

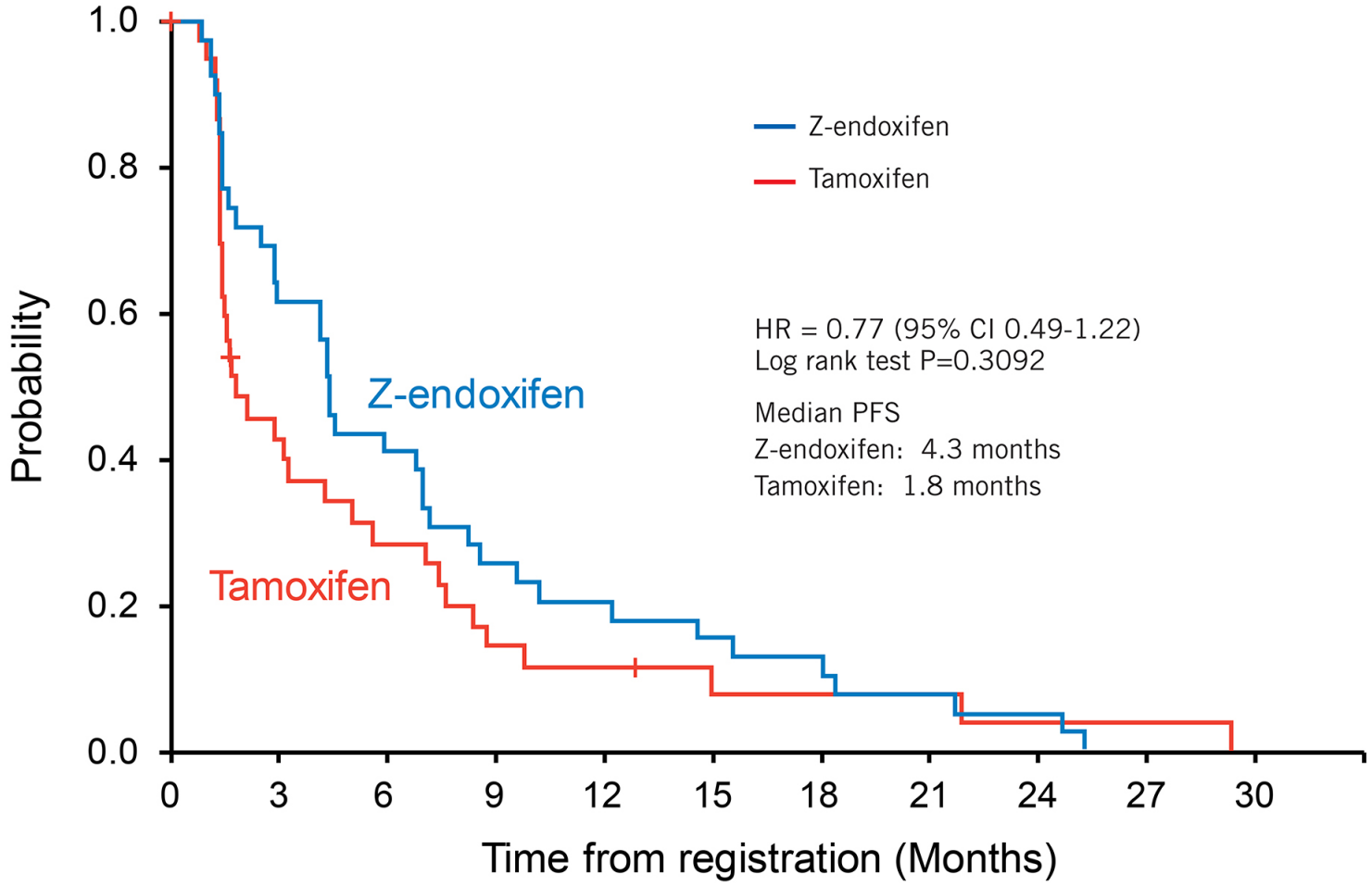
Investigational Agent: Z-Endoxifen
NSC #750393, Supplied by CTEP, DCTD, NCI

Stratification Factors:

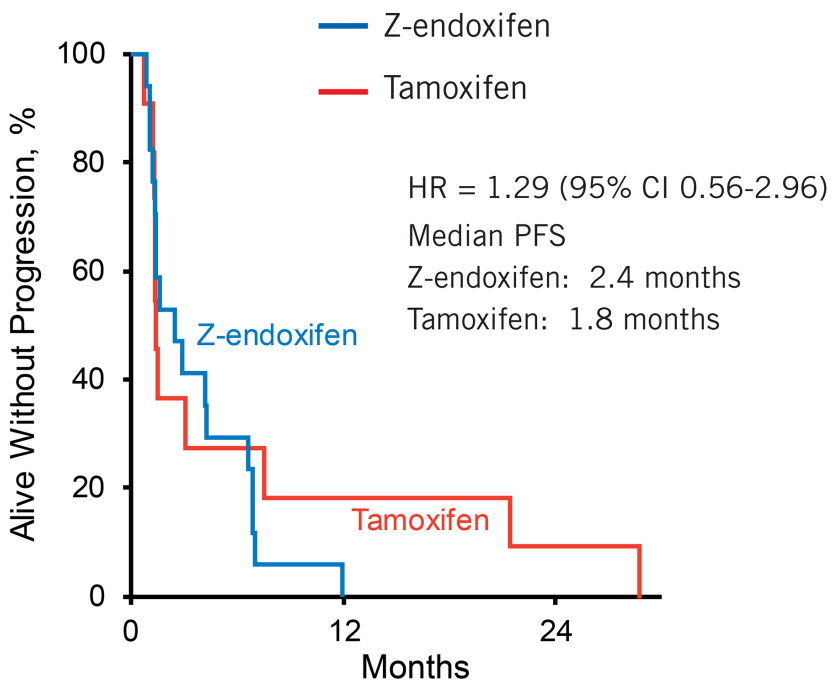
- Primary vs Secondary Endocrine Resistance
- Prior CDK 4/6 inhibitor and/or everolimus
- Measurable vs bone only disease

Statistics: With a sample size of 80 eligible patients (40 per treatment arm randomized in equal numbers) a one-sided alpha=0.10 generalized log-rank test will have at least a 90% chance of detecting a 50% decrease in hazard of disease progression with Z-Endoxifen HCl relative to tamoxifen. (corresponds to being able to detect an increase in the median PFS time from 3.0 months with tamoxifen to 6.0 months with Z-Endoxifen HCl.

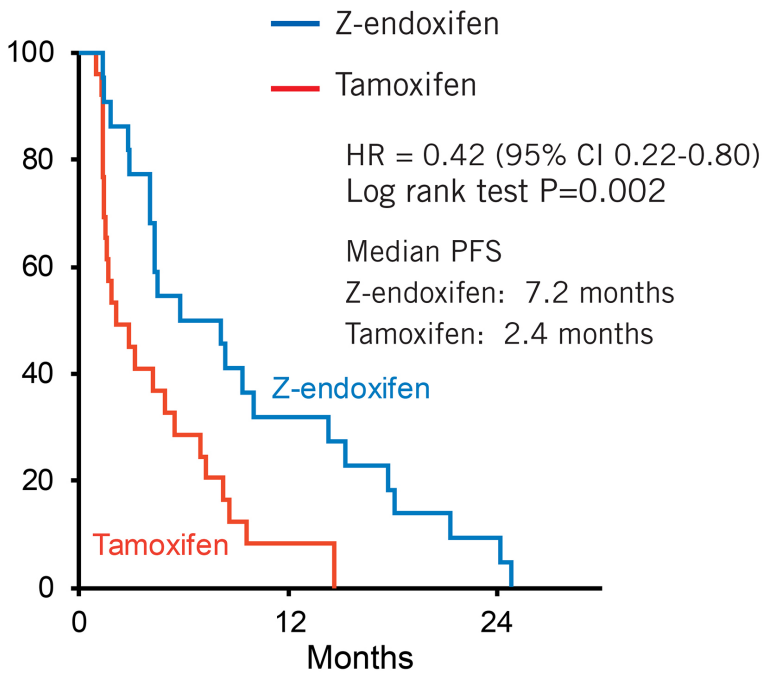
Progression-free survival



PFS According to Prior CDK 4/6 inhibitor (Data on ESR1 mutations forthcoming)



Pts at risk			
Z-endoxifen	17	1	0
Tamoxifen	11	2	1



Pts at risk			
Z-endoxifen	23	7	2
Tamoxifen	26	2	0

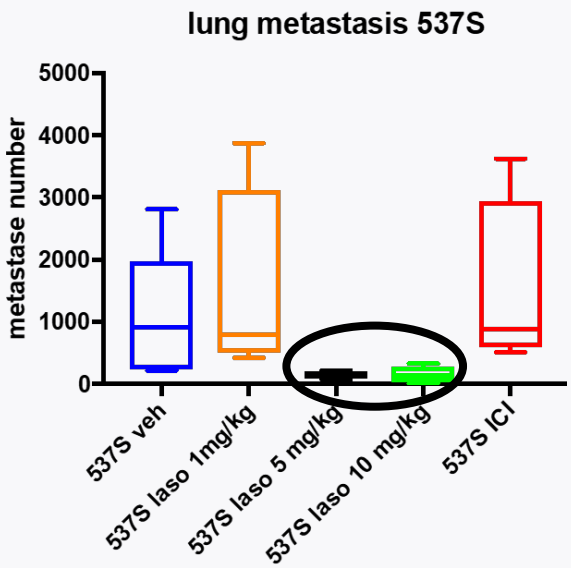
Better SERM: Lasofoxifene Binding affinity for the Y537S mutation

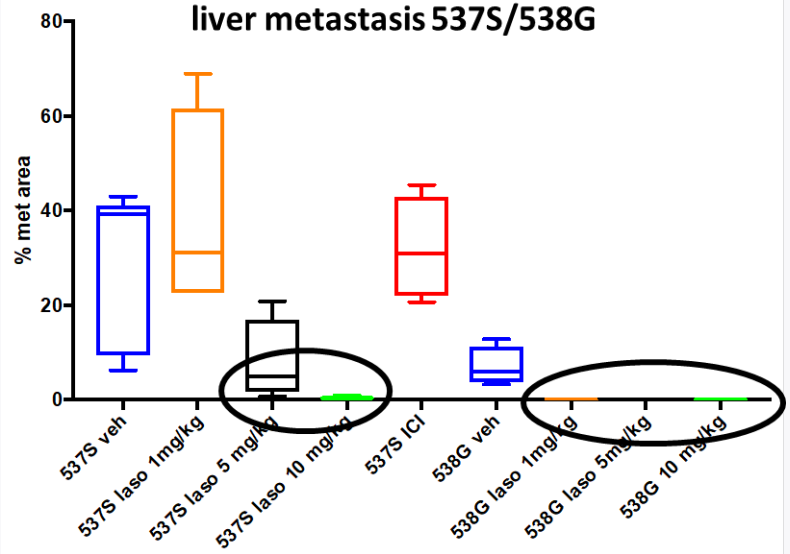
Ligand/Mutant	K_d (nM)[a]	
	WT	Y537S
Estradiol*	0.22 ± 0.11	1.40 ± 0.54
	K_i (nM)[b]	
4-OHT (SERM)*	0.12 ± 0.003	2.64 ± 0.4
Raloxifene (SERM)*	0.30 ± 0.05	3.59 ± 1.0
Bazedoxifene (SERM/SERD)*	0.37 ± 0.01	3.50 ± 0.6
Fulvestrant (SERD)*	0.13 ± 0.03	3.68 ± 0.8
Lasofoxifene	0.41 ± 0.1	0.51 ± 0.1

"a" is direct detection of binding, "d" is the competitive Ki derived from the Cheng-Prussof equation

Lasofoxifene vs fulvestrant

Lasofoxifene essentially blocked metastasis, while fulvestrant (ICI) was no different from vehicle.





Geoff Greene lab: AACR 2019

ELAINE STUDY SCHEMA

DESIGN: RANDOMIZED | OPEN LABEL | MULTICENTER

- Histological or cytological confirmation of ER+/HER2-disease assessed by a local laboratory
- Locally advanced or metastatic breast cancer with radiological or clinical progression on an AI in combination with a CDK 4/6 inhibitor for advanced breast cancer with demonstrated prior sensitivity to endocrine therapy
    - Recurrence or progression after at least 12 months of treatment in the metastatic setting
- Subjects who have not received cytotoxic chemotherapy or who have received one cytotoxic chemotherapy regimen in the neo-adjuvant or adjuvant setting prior to entry into the trial; and/or no more than one chemotherapy regimen for metastatic breast cancer.
- ECOG 0 – 1

R
1:1
N=100

Experimental – Lasofoxifene (Oral)
5 mg per day

Comparator – Fulvestrant (Intramuscular)
500 mg on Day 1, 15, and 29 then monthly

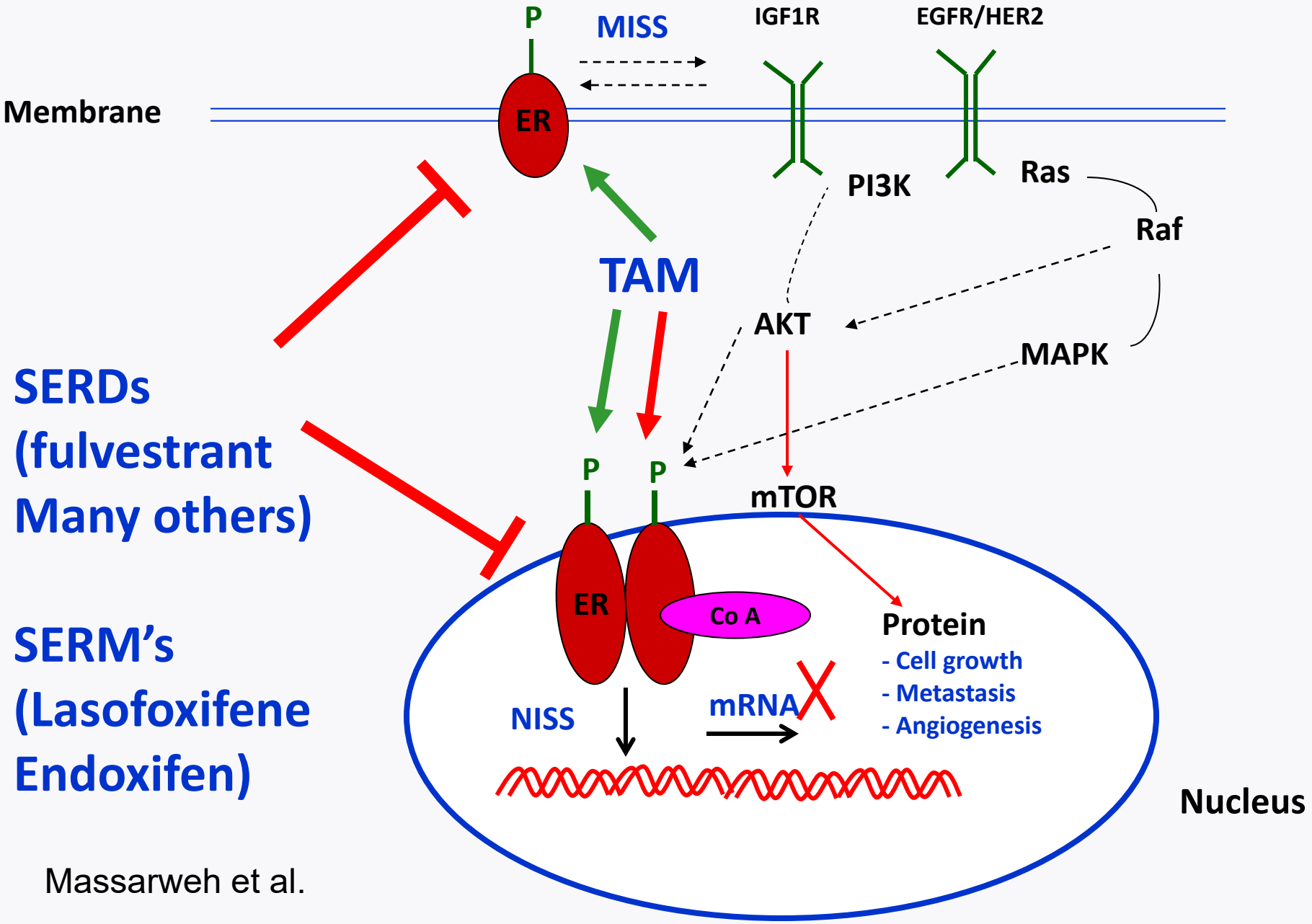
THE Elaine STUDY
EVALUATING LASOFOXIFENE IN ESR1 MUTATIONS

PRIMARY OUTCOME
- Progression Free Survival

SECONDARY OUTCOMES
- Clinical Benefit Rate
- Objective Response Rate
- Duration of Response
- Time to Response
- Incidence of AEs and Serious AEs
- Quality of Life

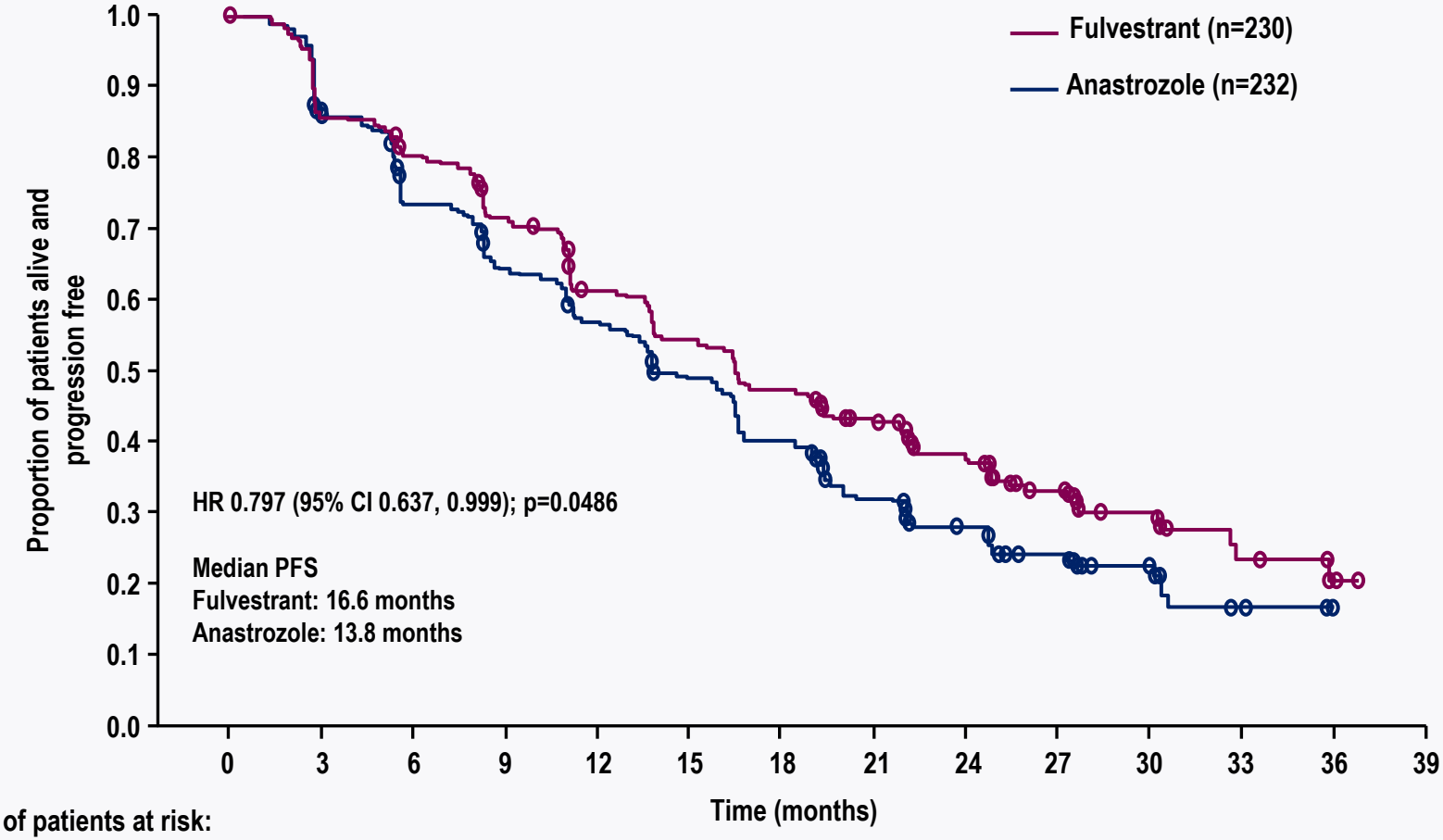
CONFIDENTIAL

SERDs: Targeting the Mutated ER



Massarweh et al.

Comparison of SERD (Fulvestrant) with Anastrozole



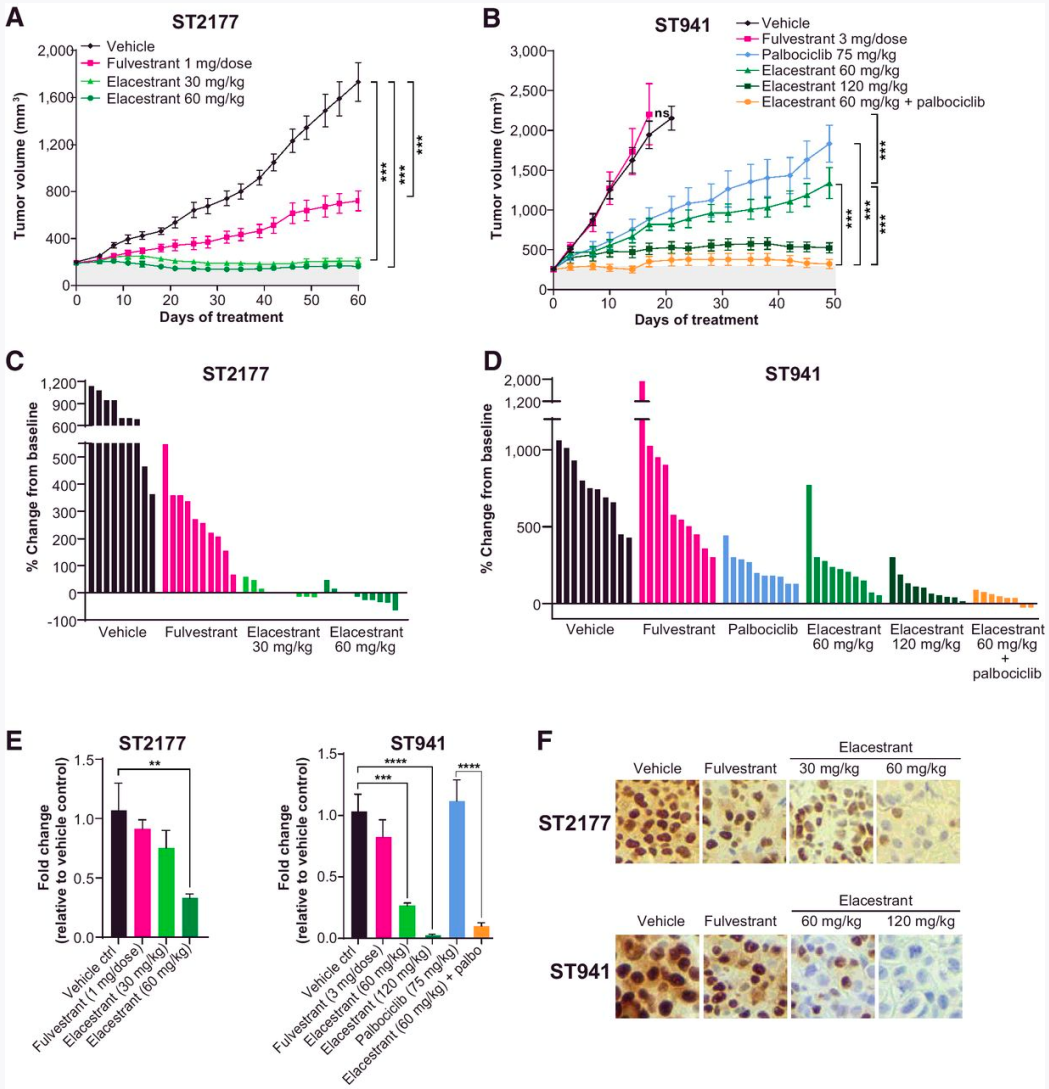
Number of patients at risk:														
Fulvestrant	230	187	171	150	124	110	96	81	63	44	24	11	2	0
Anastrozole	232	194	162	139	120	102	84	60	45	31	22	10	0	0

A circle represents a censored observation

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- Better SERD's
  - Multiple New Oral SERD's developed by many different companies with ongoing phase I, II, and III studies

Elacestrant (Oral SERD) in ER+ Breast Cancer Patient-derived Xenograft Models

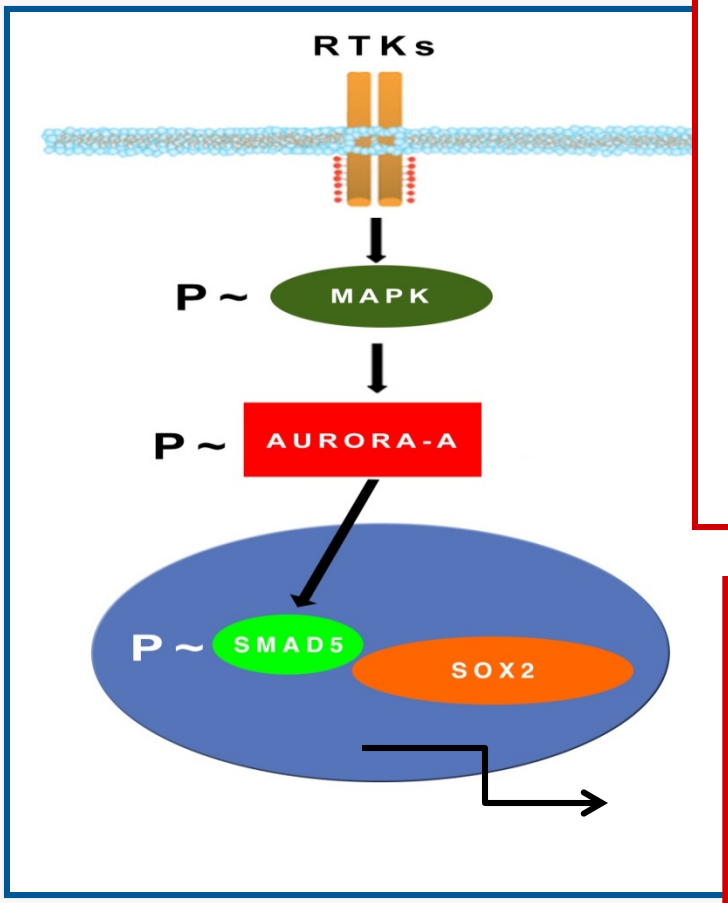


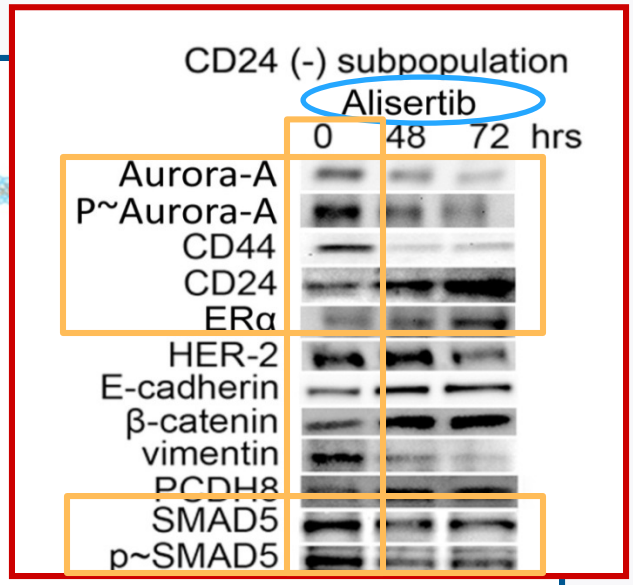
Bihanni et al. Clin Canc Res 2017

New Therapeutic Strategies for ER+ Breast Cancer: Important New Targets

- Cyclin-dependent kinases 4 and 6 (CDK 4/6 inhibitors)
  - Metastatic
  - Adjuvant
- PI3K/AKT/mTOR pathway
- The mutated ER: Targeting the ER for Degradation (SERD's)
  - *ESR1* gene mutations
- **Other important drugs/drug targets (AURKA, Alisertib; BCL-2)**

Aurora-A Kinase is associated with ERα downregulation and endocrine resistance





Activation of EMT reprogramming

↓

Expansion of CD44+/CD24- tumor initiating cells

- Decreased ERα expression
- Endocrine resistant

D'Assoro AB et al. Oncogene 2014, 33:599-610; Opyrchal M et al. PLoS ONE 2014, 9:e96995

TBCRC-041: Phase II Trial to Evaluate Alisertib Alone or Combined with Fulvestrant for Women with Advanced, Endocrine-resistant Breast Cancer

Tufia C Haddad MD
Matthew P Goetz MD
Vera J Suman PhD
James N Ingle MD
Antonio B D'Assoro MD PhD
Minetta C Liu MD
John R Hawse PhD
Liewei Wang MD PhD

To be presented at the 2020 San Antonio Breast Cancer Symposium.
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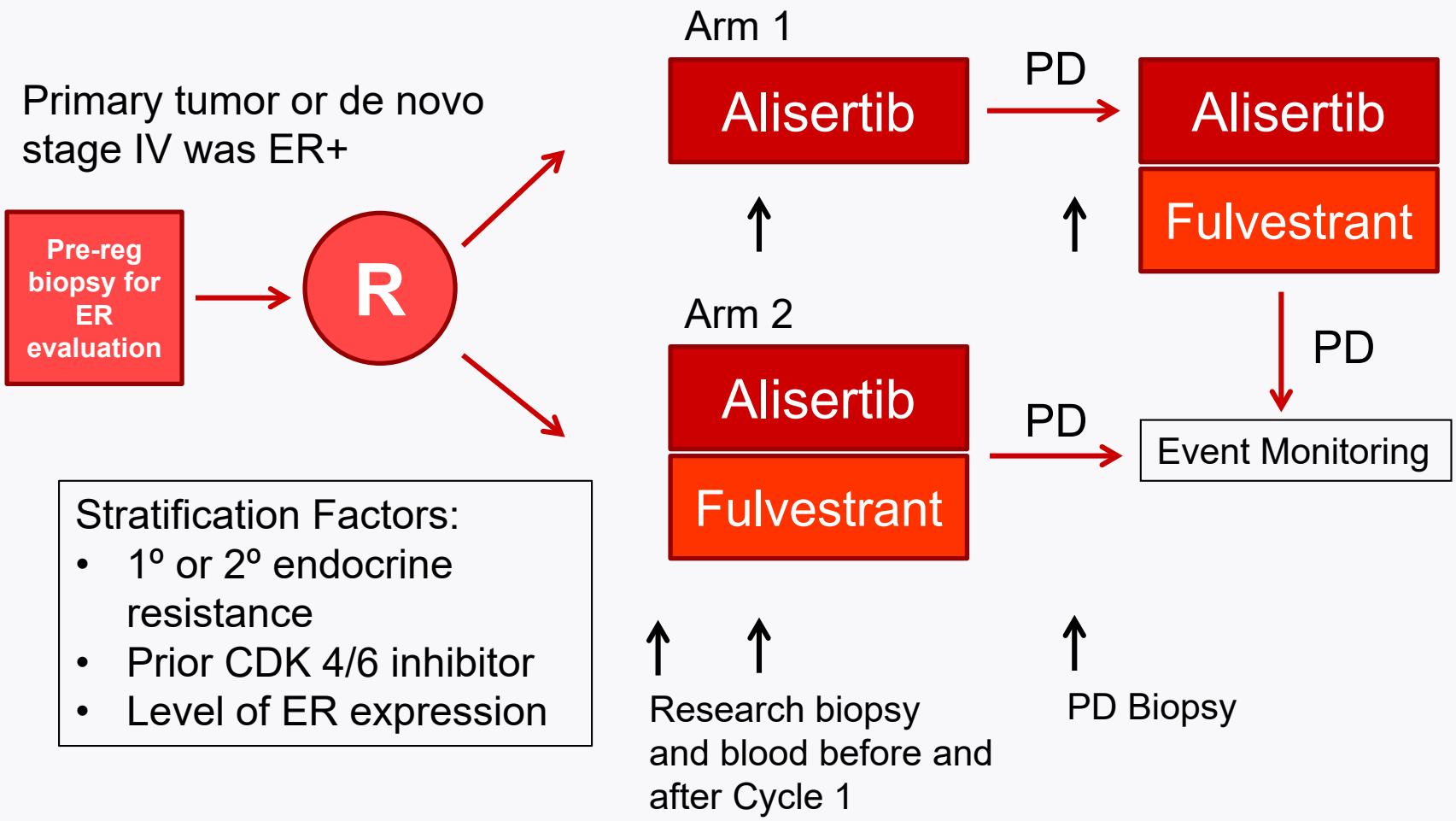
Phase II trial of alisertib alone or combined with fulvestrant for endocrine-resistant, metastatic breast cancer



Stratification Factors:

- 1º or 2º endocrine resistance
- Prior CDK 4/6 inhibitor
- Level of ER expression

MC1431 / TBCRC041 / NCT02860000

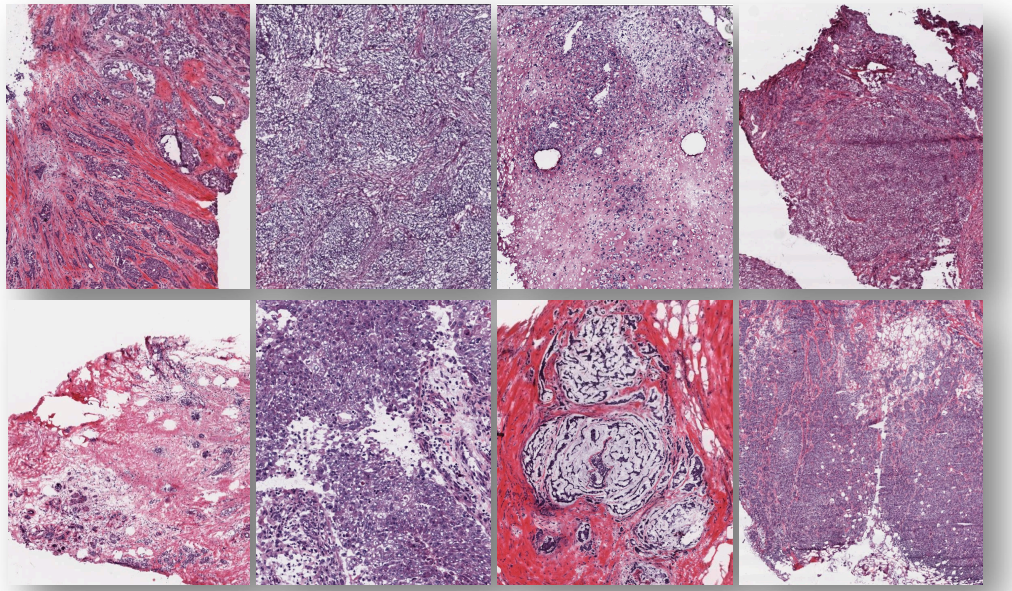
11/22/2020

Clinical Outcomes

	Alisertib (n=45)	Alisertib + Fulvestrant (n=45)
Confirmed Responses	8 PR	1 CR; 8 PR
Objective Response Rate	17.8% (90% CI: 9.2-29.8%)	20.0% (90% CI: 10.9-32.3%)
Clinical Benefit Rate (24-week)	42.2% (90% CI: 29.7-55.6%)	28.9% (90% CI: 18.0-42.0%)
Median PFS (months)	5.6 (95%CI: 3.9 – 9.3)	5.1 (95%CI: 3.8 – 7.6)
Deaths 6-month OS rate	n=10 90. 6% (95% CI: 82.2-99.8%)	n=14 75.6% (95% CI: 63.9-90.2%)

Summary

- Substantial gains in mortality have been observed over time in women with both localized and metastatic breast cancer
- New drug targets and drugs rapidly being identified to address both primary and secondary resistance



New Approaches to Triple-Negative Breast Cancer

JENNIFER A. PIETENPOL, PhD

B.F. Byrd Jr. Professor of Oncology
Director, Vanderbilt-Ingram Cancer Center
Executive Vice President for Research
Vanderbilt University Medical Center
Professor of Biochemistry and Otolaryngology

November 11, 2020

NCI Designated Comprehensive Cancer Center

VANDERBILT-INGRAM CANCER CENTER

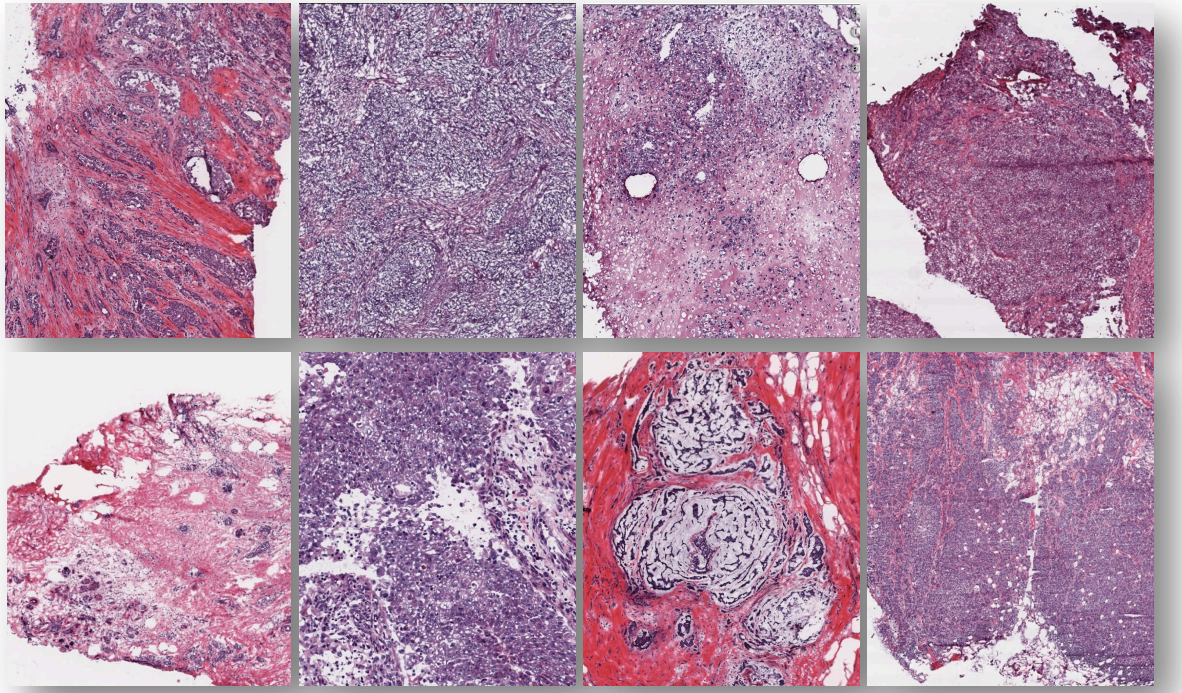
Conflicts of Interest Disclosure

1. Grant/Research Support from Incyte Corporation

2. Other financial/material interest in Insight Genetics, Inc.

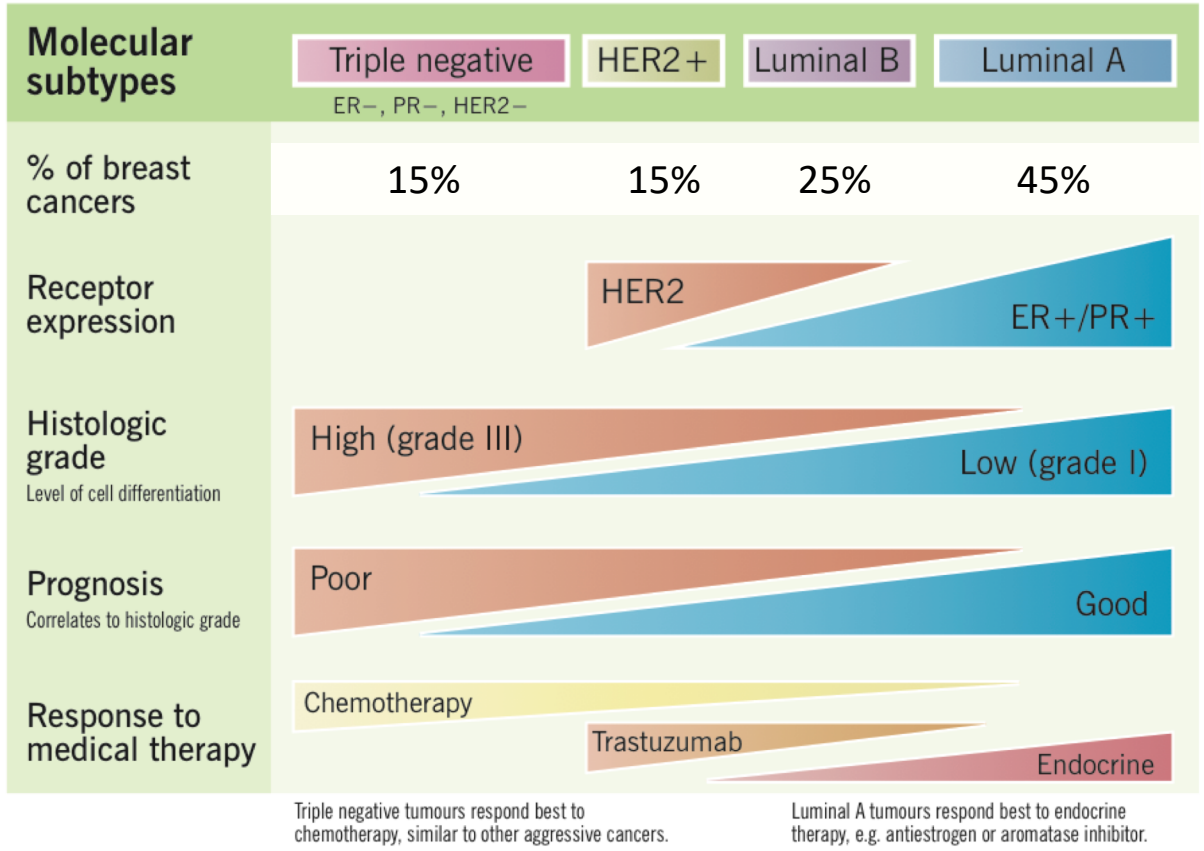
   Jennifer Pietenpol: Inventor (PCT/US2012/065724) of intellectual property (TNBCType) licensed by Insight Genetics Inc.

Limited targeted therapy for triple-negative breast cancer – we are trying to change that



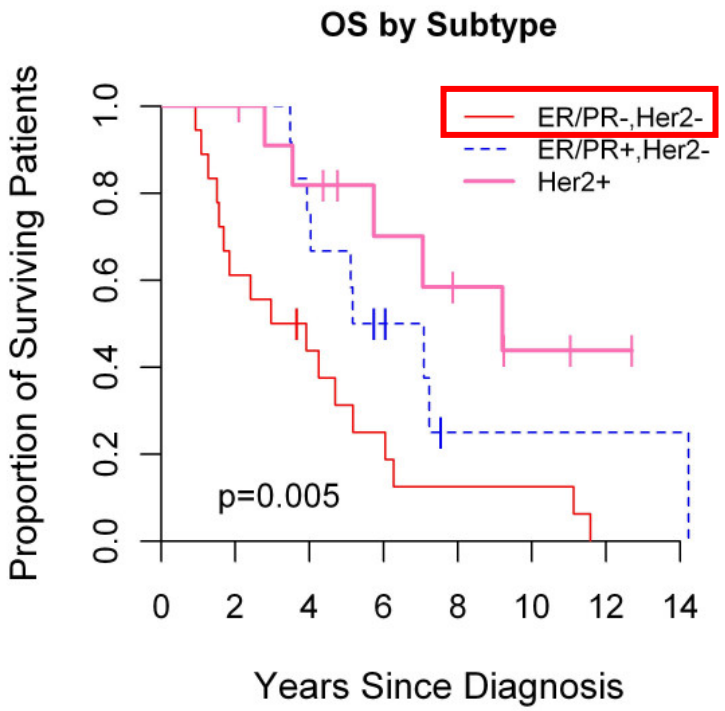
Breast Cancer Subtypes

TNBC Accounts for 25% of Breast Cancer Mortality



Adamo *et al.*, 2011

Overall Survival Depends on Breast Cancer Subtype



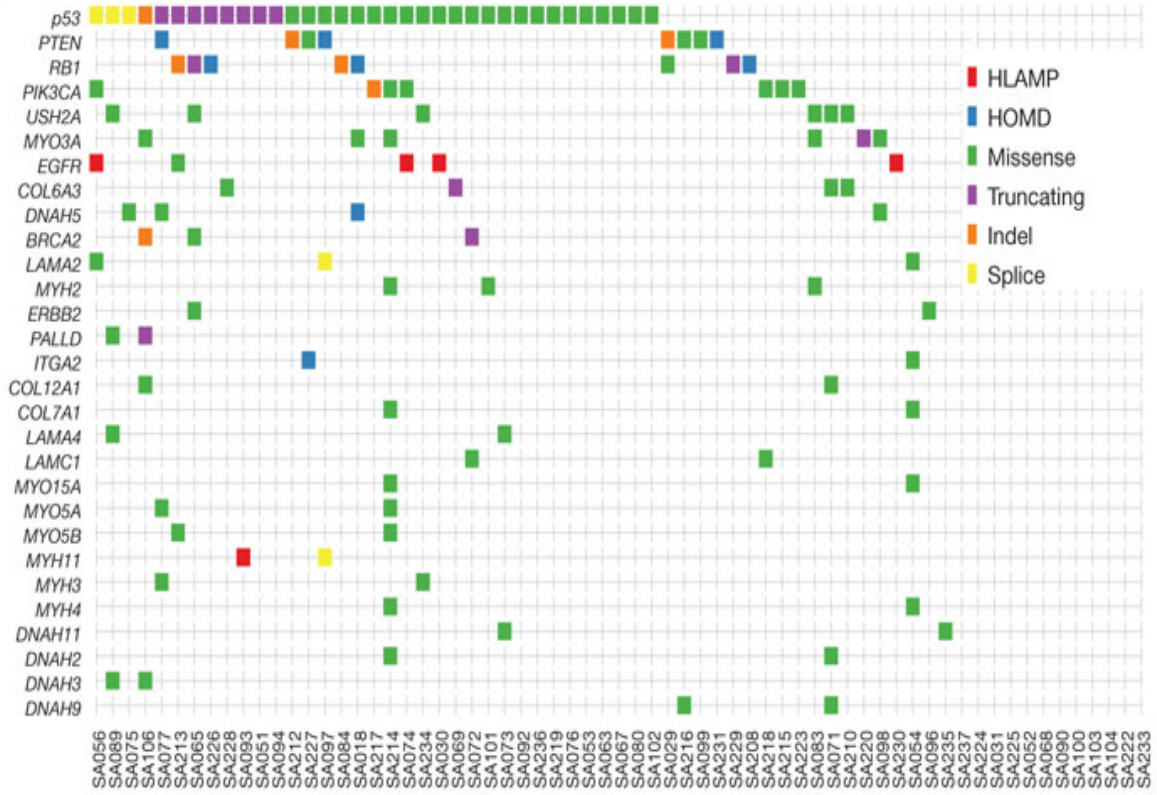
Inter- and intra-tumor heterogeneity presents significant clinical challenges

Need to expand the spectrum of targetable molecular features in TNBC

- A significant fraction of TNBCs lack high-frequency "driver" alterations amenable to therapeutic intervention

- *TP53* mutations – only alteration occurring in a majority of TNBC

- TNBCs exhibit a high frequency of genomic instability



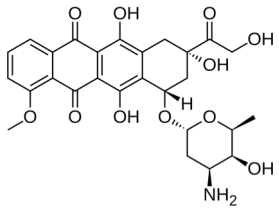
Shah *et al*. *Nature* 2012

Chemotherapy remains the stand-of-care for primary TNBC

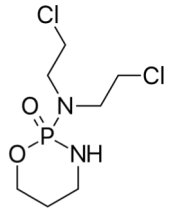
Primary, treatment-naïve tumors

Chemotherapy

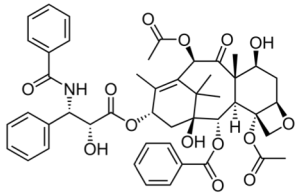
Anthracyclines, cyclophosphamide, platins & taxanes



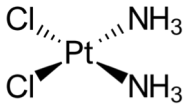
doxorubicin



cyclophosphamide



paclitaxel



cisplatin

DNA damage

Microtubule damage

Majority of TNBC tumors progress/recur

Advanced/metastatic tumors

Targeted therapy

PARP inhibitor

olaparib
talazoparib
veliparib

***BRCA1/2*-mutant tumors**

Immunotherapy

Atezolizumab* + nab-paclitaxel
Pembrolizumab + drug 1, 2 ,3
pembrolizumab + eribulin

PD-L1* expression

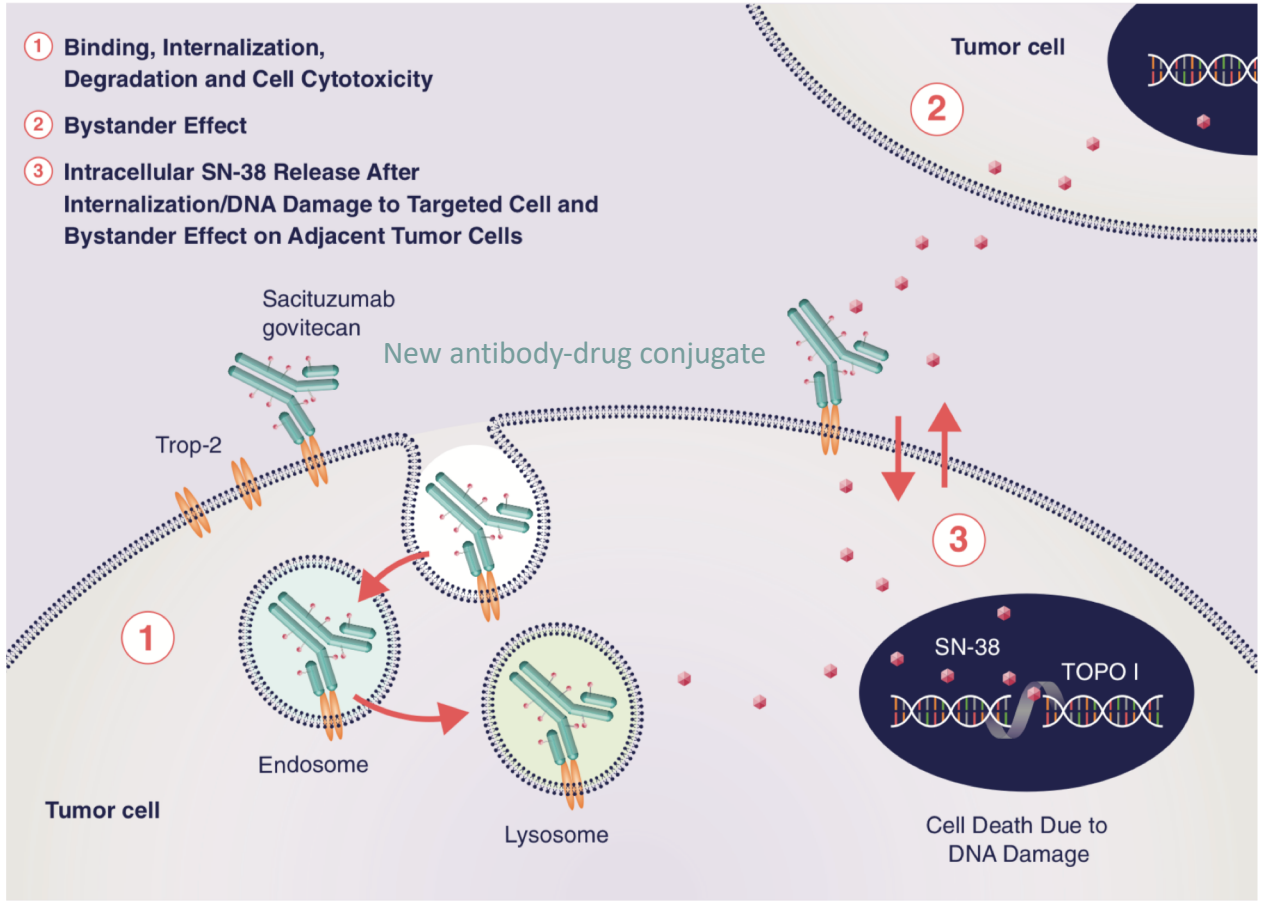
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

February 21, 2019
N Engl J Med 2019; 380:741-751
DOI: 10.1056/NEJMoa1814213

Sacituzumab Govitecan-hziy in Refractory Metastatic Triple-Negative Breast Cancer

Aditya Bardia, M.D., Ingrid A. Mayer, M.D., Linda T. Vahdat, M.D., M.B.A., Sara M. Tolaney, M.D., M.P.H., Steven J. Isakoff, M.D., Ph.D., Jennifer R. Diamond, M.D., Joyce O'Shaughnessy, M.D., Rebecca L. Moroose, M.D., Alessandro D. Santin, M.D., Vandana G. Abramson, M.D., Nikita C. Shah, M.D., Hope S. Rugo, M.D., et al.



- The primary efficacy outcome measures were investigator assessed overall response rate (ORR) using RECIST 1.1 and response duration
- The ORR was 33.3% (95% CI: 24.6, 43.1)
- The median response duration was 7.7 months (95% CI: 4.9, 10.8)

Potential for other antibody-drug conjugates to follow:

- Ladiratuzumab vedotin (SGN-LIV1A)
- Trastuzumab deruxtecan (DS-8201a/T-DXd)

Now and Future – Combination Strategies

- Exciting new treatment approaches to triple-negative breast cancer
  - New targets
  - New antibody-drug conjugates (1 FDA approved; several under investigation)
  - New combinations with immunotherapy (chemo + targeted agents)

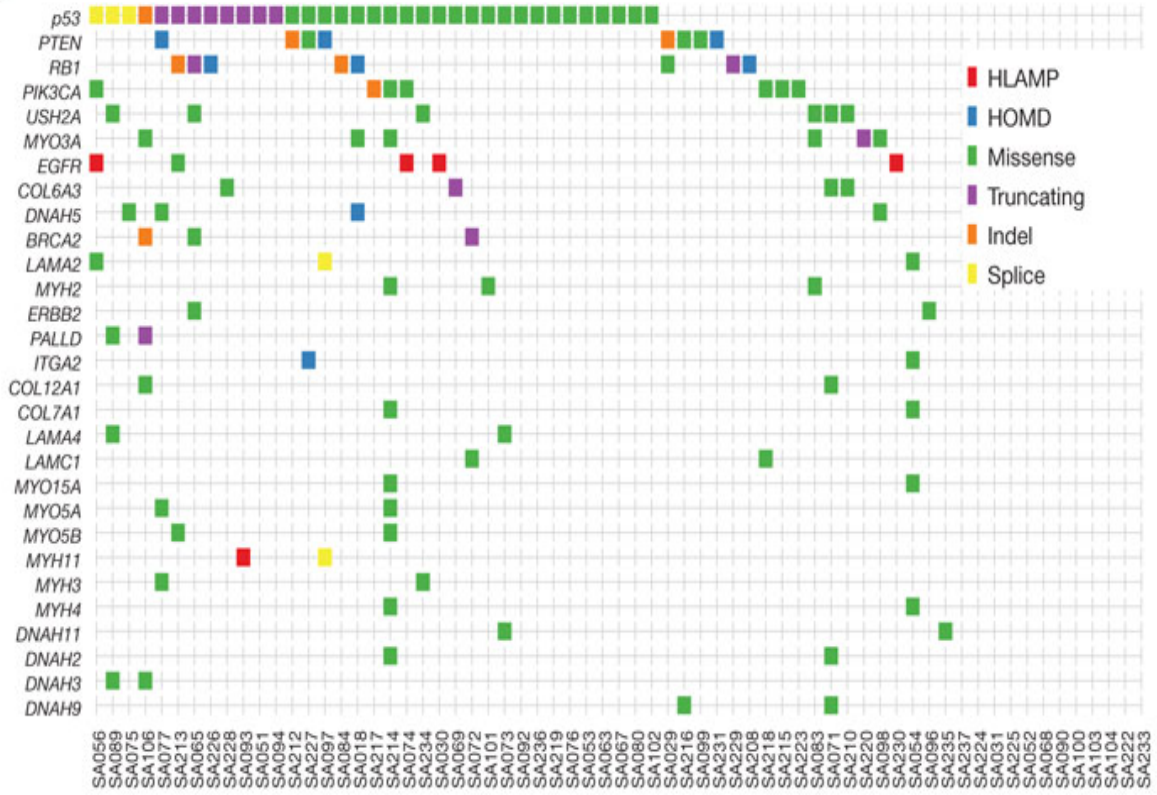
- Chemotherapy combined with immunotherapy is the new standard of care treatment for PD-L1^{+} TNBC, but a large proportion of patients do not respond to these therapies

- Several combination studies are ongoing focusing on strategies to improve response rates to immunotherapy-based treatment

Expanding the spectrum of targetable molecular features in TNBC

- Can we identify TNBC subtypes and targetable features of the “adapted states” of subtypes, including immunomodulation?

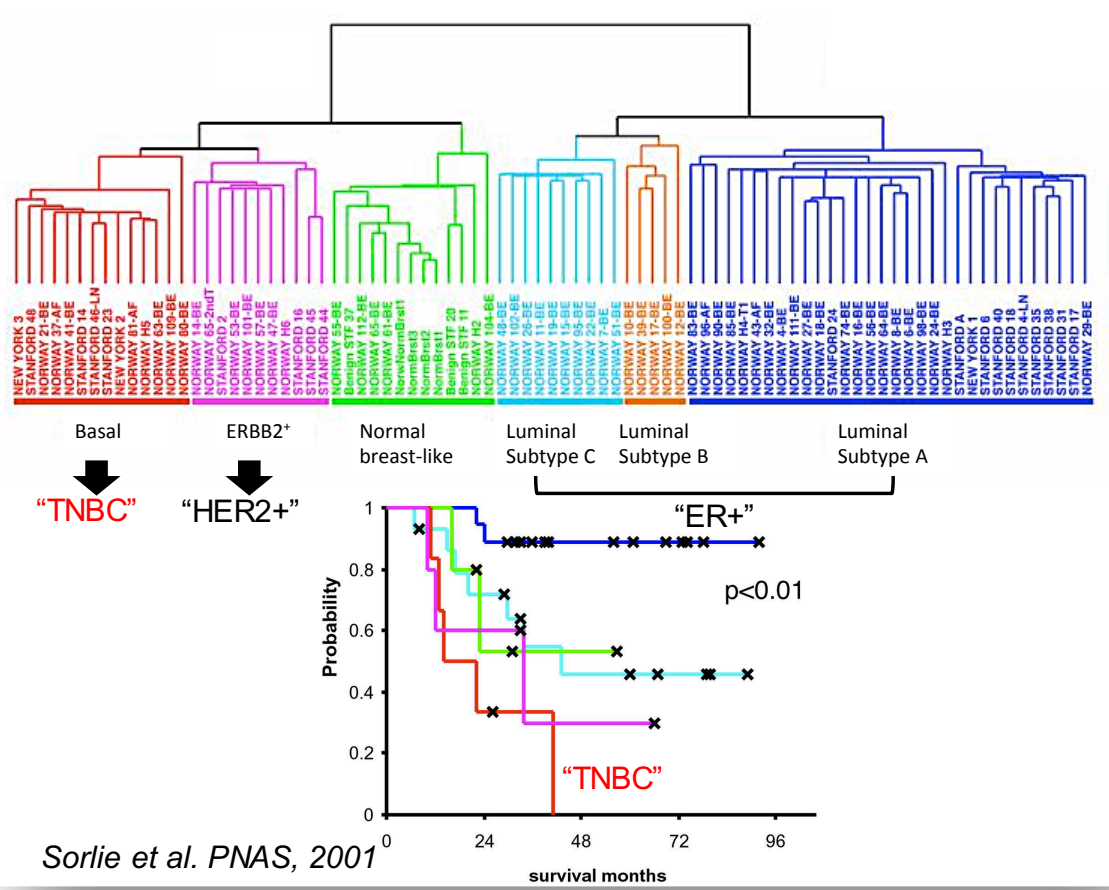
- What is the extent of intra-tumor heterogeneity in TNBC and is it targetable?



Shah *et al*. *Nature* 2012

Diversity within Triple-Negative Breast Cancer: TNBC Subtypes

Breast Cancer is a Heterogeneous Disease



Sorlie et al. PNAS, 2001

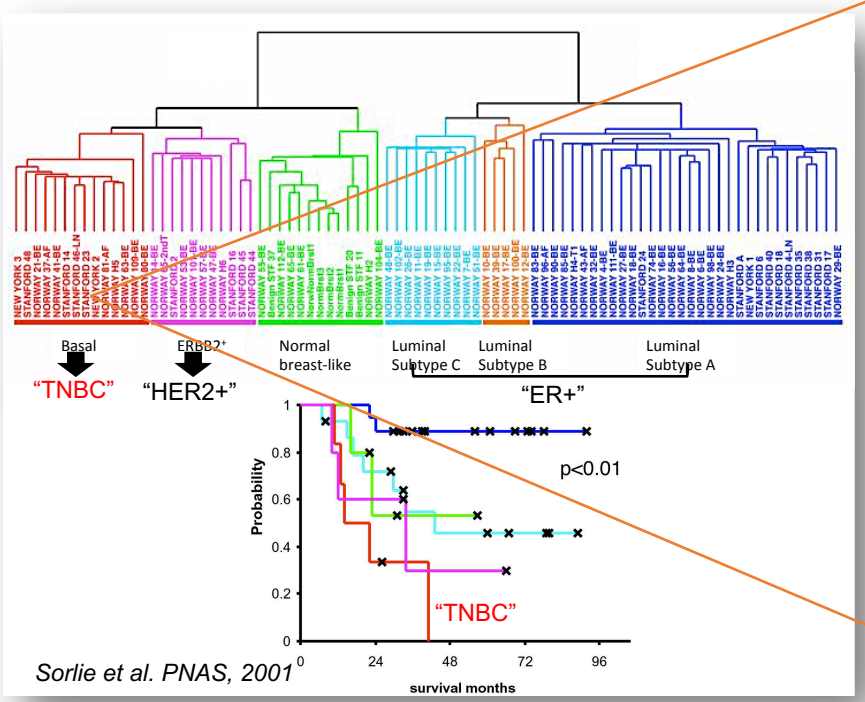
Leveraged Worldwide Data

21 breast cancer data sets
8 countries
3247 cases; 587 TNBC

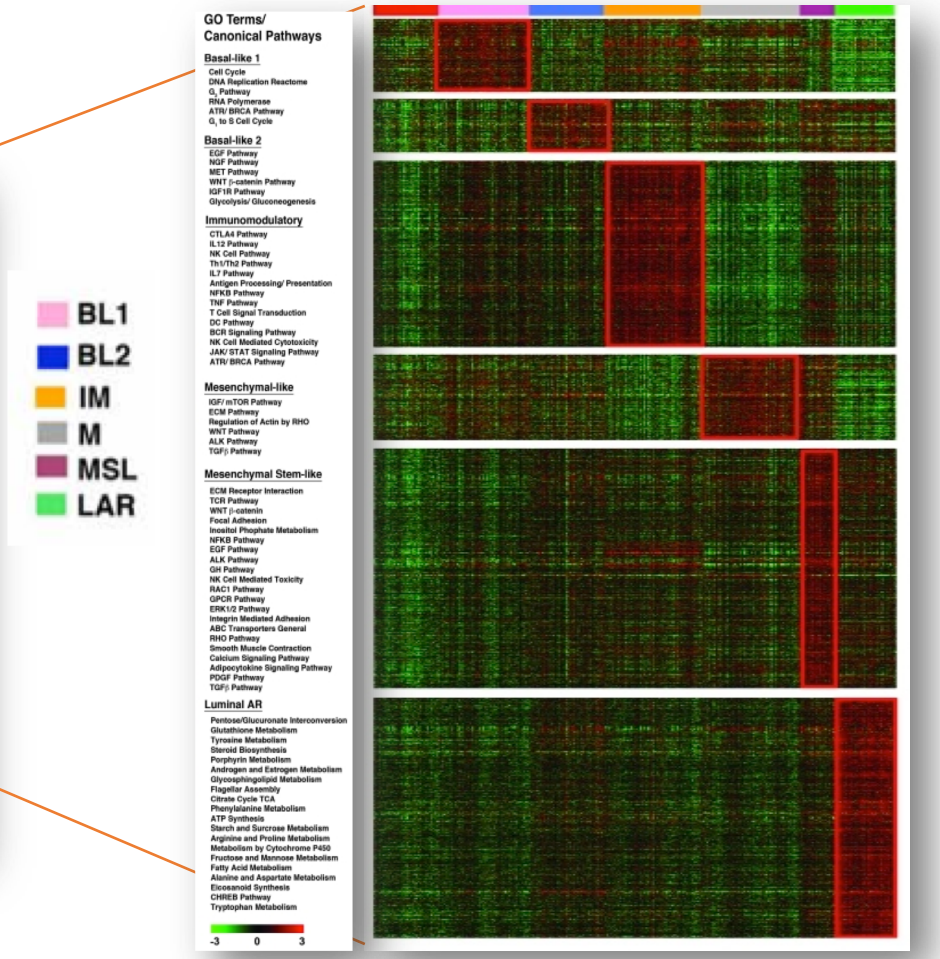
Diversity within Triple-Negative Breast Cancer: TNBC Subtypes

We identified distinct molecular subtypes of TNBC by profiling genes that were expressed

Breast Cancer is a Heterogeneous Disease

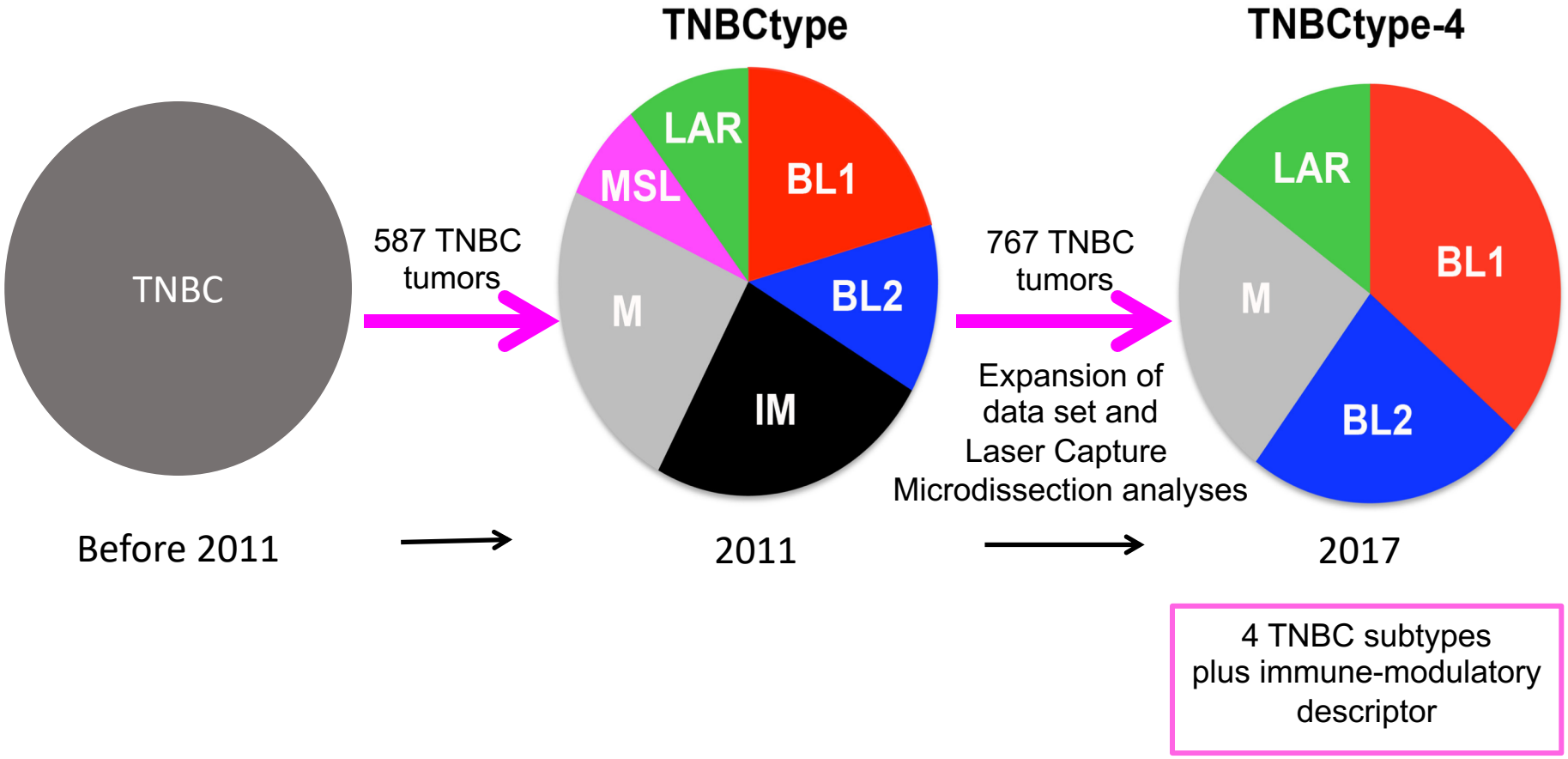


Sorlie et al. PNAS, 2001



Lehmann, Bauer, Chen, Sanders, Shyr, Pietenpol; *JCI,* 2011

Evolution of TNBC Subtyping

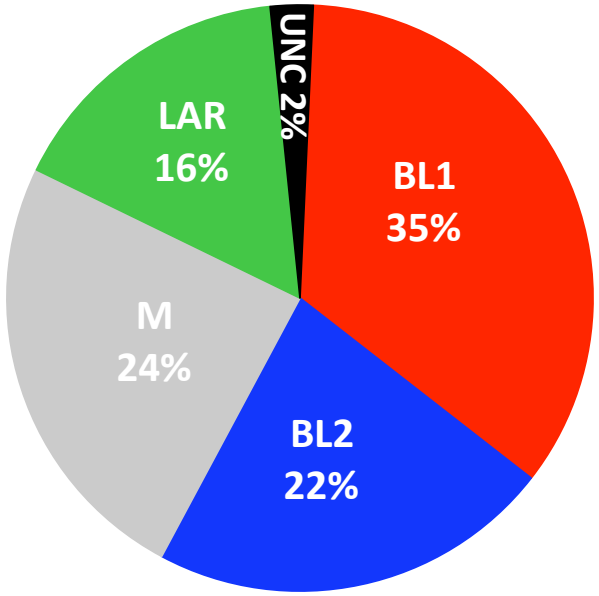


Lehmann, Bauer, Chen, Sanders, Shyr, Pietenpol; *JCI,* 2011

Lehmann, Jovanovic Chen, Shyr, Pietenpol; *PLoS One,* 2016

Gene expression-based TNBC subtypes have specific drug sensitivities

Gene expression analysis of 767 TNBC tumors



N=767

TNBC subtype	Gene Ontology	Mutation enrichment	Drug Sensitivity
BL1	Cell cycle/DNA damage response features	BRCA1/2	Platinum agents (NAC*)/PARP inhibition
BL2	MET/EGFR signaling and myoepithelial features		mTOR, growth factor receptors
M	NOTCH/TGFß and trans-differentiation features		mTOR, growth factor receptors, Src inhibition
LAR	Luminal androgen receptor signaling	PIK3CA	AR antagonist/PI3K inhibition
IM	Immune-modulatory descriptor		Chemo- and immuno-therapy

J Clin Invest. 2011 Jul;121(7):2750-67
PloS One. 2016 Jun 16;11(6)

*Neoadjuvant chemotherapy

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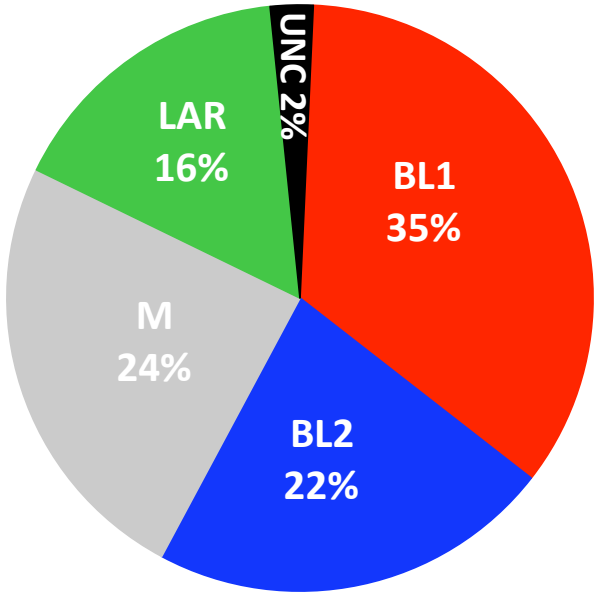

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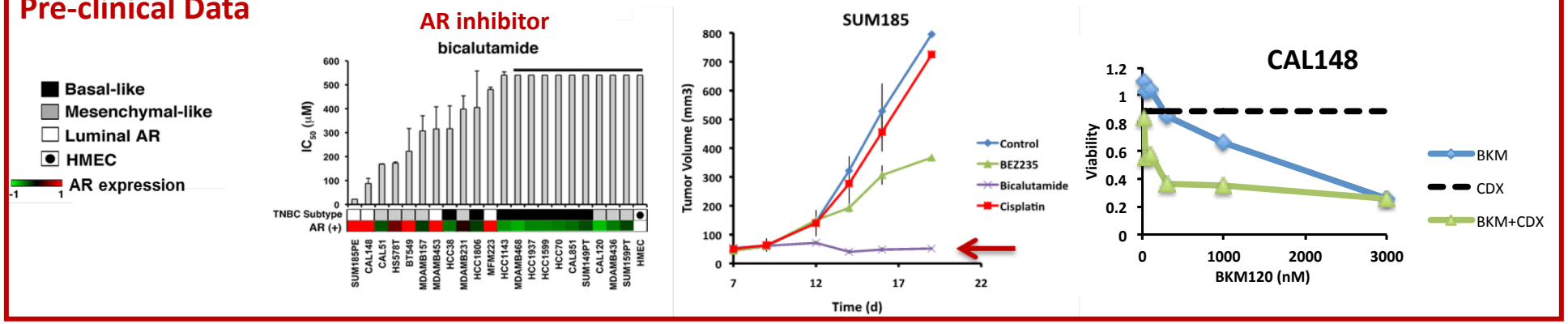
J Clin Invest. 2011 Jul;121(7):2750-67
PloS One. 2016 Jun 16;11(6)

*Neoadjuvant chemotherapy

Luminal Androgen Receptor Subtype Vulnerabilities

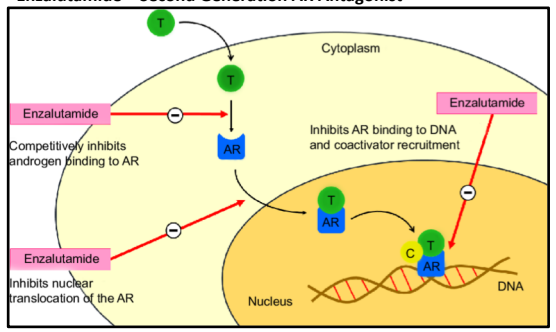
"Rethinking hormone therapy in TNBC"

Pre-clinical Data



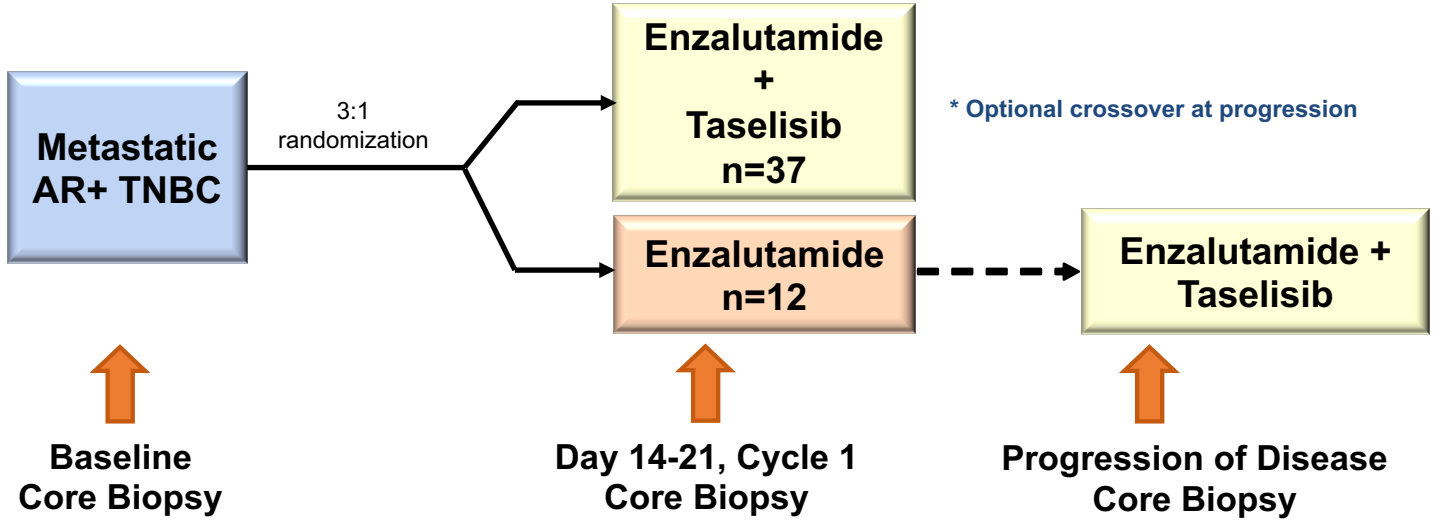
Lehmann et al., *JCI,* 2011
Lehmann et al., *BCR,* 2014

Enzalutamide – Second Generation AR Antagonist



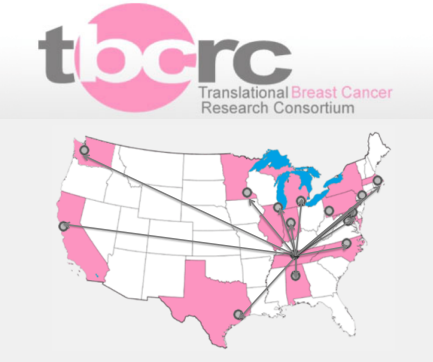
Rodriguez-Vida A *et al. Drug Des Devel Ther,* 2015

NCT02457910 (TBCRC 032): Phase Ib/II Study of PIK3CA Inhibitor Taselisib with Enzalutamide in AR+ TNBC

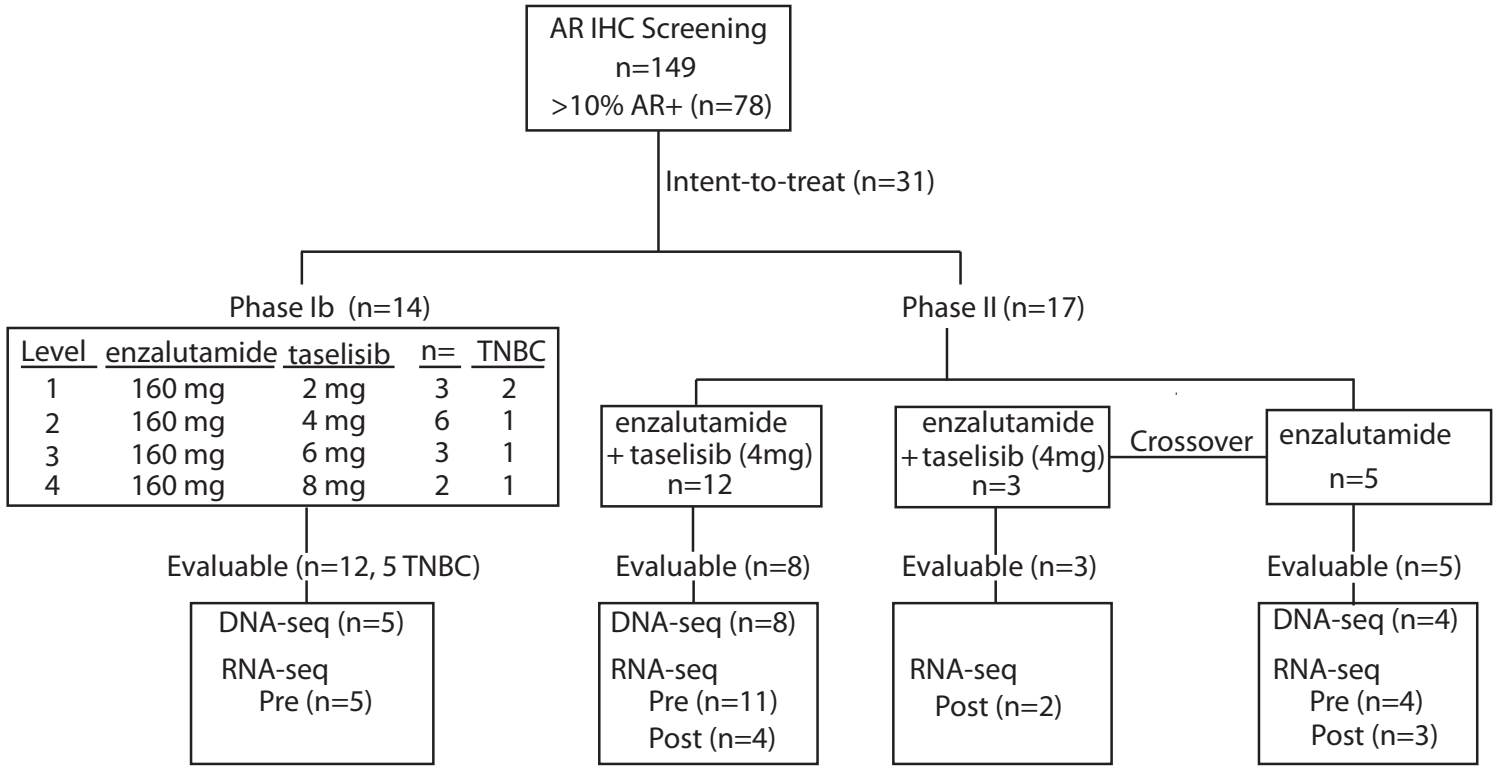


Vandana Abramson, MD

TBCRC 032 Patient accrual and correlative analyses



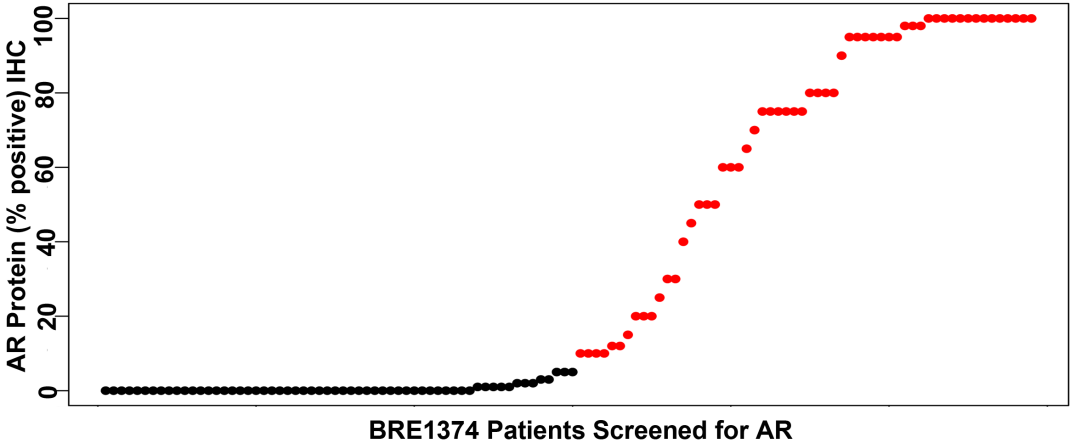
Patient#	Subject	Age on Study	Arms	Taselisib(mg)	TNBC
1	1009	46	PH1b E+T	4	NO
2	1012	62	PH1b E+T	6	NO
3	1003	70	PH1b E+T	2	NO
4	1010	66	PH1b E+T	4	NO
5	1011	43	PH1b E+T	4	NO
6	1015	54	PH1b E+T	6	NO
7	1007	59	PH1b E+T	4	NO
8	1016	57	PH1b E+T	8	YES
9	1001	62	PH1b E+T	2	YES
10	1006	58	PH1b E+T	4	YES
11	1014	60	PH1b E+T	6	YES
12	1017	59	PH1b E+T	8	YES
13	1002	60	PH1b E+T	2	YES
14	1020	60	PHII E+T	4	YES
15	1021	36	PHII E+T	4	YES
16	2001	59	PHII E+T	4	YES
17	2004	64	PHII E+T	4	YES
18	4001	38	PHII E+T	4	YES
19	5001	54	PHII E+T	4	YES
20	5002	63	PHII E+T	4	YES
21	7001	71	PHII E+T	4	YES
22	2005	69	PHII E+T	4	YES
23	3001	58	PHII E+T	4	YES
24	2002	68	PHII E+T	4	YES
25	1019	51	PHII E+T	4	YES
26	3002	74	PHII E	NA	YES
27	4002	72	PHII E	NA	YES
28	2003	62	PHII E; _CO E+T	NA	YES
29	3003	60	PHII E; _CO E+T	NA	YES
30	1018	59	PHII E; PHII E+T	NA	YES



Lehmann, Abramson, Sanders et al. & Pietenpol, *Clin Can Res*, 2019

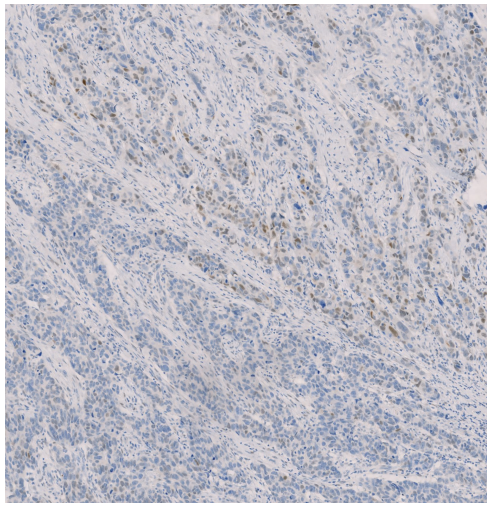
Androgen Receptor Immunohistochemistry Screening

Melinda Sanders, MD

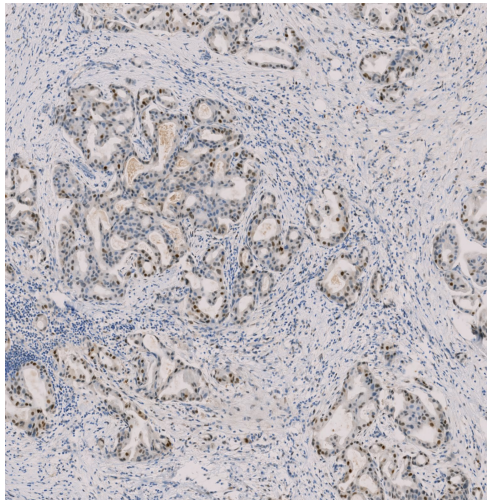


149 patients screened
48% AR positive
>10% nuclear AR

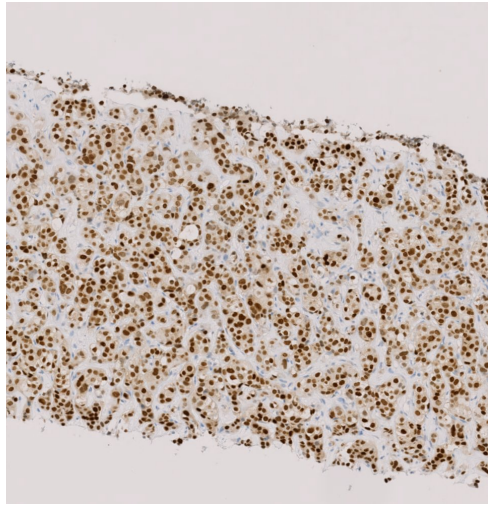
10% AR



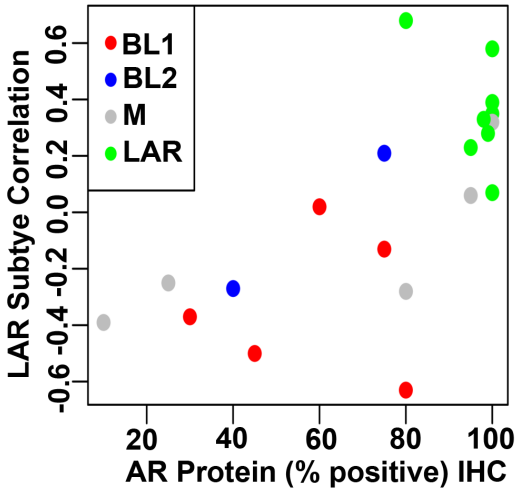
40% AR



100% AR

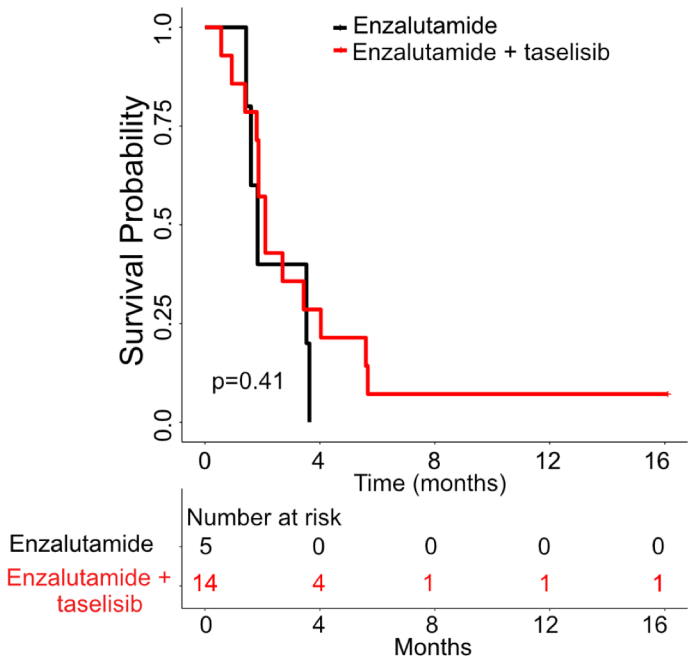


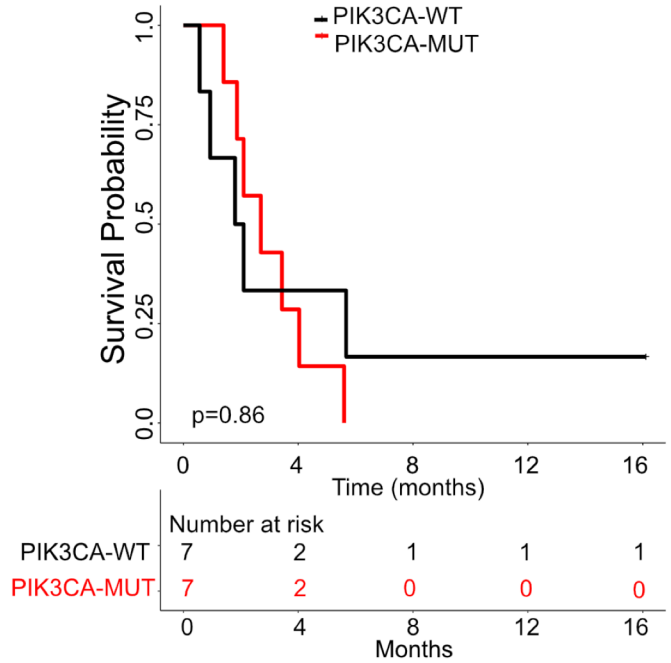
BL1=Basal-like 1
BL2=Basal-like 2
M=Mesenchymal
LAR=Luminal AR

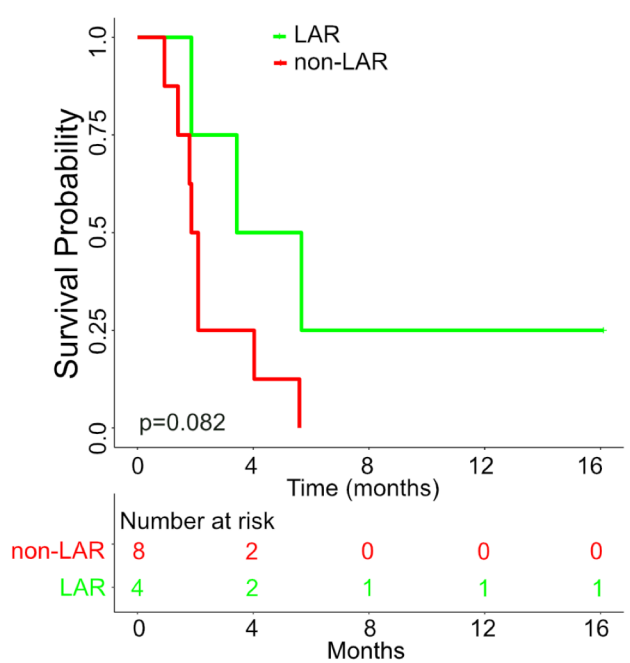


Lehmann, Abramson, Sanders et al. & Pietenpol, *Clin Can Res*, 2019

Patients with Luminal AR subtype had better progression-free survival after enzalutamide + taselisib combination







AR-target genes decreased after enzalutamide treatment in patient's tumors

Lehmann, Abramson, Sanders et al. & Pietenpol, *Clin Can Res*, 2019

Patients with LAR subtype had greater clinical benefit rate at 16 wk compared to patients with other subtypes

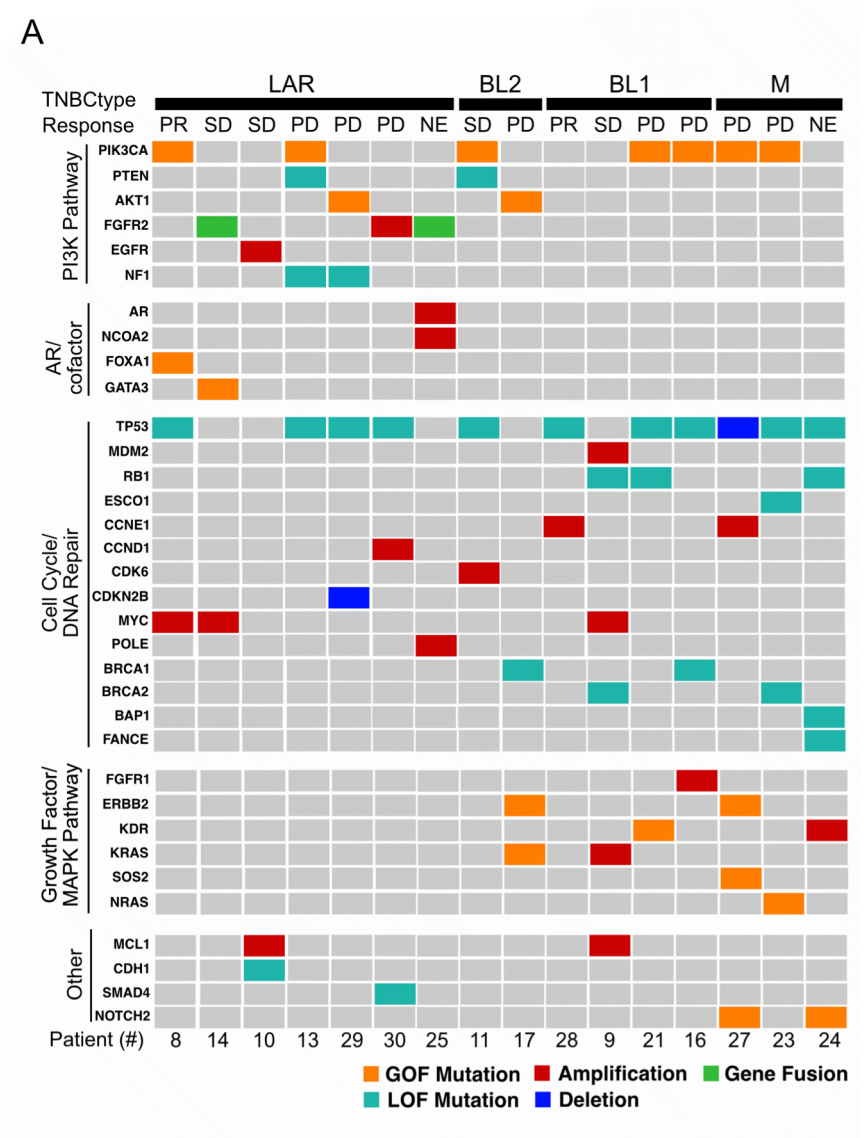
Arm	Subgroup	CBR16 (%)	OR	N=	PR	SD	PD	p-value
Enzalutamide	All patients	0		5	0	0	5	
Enzalutamide + taselisib	All patients	35.7		14	1	4	9	
	PIK3CA mutation							1.00
	wild-type	28.6		7	0	2	5	
	mutant	42.9	1.8	7	1	2	4	
	TNBCtype							0.06
	LAR	75.0	14.2	4	1	2	1	
	other subtype	12.5		8	0	1	7	
	NA	50		2	0	1	1	

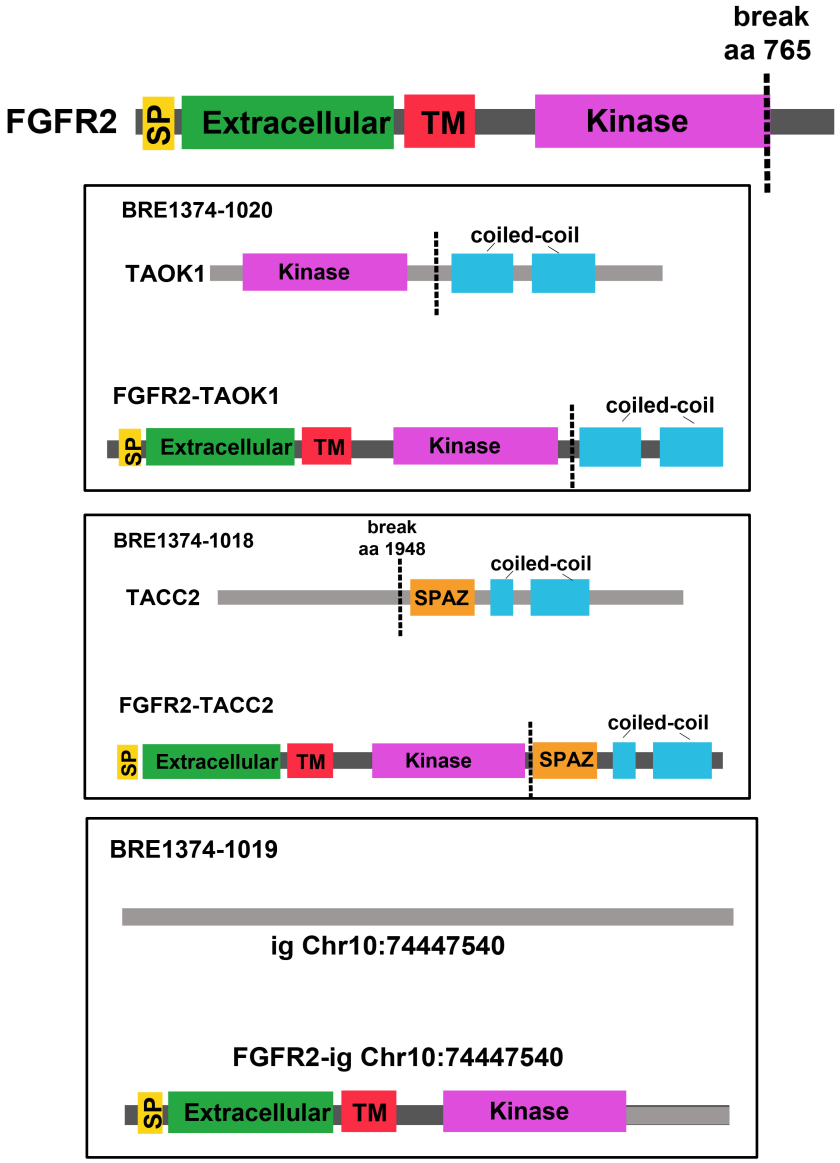
Abbreviations: CBR16, clinical benefit rate at 16 weeks; OR, odds ratio; PR, partial response; SD, stable disease; PD, progressive disease; NA, not available

Clinical benefit rate=%[partial response + stable disease]

Mutation Status of Patients' Tumors

PI3K Pathway Mutations & FGFR2 gene rearrangements enriched in LAR subtype





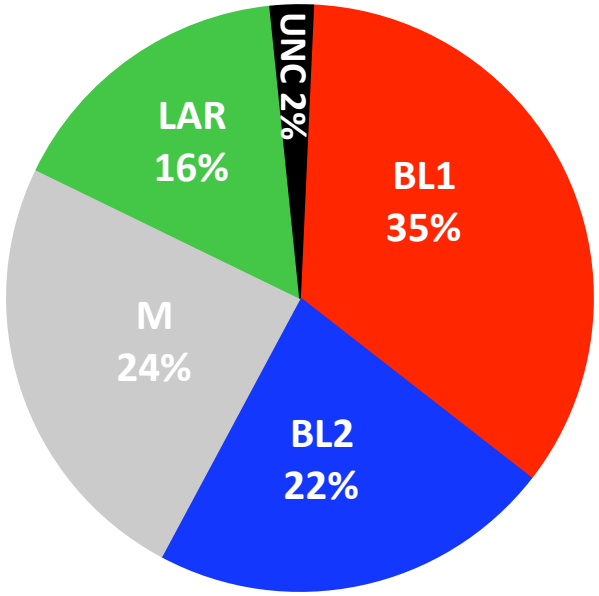
Lehmann, Abramson, Sanders et al. & Pietenpol, *Clin Can Res*, 2019

Summary

- **LAR subtype tumors displayed high percentage (>80%) of AR+ cells**
- LAR gene signature predictive of response and tumor decreases post-treatment in responding patients
- PIK3CA mutations not predictive of response to enzalutamide + taselisib
- AR splice variants (AR-V7) lacking the ligand binding domain may confer resistance to enzalutamide (similar to prostate cancer)
- FGFR2 alterations enriched in LAR subtype and provides evidence for future combinatorial targeted therapy trials

Gene expression-based TNBC subtypes have specific drug sensitivities

Gene expression analysis of 767 TNBC tumors



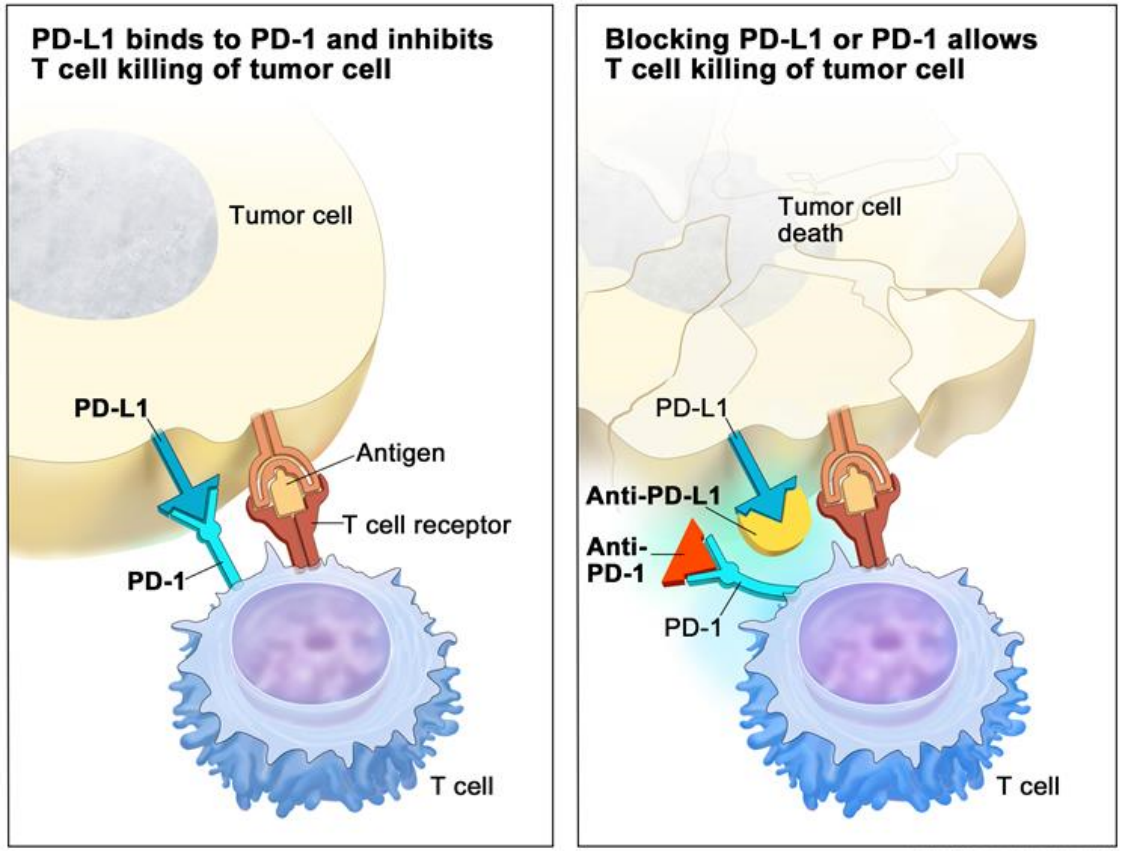
N=767

TNBC subtype	Gene Ontology	Mutation enrichment	Drug Sensitivity
BL1	Cell cycle/DNA damage response features	BRCA1/2	Platinum agents (NAC*)/PARP inhibition
BL2	MET/EGFR signaling and myoepithelial features		mTOR, growth factor receptors
M	NOTCH/TGFß and trans-differentiation features		mTOR, growth factor receptors, Src inhibition
LAR	Luminal androgen receptor signaling	PIK3CA	AR antagonist/PI3K inhibition
IM	Immune-modulatory descriptor		Chemo- and immuno-therapy

Approaches for other 84% of TNBC

J Clin Invest. 2011 Jul;121(7):2750-67
PloS One. 2016 Jun 16;11(6)

Immunotherapy in TNBC?



- Immune checkpoint inhibitors are effective in a variety of solid tumors
- In the metastatic setting (single agent):

  ~20% objective response rate in TNBC
- Atezolizumab plus nab-paclitaxel prolonged progression-free survival among patients with metastatic TNBC[1]

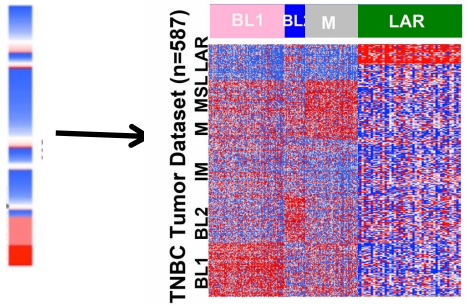
- <u>Immunotherapy combination strategies</u>:
  - ➢ Increase immune recognition through enhanced antigen presentation (BL1)

    and/or
  - ➢ Increased T-cell homing may increase immunotherapy response in recalcitrant TNBC (BL2 and M)

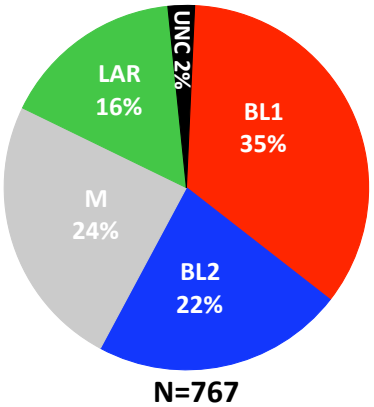
[1]Emens et al, AACR 2015; Nanda et al, AACR 2015; *N Engl J Med*. 2018
Rugo et al, KEYNOTE-028 trial, SABCS 2015;
Lefebvre et al, PLoS Medicine, 2016

Which TNBC patients will benefit from immunotherapy?

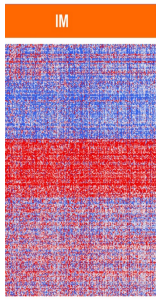
TNBC tumor

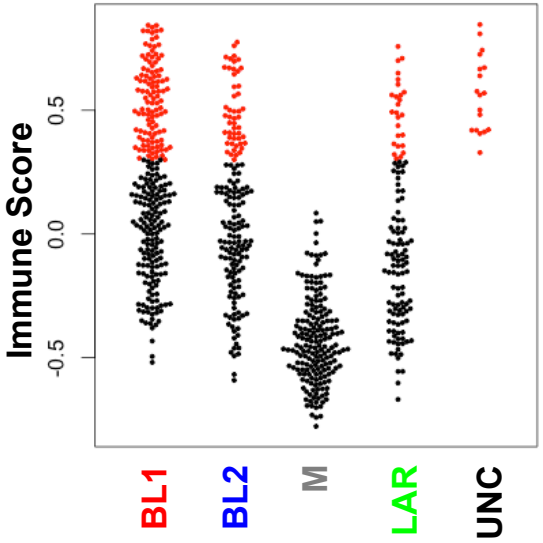


Assign subtype



Analyze immune features – immune descriptor





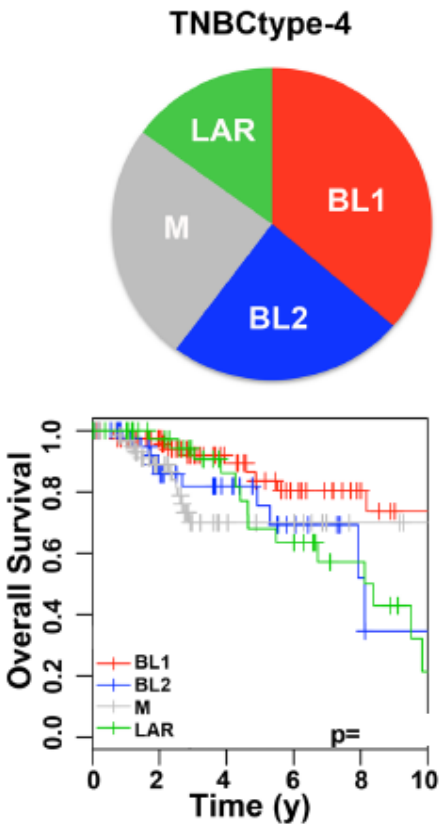
Immune descriptor/ phenotype

M. Sanders

B. Lehmann

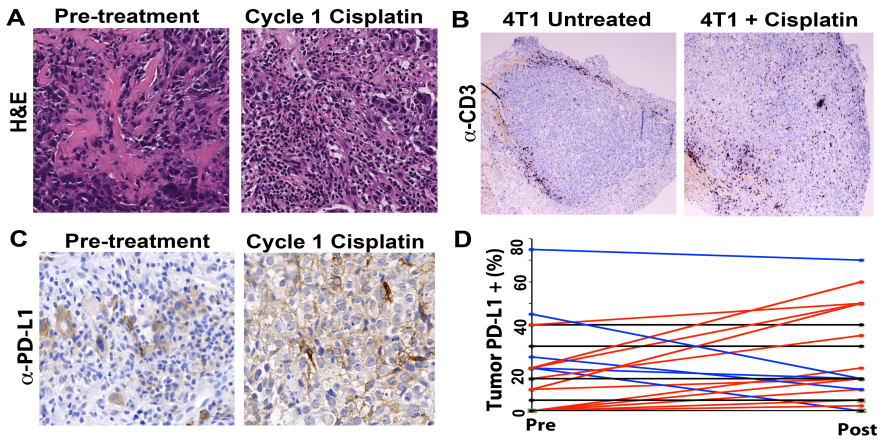
Lehmann, Chen, Shyr, Sanders, Pietenpol; PLoS One, 2016
Shaver, Lehmann, Beeler, Shyr, Pietenpol; Cancer Res, 2016

Neo-adjuvant Chemotherapy Induces Changes in Immune Cell Infiltration



Pre-Clinical and Clinical Data

Tumor-infiltrating lymphocytes and tumor PD-L1 expression in tumors after cisplatin treatment



(BRE0904; NCT00930930)

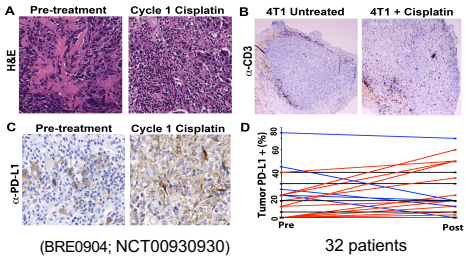
32 patients

Lehmann, Chen, Shyr, Sanders, Pietenpol; PLoS One, 2016

Jovanovic, Mayer, et al., Pietenpol., *Clin Cancer Res*, 2017

Chemoimmunotherapy in 1st/ 2nd line Metastatic TNBC
NCT03206203 (TBCRC030)

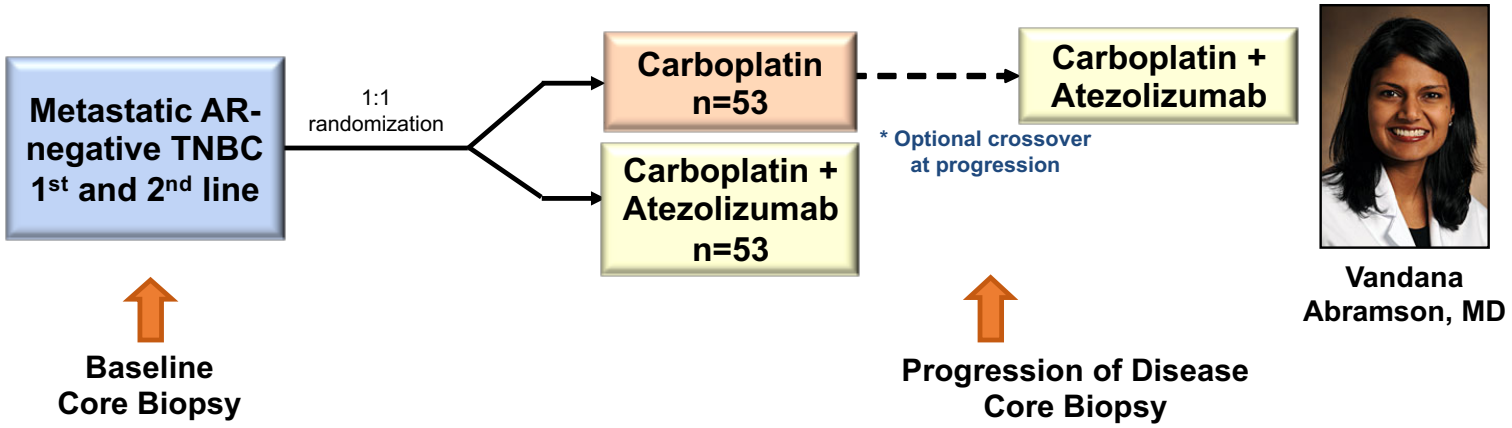
Pre-Clinical and Clinical Data
Tumor-infiltrating lymphocytes and tumor PD-L1 expression in tumors after cisplatin treatment



(BRE0904; NCT00930930)

32 patients

TBCRC 030: Phase Ib/II Study of PD-L1 Inhibitor Atezolizumab with Carboplatin in AR-negative TNBC



Vandana Abramson, MD

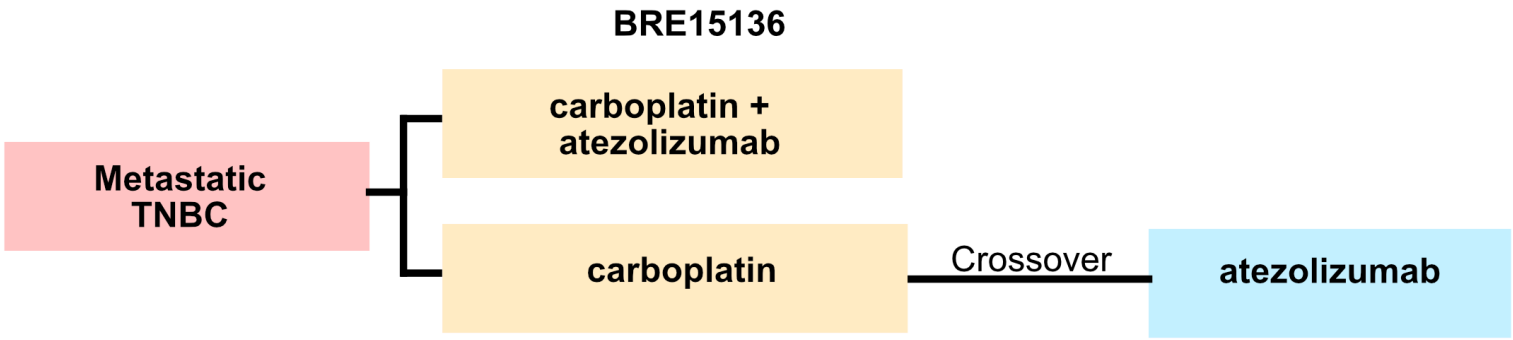
Goals:

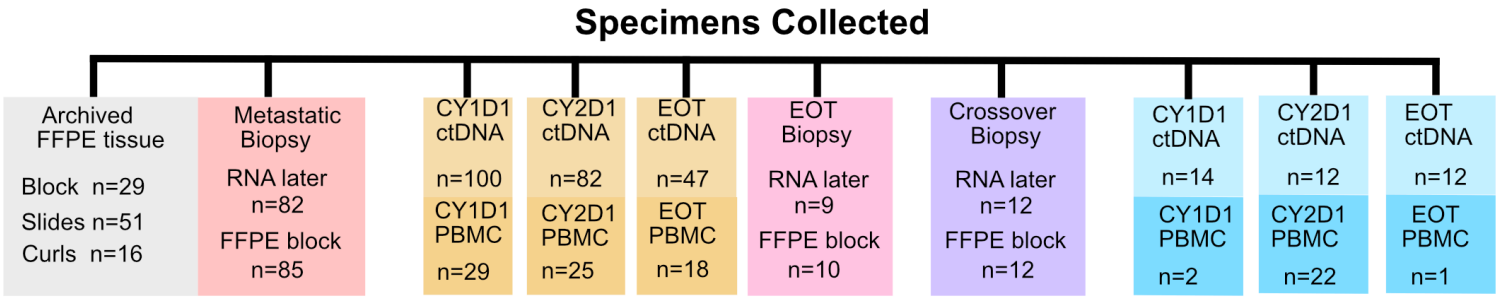
- Clinical efficacy of treatment; comparison to IMpassion130 data
- Immunophenotyping
- Identification of biomarkers for response and mechanism
- Analysis of response relative to TNBC subtype

Accrual Complete: Determine mechanisms by which carboplatin modulates tumor immune response in combination with checkpoint immunotherapy

Team work across NCI Centers

Center	Accrual #
Vanderbilt	31
U Penn	18
Georgetown	14
Indiana	18
Johns Hopkins	5
UNC	20
	106





EOT= End of treatment

Analysis of biospecimens from patients (3000 samples)

Analysis for:

(a) tumor size
(b) neo-antigenic burden
(c) PD-L1 expression
(d) TNBC subtype
(e) antigen presentation
(f) microenvironment composition
(g) tumor and blood immune cell composition

Tumor Tissue

Pretreatment	EOT	crossover
RNA later n=82	n=9	n=12
FFPE curls n=16		

Tumor genomics

Assays	Biomarker	Core
RNA-seq	TNBC type	TEMPUS/ VANTAGE
DNA-seq	Mutation load	

Tumor Tissue

Pretreatment	EOT	crossover
FFPE n=85	n=9	n=12
FFPE slides n=51		

Intra-tumor immune cell characterization

Slide	Assays	Biomarkers	Core
1	PD-L1	PD-L1	HistogeneX
2	H&E	TILs,TIME	PITC
3-10	Multiplex-IF	CD8,CD4, CD68 PD-L1, B7-H4 FOXP3, pan CK HLA-A, GZMB	PITC

Peripheral blood monuclear cells PBMCs

CYCLE 1	CYCLE 2	EOT	CYCLE 1	CYCLE 2	EOT

Peripheral immune cell characterization

Assays	Biomarkers	Core
Flow cytometry	CD8, CD4, CD3, CD68,PD-L1, IFNG, TIGIT, FOXP3, GZMB, ICOS, Tim-3	Immunophentyping Core
CyTOF		Immunophentyping Core

Blood plasma

CYCLE 1	CYCLE 2	EOT	CYCLE 1	CYCLE 2	EOT

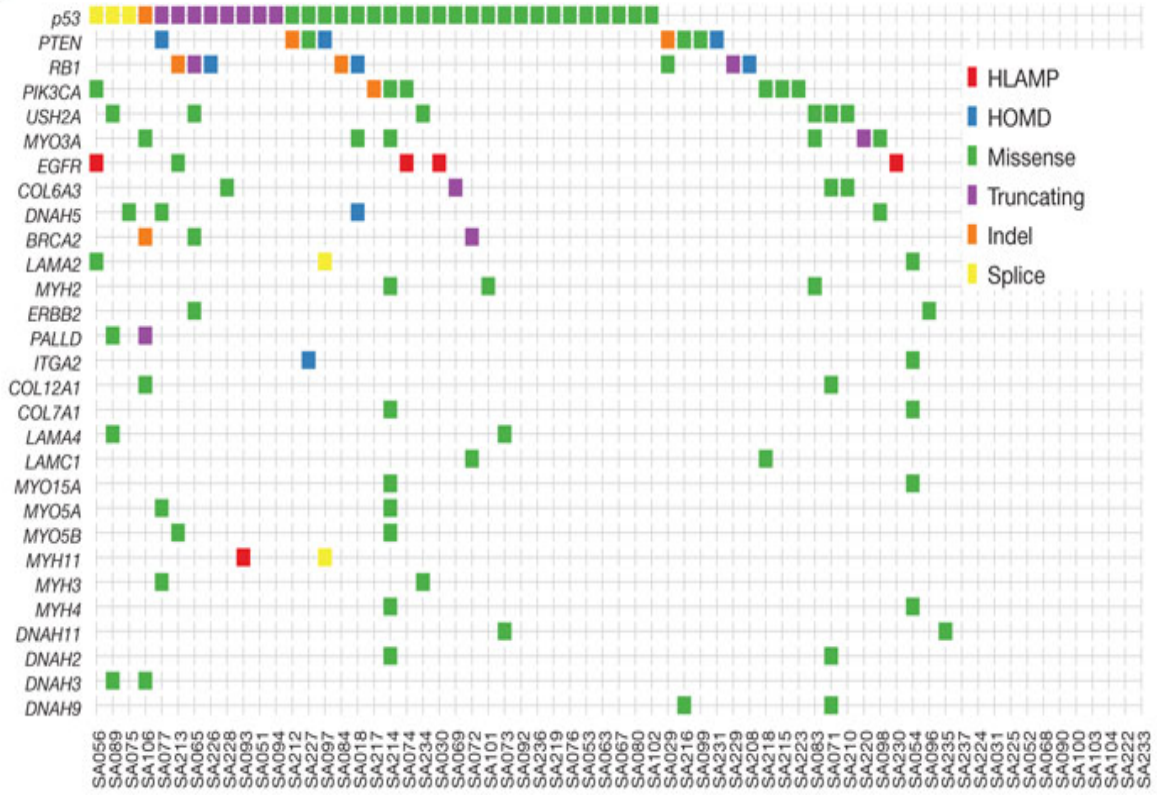
Circulating tumor DNA analysis

Assays	Biomarkers	Core
ctDNA	Process to be dictated by Ben Ho Park	Park Lab
ELISA	B7-H4	Pietenpol Lab

Expanding the spectrum of targetable molecular features in TNBC

- Can we identify TNBC subtypes and targetable features of the “adapted states” of subtypes, including immunomodulation?

- What is the extent of intra-tumor heterogeneity in TNBC and is it targetable?



Shah *et al*. *Nature* 2012

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Targeting MYCN-expressing triple-negative breast cancer with BET and MEK inhibitors

Johanna M. Schafer[1], Brian D. Lehmann[2,3]*, Paula I. Gonzalez-Ericsson[3]*, Clayton B. Marshall[1,3]*, J. Scott Beeler[1], Lindsay N. Redman[1], Hailing Jin[3], Violeta Sanchez[3], Matthew C. Stubbs[4], Peggy Scherle[5], Kimberly N. Johnson[3], Quanhu Sheng[6], Joseph T. Roland[7], Joshua A. Bauer[1,8], Yu Shyr[6], Bapsi Chakravarthy[3,9], Bret C. Mobley[10], Scott W. Hiebert[1,3], Justin M. Balko[2,3], Melinda E. Sanders[3,10], Phillip C. C. Liu[11], Jennifer A. Pietenpol[1,3]†

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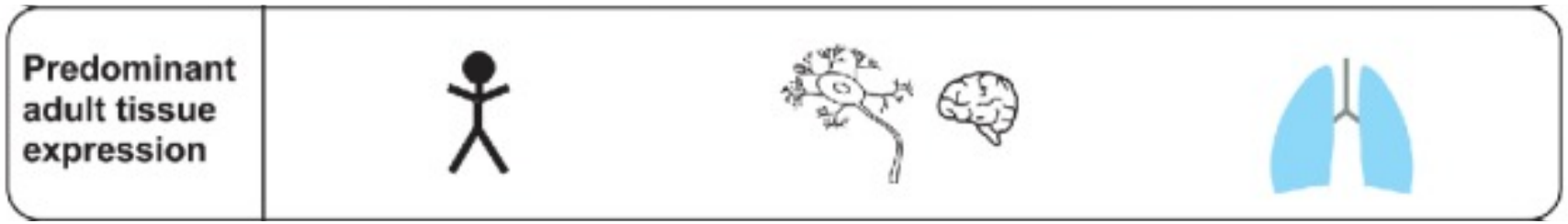
Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer that does not respond to endocrine therapy or human epidermal growth factor receptor 2 (HER2)–targeted therapies. Individuals with TNBC experience higher rates of relapse and shorter overall survival compared to patients with receptor-positive breast cancer subtypes. Preclinical discoveries are needed to identify, develop, and advance new drug targets to improve outcomes for patients with TNBC. Here, we report that MYCN, an oncogene typically overexpressed in tumors of the nervous system or with neuroendocrine features, is heterogeneously expressed within a substantial fraction of primary and recurrent TNBC and is expressed in an even higher fraction of TNBCs that do not display a pathological complete response after neoadjuvant chemotherapy. We performed high-throughput chemical screens on TNBC cell lines with varying amounts of MYCN expression and determined that cells with higher expression of MYCN were more sensitive to bromodomain and extraterminal motif (BET) inhibitors. Combined BET and MEK inhibition resulted in a synergistic decrease in viability, both in vitro and in vivo, using cell lines and patient-derived xenograft (PDX) models. Our preclinical data provide a rationale to advance a combination of BET and MEK inhibitors to clinical investigation for patients with advanced MYCN-expressing TNBC.

Johanna Schafer, PhD

Melinda Sanders, MD

Paula Gonzalez-Ericsson, MD

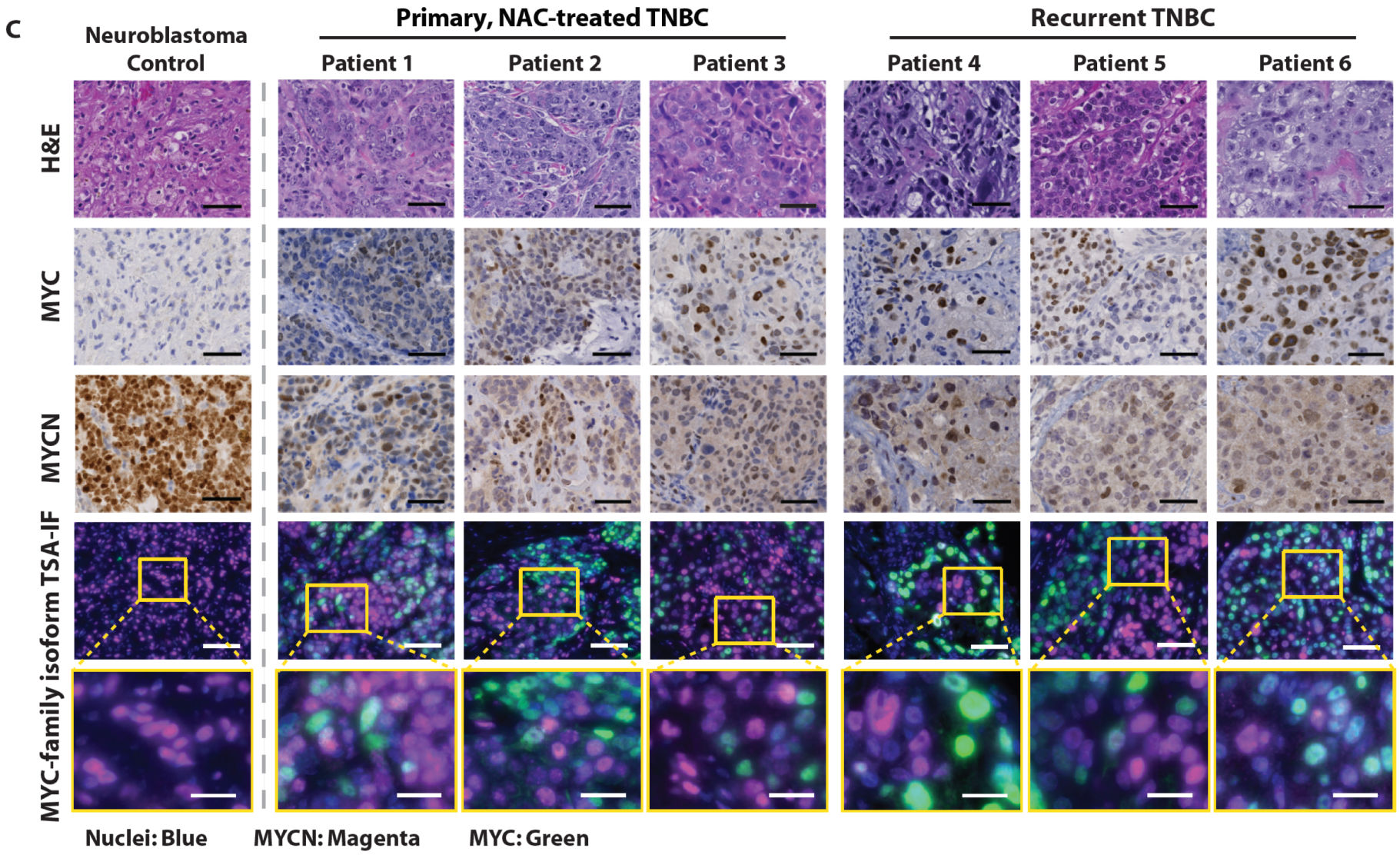
MYC-family isoform expression in human tissue & tumors



	MYC	*MYCN*	*MYCL*
In tumors:	**Nearly all tissue types**	**Sympathetic and central nervous system**	**Lung cancer**

Cell Cycle. 2017. 16(16): 1489-1498.

Intra-tumoral heterogeneity of MYCN and MYC expression in TNBC



Tyramide signal-amplified immunofluorescence TSA-IF

MYCN is expressed in neuronally-derived & non-neuronal tumors Correlates with a poor prognosis

Table 1. N-myc–amplified tumors

Tumor type	Frequency of N-myc alteration	Clinical implications	References
Neuroblastoma	Amplification in 20%	Poor prognosis, selection for aggressive treatment	Seeger et al. (13), Brodeur et al. (14), Look et al. (15), and Schneiderman et al. (17)
Medulloblastoma	Amplification in 5%	Poor prognosis	Aldosari et al. (92) and Swartling et al. (19)
Glioblastoma multiforme	Overexpression in a subset	Histone 3.3 mutations associated with overexpression of N-myc	Hodgson et al. (93) and Bjerke et al. (24)
Retinoblastoma	Amplification in <5%	Early onset, unilateral, nonhereditary, poor prognosis	Lee et al. (25) and Rushlow et al. (28)
Alveolar rhabdomyosarcoma	Amplification in 25%, overexpressed in 55%	Correlates with presence of PAX3-FOXO1 or PAX7-FOXO1 fusion genes, poor prognosis	Tonelli et al. (94)
Small-cell lung cancer	Amplification in 15%–20%	Poor response to chemotherapy, shorter survival	Nau et al. (32) and Funa et al. (34)
Prostate cancer	Amplification in 40% of neuroendocrine prostate cancer, 5% of prostate adenocarcinoma	Clinical aggressiveness	Beltran et al. (35) and Mosquera et al. (36)

Neuronally-derived cancers

Non-neuronally-derived cancers

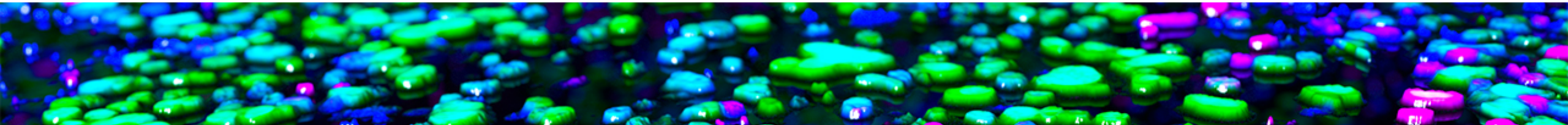
MYCN-expressing triple-negative breast cancer: Implications for therapeutic strategies

Assess levels of MYCN expression during TNBC disease progression

- At diagnosis (primary, treatment-naïve)
- After standard-of-care chemotherapy (residual disease)
- After recurrence (locally-advanced/metastatic)

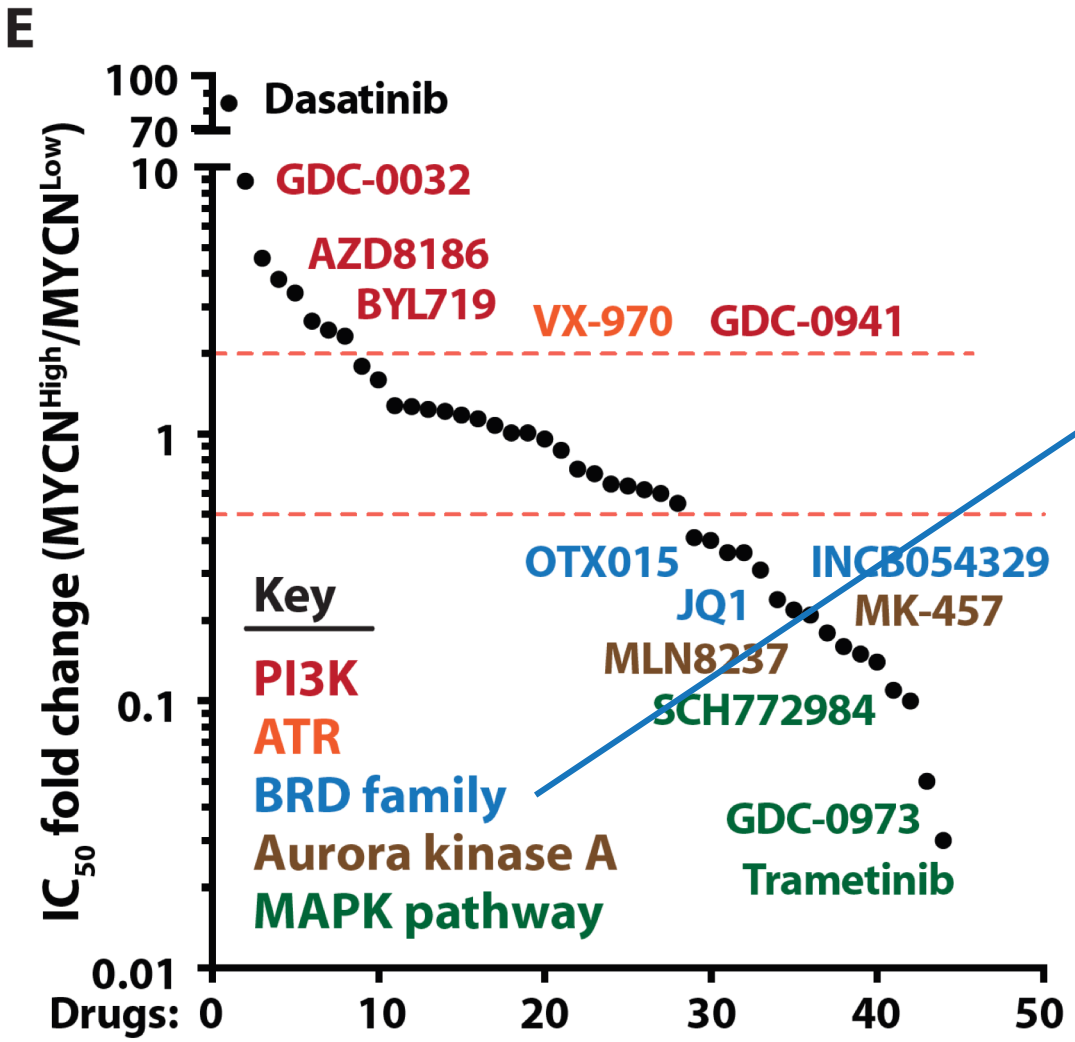
Determine and characterize MYCN-associated drug sensitivities

- *In vitro* cell line models
- *In vivo* with human TNBC tumors (patient-derived xenografts)



Evaluation of MYCN-expressing TNBC cell lines for drug sensitivity

National Cancer Institute (NCI) U.S. Food and Drug Administration (FDA)–approved oncology drug (AOD) library

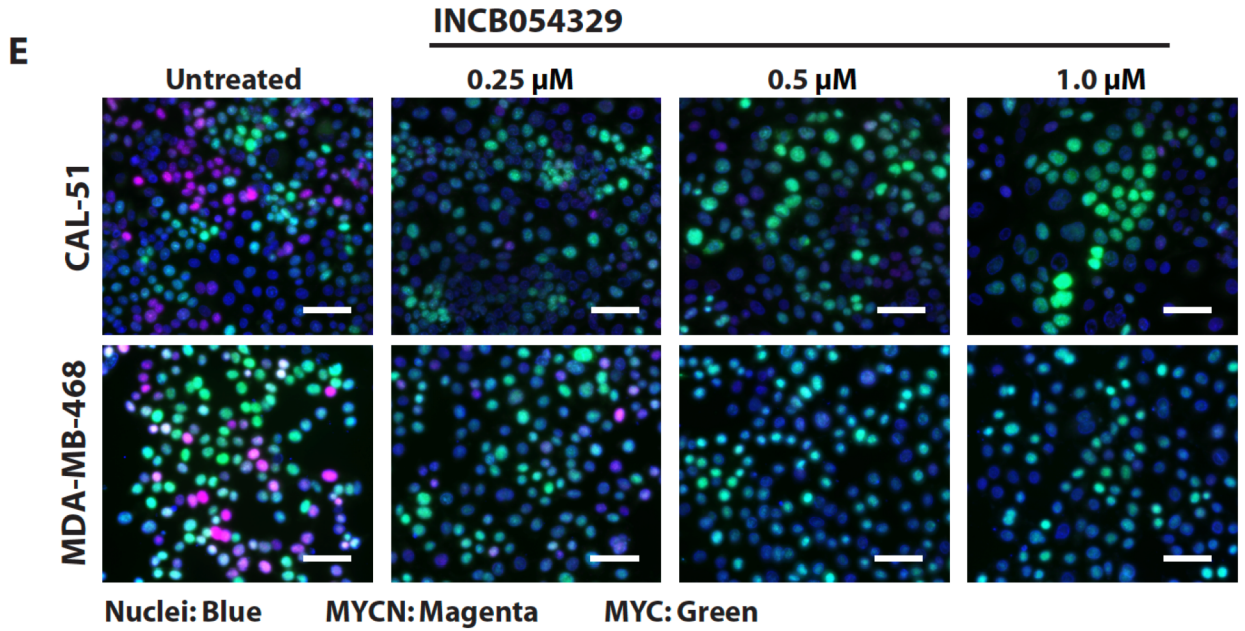


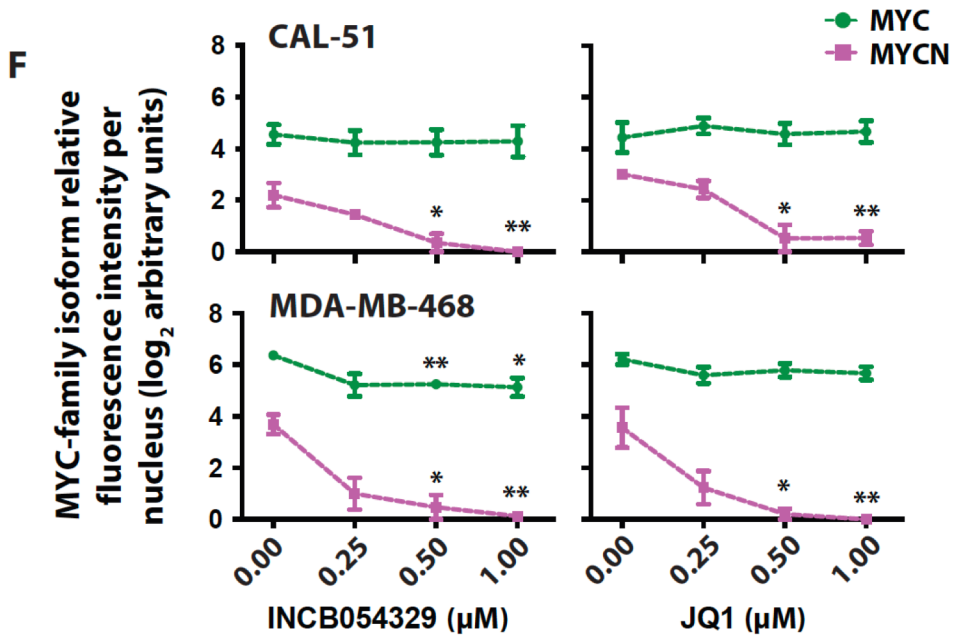
What is the BRD protein family?

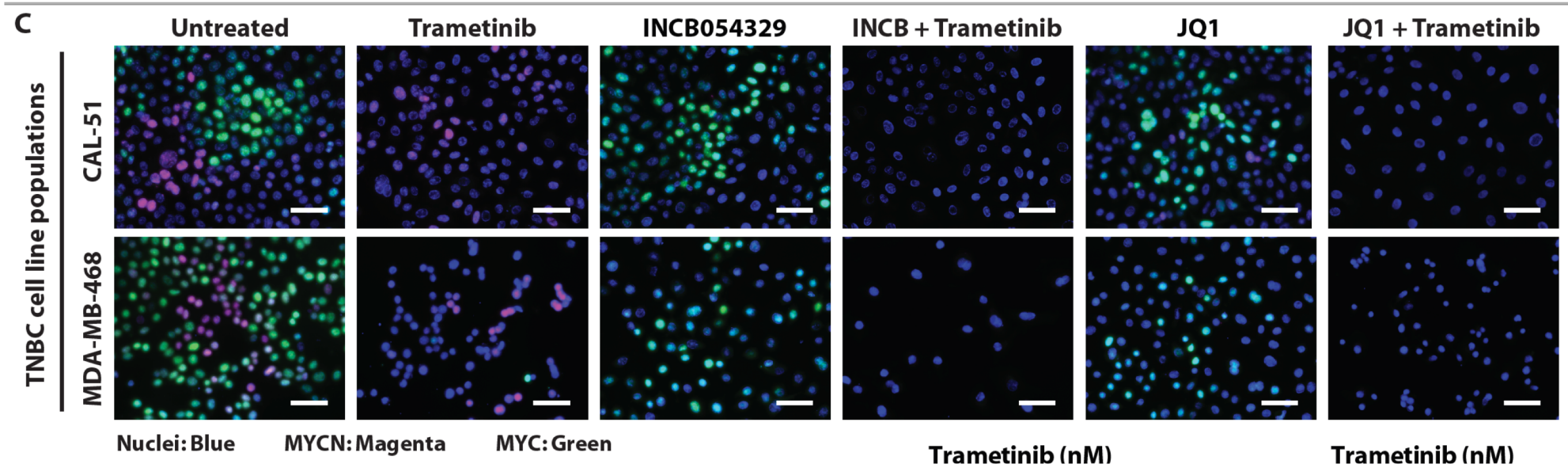
Bromodomain proteins are involved in a diverse range of functions, such as acetylating histones, remodeling chromatin, and recruiting other factors necessary for transcription. These proteins thus play a critical role in the regulation of transcription.

Inhibitors: JQ-1, INCB054329

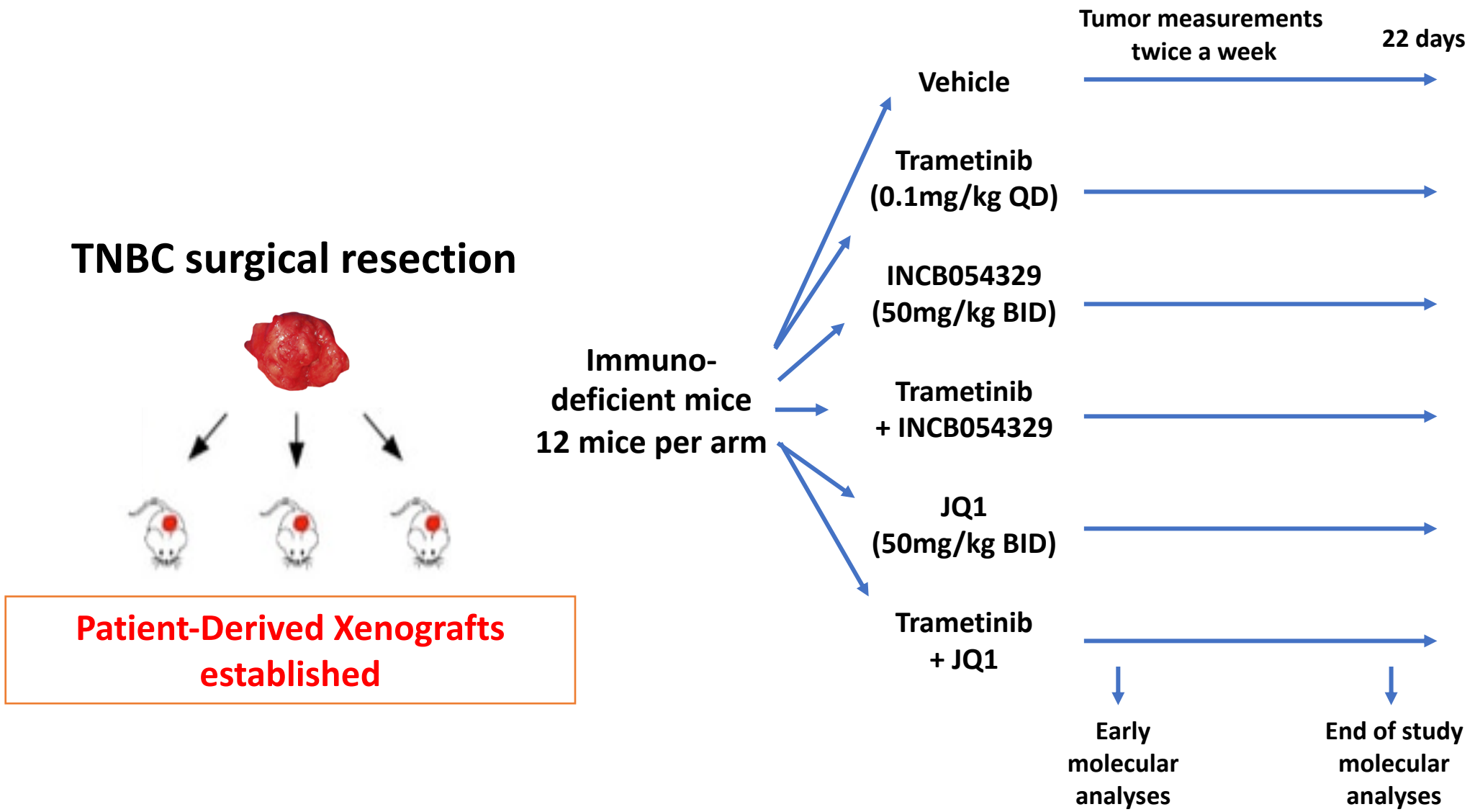
Evaluation of MYC family isoform expression after drug treatment





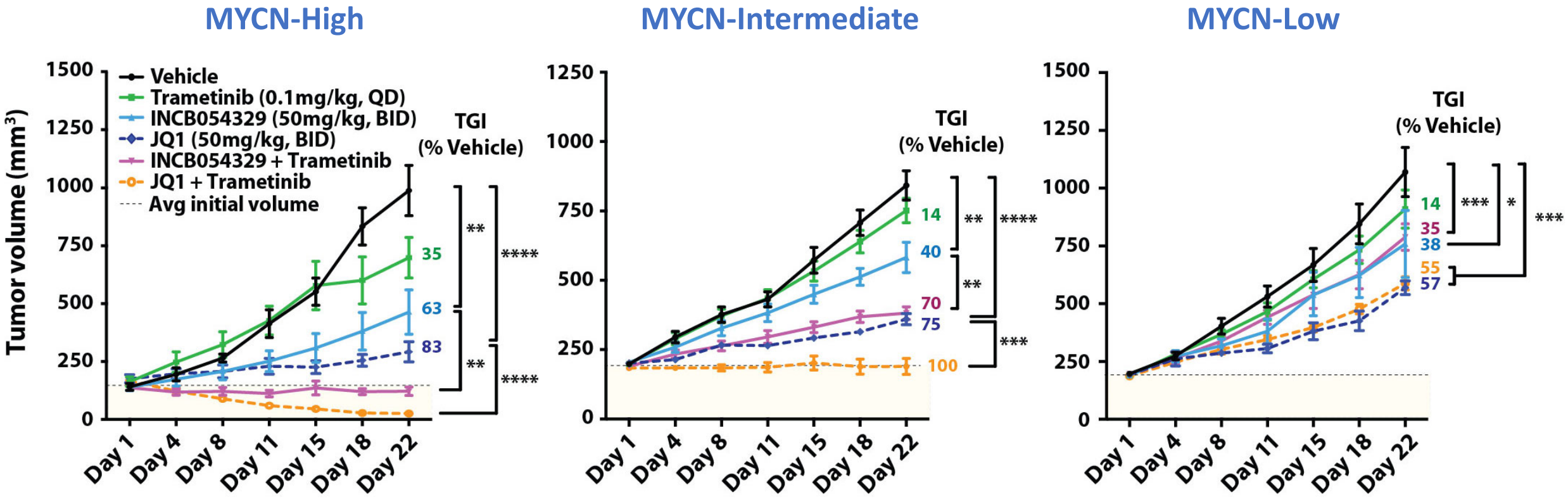


Schema for Patient-derived Xenograft (PDX) Murine "Clinical Trial"



Clayton Marshall, PhD
Kimberly Johnson

MYCN-low expressing PDX tumors do not have a synergistic decrease in viability after BETi and MEKi combination treatments *in vivo*



TGI = tumor growth inhibition

Clayton Marshall, PhD
Kimberly Johnson

Summary

- **MYCN-expressing cells are present in a significant fraction of TNBC tumors** (treatment-naïve, chemotherapy-treated, recurrent) and correlate with a less favorable prognosis

- Nearly half of TNBC tumors heterogeneously express MYCN and MYC with tumor-cell nuclei robustly express only one isoform

- MYCN-expressing cell have increased sensitivity to BET inhibitors, and BET inhibitors decrease MYCN expression

- Combined BET and MEK inhibitors synergistically decrease tumor-cell growth in cell lines and tumor models that express both MYCN and MYC

Expanding the spectrum of TNBC targetable states
TNBC-MATCH

Current practice

Therapy	Leading Compounds	Target	Tumor Biomarker	FDA-Approval
Chemotherapy	Anthracyclines Cis/Carboplatin Taxanes	Genomic Instability Microtubules	NA	All stages
PARP inhibitors	Olaparib Talazoparib	PARP PARP	Germline BRCA1/2 mutant	Advanced or metastatic
Immunotherapy Combination	Atezolizumab Taxane	PD-LI	PD-L1 expression	Advanced or metastatic

Unmet needs remain in TNBC treatment

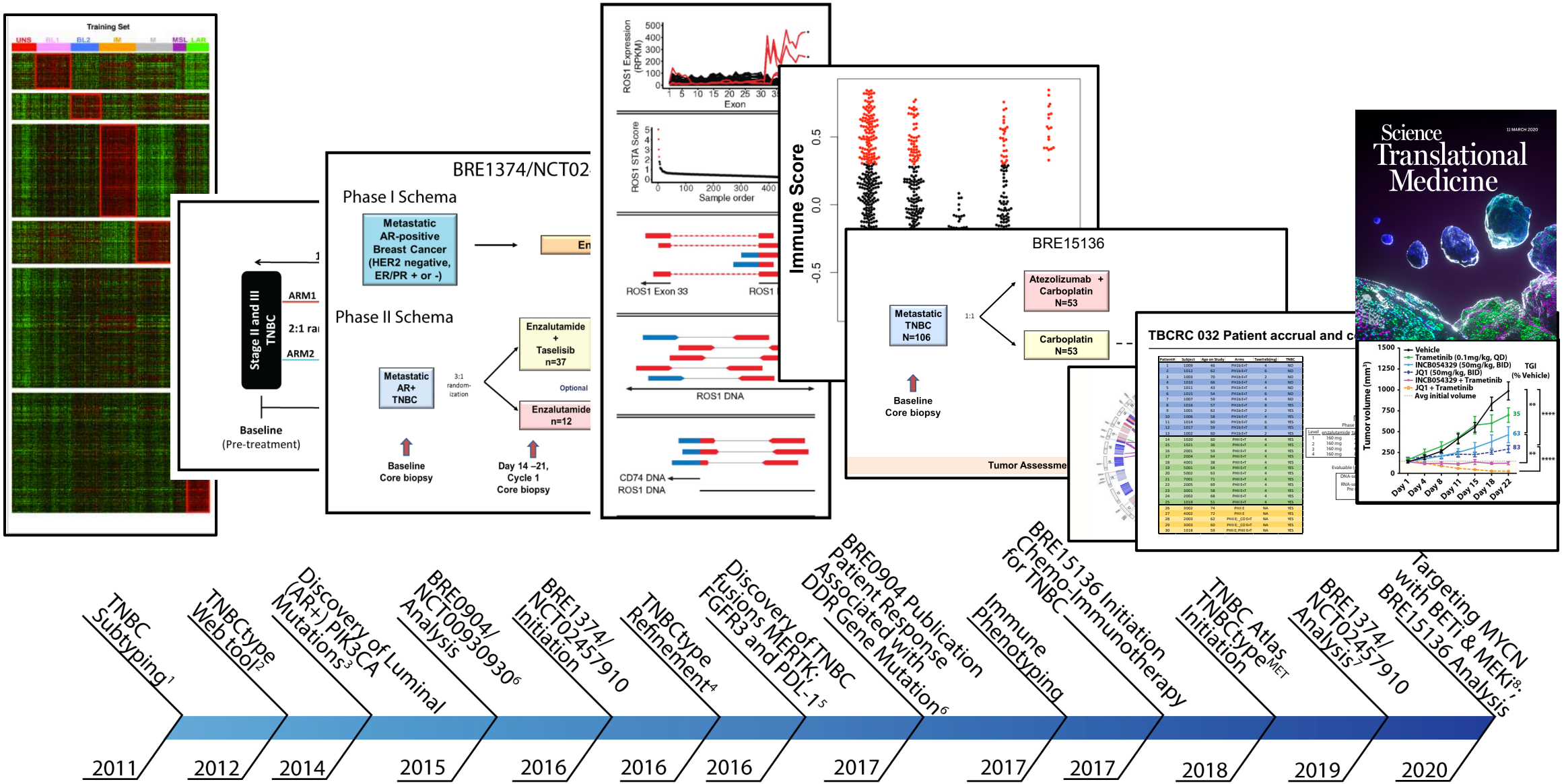
1. More durable and effective therapies in the management of patients who relapse early or are resistant to chemotherapy

2. Determine why immune reactions are suppressed as patients get further into their disease course with later lines of therapy, important for future trial combinations

Path Forward

Improve detection strategies to identify clinically relevant "states" in individual patients with TNBC tumors

- **Dependence on known targetable pathways**
  - BRCA1/2 alterations or "BRCAness" (PARP inhibitors)
  - LAR gene expression and PIK3CA alterations (AR antagonists and kinase inhibitors)
  - Heterogeneity – combinations of BETi, MEKi, PI3Ki
- **High frequency non-synonymous mutations / neo-antigens facilitating immunotherapy**; combination approach to target tumor and microenvironment; faulty DNA repair and expression of immune-oncology biomarkers (e.g. PD-L1)
- **Lower-frequency targetable alterations**
  - Gene fusions; high sensitivity detection can identify targetable alterations in a heterogeneous background (e.g. FGFR); proof-of-concept in mouse models



[1]Lehmann BD and Bauer JA *et al.*, *J Clin Invest*. 2011
[2]Chen X *et al.*, *Cancer Inform*. 2012
[3]Lehamann BD *et al.*, *Breast Cancer Res*, 2014
[4]Lehmann BD *et al.*, *PLos One*, 2016
[5]Shaver TM and Lehmann BD *et al.*, *Cancer Res*, 2016
[6]Jovanovic B *et al.*, *Clin Cancer Res*, 2017
[7]Lehmann BD and Abramson, B *et al.*, *Clin Cancer Res*, 2019
[8]Schafer JM et al., *Sci Trans Med*, 2020

Vanderbilt-Ingram Breast Cancer Patient Advocacy Program

Patty Lee

Lynn Cargen

Linda Horton

Janet Piper

Team Acknowledgements

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Jennifer Rosenbluth
Bojana Jovanovic

Biostatistics
Steven Chen
Qi Liu
Yu Shyr

Hematology/Oncology
Ingrid Mayer
Vandana Abramson

Pathology
Melinda Sanders
Paula Gonzalez-Ericsson

Radiation Oncology
Bapsi Chakravarthy

Incyte Corporation
Phillip Liu
Matthew Stubbs
Peggy Scherle

Patients who enrolled or will enroll in trials
Patient Advocates

NATIONAL CANCER INSTITUTE®

NCI Designated Comprehensive Cancer Center

NCI grants:
CA105436
CA068485
CA098131
CA183531

susan G. komen®
SAC110030

DEPARTMENT OF DEFENSE · UNITED STATES OF AMERICA

T.J. Martell Foundation

CANCER

Targeting MYCN-expressing triple-negative breast cancer with BET and MEK inhibitors

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Johanna M. Schafer[1], Brian D. Lehmann[2,3]*, Paula I. Gonzalez-Ericsson[3]*, Clayton B. Marshall[1,3]*, J. Scott Beeler[1], Lindsay N. Redman[1], Hailing Jin[3], Violeta Sanchez[3], Matthew C. Stubbs[4], Peggy Scherle[5], Kimberly N. Johnson[3], Quanhu Sheng[6], Joseph T. Roland[7], Joshua A. Bauer[1,8], Yu Shyr[6], Bapsi Chakravarthy[3,9], Bret C. Mobley[10], Scott W. Hiebert[1,3], Justin M. Balko[2,3], Melinda E. Sanders[3,10], Phillip C. C. Liu[11], Jennifer A. Pietenpol[1,3]†

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer that does not respond to endocrine therapy or human epidermal growth factor receptor 2 (HER2)–targeted therapies. Individuals with TNBC experience higher rates of relapse and shorter overall survival compared to patients with receptor-positive breast cancer subtypes. Preclinical discoveries are needed to identify, develop, and advance new drug targets to improve outcomes for patients with TNBC. Here, we report that MYCN, an oncogene typically overexpressed in tumors of the nervous system or with neuroendocrine features, is heterogeneously expressed within a substantial fraction of primary and recurrent TNBC and is expressed in an even higher fraction of TNBCs that do not display a pathological complete response after neoadjuvant chemotherapy. We performed high-throughput chemical screens on TNBC cell lines with varying amounts of MYCN expression and determined that cells with higher expression of MYCN were more sensitive to bromodomain and extraterminal motif (BET) inhibitors. Combined BET and MEK inhibition resulted in a synergistic decrease in viability, both in vitro and in vivo, using cell lines and patient-derived xenograft (PDX) models. Our preclinical data provide a rationale to advance a combination of BET and MEK inhibitors to clinical investigation for patients with advanced MYCN-expressing TNBC.

INTRODUCTION

Triple-negative breast cancer (TNBC) affects younger women and is characterized by increased rates of relapse, more frequent metastasis, and shorter survival compared to the other breast cancer subtypes (*1*). Although TNBC only represents ~15% of all breast cancer cases, it accounts for ~25% of all breast cancer–related deaths (*2*), with treatment options for most patients limited to cytotoxic chemotherapy. Prognosis is unfavorable for patients with metastatic TNBC, as >50% of patients with metastatic disease die within 1 year of diagnosis (*2*). Development of targeted therapies for TNBC is challenging because of its molecular heterogeneity and lack of therapeutically targetable, high-frequency driver alterations (*3*). Understanding the heterogeneity within TNBC and molecular mechanisms that contribute to the emergence of treatment-resistant, metastatic disease may inform the development of more effective therapeutics and address an unmet medical need in breast cancer.

Aside from *TP53*, most of the mutations found in TNBC are within the phosphoinositide 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) kinase 1/2 (MEK1/2) signaling pathways. The most frequent oncogenic mutations in TNBC occur in "hotspot" regions of the *PIK3CA* gene (E545 helical domain and H1047 kinase domain) (*4*), and the most frequently amplified oncogene is *MYC* (*5*, *6*). MYC family members—MYC, MYCN, and MYCL—are transcription factors that regulate the expression of genes involved in normal development, cell growth, proliferation, metabolism, and survival (*7*). Aberrant expression of MYC family members has been considered tumorigenic in a tissue-specific manner [*MYCN* in neuronal (*8*, *9*) or neuroendocrine (NE) tumors (*10*, *11*) and *MYCL* in lung (*7*)]. However, recent reports have shown elevated *MYCN* expression in nonneuronal tissues, such as ovarian (*12*) and prostate cancer (*13*), as well as hematopoietic cells that give rise to acute lymphoblastic (*14*) and myeloid (*15*) leukemias. Further, there is increasing evidence that *MYCN* expression is deregulated in a subset of breast cancers with unfavorable prognostic features and clinical outcomes (*16*–*18*). *MYCN* transcript has been found in circulating breast tumor cell clusters within the bloodstream of breast cancer patients (*19*) and is associated with a stem cell program found in tumor-initiating metastatic cells (*18*), implicating a role for *MYCN* in the recurrence and metastatic spread of breast cancer.

To determine the overall frequency of MYCN-expressing tumors in primary TNBC and whether MYCN expression changes in response to neoadjuvant chemotherapy (NAC), we evaluated TNBC patient cohorts composed of primary, treatment-naïve tumors or primary, NAC-treated tumors. We also evaluated the quantity of MYCN RNA and protein in the metastatic setting. In parallel, we investigated the biological relevance of MYCN versus MYC expression in TNBC cells and whether MYCN expression was associated with response to compounds currently or previously under clinical development [including the National Cancer Institute (NCI) U.S. Food and Drug Administration (FDA)–approved oncology drug (AOD) library]. Top "hits"

[1]Department of Biochemistry, Vanderbilt University, Nashville, TN 37232, USA. [2]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [3]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [4]Incyte Corporation, Wilmington, DE 19803, USA. [5]Prelude Therapeutics, Wilmington, DE 19803, USA. [6]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [7]Section of Surgical Sciences, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [8]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37232, USA. [9]Department of Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [10]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [11]Kymera Therapeutics, Cambridge, MA 02139, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: j.pietenpol@vumc.org

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from the drug screen were examined as single agents and in combination, in vitro, and in mice harboring TNBC patient-derived xenografts (PDXs) with differing amounts of MYCN. We found that combined bromodomain and extraterminal motif (BET) and MEK inhibition synergistically inhibited growth of MYCN-expressing PDX TNBC tumors.

RESULTS

A substantial fraction of primary TNBCs express MYCN

To evaluate *MYCN* expression in TNBC, we first identified TNBC tumors from primary, treatment-naïve cases in The Cancer Genome Atlas (TCGA) Breast Invasive Carcinoma (BRCA) dataset (fig. S1A) (*4*). *MYCN* transcript was expressed in all tumors [transcript per million (TPM) >0] and elevated (>12 TPM, >1 SD above the mean) in 10.2% (20 of 197) of cases (Fig. 1A). Likewise, we detected elevated *MYCN* expression in a similar proportion of primary TNBC cases (fig. S1B) in two other datasets, TNBC587 (>0.65 median-centered $\log_2$ normalized, $n = 65$ of 587) (*20*) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (>7 $\log_2$ normalized, $n = 48$ of 323) (fig. S2, A and B) (*21*). To gain insight into the biological relevance of *MYCN* expression in TNBC, we compared the amount of *MYCN* transcript in primary, treatment-naïve TNBC (source: TCGA, BRCA) to transcript expressed in known MYCN-driven cancers (Fig. 1B) (*22*). Cancers with *MYCN* gene amplifications such as neuroblastoma (NB), glioblastoma multiforme (GBM), and acute myeloid leukemia (AML) originate from migrating neural crest cells, neural stem cells, or hematopoietic stem cells, respectively (*22*). *MYCN* is also amplified or overexpressed in at least 20 and 60% of adenocarcinoma (Adeno) and NE castration-resistant prostate cancer (CRPC) cases, respectively (*10*, *13*). Although the amount of transcript in TNBC was not as high as in NB (*23*), AML (source: TCGA, LAML), or GBM (source: TCGA, GBM), *MYCN* expression was similar to NE-CRPC and significantly higher ($P < 0.0001$) than Adeno-CRPC (Fig. 1B and data file S1) (*24*, *25*). Further, elevated *MYCN*-expressing TNBC cases identified in TCGA (Fig. 1A) had higher *MYCN* expression than the top *MYCN*-expressing NE-CRPC tumors (Fig. 1B and data file S1).

Because the *MYCN* transcript in clinical specimens could have originated from tumor or tumor-infiltrating immune or stromal cells, we performed MYCN immunohistochemistry (IHC) to identify the cellular distribution of MYCN protein in an independent cohort of 191 primary, treatment-naïve TNBC tumors, curated at Vanderbilt University Medical Center (VUMC) and US Biomax. IHC demonstrated that 45% of specimens contained nuclear MYCN within tumor cells, and similar to our RNA analyses, 11.5% of cases had high expression (H-score > 30, >1 SD above the mean) (Fig. 1, C and D, and data file S2). IHC specificity was confirmed with positive and negative controls from PDXs and cell line–derived xenografts (CDXs), including SK-N-BE(2)C, a validated *MYCN*-amplified NB CDX (fig. S3, A and B; table S1A; and data file S1) (*26*). The relative amounts of *MYCN* transcript highly correlated with IHC protein quantities (H-score) across two PDX cohorts (cohort1, $R^2 = 0.968$; cohort2, $R^2 = 0.822$), further validating antibody specificity (fig. S3, C and D; table S1, A and B; and data file S1). Collectively, these data demonstrated the prevalence of MYCN protein in TNBC tumor cell nuclei and provided rationale to further characterize MYCN-expressing cells in the context of disease etiology.

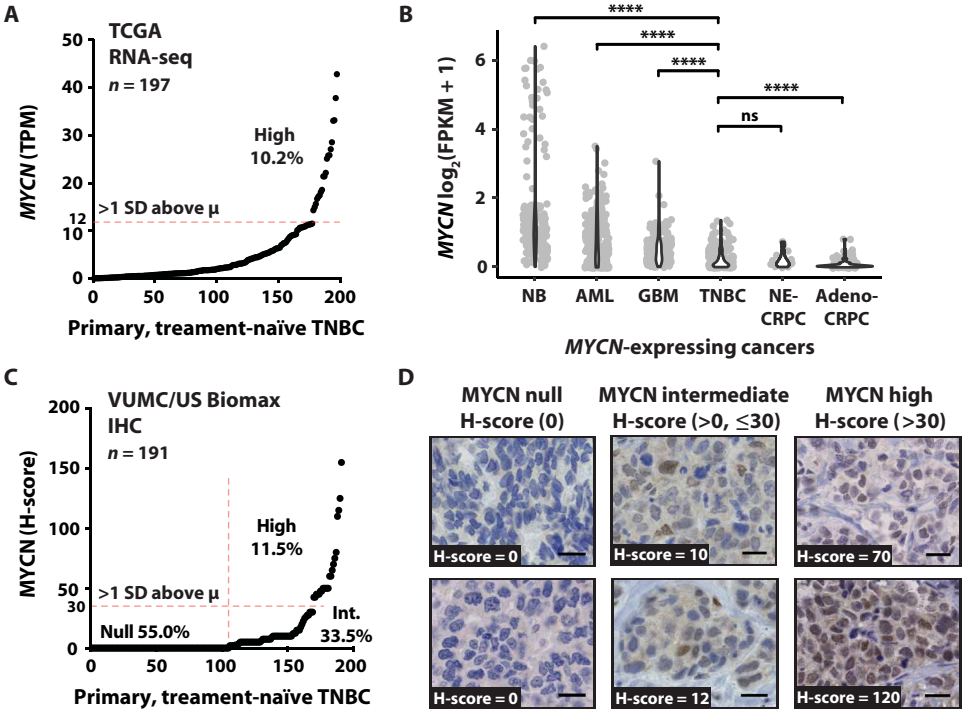

Fig. 1. *MYCN* RNA and MYCN protein expression in primary, treatment-naïve TNBC. (**A**) *MYCN* transcript (TPM) from 197 primary, treatment-naïve TNBCs (source: TCGA, BRCA). μ, mean. (**B**) Violin plot showing *MYCN* expression in TNBC (source: TCGA, BRCA; $n = 197$) compared to neuroblastoma (NB; $n = 161$) (*23*), acute myeloid leukemia (AML) (source: TCGA, LAML; $n = 173$), glioblastoma multiforme (GBM) (source: TCGA, GBM; $n = 156$), and castration-resistant prostate cancer (CRPC), including neuroendocrine (NE; $n = 15$) and adenocarcinoma (Adeno; $n = 123$) (*24*, *25*). Wilcoxon rank sum test comparing TNBC to the other cancer types. *P* values were adjusted by false discovery rate (FDR), ****$P < 0.0001$. ns, not significant. (**C**) MYCN protein quantities (H-scores) from 191 primary, treatment-naïve TNBCs [source: Vanderbilt University Medical Center (VUMC) and US Biomax]. Int., intermediate. (**D**) Representative MYCN IHC images in TNBC specimens devoid of MYCN protein expression (H-score = 0), which contain intermediate amounts of MYCN (H-score between >0 and ≤30) or have high MYCN (H-score > 30). Scale bars, 20 μm.

Increased fraction of MYCN-expressing cells in residual TNBC after NAC

Because of the lack of therapeutic targets in TNBC, patients are primarily treated with combination chemotherapy, and less than 30% of patients achieve a pathological complete response (pCR) after NAC (*27*, *28*). Patients with residual disease after NAC exhibit poor overall survival due to an enrichment of chemotherapy-resistant tumor cells and a lack of subsequent therapeutic options (*29*, *30*). To evaluate MYCN expression in residual tumor cells after NAC, we performed IHC for MYCN on a primary TNBC cohort ($n = 115$) with residual disease surgically resected after NAC (Fig. 2A, table S2A, and data file S2) (*6*). MYCN expression

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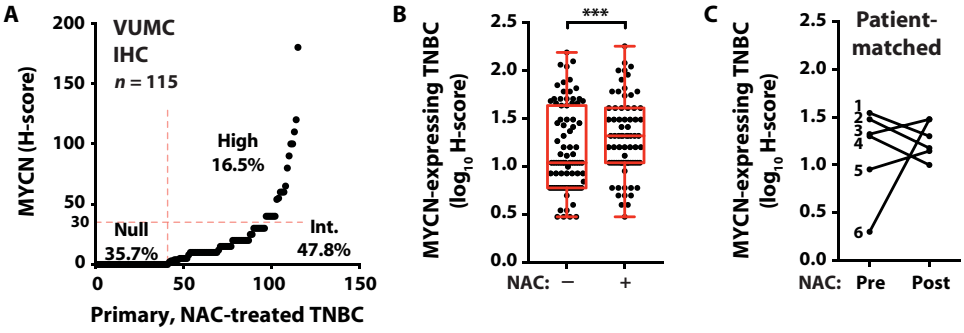

Fig. 2. Increased percentage of MYCN-expressing cells in residual disease after NAC. (**A**) MYCN H-scores in residual disease from 115 primary, NAC-treated TNBCs (source: VUMC). Null, H-score = 0; Int., H-score >0 to ≤30; High, H-score > 30. (**B**) Box plot showing MYCN H-scores in primary, treatment-naïve TNBC cases (n = 191; see Fig. 1C) compared to residual disease from primary, NAC-treated TNBC cases [n = 115; see (A)]. Wilcoxon rank sum test, ***P = 0.0001. (**C**) MYCN H-scores in patient-matched TNBC cases before and after NAC (n = 6; see table S2C for treatments and patient characteristics).

was significantly (P = 0.001) higher in the post–NAC-treated TNBC cohort (Fig. 2A and data file S2) compared to cases in the treatment-naïve TNBC cohort (Fig. 1C), with 65% versus 45% of cases having an H-score greater than zero (Fig. 2, A and B, and table S2B). Most (90%) of the patients in the NAC-treated TNBC cohort had stage III disease at the time of diagnosis, whereas the treatment-naïve cohort consisted primarily of patients with stage I (11%) and stage II (70%) disease (table S2A). To remove a potential bias due to differences in clinical stage between cohorts, we restricted the comparison of MYCN expression to tumors from patients with stage III disease from each cohort; MYCN expression (H-score > 0) remained significantly (P = 0.014) higher in the residual disease of patients after NAC treatment (65%, 54 of 83) compared to treatment-naïve patients (40%, 10 of 25) (table S2B). Because the primary treatment-naïve and NAC-treated TNBC cohorts were independently assembled, we examined MYCN expression in patient-matched TNBC before and after NAC treatment (n = 6) (table S2C). Compared to the quantity of MYCN protein before treatment, MYCN protein expression was similar or increased after NAC, demonstrating that MYCN-expressing cells remained after treatment (Fig. 2C and data file S1). These data suggest that either MYCN expression was induced or preexisting MYCN-expressing tumor cells persisted in the TNBC cell populations after chemotherapy.

Primary and metastatic TNBC display heterogeneous MYCN and MYC protein expression

Despite better initial responses to NAC in TNBC compared to the other breast cancer subtypes, patients with TNBC experience higher rates of relapse and a worse overall survival in the metastatic setting (*27*). Given that nearly all women with metastatic TNBC ultimately die of their disease (*31*), we evaluated *MYCN* expression in the context of disease recurrence. We analyzed the TNBC cases from a recent study evaluating transcriptional changes between primary and metastatic breast cancer (fig. S4A) (*32*). *MYCN* transcript was increased or similarly expressed in nearly all metastatic specimens compared to matched primary TNBC, and *MYCN* was expressed at all metastatic sites evaluated [adrenal gland, lymph node, liver, lung, chest (chest wall, rib, pleura, and mediastinum), neural tissue (brain and spine), kidney, and skin] (fig. S4B and data file S1). Similarly, we performed MYCN IHC on 10 locally recurrent (5 chest wall and 5 skin) and 28 metastatic (5 lung and 23 brain) surgically resected TNBC tumors and detected MYCN protein expression (H-score > 0) in 55% (21 of 38) of the recurrent TNBC tumors analyzed [lung, 80% (4 of 5); skin, 80% (4 of 5); chest wall, 60% (3 of 5); brain, 43% (10 of 23)] (Fig. 3A and data file S2).

Because *MYCN* expression has been shown to be elevated in newly seeded metastatic TNBC lesions that differentiate into high *MYC*-expressing proliferative tumors (*18*), we investigated the relationship between MYC family isoforms (MYCN and MYC) in both primary and recurrent TNBCs. We performed MYC IHC on tissue representing each of our TNBC patient cohorts [primary, treatment-naïve TNBC (Fig. 1C); primary, NAC-treated TNBC (Fig. 2A); and recurrent TNBC (Fig. 3A)] previously analyzed for MYCN. Thirty-four percent (30 of 88) of primary, treatment-naïve TNBC; 49% (56 of 114) of primary, NAC-treated TNBC; and 50% (19 of 38) of recurrent TNBC expressed both MYC family isoforms (Fig. 3B). *MYCN* and *MYC* can be expressed both spatially and temporally in a mutually exclusive manner during normal tissue development (*33*); thus, we assessed the distribution of these proteins in individual cells within a given tumor section using dual MYC family isoform tyramide signal–amplified immunofluorescence (TSA-IF). We found that both MYCN and MYC were heterogeneously expressed in tumor cells throughout the sections, and most of the cell nuclei robustly expressed only one MYC family member (Fig. 3C and fig. S5). These data demonstrate the cell-to-cell heterogeneity of MYC family isoform expression in TNBC and the dynamic distribution of expression of these oncogenes at both primary and metastatic sites.

Preclinical models of MYCN-expressing TNBC

To identify MYCN-expressing TNBC cell line models for preclinical evaluation, we assessed *MYCN* expression across TNBC cell lines in the Cancer Cell Line Encyclopedia (*34*). CAL-51 and MDA-MB-468 displayed the highest amounts of *MYCN* transcript (fig. S6A). Given that TNBC clinical specimens displayed heterogeneous MYCN and MYC expression (Fig. 3C), we evaluated whether this heterogeneity existed within TNBC cell line models. We adapted our TSA-IF staining procedure used on formalin-fixed, paraffin-embedded tumor sections to cells fixed in situ after growth as adherent cultures and analyzed cellular MYCN and MYC expression within the CAL-51 and MDA-MB-468 cell populations. Individual cells in either cell line culture robustly expressed either nuclear MYCN or MYC (Fig. 4A), consistent with observed MYC family isoform heterogeneity in clinical specimens (Fig. 3C). To further evaluate the biological characteristics of MYCN-expressing tumor-derived cells, we isolated single cells from the CAL-51 parental cell line and generated clonally derived cell lines. Individual clones displayed varying MYCN and MYC protein expression, with 6% (2 of 33) of cells exhibiting elevated MYCN (Fig. 4B). MYCN and MYC protein quantities were consistent with the relative *MYCN* and *MYC* transcript in six of the clonal cell lines evaluated (Cln3, Cln5, Cln8, Cln15, Cln37, and Cln39; fig. S6, B and C), and individual MYC family isoform RNA and protein

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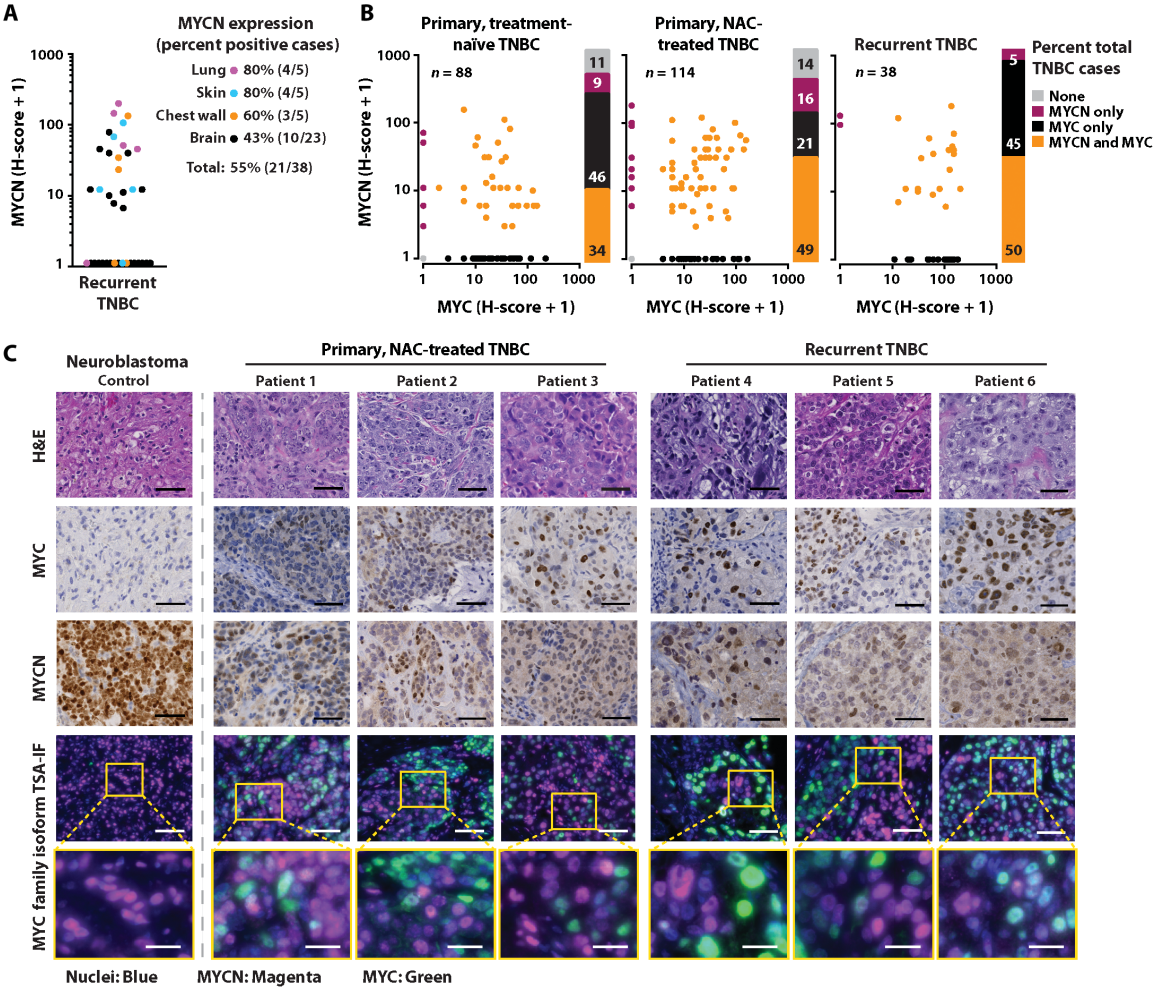

Fig. 3. Intratumoral heterogeneity of MYCN and MYC expression in TNBC. (**A**) MYCN H-scores from 38 recurrent TNBC cases with quantification of percent positive cases (H-score > 0) for each site of recurrence, labeled by color [lung (magenta), skin (blue), chest wall (orange), and brain (black)]. (**B**) MYCN and MYC H-scores for each of the 88 primary, treatment-naïve; 114 primary, NAC-treated; and 38 recurrent TNBC cases. Stacked bar graphs represent the percentages of total cases expressing (H-score) each MYC family isoform [alone (MYCN only and MYC only), both isoforms (MYCN and MYC), or neither isoform (None)]. (**C**) Representative hematoxylin and eosin (H&E), IHC, and TSA-IF stains of MYCN and MYC in primary and recurrent TNBC. Individual fluorescence images for cell nuclei (blue), MYCN (magenta), and MYC (green) can be found in fig. S5. The dashed lines separating a *MYCN*-amplified NB-positive control from TNBC cases represent the same exposure times for all samples but a decreased brightness adjustment for MYCN in the NB control due to overexpression of MYCN. Tumor images do not represent serial sections. Scale bars, 50 μm (top four rows) and 20 μm (bottom row).

were expressed at higher quantities in the clonal lines as compared to the CAL-51 cell population (fig. S6, B and C). Thus, the CAL-51 cell line is composed of a heterogeneous population of cells with varying MYC family isoform expression.

CAL-51 cells harbor an activating *PIK3CA* mutation (E542K), and their growth is dependent on PI3K pathway signaling (*35*). Given the frequent evolution of tumor cell drug resistance in response to PI3K-targeted cancer therapies (*36*), we hypothesized that MYCN-expressing cells in the CAL-51 population (Fig. 4, A and B) would have a growth advantage under selective pressure with PI3K inhibitor (PI3Ki) treatment. To test this hypothesis, we treated CAL-51 with increasing concentrations of the PI3Ki taselisib (GDC-0032) over time to generate PI3Ki-resistant cells (CAL-51^{PI3KiR}). After 6 months, single cells from CAL-51^{PI3KiR} were isolated to generate clonally derived PI3Ki-resistant cell lines. To determine whether the individual CAL-51^{PI3KiR} clonal cell lines displayed durable resistance to PI3Ki, we treated CAL-51^{PI3KiR} cells with taselisib or another PI3Ki, pictilisib (GDC-0941), after the lines were cultured for 2 weeks in the absence of drug (a "drug holiday"). Five of the seven CAL-51^{PI3KiR} clonal cell lines maintained resistance to PI3K inhibition, whereas two of the lines reverted back to a PI3Ki-sensitive state (Fig. 4C and data file S1). CAL-51^{PI3KiR} clonal cell lines were evaluated for MYC family isoform expression, and those lines that had acquired durable resistance to PI3Ki also displayed higher MYCN protein expression (compare Fig. 4, C and D). In contrast to 6% (2 of 33) of the parental clonal cell lines, most (86%, 12 of 14) of the CAL-51^{PI3KiR} clonal cell lines expressed MYCN (Fig. 4D), suggesting that MYCN expression conferred a growth advantage to CAL-51 cells under the continuous selective pressure of PI3Ki treatment. For all subsequent description of results presented herein, we refer to the clonally derived CAL-51 low and high MYCN-expressing cell lines as MYCN^{Low} and MYCN^{High}, respectively.

MYCN-expressing TNBC cells have increased sensitivity to BET inhibitors

The heterogeneity of MYC family isoform expression in the CAL-51 and MDA-MB-468 cell lines, which is consistent with the heterogeneity observed in TNBC clinical specimens (Fig. 3C), supports the use of these two cell lines as preclinical tools to investigate differential drug sensitivity of MYCN-expressing cells. Because the MYC family members are basic helix-loop-helix (bHLH) transcription factors lacking catalytic domains, strategies to inhibit their activity

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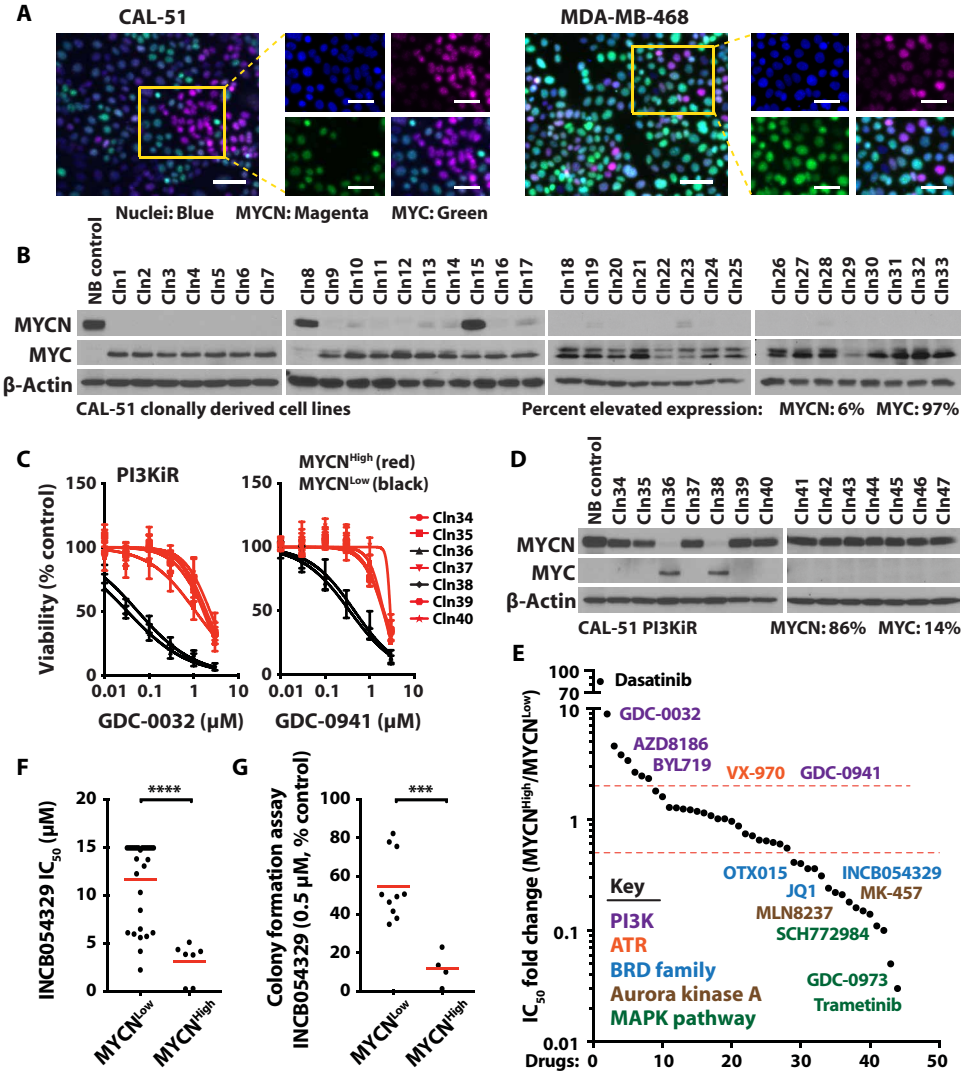

Fig. 4. Evaluation of MYCN-expressing TNBC clonal cell line drug sensitivity. (**A**) Representative TSA-IF stains of MYCN and MYC in the CAL-51 and MDA-MB-468 TNBC cell lines. Colors represent cell nuclei (blue), MYCN (magenta), and MYC (green). Scale bars, 50 μm [overlay fluorescence images at ×20 magnification (left panel for each cell line)] and 20 μm [individual fluorescence images at ×40 magnification (right panels for each cell line)]. (**B**) Immunoblot analysis of MYCN, MYC, and β-actin in the indicated 33 clonally derived cell lines established from CAL-51. NB control, MYCN-amplified SK-N-BE(2)C cell lysate. (**C**) Viability of PI3Ki-resistant (PI3KiR) CAL-51 clonally derived cell lines after treatment with escalating doses of GDC-0032 or GDC-0941 for 72 hours. Black and red dose-response curves represent the indicated MYCN^{Low} and MYCN^{High} clonally derived cell lines, respectively. Data shown represent the means ± SEM of three biological replicates. (**D**) Immunoblot analysis of MYCN, MYC, and β-actin in the 14 indicated CAL-51^{PI3KiR} clonally derived cell lines. (**E**) IC_{50} of 40 compounds used in a secondary screen to treat five MYCN^{Low} and MYCN^{High} cell lines for 72 hours. Colors associate with drug class [PI3K (purple), ATR (orange), BRD family (blue), Aurora kinase A (brown), and MAPK pathway (green)]. Horizontal red dashed lines represent a separation of compounds that had a greater or less than twofold difference in IC_{50} between MYCN^{Low} and MYCN^{High} cell lines. (**F**) IC_{50} of 31 CAL-51 clonally derived cell lines after treatment with escalating doses of INCB054329 for 72 hours. Red lines represent means. Student's *t* test, ****$P < 0.0001$. (**G**) Quantification of crystal violet–stained colonies compared to control for 10 MYCN^{Low} and 4 MYCN^{High} cell lines treated with 0.5 μM INCB054329 for 6 days. Red lines represent means. Student's *t* test, ***$P < 0.001$.

have been limited to indirect targeting of proteins that regulate MYC family isoform stability or expression; these include the bromodomain (BRD)–containing family of transcriptional regulators, PIM1, MEK1/2, and Aurora kinase A (*9*, *37*–*40*). To gain insight into potential strategies for targeting MYCN-expressing TNBC, we performed a high-throughput drug sensitivity screen on two MYCN^{Low} and two MYCN^{High} clonally derived cell lines (described in the previous section) for sensitivity to a library of 158 compounds, containing the 114 compounds in the NCI FDA-AOD library and 44 additional compounds of interest. Analysis of half-maximal inhibitory concentrations (IC_{50}) demonstrated similar drug sensitivities between each clonal cell line set (MYCN^{Low}, $R^2 = 0.9476$; MYCN^{High}, $R^2 = 0.9439$), with MYCN^{High} cell lines having greater sensitivity to compounds that target the BRD family, Aurora kinase A, and MAPK pathway proteins (fig. S7 and data file S3). We performed a secondary screen on MYCN^{Low} ($n = 5$) and MYCN^{High} ($n = 5$) cell lines with inhibitors that demonstrated a >2-fold increase or decrease in IC_{50}, plus additional related compounds of interest. Again, MYCN^{High} cell lines displayed greater sensitivity to compounds previously shown to regulate MYC family isoform expression or activity (Fig. 4E and data file S4), including compounds targeting the BRD family of transcriptional regulators (JQ1, INCB054329, and OTX-015) (*41*–*43*).

BET inhibitors (BETis) are a class of compounds currently under clinical development that broadly target the BRD family (predominantly BRD2, BRD3, and BRD4) (*44*). Preclinical studies have demonstrated that BETis are a promising strategy to target *MYCN*-amplified NB because BRD4 regulates the transcription of *MYCN* and occupies MYCN target gene enhancers and super-enhancers (*8*, *9*). Because BETi sensitivity has been reported to have a stronger positive correlation with *MYCN* expression than with *MYC* expression in both hormonally (*12*) and nonhormonally regulated malignancies (*8*, *9*), we investigated BETis further using our MYCN-expressing TNBC preclinical models. By treating additional CAL-51 clonally derived cell lines that had differing expression of MYCN ($n = 26$) with BETi, we validated results from our earlier drug screens, showing that high MYCN-expressing cells were more sensitive ($P < 0.0001$) to BETi (Fig. 4F and data file S1). Further, we performed longer-term drug treatments and evaluated the colony-forming ability of a subset of clonal cell lines ($n = 14$) differing in MYCN and MYC expression. Again, high MYCN-expressing cells were more sensitive to BETi, and longer-term treatments resulted in more profound differential sensitivity ($P < 0.001$) (Fig. 4G and data file S1). MYCN^{High} cell lines had a ≥5-fold decrease in cell

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growth compared to MYCN^{Low} cell lines in both short-term metabolic and long-term colony formation assays, demonstrating an association between MYCN expression and BETi sensitivity in TNBC.

Changes in MYC family isoform expression in response to BETi treatment

To determine whether the increased sensitivity of MYCN-expressing cells to BETi was MYCN dependent, MYCN^{Low} and MYCN^{High} lines were subjected to *MYCN* small interfering RNA (siRNA)–mediated knockdown. siRNAs targeting *MYCN* RNA decreased MYCN protein and decreased viability in a dose-dependent manner only in the MYCN^{High} cell lines, without altering the amount of MYC in MYCN^{Low} cells (Fig. 5A). MYC expression increased with MYCN knockdown in MYCN^{High} cells (Fig. 5A), suggesting a feedback signaling mechanism between the MYC family members to ensure cell survival under normal growth conditions. To determine whether *MYCN* is a downstream target of BRD-mediated transcriptional regulation, we performed precision nuclear run-on sequencing (PRO-seq) on two MYCN^{High} and two MYCN^{Low} cell lines treated with BETi (0.5 μM INCB054329) for 15 min. Nascent RNA at the *MYCN* locus was observed only in MYCN^{High} cells, and *MYCN* transcripts were reduced after BETi treatment (Fig. 5B). Nascent RNA at the *MYC* locus decreased in the MYCN^{Low} cell lines after BETi treatment, consistent with reported responses to BETi in previous studies (Fig. 5B) (*37*, *45*). However, the *MYC* RNA increased to basal quantities by 4 hours [RNA sequencing (RNA-seq); Fig. 5C] in the MYCN^{Low} cells, and protein amounts were increased at 24 hours (immunoblot; Fig. 5D) in the MYCN^{High} cells, in parallel experiments. Gene set enrichment analyses (GSEAs) performed on RNA samples harvested after 4 hours of BETi treatment demonstrated that MYC target genes were significantly down-regulated in response to BETi treatment in the MYCN^{High} cells [Hallmark MYC targets V1: $P < 0.0001$, false discovery rate (FDR) $q < 0.0001$; Hallmark MYC targets V2: $P < 0.0001$, FDR $q < 0.0001$; fig. S8], consistent with BETi-mediated down-regulation of MYCN-mediated transcriptional activity.

To evaluate MYC family isoform dynamics in individual cells after BETi exposure, CAL-51 and MDA-MB-468 were treated with increasing doses of BETi (INCB054329 or JQ1) for 24 hours and TSA-IF was performed for MYCN and MYC detection. Similar to MYC family isoform expression changes observed in the CAL-51 clonal cell lines (Fig. 5, C and D), BETi treatment decreased MYCN expression in a dose-dependent manner in the heterogeneous CAL-51 and MDA-MB-468 parental populations (Fig. 5, E and F, and data files S5 and S6). In addition to the activating *PIK3CA* mutation in CAL-51, both CAL-51 and MDA-MB-468 lack PTEN protein, a negative regulator of PI3K pathway signaling (*35*). BETi treatment resulted in little to no change in MYC expression in both CAL-51 and MDA-MB-468 (Fig. 5, E and F, and data files S5 and S6), which is consistent with a previous study demonstrating that BETi treatment had little effect on MYC expression in PI3K pathway–mutant breast cancer (*46*).

Combination BETi and MEK inhibitor treatment in MYCN-expressing TNBC cell lines

Given that most of the MYCN-expressing TNBCs also contain MYC-expressing tumor cells (Fig. 3B), we identified drug combinations that would decrease expression of both isoforms and thereby inhibit cell proliferation and tumor development. We performed differential gene expression analyses using TNBC tumors from the TNBC587 dataset (*20*) with high *MYCN* expression and low *MYC* (*MYCN*^{RatioHigh}) compared to tumors with high *MYC* expression and low *MYCN* (*MYC*^{RatioHigh}). Selecting tumors on the basis of expression ratios allowed us to minimize the inclusion of heterogeneous tumors coexpressing both isoforms that would confound the results. The optimal number of tumors used for comparative analyses was determined by comparing the number of differentially expressed genes for different percentages of *MYCN*^{RatioHigh} and *MYC*^{RatioHigh} tumors compared to random samplings (fig. S9, A and B). To determine the degree of variance among all *MYCN*^{HighRatio} and *MYC*^{HighRatio} tumors selected for analysis, we performed a principal components analysis (PCA). *MYCN*^{HighRatio} and *MYC*^{HighRatio} tumors clustered apart from each other, indicating that tumors within each respective group have a greater similarity (fig. S9C). GSEA comparing *MYCN*^{HighRatio} and *MYC*^{HighRatio} tumors demonstrated a positive association between *MYCN* expression and MEK signaling (El-Ashry MEK Up V1 Up: $P < 0.001$, FDR $q \leq 0.001$; fig. S9D), providing rationale to explore the regulation of MYCN and MYC expression by MAPK pathway signaling.

MYC protein stability can be regulated by the MAPK pathway (*47*), and inhibition of MAPK pathway signaling can cause MYC instability and proteasomal degradation (*48*). Given that MAPK pathway inhibitors are also under preclinical investigation to treat aggressive relapsed MYCN-driven NB (*49*, *50*) and were among the top hits in our previously described drug screens (Fig. 4E and fig. S7), we evaluated whether MAPK pathway inhibition would alter MYCN protein quantities and/or be effective at decreasing MYC expression when combined with BRD inhibition. MYCN^{High} and MYC-expressing MYCN^{Low} CAL-51 clonal cell lines were treated with inhibitors targeting proteins in the MAPK pathway, including epidermal growth factor receptor (erlotinib), RAF (TAK-632), MEK1/2 (trametinib and GDC-0973), and extracellular signal–regulated kinase 1/2 (ERK1/2) (SCH772984). MEK inhibitors (MEKis) were most effective at inhibiting MAPK pathway signaling, as evidenced by decreased ERK1/2 phosphorylation and decreased MYC and MYCN in each line that expressed a given isoform (Fig. 6A). Because the FDA-approved MEKi trametinib demonstrated the greatest decrease in MYC and MYCN, we evaluated the effects of trametinib treatment alone or in combination with BETi. MYCN decreased while MYC increased in CAL-51 MYCN^{High} clonal cell lines treated with either BETi agent alone (INCB054329 or JQ1; Fig. 6B). However, trametinib in combination with either BETi attenuated MYC up-regulation, thereby decreasing the amount of both MYC family isoforms (Fig. 6B).

To expand our analysis of effects of BETi and MEKi combination treatment on heterogeneous populations of MYCN-expressing TNBC, we treated CAL-51 and MDA-MB-468 cells with trametinib, INCB054329, or JQ1 as single agents, or with either BETi in combination with trametinib, for 48 hours and examined MYC and MYCN expression. Treatment with either BETi alone decreased MYCN expression in both TNBC cell lines (Fig. 6, C and D, and data file S7), consistent with previous single-agent results (Fig. 5, E and F). Whereas BETi treatment resulted in little to no change in MYC, single-agent trametinib decreased MYC expression to a greater extent than MYCN in both cell lines; when trametinib was combined with either BETi, MYC and MYCN decreased to a larger extent than with either agent alone (Fig. 6, C and D, and data file S7).

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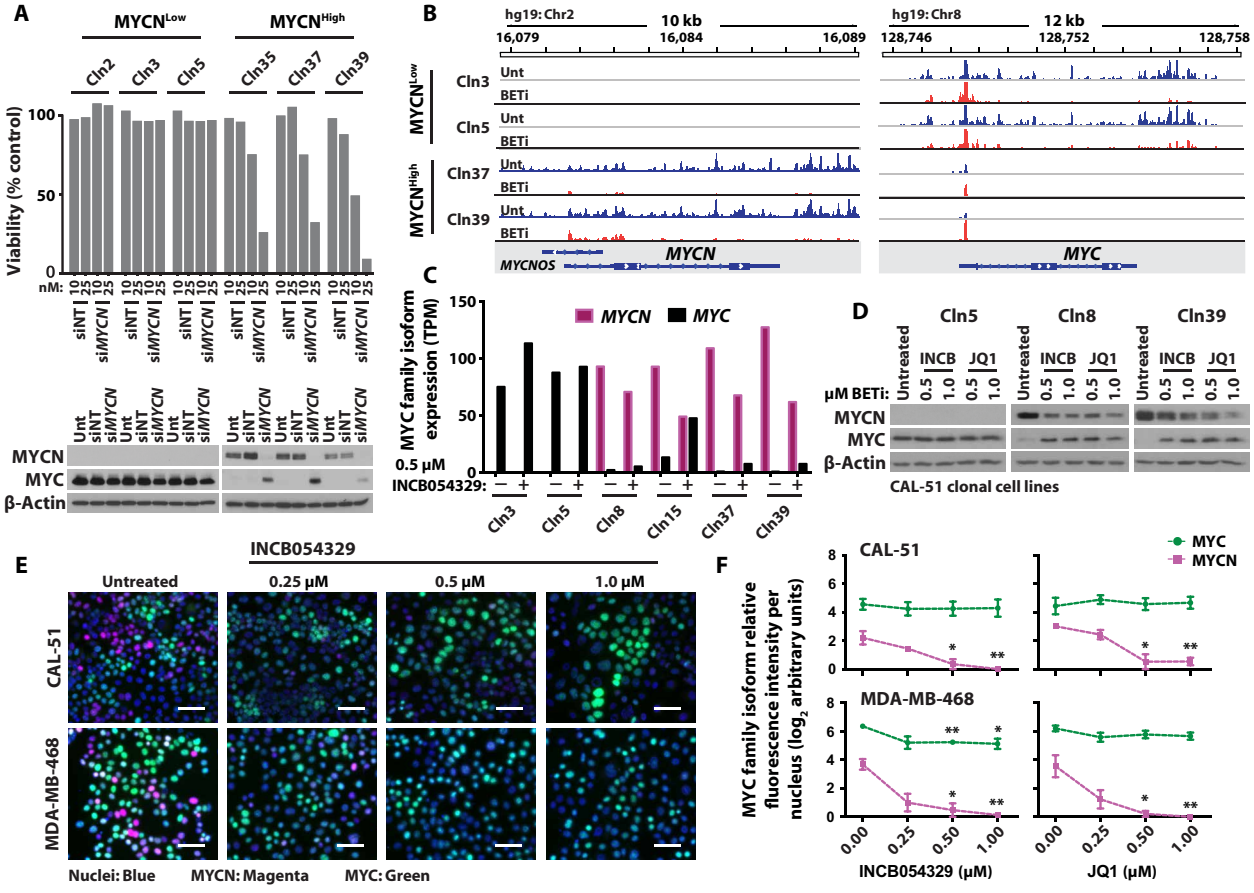

Fig. 5. Evaluation of MYC family isoform expression after BETi treatment. (**A**) Top: Viability of MYCN^{Low} and MYCN^{High} cell lines after siRNA-mediated knockdown using nontargeting (siNT) or anti-*MYCN* (si*MYCN*) siRNAs for 96 hours. Data shown represent the mean of three technical replicates. Bottom: Immunoblot analysis of MYCN, MYC, and β-actin in MYCN^{Low} and MYCN^{High} cell lines after the described knockdown with 25 nM siRNAs. (**B**) Genome viewer showing sequencing alignment tracks of nascent transcript PRO-seq mapping at the *MYCN* and *MYC* gene loci for the two indicated MYCN^{Low} and MYCN^{High} cell lines after treatment with dimethyl sulfoxide control (Unt, blue) or 0.5 μM INCB054329 (BETi, red) for 15 min. (**C**) *MYCN* and *MYC* expression (TPM) in the two indicated MYCN^{Low} (Cln3 and Cln5) and four MYCN^{High} (Cln8, Cln15, Cln37, and Cln39) cell lines after treatment with 0.5 μM INCB054329 for 4 hours. (**D**) Immunoblot analysis of MYCN, MYC, and β-actin in three of the cell lines described in (C) after treatment with 0.5 and 1.0 μM INCB054329 or JQ1 for 24 hours. (**E**) MYC family isoform TSA-IF on two MYCN-expressing TNBC cell lines (MDA-MB-468 and CAL-51) after 0, 0.25, 0.5, or 1.0 μM INCB054329 or JQ1 for 24 hours. Colors represent cell nuclei (blue), MYCN (magenta), and MYC (green). Scale bars, 50 μm. (**F**) Quantification of fluorescence intensity per nucleus for MYCN and MYC after BETi treatments described in (E). Data shown represent the median ± SEM of three biological replicates. Student's *t* test between untreated and BETi-treated cells, $^{*}P < 0.05$, $^{**}P < 0.01$.

MDA-MB-468 and CAL-51 cell populations were treated with a range of low-dose BETi and MEKi concentrations to evaluate growth and viability in response to BETi and MEKi treatment. Both TNBC cell lines were treated with escalating doses of INCB054329 or JQ1, as single agents, or in combination with increasing doses of trametinib, and colony-forming ability was assessed after 6 days (Fig. 6E and data file S1). MDA-MB-468, the higher MYCN-expressing cell line (Figs. 5F and 6D), displayed greater sensitivity to single-agent BETi treatments compared to CAL-51, and the combination of BETi and MEKi resulted in a synergistic decrease in cell growth, as determined by Bliss independence analyses (*51*, *52*), in both MYCN-expressing lines (Fig. 6E and data file S1). These data demonstrate that low-dose BETi and MEKi combinations are effective in MYCN-expressing TNBC cell populations and provide rationale to further evaluate the combination using in vivo model systems of MYCN-expressing TNBC.

Combination BETi and MEKi treatment is effective at inhibiting in vivo growth of MYCN-expressing TNBC PDXs

To evaluate the preclinical efficacy of BET and MEK inhibition in vivo, we first confirmed MYCN and MYC protein expression in three TNBC PDX models with differing *MYCN* and *MYC* RNA expression (Fig. 7A and table S1A). The TM00096 PDX model was derived from a TNBC metastatic lung lesion (table S1A) (*53*) and expresses MYCN and MYC in ~37 and ~51% of the tumor cells, respectively (Fig. 7B). PDX models TM01273 and TM00090 both have a low percentage of MYCN-expressing cells (~2 and <1%, respectively) relative to MYC-expressing cells (~63 and ~32%, respectively) (Fig. 7B). For all three models, a 2-mm^3 tumor was subcutaneously implanted into NOD (nonobese diabetic) *scid* gamma (NSG) mice, and when xenograft tumor volumes reached ~150 mm^3, mice were treated with vehicle control, trametinib [0.1 mg/kg, once daily (QD)], INCB054329 [50 mg/kg, twice daily (BID)], or the

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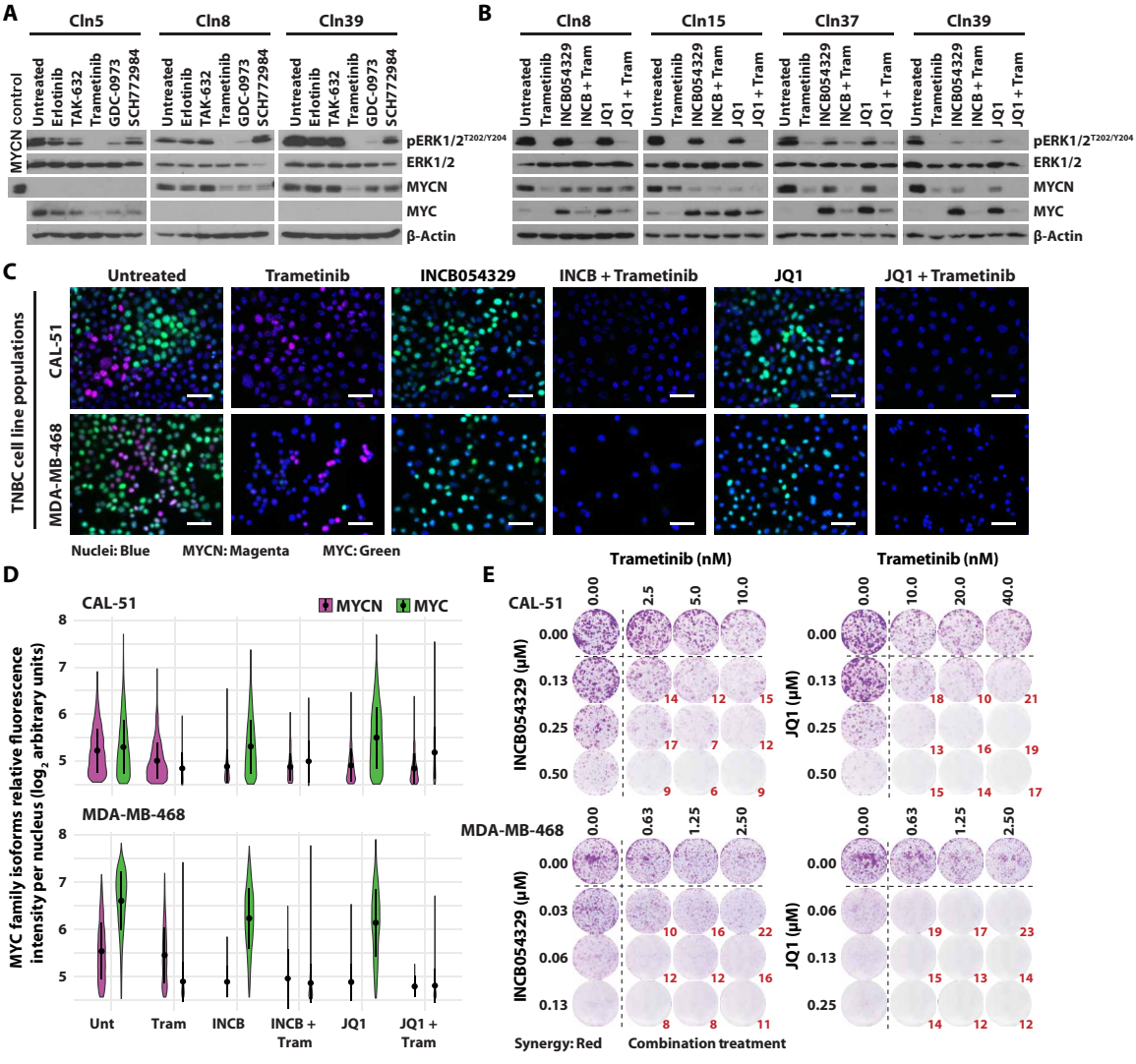

Fig. 6. Effect of BETi and MEKi combination treatment on MYC family isoform expression and cell viability of MYCN-expressing TNBC cell lines. (**A**) Immunoblot analysis for pERK1/2, total ERK1/2, MYCN, MYC, and β-actin in MYCN^{Low} and MYCN^{High} cell lines after treatment with MAPK pathway inhibitors at 0.25 μM for 24 hours. (**B**) Immunoblot analysis of pERK1/2, total ERK1/2, MYCN, MYC, and β-actin in four MYCN^{High} CAL-51 cell lines after treatment with 0.25 μM trametinib (Tram), 0.5 μM INCB 054329 (INCB), 0.5 μM JQ1, or the combination of trametinib with either BETi for 48 hours. All immunoblot experiments shown are representative of at least two biological replicates. (**C**) MYC family isoform TSA-IF on two MYCN-expressing TNBC cell lines (MDA-MB-468 and CAL-51) after 0.25 μM trametinib, 0.5 μM INCB054329, 0.5 μM JQ1, or the combination of trametinib with either BETi for 48 hours. Colors represent cell nuclei (blue), MYCN (magenta), and MYC (green). Scale bars, 50 μm. (**D**) Violin plots showing quantification of fluorescence intensity for MYCN and MYC per nucleus after BETi treatments described in (C). TSA-IF images and quantification are representative of three biological replicates. (**E**) Crystal violet colony formation assays for MDA-MB-468 and CAL-51 after treatment with the indicated concentrations of trametinib, INCB054329, or JQ1 alone, or either BETi in combination with trametinib, for 6 days. Values in red represent mean delta Bliss synergy for combination treatments of three biological replicates.

combination of the two agents at the indicated doses for 14 days. Compared to vehicle-treated controls, combined BETi and MEKi treatment resulted in a synergistic and significant ($P < 0.01$) reduction in tumor growth only in the high MYCN-expressing PDX model [delta Bliss synergy (Syn) and tumor growth inhibition (TGI): TM00096: Syn = 38, TGI = 97%; TM01273: Syn = −4, TGI = 58%; TM00090: Syn = −8, TGI = 35%] (Fig. 7C and data file S1). These in vivo results were consistent with our in vitro observations and further confirmed an association between MYCN expression and efficacy of BETi and MEKi combination treatment.

To expand and reproduce our in vivo findings, we performed another PDX "trial" with TM00096 (MYCN^{High}) alongside two additional TNBC PDX models, HBCx1 and BCM-2147, that have an intermediate (MYCN^{Intermediate}) or low (MYCN^{Low}) percentage of MYCN-expressing cells (~20 and ~2%, respectively) relative to MYC-expressing cells (~80 and ~95%, respectively) (Fig. 7D). All three models were treated for 22 days with trametinib, INCB054329, or JQ1 (50 mg/kg, BID) as single agents or with the indicated BETi combined with trametinib. All compounds administered were well tolerated, and all animals completed the study without excess weight loss (fig. S10 and data file S1) or limiting morbidities. In response to either single-agent BETi treatment, we observed the greatest statistical difference from vehicle in the MYCN^{High} model (TM00096), with a 63% TGI in response to INCB054329 treatment (compared to 40 and 38% in the MYCN^{Intermediate} and MYCN^{low} models, respectively) and an 83% TGI in response to JQ1 (compared to 75 and 57% in the MYCN^{Intermediate} and MYCN^{low} models, respectively; Fig. 7E and data file S1). Combined MEKi and BETi resulted in a synergistic TGI in mice harboring either MYCN^{High} or MYCN^{Intermediate} tumors (INCB054329 and trametinib: Syn = 21 and 15, respectively; JQ1 and trametinib: Syn = 18 and 16, respectively; Fig. 7E and data file S1) and an 11 and 85% reduction in tumor volume, compared to the starting treatment-naïve tumor volume, in the MYCN^{High} PDX model when trametinib was combined with either INCB054329 or

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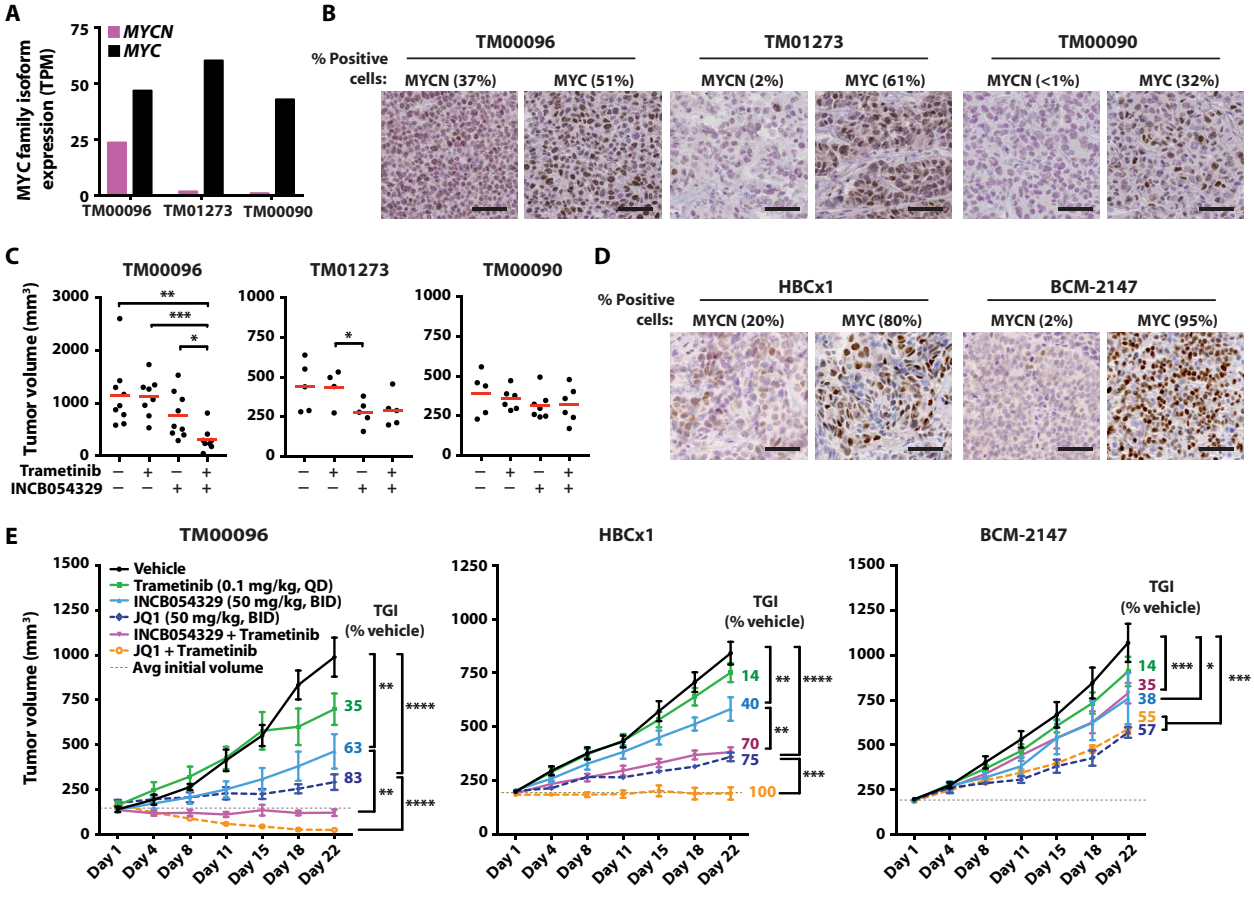

Fig. 7. Evaluation of tumor growth after BETi and MEKi combination treatment in vivo. (**A**) *MYCN* and *MYC* expression (TPM) in three TNBC PDX models (TM00096, TM01273, and TM00090). (**B**) Representative IHC and quantification of percent positive cells for MYCN and MYC in TM00096, TM01273, and TM00090 sections. Scale bars, 20 μm. (**C**) Tumor volume (in cubic millimeters) of TM00096, TM01273, and TM00090 treated with trametinib [0.1 mg/kg, once daily (QD)] or INCB054329 [50 mg/kg, twice daily (BID)] alone or in combination for 14 days. Red bars represent means. (**D**) Representative IHC and quantification of percent positive cells for MYCN and MYC in HBCx1 and BCM-2147 sections. Scale bars, 20 μm. (**E**) Tumor volume (in cubic millimeters) of TM00096, HBCx1, and BCM-2147 treated with trametinib, INCB054329, or JQ1 (50 mg/kg, BID) alone, or either BETi in combination with trametinib for 22 days. The dashed lines represent the mean initial tumor volume at time of first treatment. Avg, average; TGI, tumor growth inhibition. Data shown represent the means ± SEM. Student's *t* test between vehicle, BETi, and corresponding combination-treated tissue, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

JQ1, respectively (left panel, below the gray dashed lines; Fig. 7E and data file S1).

To determine the effects of the agents on pharmacodynamic markers in vivo, tumors were resected and protein was extracted after the initial (2 days) and final (22 days) treatments during the PDX study. Through immunoblot analyses, we observed that trametinib decreased phosphorylated ERK1/2 (pERK1/2) and both BETis decreased MYC and MYCN in all three PDX models, consistent with the agents' predicted biochemical activities (fig. S11A). To determine whether decreased cell proliferation or increased apoptosis contributed to the observed decrease in tumor growth in the $MYCN^{High}$ and $MYCN^{Intermediate}$ models treated with the combination, we evaluated markers of proliferation (Ki67) and apoptosis [cleaved poly(adenosine diphosphate–ribose) polymerase (PARP) and cleaved caspase-3] by IHC and immunoblot, respectively. Unlike the $MYCN^{Low}$ PDX model, Ki67 decreased in tissue from the $MYCN^{High}$ and $MYCN^{Intermediate}$ models treated with BETi, as a single agent or in combination with MEKi, after 2 days of treatment and to a greater extent at the end of treatment (fig. S11B and data file S1). Only the $MYCN^{High}$ model displayed markers of apoptosis after 2 days of treatment with each single agent alone or in combination (fig. S11A). These data suggest that BETis decreased the quantity of both MYCN and MYC in tumor cells grown in vivo and the combination treatments that resulted in a decrease in tumor volume in both MYCN-expressing TNBC models (Fig. 7E) were due to proapoptotic mechanisms in the $MYCN^{High}$ model and antiproliferative effects in the $MYCN^{High}$ and $MYCN^{Intermediate}$ models.

Changes in MYC family isoform expression in vivo after BETi and MEKi combination treatment

To evaluate changes in cellular expression of MYCN and MYC during treatment, we performed IHC and dual MYC family isoform TSA-IF on PDX tissue collected after initial and final doses. Similar to immunoblot results at the early treatment time point (fig. S11A), single-agent BETis decreased MYC in the $MYCN^{Low}$ PDX model and both MYC and MYCN in the $MYCN^{High}$ and $MYCN^{Intermediate}$ models compared to vehicle-treated MYC family isoform expression (Fig. 8A, fig. S12, and data files S1 and S7). At the late treatment

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time point, MYCN expression was inhibited to a greater extent than MYC after treatment with either single-agent BETi in both MYCN-expressing PDX models compared to vehicle-treated tissue (Fig. 8, B and C, and data files S1 and S7). However, trametinib combined with either BETi decreased MYCN and MYC to a greater extent than with either BETi alone throughout the time course of treatment in both the $MYCN^{High}$ and $MYCN^{Intermediate}$ models (Fig. 8, B and C, and data files S1 and S7). Together, treatment with either structurally distinct BETi, INCB054329 or JQ1, when combined with MEKi, continuously inhibited MYC family isoform expression and resulted in synergistic TGI in the $MYCN^{High}$ and $MYCN^{Intermediate}$ TNBC PDX models and tumor regression in the $MYCN^{High}$ model.

DISCUSSION

The lack of therapeutically targetable, high-frequency driver alterations across TNBC creates a challenge for developing strategies to treat patients with this cancer. Here, we evaluate the expression of MYCN, a transcription factor associated with increased stemness, epithelial-to-mesenchymal transition, survival, and dormancy phenotypes in TNBC cells (*18*). Through the use of IHC, we assessed MYCN protein expression in several TNBC patient cohorts, including both primary tumors and metastatic disease, and report that a substantial fraction (45 to 64%) of tumors heterogeneously express MYCN. Further, MYCN-expressing cells are present in residual disease after NAC treatment, as well as in TNBC cell line cultures that acquired resistance to PI3Ki, suggesting that induction or maintenance of MYCN expression confers a survival advantage for cells treated with compounds that target microtubule structure (taxanes), induce DNA damage (anthracyclines), or cause metabolic stress (PI3Ki). NE prostate cancer, a tumor type considered to be driven by MYCN expression (*54*), is associated with castration and androgen inhibitor resistance and a poor prognosis (*54*, *55*). Unlike *MYCN*-amplified NB, AML, and GBM, which are tumors that have retained a same-cell lineage, NE prostate cancers are thought to have differentiated from castration-resistant prostate adenocarcinoma through MYCN-mediated mechanisms and lineage switching (*13*, *54*). Here, we found *MYCN* transcript in primary, treatment-naïve TNBC to be comparable to *MYCN* expression in NE-CRPC, suggesting that MYCN-expressing TNBC could represent a similar altered differentiation state.

In addition to TNBC tumors lacking therapeutic targets, the development of effective drug treatment strategies for TNBC patients has also been hindered by the presence of highly heterogeneous intratumoral cell populations with differing biological properties within an individual patient's tumor. Through the use of dual MYC isoform TSA-IF, we report that MYCN and its family member MYC are heterogeneously expressed in separate cell nuclei within a given tumor in at least 40% of TNBC tumors. Previous studies have demonstrated that MYCN and MYC preferentially regulate the same set of core genes involved in metabolism and cell growth, and although the MYCN allele can functionally replace MYC in murine development (*56*), MYCN and MYC have separate temporal regulation over organogenesis in early vertebrate development (*33*). *MYCN* expression is essential for initial establishment of stem and progenitor populations; over the course of organ system development, *MYCN* expression switches to low *MYC* expression to support stem and progenitor cell maintenance, and during cell lineage commitment and expansion, increased *MYC* drives highly proliferative cells until they reach terminal differentiation (*33*). We observed similar MYC family isoform switching in our clonally derived TNBC cell line models, indicating that tumor cells have retained the ability to transition between MYCN and MYC, which may account for the large range (2 to 100%) of MYCN expression within heterogeneous TNBC cell populations.

By isolating and expanding single cells from heterogeneous TNBC tumor–derived cell line populations, we generated distinct MYCN- and MYC-expressing cell cultures with a similar genetic background, thus allowing us to assign the biological relevance of MYCN versus MYC expression to sensitivity of compounds under preclinical or clinical investigation. We conducted a high-throughput 158-drug screen that included compounds from the NCI FDA-AOD library and identified inhibitors of the BRD family of transcriptional regulators (BETi) that were preferentially effective in inhibiting MYCN-expressing tumor cell growth. BETis are a class of compounds currently under early-stage clinical development that broadly target the BRD family (predominantly BRD2, BRD3, and BRD4) of transcriptional regulators (*44*). These compounds were of particular interest given previous reports that MYC family isoform signaling, including contributions from MYCN, is enriched in TNBC (*57*) and that TNBC has preferential sensitivity to BETis compared to other breast cancer subtypes (*45*). Further, efficacy of BETis has been predominantly attributed to selective disruption of super-enhancer–associated genes that deregulates transcription factor activity (*45*, *58*, *59*). BRD4 regulates transcription of *MYCN* as well as occupies MYCN-associated target genes, enhancers, and super-enhancers (*22*), and preclinical studies have suggested BETis as a promising strategy to target MYCN-driven neuronal [NB (*8*, *9*), medulloblastoma (*60*), and embryonal tumors with multilayered rosettes (*61*)] and nonneuronal [ovarian cancer (*12*) and alveolar rhabdomyosarcomas (*62*)] tumor cell growth. Whereas previous studies have focused on BRD-mediated targeting of MYC, we show that TNBC tumors are heterogeneously composed of MYC- and MYCN-expressing cells and MYCN-expressing cells have differential sensitivity to BETis in select tumor cells and model systems.

We acknowledge that limitations exist in regard to this study. As previously mentioned, MYCN-expressing cells exist within highly heterogeneous intratumoral cell populations. Our assessment of MYCN expression in TNBC tumors is limited to the tissue sections under investigation and may not be representative of the entire tumor. Thus, the number of MYCN-expressing TNBC tumors may be higher than reported here. We also demonstrate the presence of MYCN-expressing cells in residual disease after NAC and PI3Ki treatment. Whether MYCN-expressing cells were preexisting and selected for with treatment or whether epigenetic events up-regulated MYCN expression in cells initially devoid of MYCN remains unclear. Last, the restricted availability of MYCN-expressing TNBC models for in vitro and in vivo preclinical evaluation limits analyses of the effects of combined MEKi and BETi treatment across a larger cohort of MYCN-expressing TNBC.

Currently, BETis are in the initial stages of clinical assessment and have had their greatest single-agent clinical efficacy in hematopoietic and nuclear protein in testis (NUT) midline malignancies (*44*); however, favorable preclinical investigations with BETi combination treatments have catalyzed interventional trials to improve hematopoietic malignancy and solid tumor patient responses (*44*, *63*). In our study, we found that single-agent BETi and MEKi treatments decreased both MYCN and MYC expression and had a greater effect

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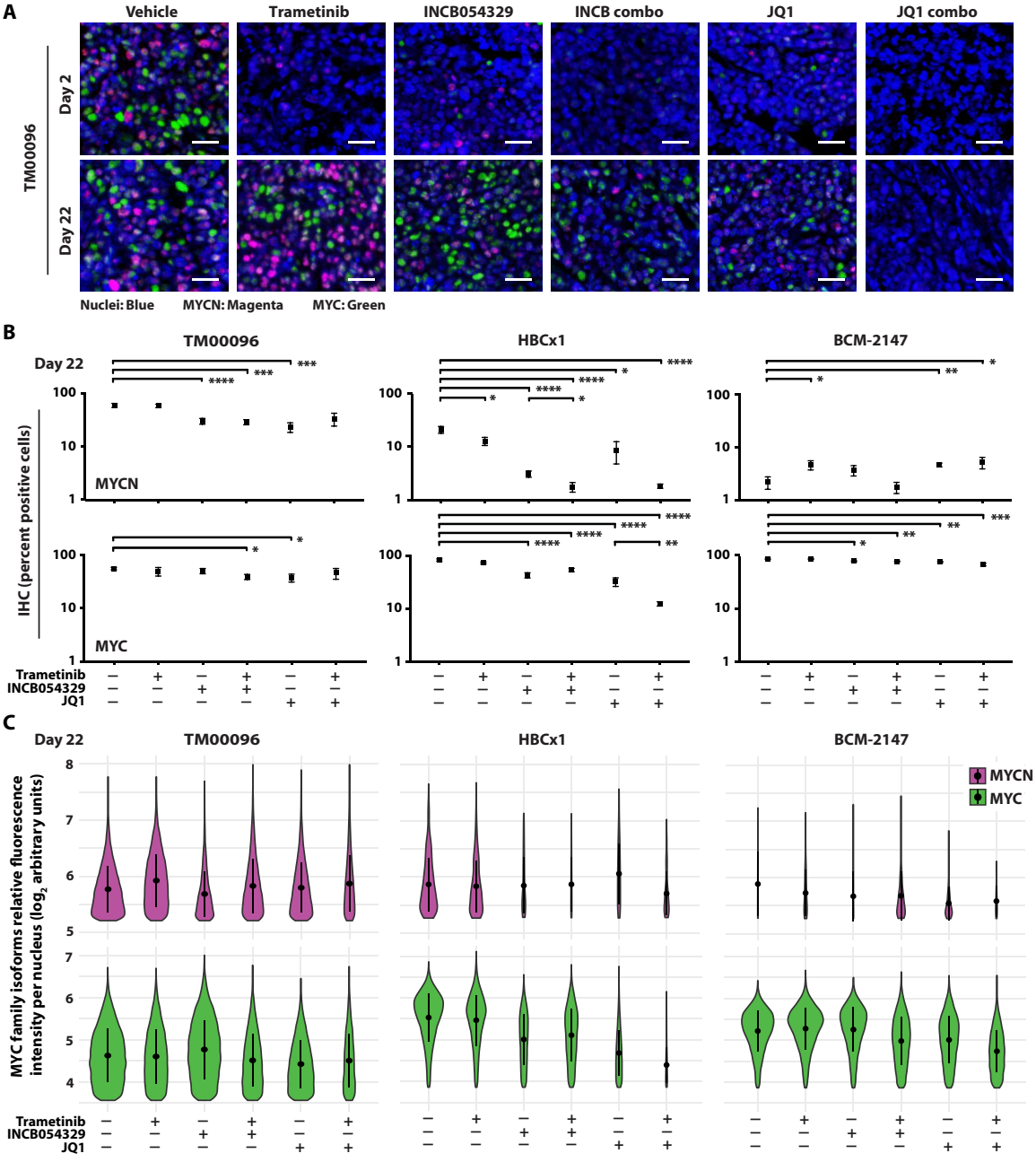

Fig. 8. Evaluation of MYC family isoform expression after BETi and MEKi combination treatment in vivo. (**A**) Representative TSA-IF of MYCN and MYC in TM00096 after 2 or 22 days of treatment with trametinib (0.1 mg/kg, QD), INCB054329 (50 mg/kg, BID), or JQ1 (50 mg/kg, BID) alone, or either BETi in combination with trametinib. Colors represent cell nuclei (blue), MYCN (magenta), and MYC (green). Scale bars, 20 μm. (**B**) Quantification of IHC (percent positive cells) and (**C**) violin plots showing the distribution of TSA-IF intensity per nucleus for MYCN and MYC in TM00096, HBCx1, and BCM-2147 sections after treatments described in (A) for 22 days. Student's *t* test between vehicle and treatment arms and between single-agent BETi and corresponding combination-treated tissue, *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$.

when used in combination. Combined low-dose BETi and MEKi displayed a synergistic decrease in tumor cell viability, both in the setting of in vitro cell cultures and in mice harboring TNBC PDXs with heterogeneous expression of both MYCN and MYC. Synergies between BETi and MEKi have been attributed to an up-regulation of MAPK pathway signaling in response to BETi treatment (*64*) and the ability of BETis to disrupt adaptive bypass mechanisms induced by MEKi treatment (*65*). Although we did not observe an up-regulation of MAPK pathway signaling after BETi treatment in either our TNBC cell lines or PDX tissue, we cannot rule out chromatin modulation or enhancer remodeling in response to treatment with either single agent given the rebound/up-regulation of MYC expression in response to BETi treatment in the CAL-51 clonal cell lines. Aurora kinase inhibitors, which are also used to target MYCN-driven tumors (*10*, *24*), were a top "hit" in our screens against MYCN-expressing TNBC. Given preferential effects of cyclin-dependent kinase (CDK) inhibitors on MYC family isoform pathway signaling (*57*), CDK and Aurora kinase inhibitors could also be evaluated as a means for targeting MYCN-expressing TNBC.

In summary, we have identified MYCN-expressing TNBC cell populations within a substantial fraction of evaluated tumors that have the ability to survive various forms of drug-induced cellular stress, have survival advantages in vitro under selective antiproliferative treatments, and transition between differentiation states (as defined by MYC family expression status). On the basis of our preclinical results using in vitro and in vivo TNBC models, we posit that BETi and MEKi combination treatment will induce regression of MYCN-expressing TNBC tumors. Given that patients with TNBC primarily receive systemic cytotoxic chemotherapies that

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frequently result in unfavorable outcomes, we propose the clinical development of combination BETi and MEKi for patients with advanced TNBC, with parallel evaluation of MYCN as a potential marker for patient selection.

MATERIALS AND METHODS

Study design

The study was designed to identify the proportion of treatment-naïve (n = 191), NAC-treated (n = 115), and recurrent TNBC tumors (n = 38) that express MYCN. Clinical specimens for IHC analyses were collected at VUMC in Nashville, TN; Instituto Nacional de Enfermedades Neoplásicas in Lima, Peru; or in conjunction with a commercial source, US Biomax. All clinical and pathologic data were retrieved under institutionally approved protocols. Protein expression (H-scores) resulting from IHC for MYCN and MYC was determined by a pathologist (P.I.G.-E.), and analyses were performed by researchers blinded to the patients' medical background and treatments received.

We also designed the study to investigate whether compounds identified through in vitro assays would induce TNBC cell growth inhibition and/or apoptosis in vivo. Mice were housed and treated in accordance with protocols approved by the Institutional Care and Use Committee for animal research at Vanderbilt University. To sufficiently power the studies at 90% ($\beta = 0.2$) and a significance level of $\alpha = 0.05$, assuming normal distributions, equal SD, and an expected effect size of 50%, five to nine mice were used for tumor measurements per arm, depending on the growth kinetics of each PDX model. Once the PDX tumors reached about 150 to 250 mm^3, mice were randomized into single-agent or combination treatment groups that consisted of a MEKi (trametinib) and/or BETi (INCB054329 or JQ1). Two additional mice per arm were included in the study for early PDX molecular analyses and were removed after 2 days of treatment. The $MYCN^{High}$ PDX model (TM00096) was evaluated twice: first, in a four-arm study with trametinib and INCB054329 treatments for 14 days and, again, in a six-arm study with all described compounds for 22 days. No data exclusion criteria were applied or outliers excluded. Early and late molecular analyses (after 2 and 22 days of treatment, respectively) on PDX tumors were performed. MYCN and MYC expression (H-scores) were quantified by a pathologist (P.I.G.-E.), and analyses were performed blinded to treatments received.

In vivo PDX experiments

Mice were housed and treated in accordance with protocols approved by the Institutional Care and Use Committee for animal research at Vanderbilt University. Female 6- to 8-week-old NSG or athymic nude mice (The Jackson Laboratory) were anesthetized with isoflurane and subjected to subcutaneous engraftment of a 2-mm^3 TNBC PDX [The Jackson Laboratory (TM00096, TM00090, TM01273), Baylor University (BCM-2147), and Xentech (HBCx1)] fragment into the lateral dorsal side of each mouse. After surgical implantation, the mice were monitored daily for 10 to 14 days. Once wound clips were removed and tumors reached about 150 to 250 mm^3, mice were randomized into single-agent and combination treatment groups. Mice were treated with a MEKi, trametinib (0.1 mg/kg, QD), in 0.5% methylcellulose with 0.2% Tween 80, and/or a BETi, INCB054329 or JQ1 (50 mg/kg, BID), in 0.5% methylcellulose with 5% N,N-dimethylacetamide, through orogastric gavage for either 14 or 22 days. Tumor volumes were calculated twice a week by caliper measurements (width^2 × length/2), and body weights were measured once a week. Tumors used for subsequent molecular analyses were snap-frozen and deposited in a liquid nitrogen storage tank.

Statistical methods

Statistical analyses were performed using GraphPad Prism software (GraphPad) and R (version 3.6, www.R-project.org/). As indicated in the figure legends, the SD, SEM, or boxplot is shown. Wilcoxon rank sum test was used to compare the amount of MYCN transcript between TNBC and the other MYCN-expressing cancer types. P values were adjusted by FDR (Fig. 1B). Wilcoxon rank sum test was also used to determine the difference in MYCN expression between treatment-naïve and NAC-treated MYCN-expressing tumors (Fig. 2B). Student's t tests were used to determine differential BETi sensitivity between $MYCN^{Low}$ and $MYCN^{High}$ CAL-51 clonal cell lines (Fig. 4, F and G) and changes in MYC family isoform relative fluorescence intensity per nucleus before and after BETi treatments (Fig. 5F). Student's t tests were also used to determine significance of differences in tumor volumes and MYC family isoform expression between MEK and/or BETi treatments in the PDX experiments (Figs. 7, C and E, and 8B). $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Identification of TNBC breast cancers in TCGA and METABRIC datasets.

Fig. S2. Distribution of *MYCN* expression across TNBC.

Fig. S3. IHC detection of MYCN in CDX and PDX tissue.

Fig. S4. *MYCN* expression in primary TNBC and patient-matched metastases.

Fig. S5. MYCN and MYC intratumoral heterogeneity in primary and recurrent TNBC.

Fig. S6. *MYCN* and *MYC* expression in TNBC cell populations and CAL-51 clonally derived cell lines.

Fig. S7. BETi sensitivity of CAL-51 $MYCN^{High}$ cell lines.

Fig. S8. Changes in MYC target gene expression in CAL-51 $MYCN^{High}$ cell lines after BETi treatment.

Fig. S9. Differential gene expression analyses between $MYCN^{RatioHigh}$ and $MYC^{RatioHigh}$ TNBC.

Fig. S10. Effect of BETi and MEKi combination treatments on weight of treated mice.

Fig. S11. Evaluation of apoptosis and proliferation after BETi and MEKi treatment in TNBC PDX models.

Fig. S12. Evaluation of MYC family isoform expression after BETi and MEKi combination treatment in vivo.

Table S1. *MYCN* and *MYC* expression in breast cancer PDX models.

Table S2. Characteristics of patients with treatment-naïve and NAC-treated primary TNBC.

Data file S1. Tabular data points for experiments with a sample size of $n < 20$.

Data file S2. IHC results for MYCN and MYC in primary, treatment-naïve; primary, NAC-treated; and recurrent TNBC cases.

Data file S3. Primary drug screen results using CAL-51 $MYCN^{Low}$ and $MYCN^{High}$ cell lines.

Data file S4. Secondary drug screen results using CAL-51 $MYCN^{Low}$ and $MYCN^{High}$ cell lines.

Data file S5. Tabular data points for MYC family isoform TSA-IF in CAL-51 after single-agent BETi treatment.

Data file S6. Tabular data points for MYC family isoform TSA-IF in MDA-MB-468 after single-agent BETi treatment.

Data file S7. Tabular data points for MYC family isoform TSA-IF in TNBC cell lines and PDX tissue after BETi and MEKi single-agent and combination treatment.

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View/request a protocol for this paper from *Bio-protocol*.

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Targeting MYCN-expressing triple-negative breast cancer with BET and MEK inhibitors

Johanna M. Schafer, Brian D. Lehmann, Paula I. Gonzalez-Ericsson, Clayton B. Marshall, J. Scott Beeler, Lindsay N. Redman, Hailing Jin, Violeta Sanchez, Matthew C. Stubbs, Peggy Scherle, Kimberly N. Johnson, Quanhu Sheng, Joseph T. Roland, Joshua A. Bauer, Yu Shyr, Bapsi Chakravarthy, Bret C. Mobley, Scott W. Hiebert, Justin M. Balko, Melinda E. Sanders, Phillip C. C. Liu and Jennifer A. Pietenpol

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BETting on combination treatment

MYCN is a well-known oncogene that plays a role in cancer aggressiveness, but it is typically associated with neuroendocrine cancers. Schafer *et al.* found a role for MYCN in triple-negative breast cancer, a particularly aggressive form of the disease, and identified a potential intervention. Although there is no standard way to target MYCN directly, the authors determined that a group of experimental drugs called BET inhibitors are effective against triple-negative breast cancers that overexpress MYCN, especially when coupled with the inhibition of MEK, another known oncogene.

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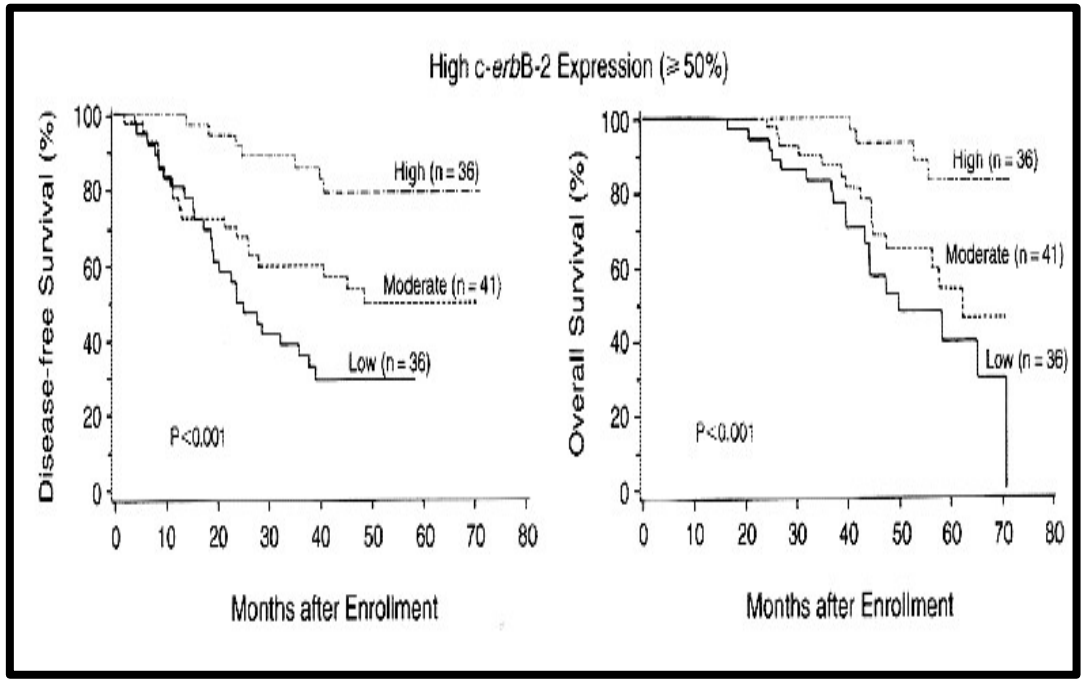
New Approaches for HER2+ Breast Cancer

Eric P. Winer, MD
Dana-Farber Cancer Institute
Boston, MA
November 11, 2020

HER2+ Breast Cancer: 1994

- HER2+ was recognized as a negative prognostic factor in adjuvant and metastatic settings
- High rate of recurrence in adjuvant setting
- Short survival in the metastatic setting
- Limited treatment options for advanced disease

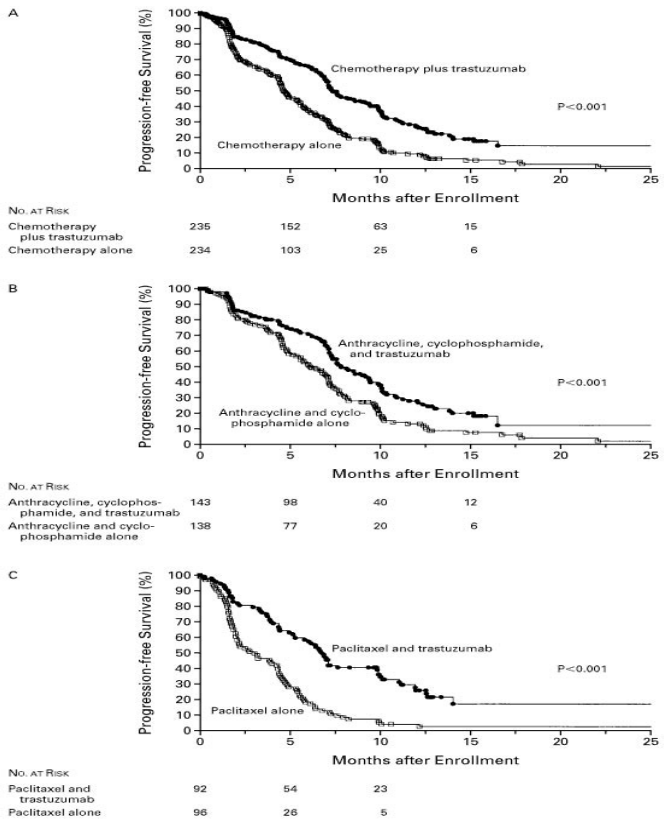
CALGB 8541: Low vs Moderate vs. High Dose CAF



High dose therapy associated with greater benefit in patients with HER2+ disease, but still relatively high risk of recurrence

Muss et al, NEJM 1994

Chemotherapy versus Trastuzumab + Chemotherapy in HER2+ MBC



	Chemotherapy (AC/paclitaxel)	Chemotherapy plus trastuzumab	P value
Response Rate	32%	50%	<0.001
PFS	4.6 months	7.4 months	<0.001
OS	20.3 months	25.4 months	0.046

Slamon et al, NEJM 2001

Why has the impact of anti-HER2 therapy been so impressive given initial results?

Problems with initial trial

1. Unexpected cardiac toxicity
2. Poor quality testing for HER2 (targeted therapy requires accurate target identification)
3. Cross-over

Advances over two decades

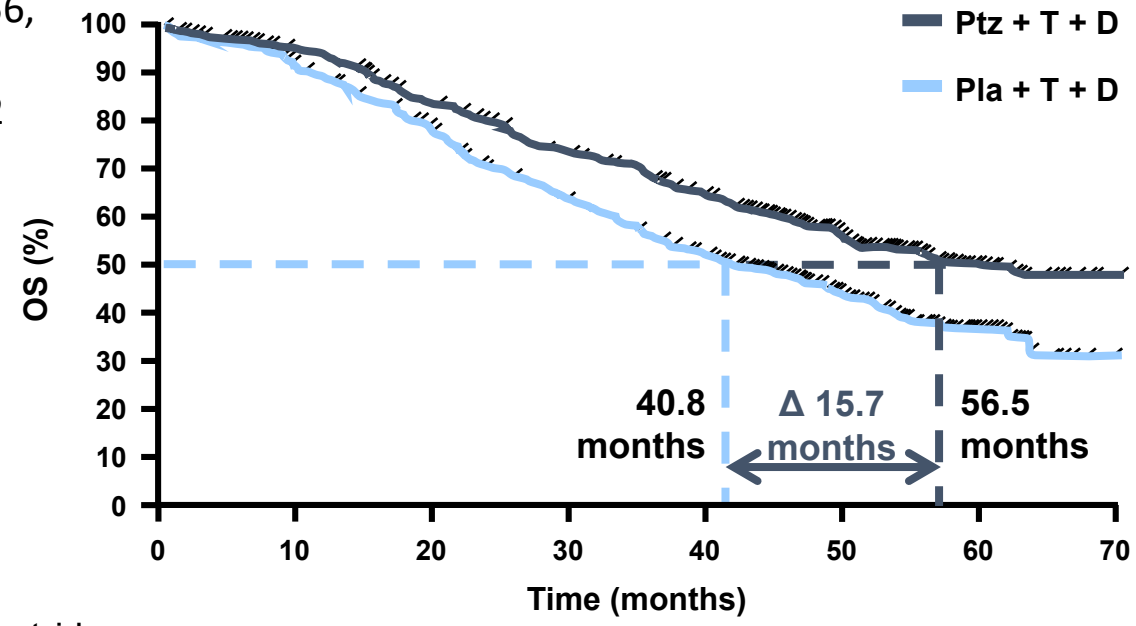
1. Indefinite continuation of HER2 directed therapy
2. Development of multiple new agents
3. Better treatment of brain metastases
4. Widespread use in early stage disease

Multiple New Agents
(showing importance of HER2 as target after initial progression)

- ~~Laptinib~~
- Pertuzumab
- T-DM1
- Neratinib
- Tucatinib
- Trastuzumab deruxtecan

CLEOPATRA: Final OS Analysis
Taxotere + Herceptin +/-Pertuzumab

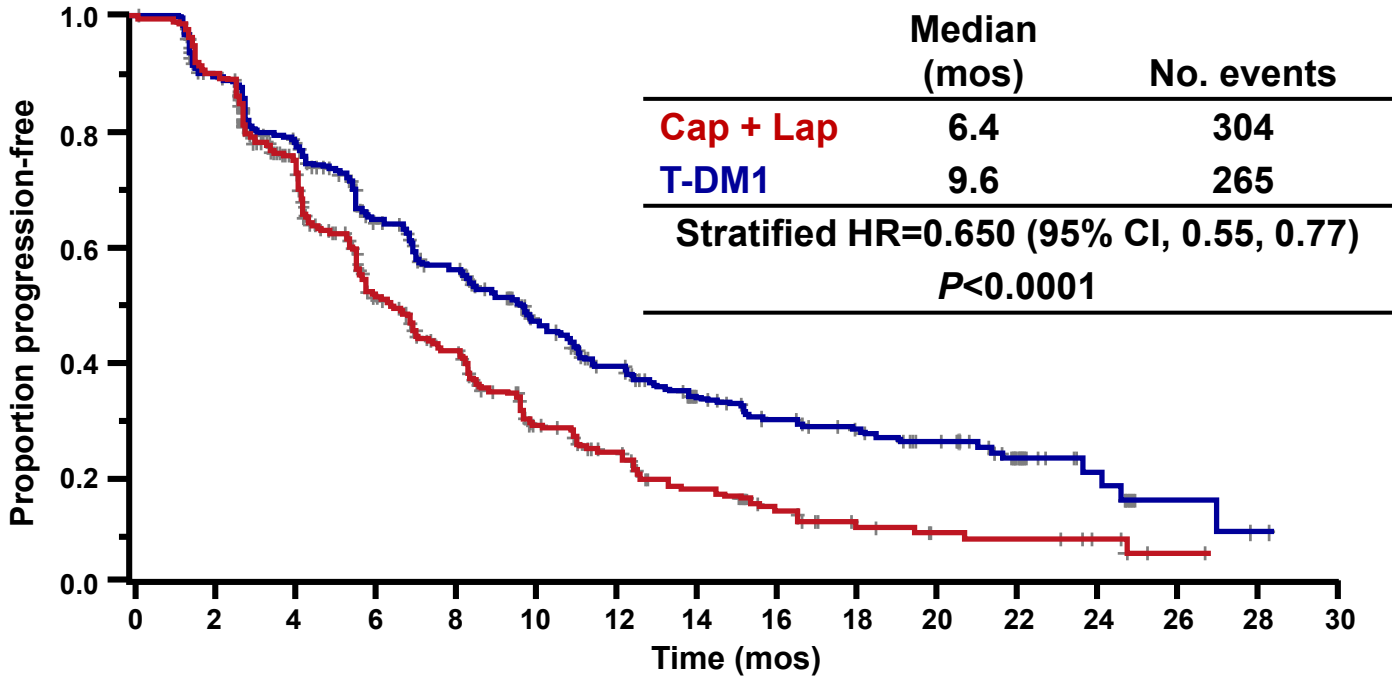
HR 0.68
95% CI = 0.56, 0.84
p = 0.0002



n at risk								
Ptz + T + D	402	371	318	268	226	104	28	1
Pla + T + D	406	350	289	230	179	91	23	0

Swain et al, ESMO 2014 and NEJM 2015

EMILIA: T-DM1 vs Capecitabine/Lapatinib in Second Line Setting

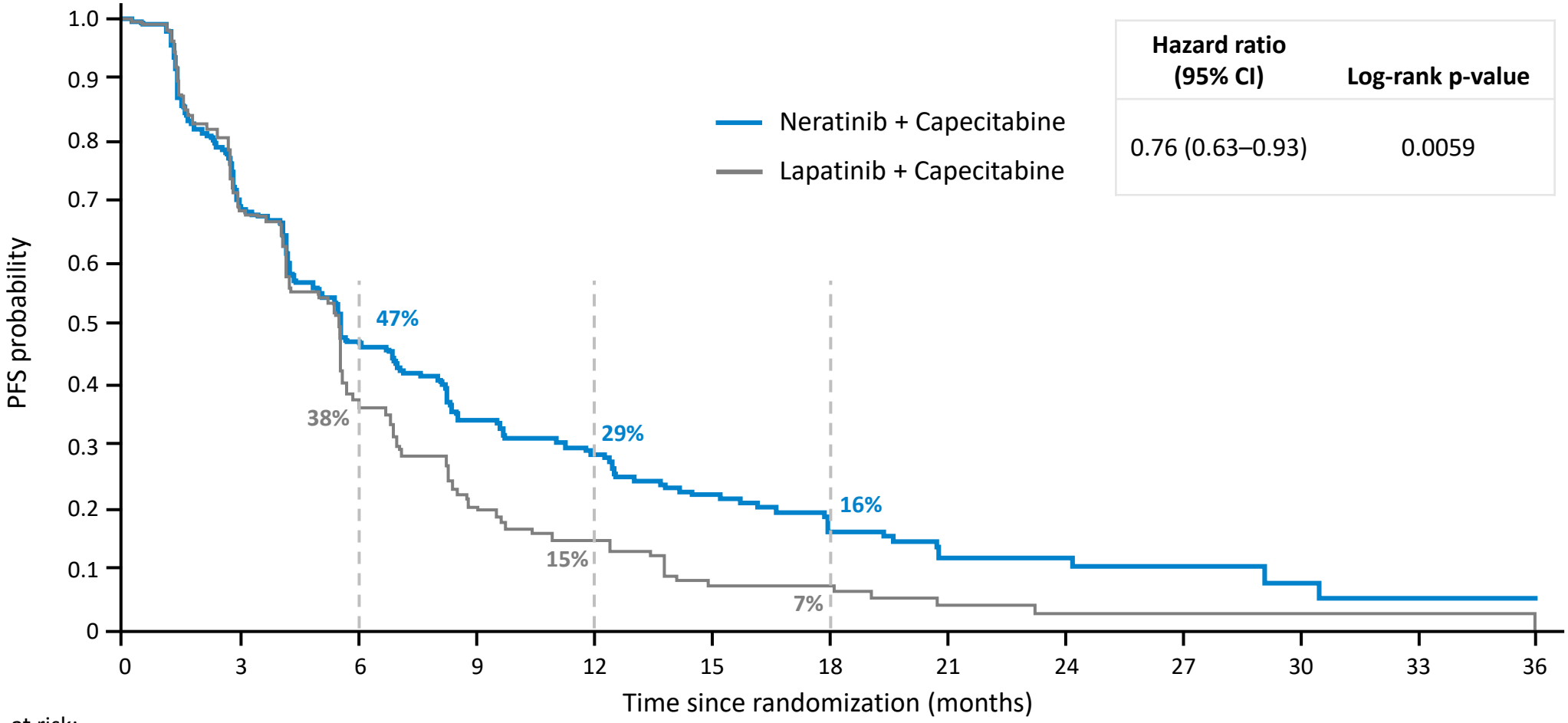


	Median (mos)	No. events
Cap + Lap	6.4	304
T-DM1	9.6	265
Stratified HR=0.650 (95% CI, 0.55, 0.77) *P*<0.0001		

Second interim analysis confirmed a statistically significant benefit in overall survival with T-DM1

Blackwell et al, NEJM 2012

Progression Free Survival: Capecitabine + Neratinib vs Lapatinib



Hazard ratio (95% CI)	Log-rank p-value
0.76 (0.63–0.93)	0.0059

No. at risk:

N+C	307	183	113	69	54	35	20	13	9	7	3	2	2
L+C	314	183	82	39	24	9	8	3	2	2	2	2	1

Brufsky et al, ASCO 2019

Tucatinib – Potent and Selective HER2 Inhibitor

- Selective small molecular tyrosine kinase inhibitor similar to Neratinib and Lapatinib
- HER2 selectivity leads to decreased potential for EGFR-related toxicities compared to dual inhibitors
- Penetrates CNS very well

Compound	Cellular Selectivity Data	
	HER2 IC_{50} (nM)	EGFR IC_{50} (nM)
Lapatinib	49	31
Neratinib	7	8
Tucatinib	8	>10,000

Moulder et al. AACR-NCI-EORTC 2011; Koch et al. AACR 2011; Borges et al, AACR Special Conference on Advances in Breast Cancer Research 2013

HER2CLIMB Trial Design

Key Eligibility Criteria

- HER2+ metastatic breast cancer
- Prior treatment with trastuzumab, pertuzumab, and T-DM1
- ECOG performance status 0 or 1
- Brain MRI at baseline
  - Previously treated stable brain metastases
  - Untreated brain metastases not needing immediate local therapy
  - Previously treated progressing brain metastases not needing immediate local therapy
  - No evidence of brain metastases

R* (2:1)

N=410

Tucatinib + Trastuzumab + Capecitabine
(21-day cycle)

Tucatinib 300 mg PO BID
+
Trastuzumab 6 mg/kg Q3W (loading dose 8 mg/kg C1D1)
+
Capecitabine 1000 mg/m^2 PO BID (Days 1-14)

N=202

Placebo + Trastuzumab + Capecitabine
(21-day cycle)

Placebo
+
Trastuzumab 6 mg/kg Q3W (loading dose 8 mg/kg C1D1)
+
Capecitabine 1000 mg/m^2 PO BID (Days 1-14)

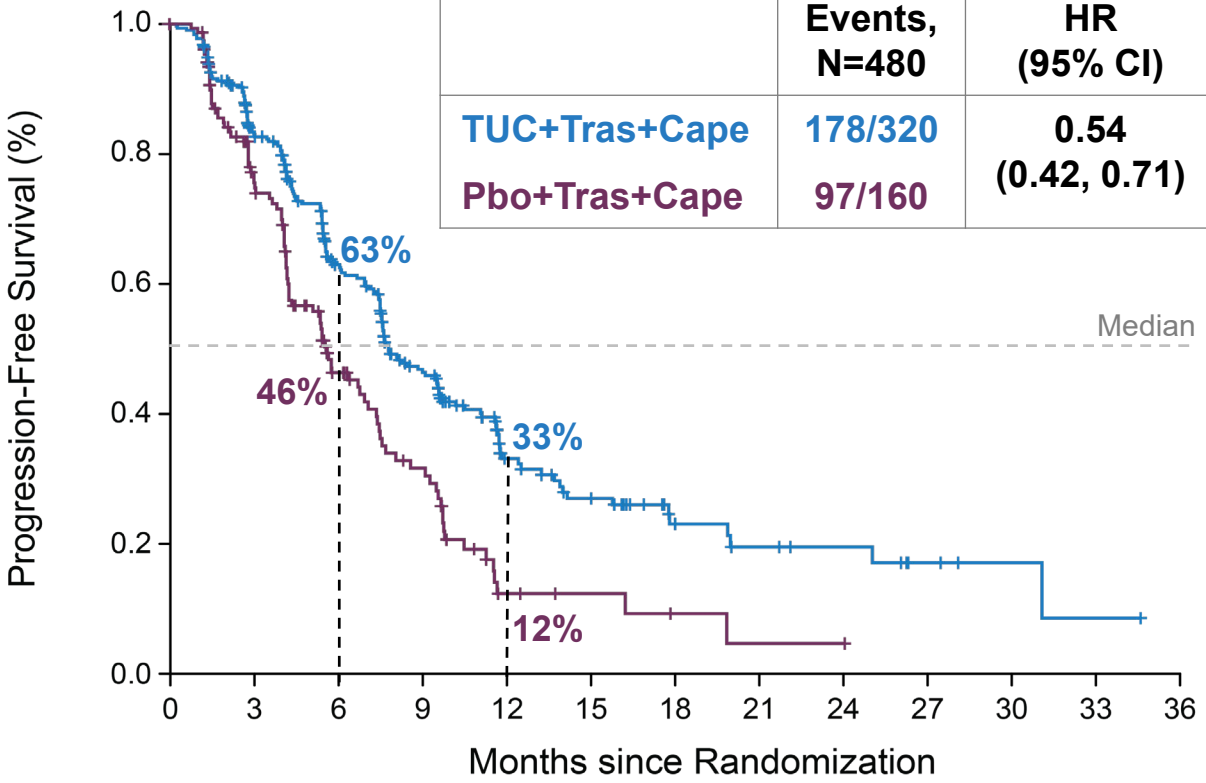
*Stratification factors: presence of brain metastases (yes/no), ECOG status (0 or 1), and region (US or Canada or rest of world)

https://clinicaltrials.gov/ct2/show/NCT02614794

Murthy…..Winer; SABCS 2019, NEJM 2020

Progression-Free Survival in the Primary Endpoint Population

	Events, N=480	HR (95% CI)	P Value
TUC+Tras+Cape	178/320	0.54 (0.42, 0.71)	<0.00001
Pbo+Tras+Cape	97/160		



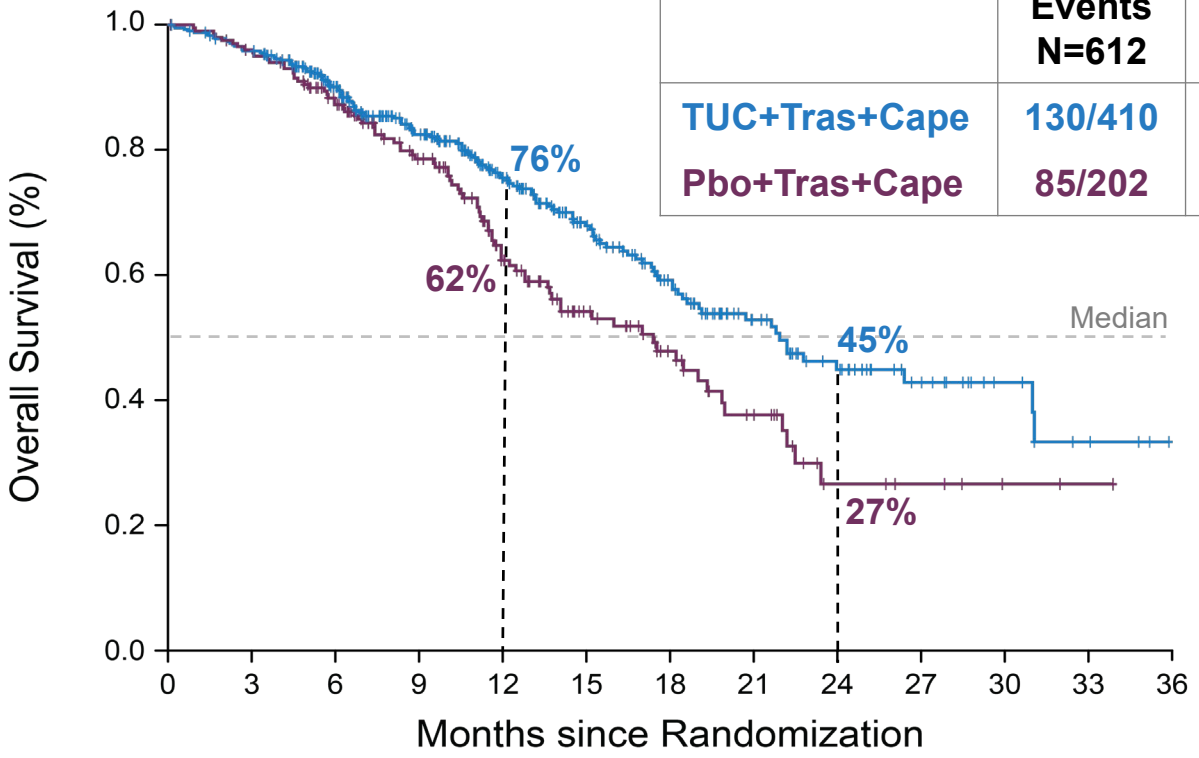
No. at Risk													
TUC+Tras+Cape	320	235	152	98	40	29	15	10	8	4	2	1	0
Pbo+Tras+Cape	160	94	45	27	6	4	2	1	1	0	0	0	0

Risk of progression or death was reduced by 46% in the primary endpoint population

One-year PFS (95% CI):	
TUC+Tras+Cape	Pbo+Tras+Cape
33% (27, 40)	12% (6, 21)
Median PFS (95% CI):	
7.8 months (7.5, 9.6)	5.6 months (4.2, 7.1)

Prespecified efficacy boundary for PFS: P=0.05
Data cut off: Sep 4, 2019

Overall Survival in the Total Study Population



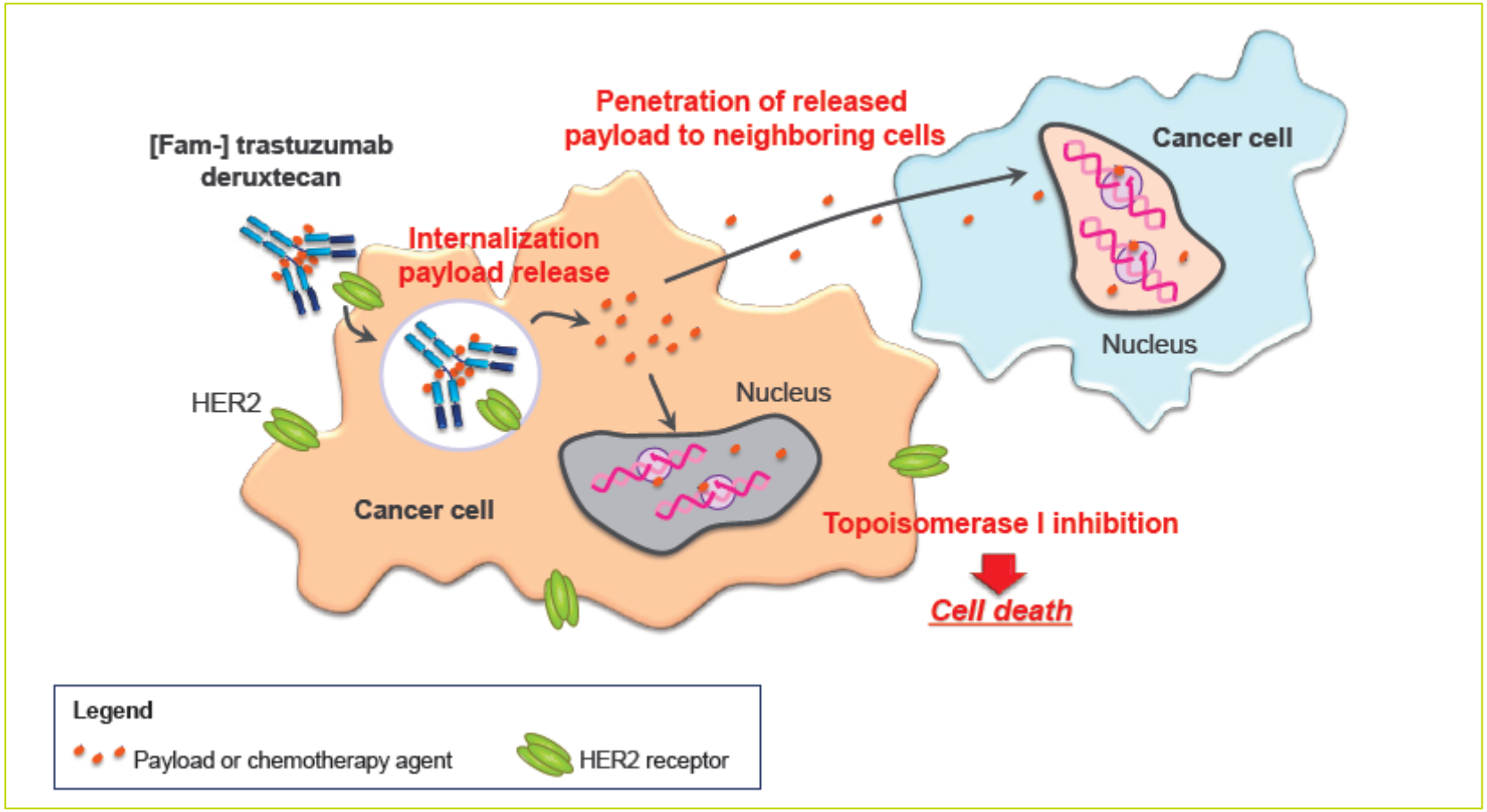
	Events N=612	HR (95% CI)	P Value
TUC+Tras+Cape	130/410	0.66 (0.50, 0.88)	0.00480
Pbo+Tras+Cape	85/202		

No. at Risk												
TUC+Tras+Cape	410	388	322	245	178	123	80	51	34	20	10	4	0
Pbo+Tras+Cape	202	191	160	119	77	48	32	19	7	5	2	1	0

Risk of death was reduced by 34% in the total population	
Two-year OS (95% CI):	
TUC+Tras+Cape 45% (37, 53)	Pbo+Tras+Cape 27% (16, 39)
Median OS (95% CI):	
21.9 months (18.3, 31.0)	17.4 months (13.6, 19.9)

was met at the first interim analysis.
Data cut off: Sep 4, 2019

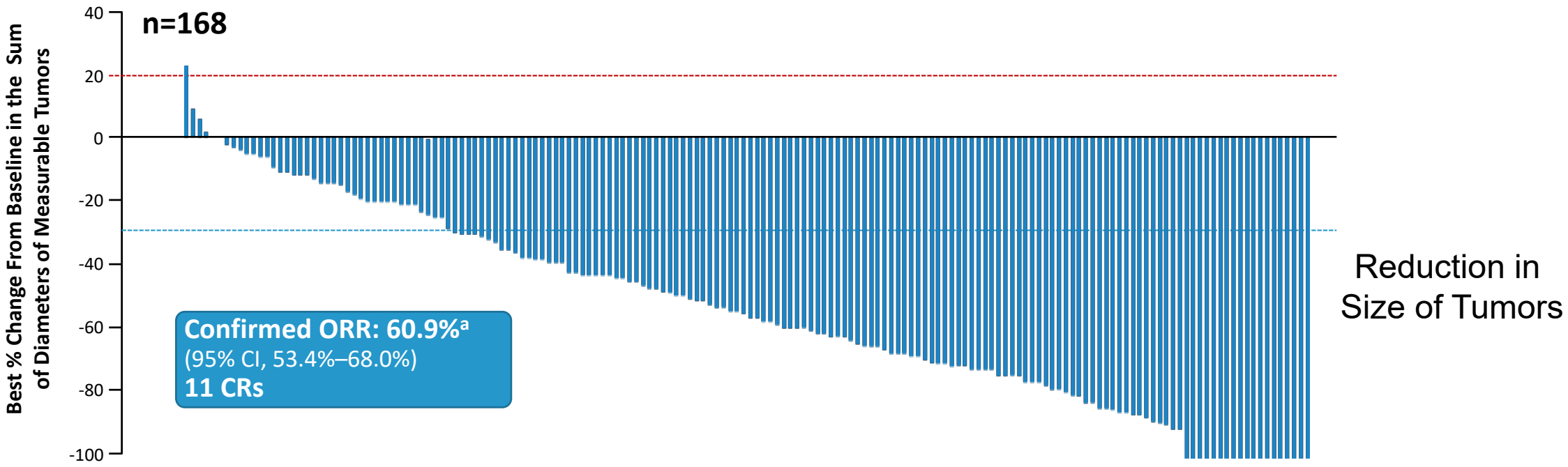
Trastuzumab Deruxtecan: The membrane-permeable payload can attack neighbouring cancer cells (i.e. a bystander effect)



ADCC= antibody-dependent cellular cytotoxicity; HER2=human epidermal growth factor receptor 2; Topo-1=topoisomerase I.

1. Ogitani Y et al. *Cancer Sci*. 2016;107:1039–1046 . 2. Ogitani Y et al. *Clin Cancer Res*. 2016;22:5097–5108.

Phase II Trial of Trastuzumab Deruxtecan



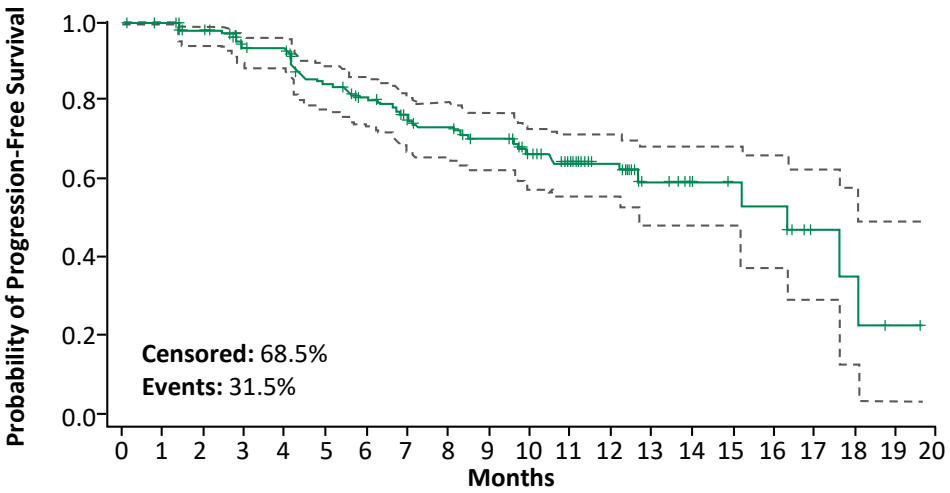
By independent central review.
The line at 20% indicates progressive disease; the line at −30% indicates partial response.
[a] Includes all patients who received T-DXd 5.4 mg/kg (intent-to-treat analysis; N=184).

Modi….Krop, SABCS 2019, NEJM 2020

Phase II Trial of Trastuzumab Deruxtecan

Progression-Free Survival

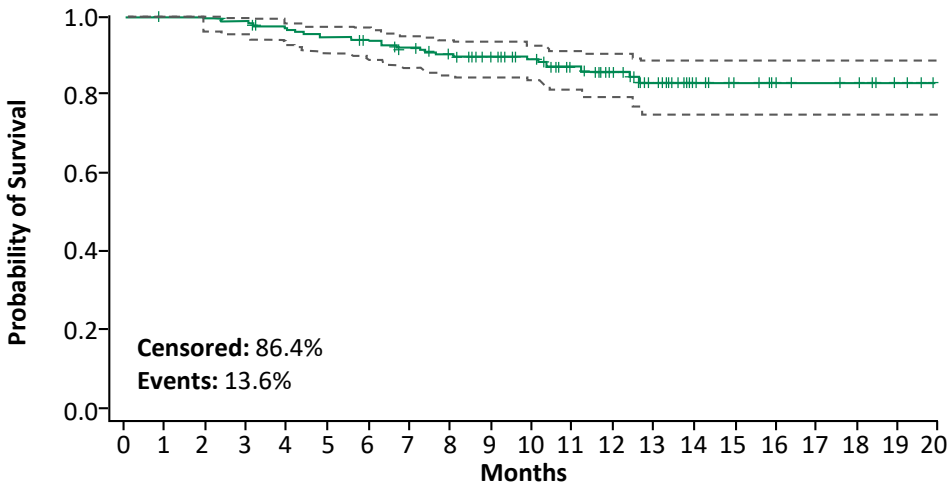
Median: 16.4 months (95% CI, 12.7-NE)



No. at risk: 184 182 174 155 153 135 121 107 103 94 69 54 38 17 11 10 9 4 3 1 0

Overall Survival

Median: Not reached (95% CI, NE-NE)



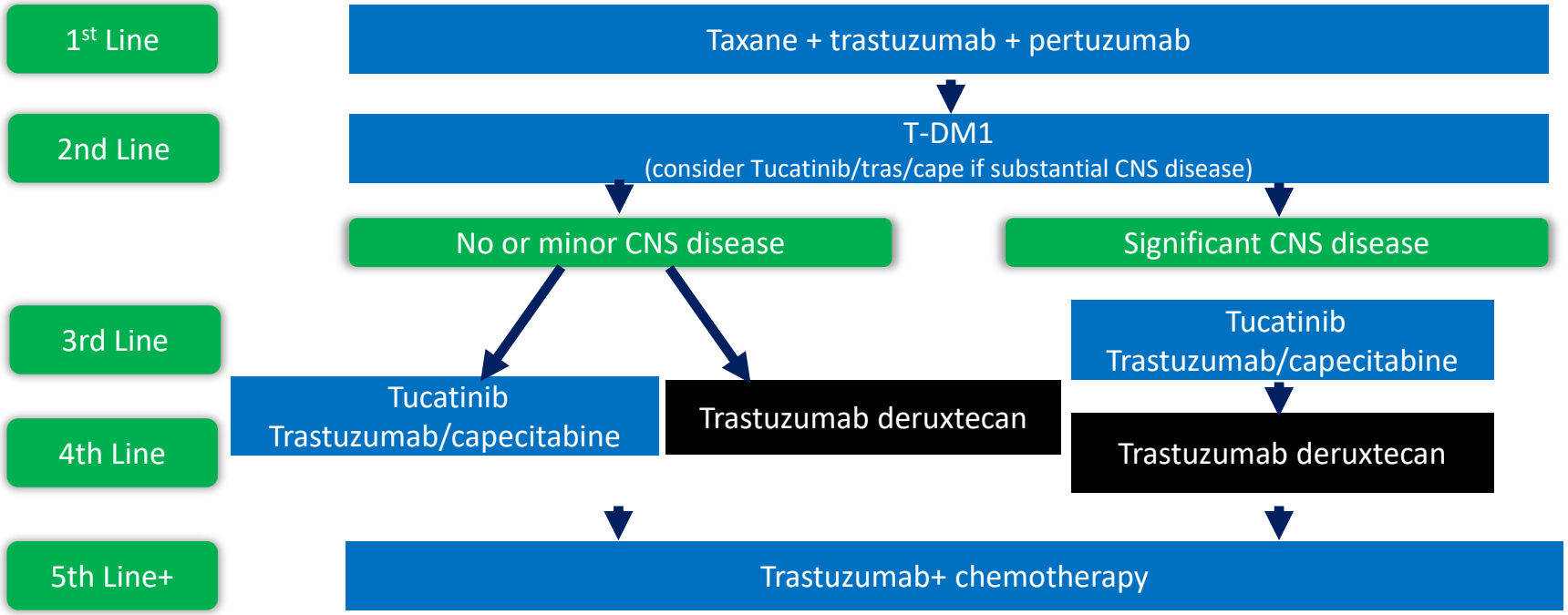
No. at risk: 184 183 182 179 174 171 167 161 155 147 133 101 66 36 21 16 12 9 8 4 0

- Median follow-up, 11.1 months (range, 0.7-19.9 months)
- Median PFS in the 24 patients with brain metastases was 18.1 months (95% CI, 6.7-18.1 months)

Patients who received T-DXd 5.4 mg/kg.
CI, confidence interval; NE, not estimable.

Modi….Krop, SABCS 2019, NEJM 2020

Approach to Therapy for Metastatic HER2+ disease: Move to Personalization



Do Not Forget Endocrine Therapy Combinations in ER+ Disease

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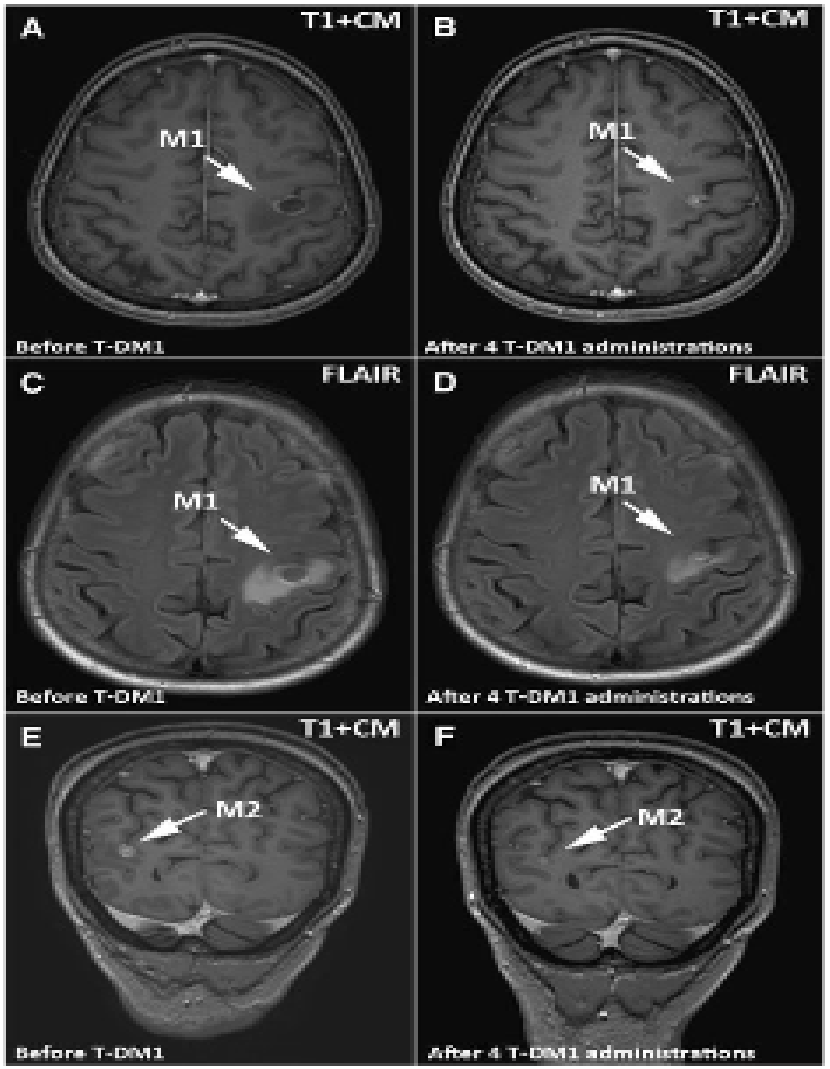
CNS Response to Large HER2-Directed Molecules

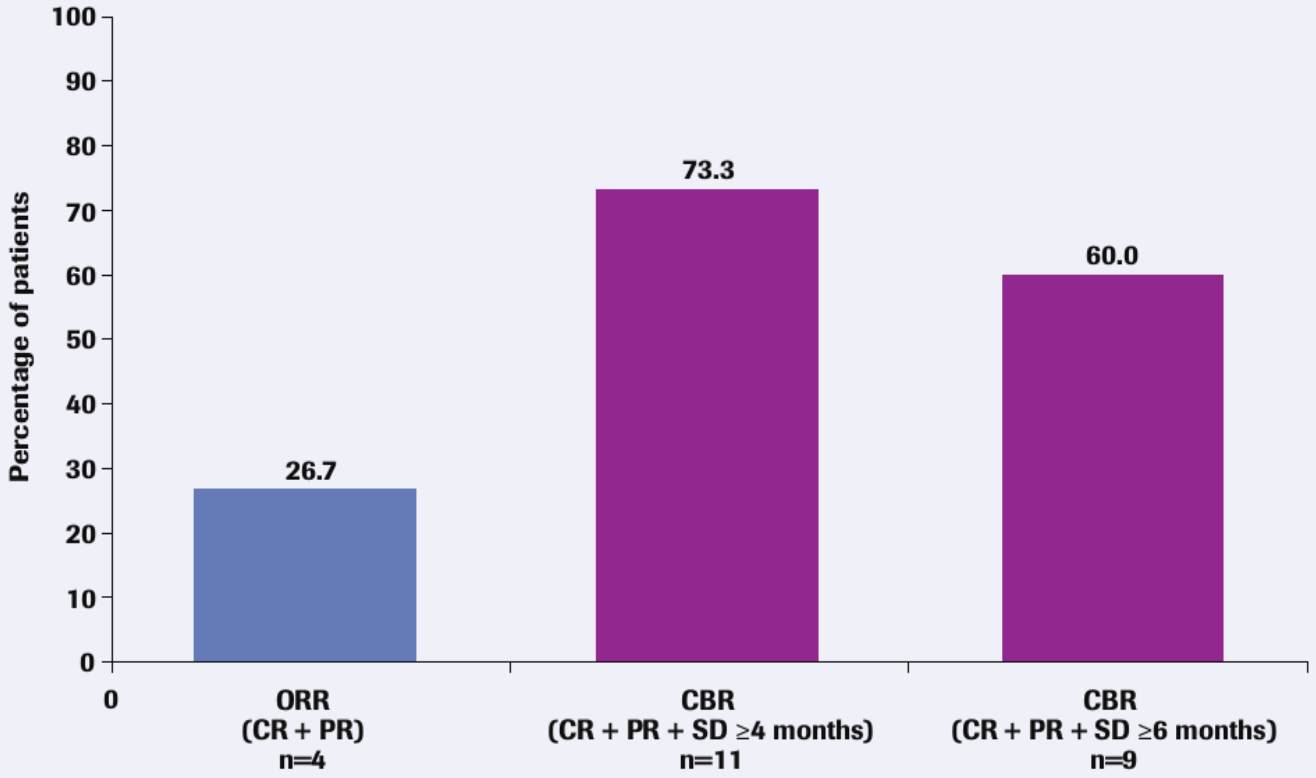
Pre-T-DM1 Post-T-DM1



J Neurooncol (2014) 116:205–206

High Dose Trastuzumab + Pertuzumab

Figure 4. Efficacy within the CNS per RANO-BM criteria (N=15)



CBR, clinical benefit rate; CNS, central nervous system; CR, complete response; ORR, objective response rate; PR, partial response; RANO-BM, Response Assessment in Neuro-Oncology Brain Metastases; SD, stable disease.

Lin et al, ASCO 2017

Lapatinib + Capecitabine for HER2+ BCBM

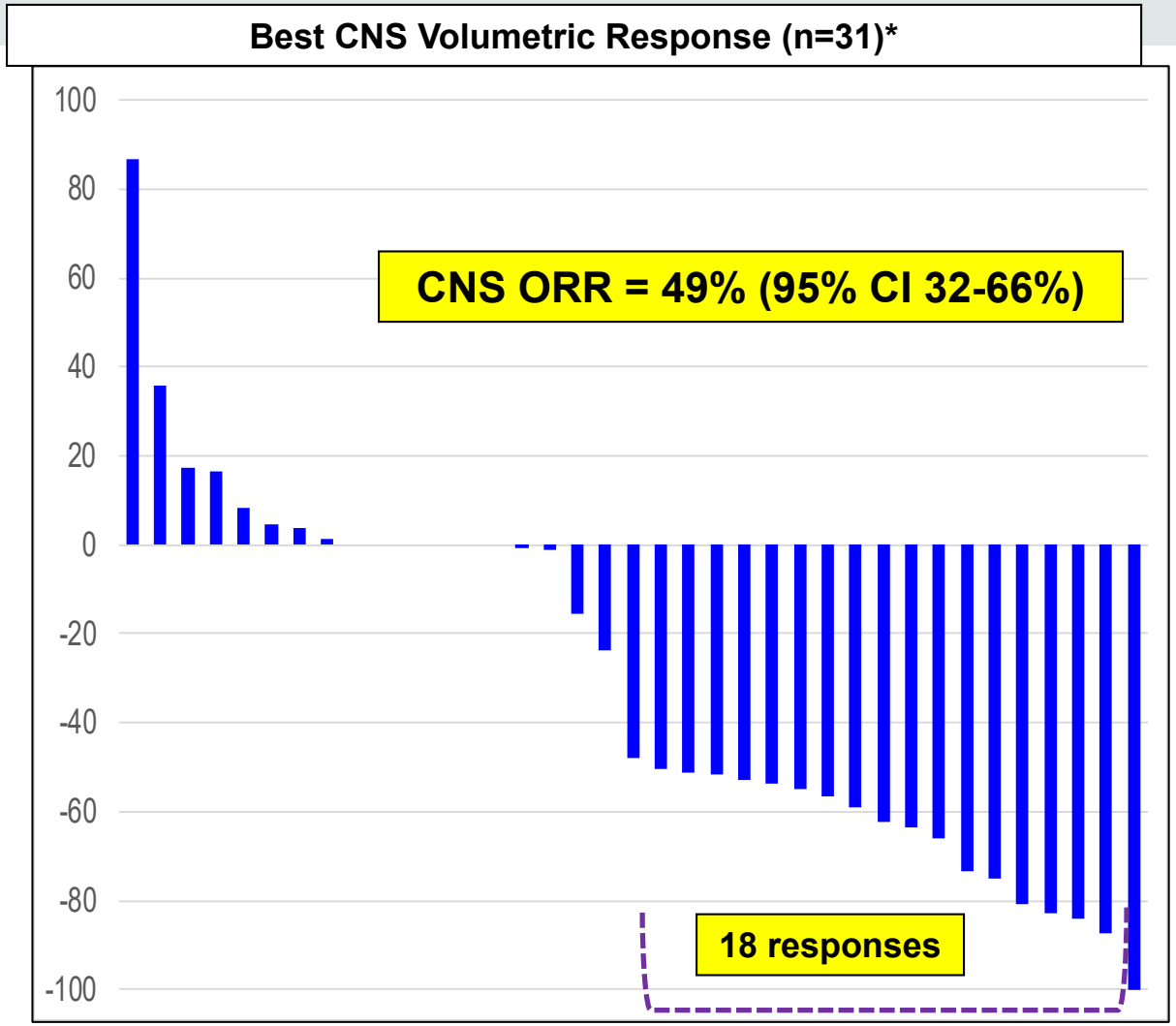
Study	N	Prior RT	CNS ORR	TTP/PFS	OS
Lin et al CCR 2009*	50	100%	**20%**	**3.6 mo**	NR
Boccardo et al, ASCO 2008 (LEAP)	138	NR	**18%**	Median time on study 2.8 mo	NR
Sutherland et al, Br J Ca 2010 (LEAP)	34	94%	**21%**	**5.1 mo**	NR
Metro et al, Ann Oncol 2011	22	86%	**32%**	**5.1 mo**	11 mo from start of LC
Lin et al, J Neuro-Oncol 2011*	13	100%	**38%**	NR	NR
Bachelot et al, Lancet Oncol 2013*	45	0%	**66%**	**5.5 mo**	91% alive at 6 mo

As a single agent, CNS ORR to lapatinib is only ~ 6%
(Lin et al, CCR 2009)

In pre-treated patients, **lapatinib-cape** results in CNS **ORR 18-38%**

In the **upfront setting** (*instead* of RT), **lap-cape** results in **CNS ORR 66%**

Capecitabine and Neratinib: Volumetric Response

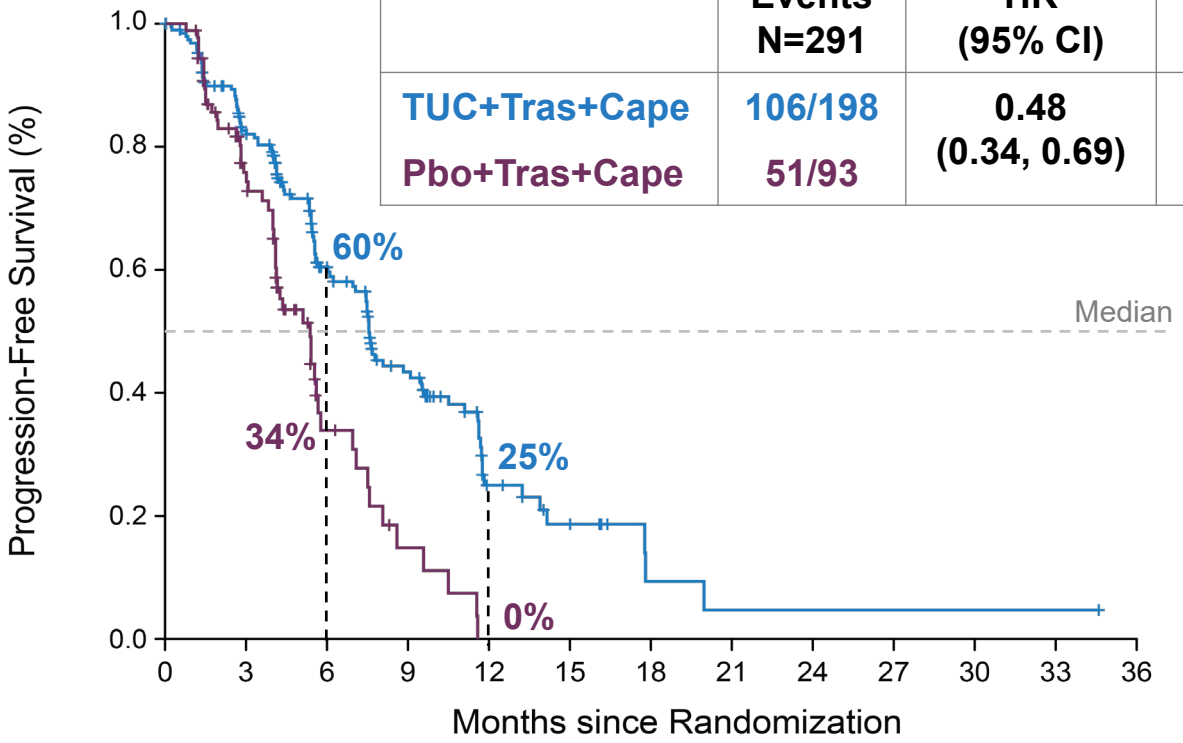


Freedman et al, ASCO 2017, JCO 2019

Led to compendium listing by NCCN

Progression-Free Survival for Patients with Brain Metastases

	Events N=291	HR (95% CI)	P Value
TUC+Tras+Cape	106/198	0.48 (0.34, 0.69)	<0.00001
Pbo+Tras+Cape	51/93		



No. at Risk													
TUC+Tras+Cape	198	144	78	45	14	8	2	1	1	1	1	1	0
Pbo+Tras+Cape	93	49	12	4	0	0	0	0	0	0	0	0	0

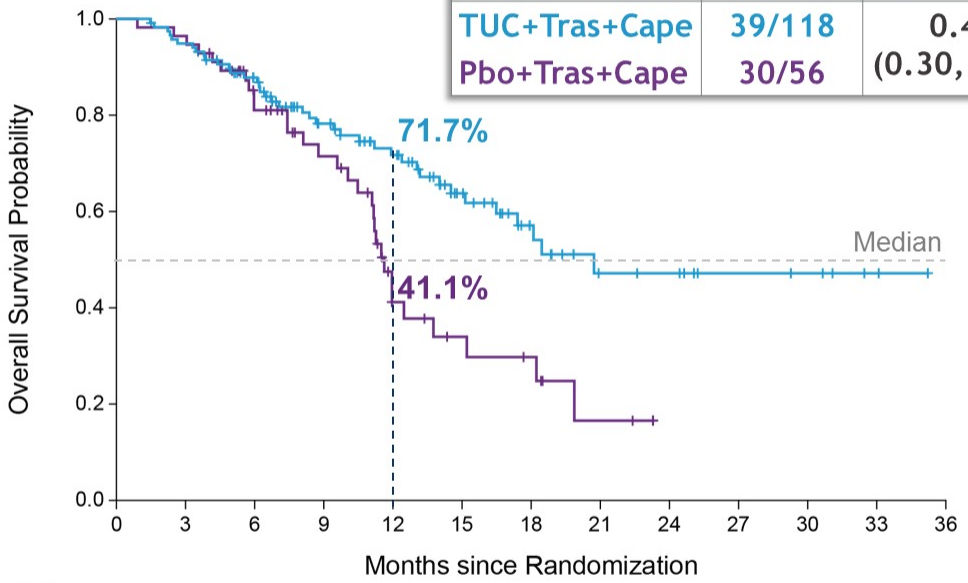
Risk of progression or death in patients with brain metastases was reduced by 52% in the total population

One-year PFS (95% CI):	
TUC+Tras+Cape 25% (17, 34)	Pbo+Tras+Cape 0%
Median PFS (95% CI):	
7.6 months (6.2, 9.5)	5.4 months (4.1, 5.7)

(P=0.0080) was met at the first interim analysis.
Data cut off: Sep 4, 2019

OS Benefit in Patients with Active Brain Metastases

	Events N=174	HR (95% CI)	P Value
TUC+Tras+Cape	39/118	0.49 (0.30, 0.80)	0.004
Pbo+Tras+Cape	30/56		



No. at Risk

TUC+Tras+Cape	118	111	89	66	51	33	19	11	10	6	5	2	0
Pbo+Tras+Cape	56	54	39	29	12	8	6	2	0	0	0	0	0

HR: hazard ratio computed from Cox proportional hazards model using stratification factors (ECOG performance status: 0/1, and Region of world: North America/Rest of World) at randomization. All P values are nominal.

Risk of death was reduced by 51% in patients with active brain metastases

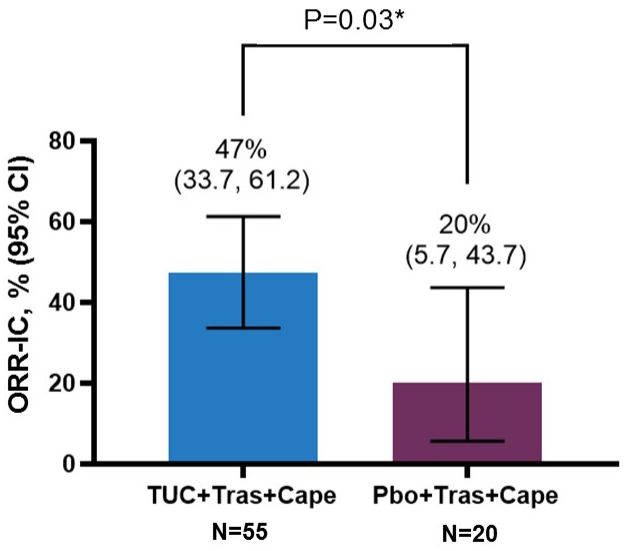
One-year OS (95% CI):	
TUC+Tras+Cape 71.7% (61.4, 79.7)	Pbo+Tras+Cape 41.1% (25.5, 56.1)
Median OS (95% CI):	
20.7 months (15.1, NE)	11.6 months (10.5, 13.8)

NE: not estimable

Lin et al. ASCO 2020

Intracranial Response Rate (ORR-IC) in Patients with Active Brain Metastases and Measurable Intracranial Lesions at Baseline

Confirmed Objective Response Rate (RECIST 1.1)



*Stratified Cochran-Mantel-Haenszel P value

	TUC+Tras+Cape (N=55)	Pbo+Tras+Cape (N=20)
Best Overall Intracranial Response[a], n (%)		
Complete Response (CR)	3 (5.5)	1 (5.0)
Partial Response (PR)	23 (41.8)	3 (15.0)
Stable Disease (SD)	24 (43.6)	16 (80.0)
Progressive Disease (PD)	2 (3.6)	0
Not Available[b]	3 (5.5)	0
Subjects with Objective Response of Confirmed CR or PR, n	26	4
Duration of Intracranial Response (DOR-IC)[e] (95% CI)[f], months	6.8 (5.5, 16.4)	3.0 (3.0, 10.3)

(a) Confirmed Best overall response assessed per RECIST 1.1. (b) Subjects with no post-baseline response assessments. (c) Two-sided 95% exact confidence interval, computed using the Clopper-Pearson method (1934). (d Cochran-Mantel-Haenszel test controlling for stratification factors (ECOG performance status: 0/1, and Region of world: North America/Rest of World) at randomization. (e) As estimated using Kaplan-Meier methods. (f) Calculated using the complementary log-log transformation method (Collett, 1994).

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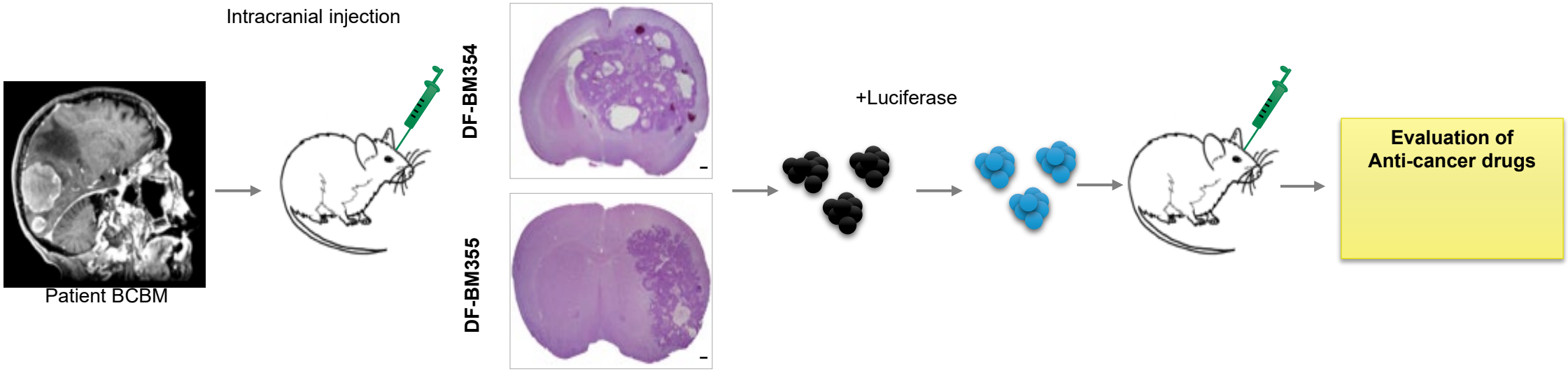
PRESENTED BY: Nancy Lin, nlin@partners.org

Orthotopic PDX Models of Brain Metastases



*Note: Initial experiments done by intracranial injection—we have subsequently show success using intra-carotid or intra-cardiac injection

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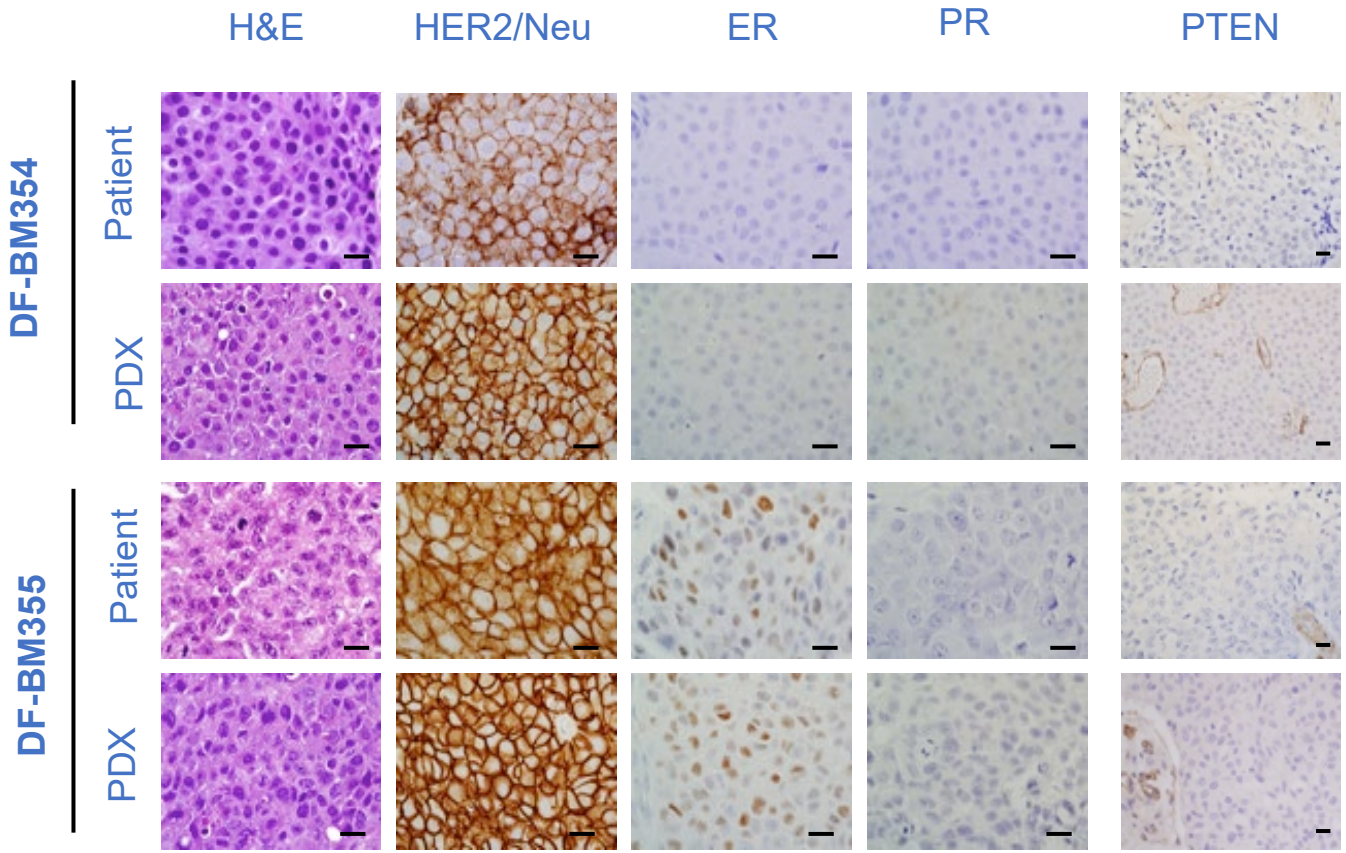
Courtesy of Jean Zhao

PDXs maintain the pathological and molecular profiles of primary tumors



BM-ID#	IHC		
	HER2	ER	PTEN
354	+	-	-
355	+	+	-
463	+	+	-
503	+	+	-
507	+	-	-
590	+	-	-
623	+	+	-
686	+	+	-
727	+	+	+
751	+	+	-
794	+	-	-
Ni-7	+	+	-
Ni-17	+	-	-

J Ni, & S. Ramkissoon

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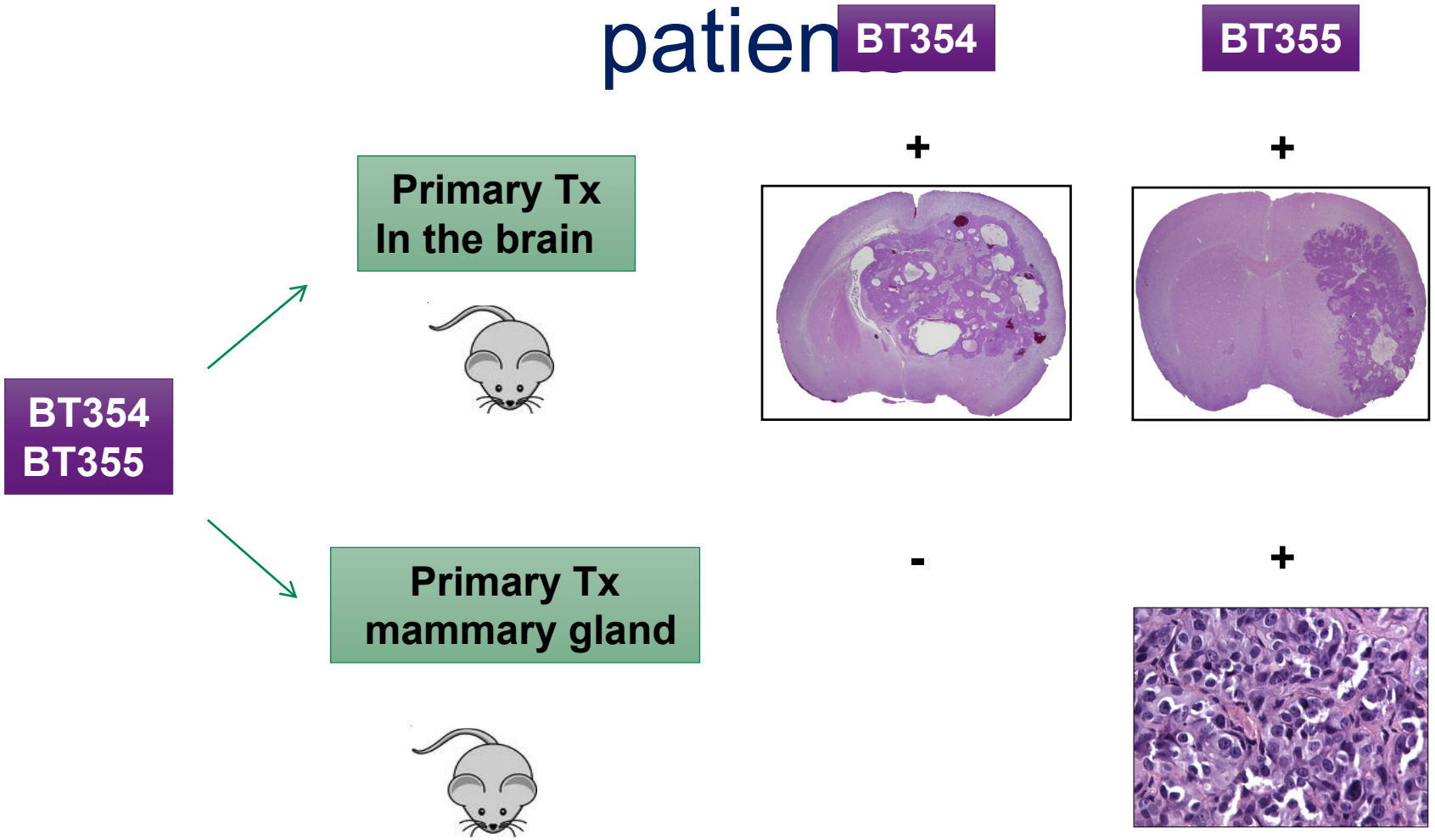
PDX models of brain metastases derived from HER2 breast cancer patients



In general, brain mets have a substantially higher take rate in brain than mammary fat pad

J Ni, & S. Ramkissoon (Zhao laboratory)

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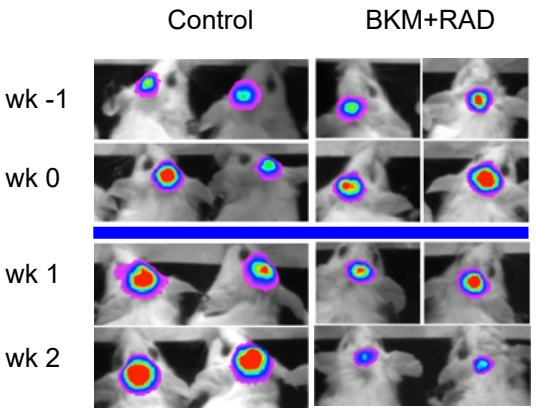
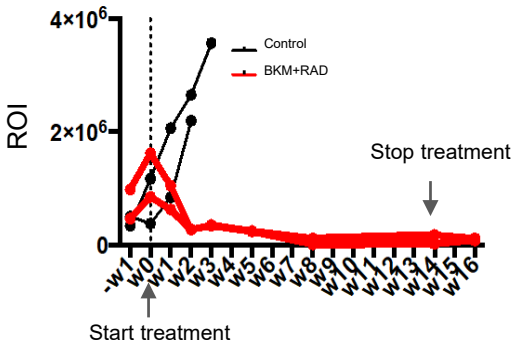
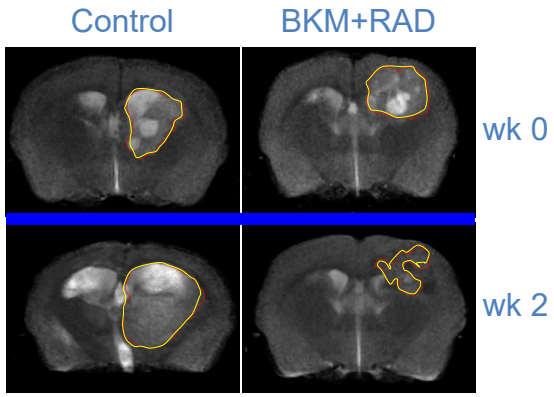
Evaluation of Rational Targeted Therapies

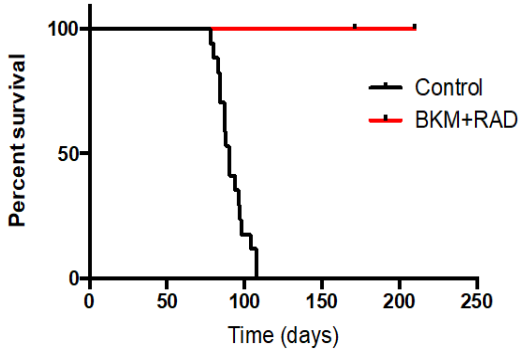
Single Agents with Minimal Activity
- -- lapatinib
- -- BKM 120
- -- bromodomain inhibitor
- -- RAD 001

Multiple Combinations Tested With Minimal Activity Except BKM + RAD 001









Ni et al, Nat Med 2016

Perhaps more than in any other area of HER2+ disease, progress will only be made as a result of close collaboration between laboratory and clinic

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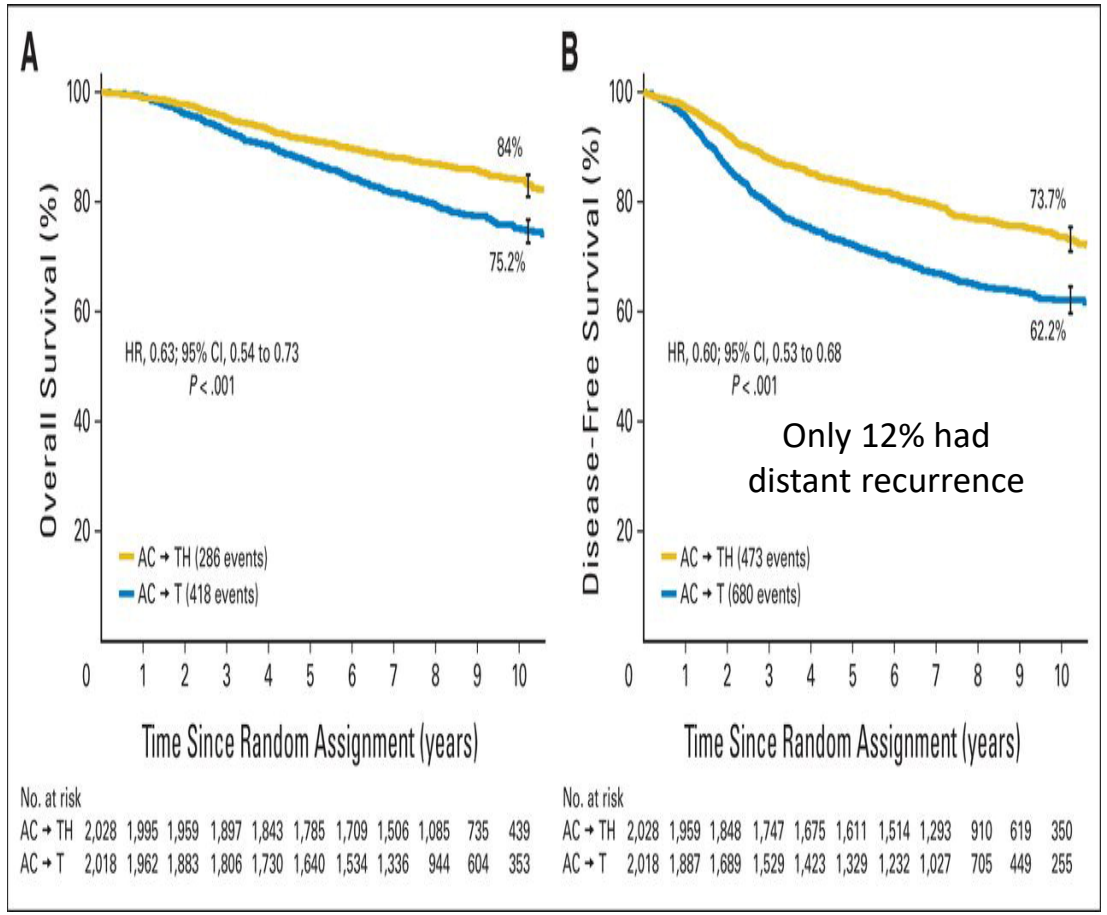
Major Question For HER2+ Metastatic Disease: Are Some Patients Curable?

- Clearly tail on the curve in most studies
- Patients with *de novo* disease most likely to have long term disease free survival
- With new non-cross resistant drugs, can we develop curative approaches?
- What will be the role of local therapy?
- Planned TBCRC study is trying to tackle this challenge

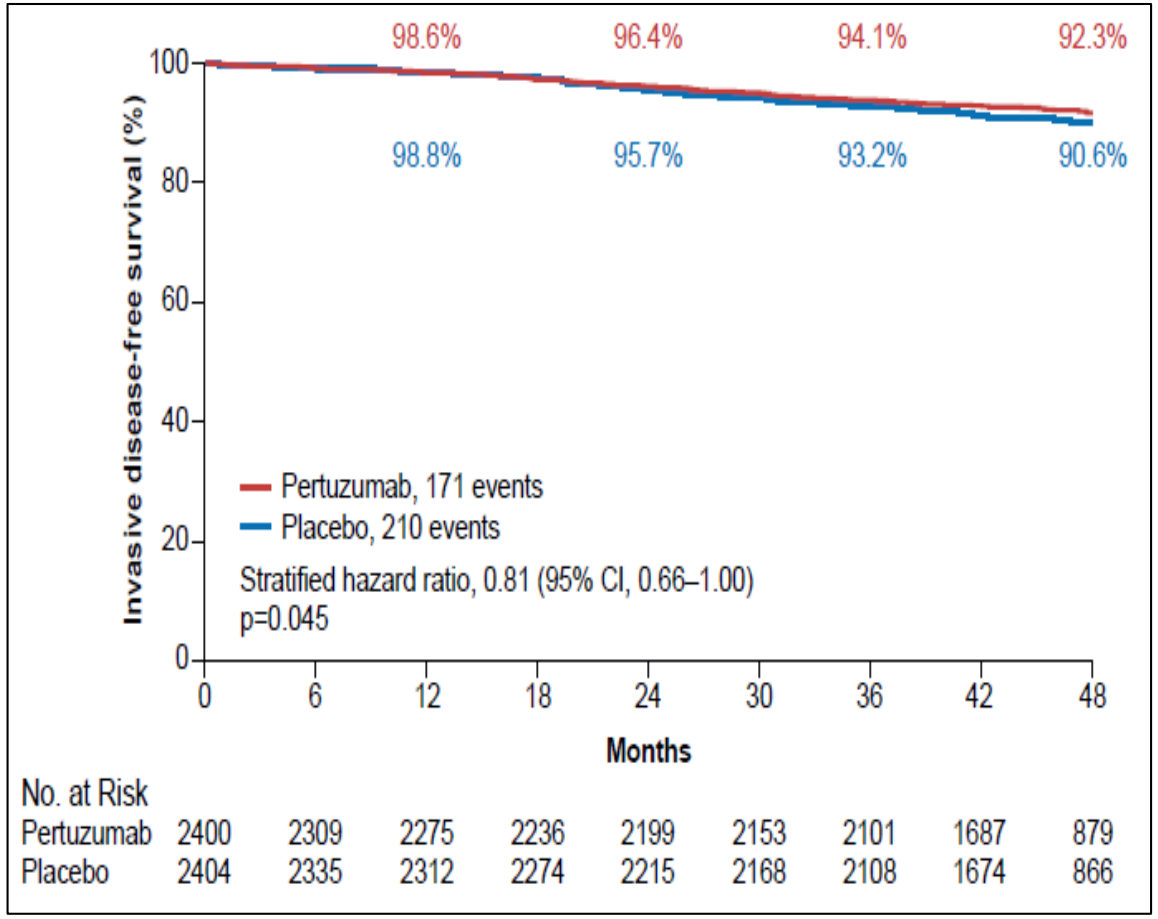
Outcomes in stage I-III Disease with Chemotherapy and Trastuzumab

Joint Analysis



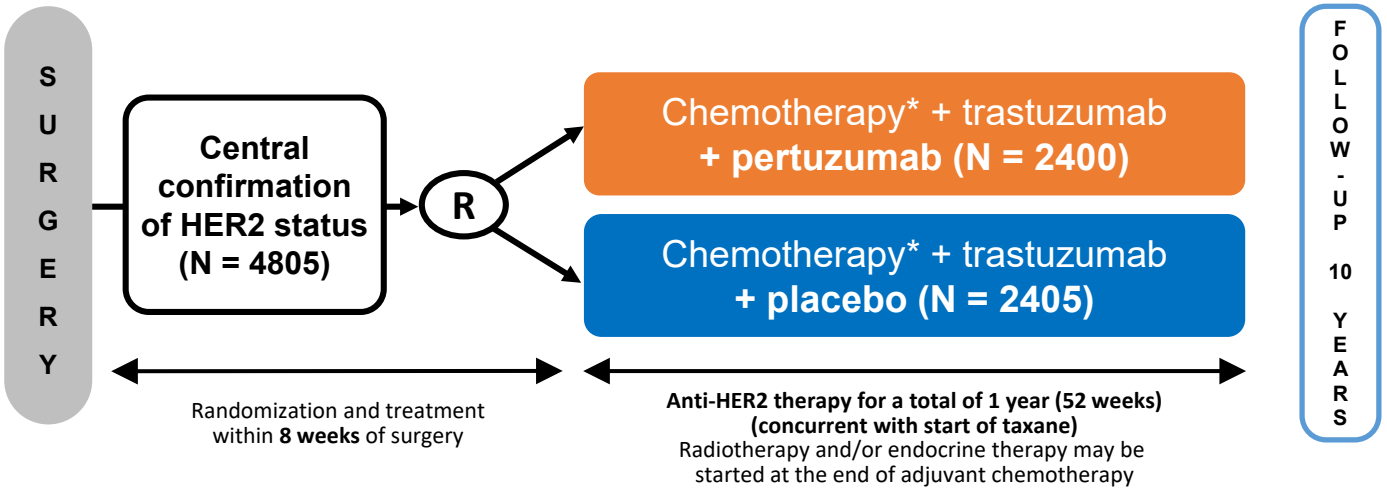
Perez et al, JCO 2014

Aphinity



von Minckwitz et al NEJM 2017

APHINITY: A Phase III adjuvant study investigating the benefit of pertuzumab when added to trastuzumab + chemotherapy



* Standard anthracycline or non-anthracycline (TCH) regimens were allowed: 3–4 x FEC (or FAC) → 3–4 x TH; 4 x AC (or EC) → 4 x TH; 6 x TCH

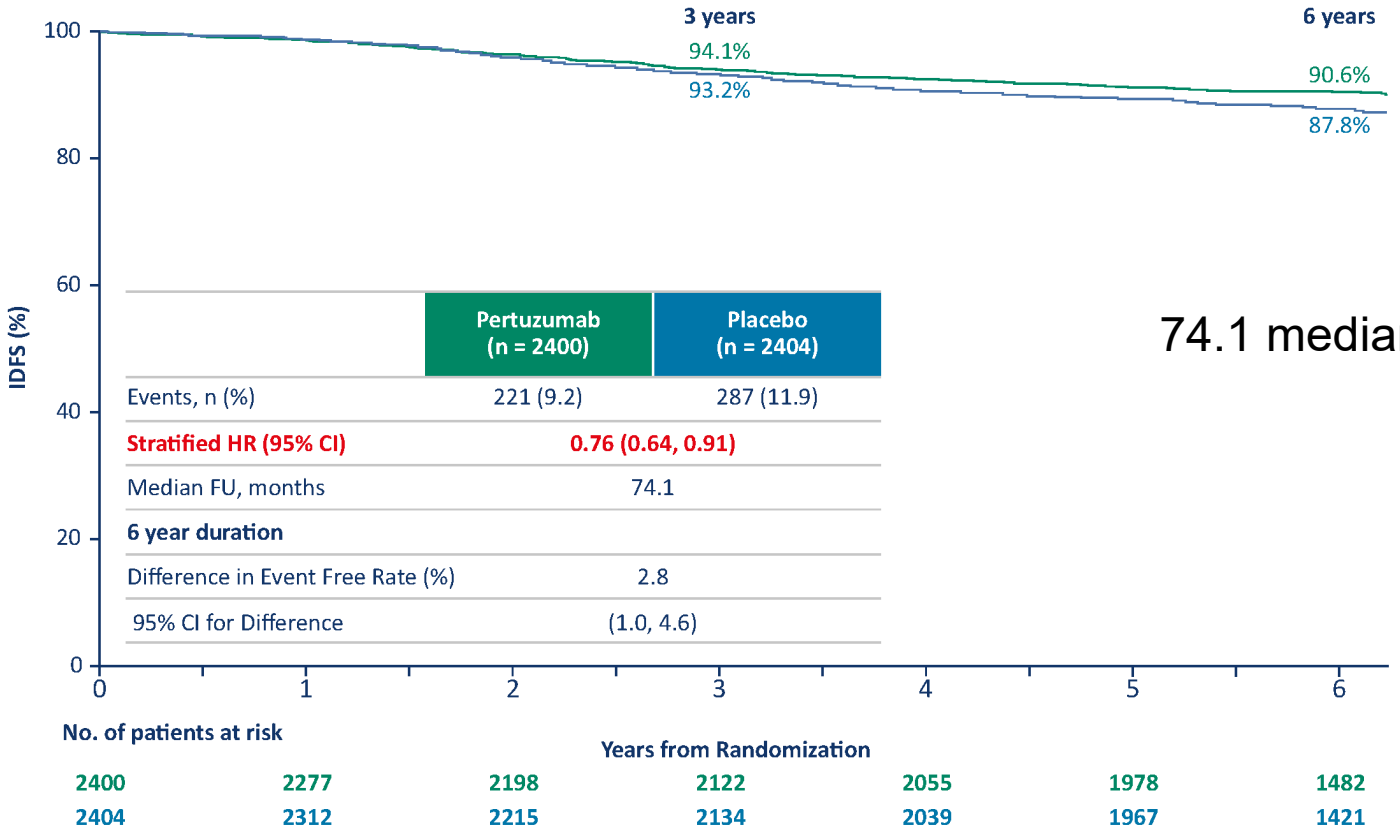
- **Primary endpoint:** IDFS (APHINITY definition differs from STEEP definition)
- **Secondary endpoint:** IDFS with 2nd primary non-breast primary cancers included, DFS, OS, RFI, DRFI, safety, and HRQoL
- **Stratification factors:** nodal status, HR status, chemotherapy regimen, geographic region, Protocol version (A vs. B)
- **Clinical cut off date (CCOD)** at the time of primary analysis was 19 Dec 2016, median follow up of 45.4 months

DFS, disease-free survival; DRFI, distant relapse-free interval; HR, hormone receptor; HRQoL, health-related quality of life; IDFS, invasive disease-free survival; OS, overall survival; RFI, relapse-free interval

Adapted from von Minckwitz et al. N Engl J Med 2017
www.clinicaltrials.gov/ct2/show/NCT01358877

Piccart et al, SABCS 2019

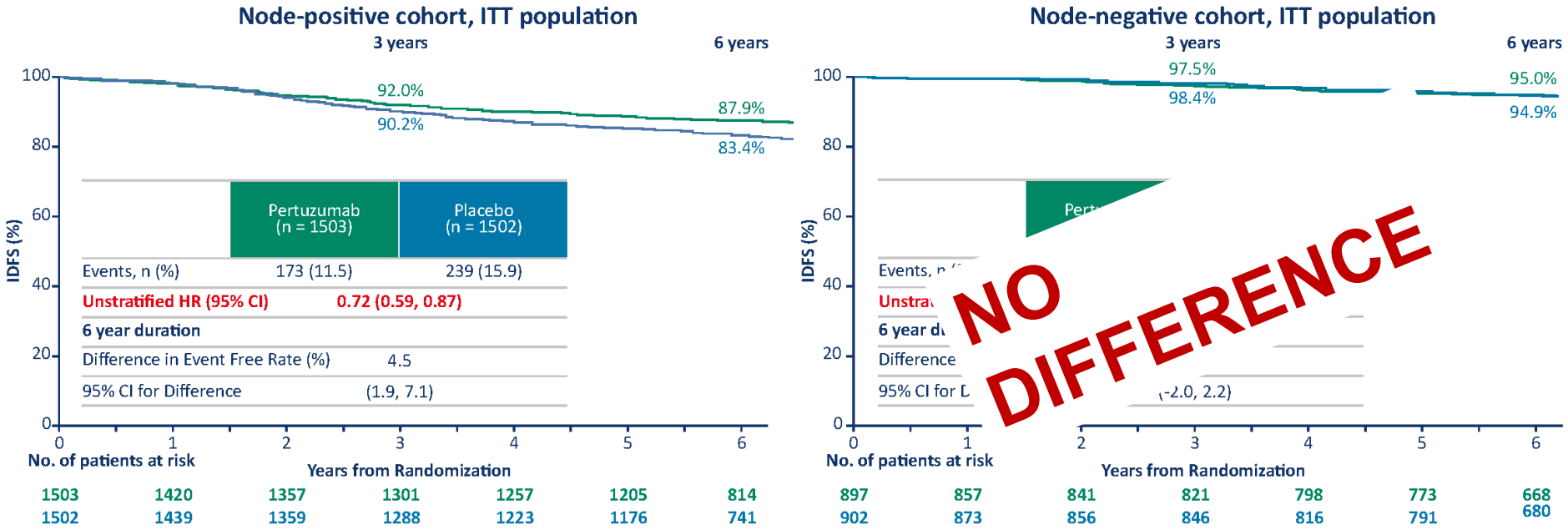
APHINITY Updated Analysis
Time to first IDFS event by treatment regimen (ITT population)



	Pertuzumab (n = 2400)	Placebo (n = 2404)
Events, n (%)	221 (9.2)	287 (11.9)
Stratified HR (95% CI)	**0.76 (0.64, 0.91)**	
Median FU, months	74.1	
6 year duration		
Difference in Event Free Rate (%)	2.8	
95% CI for Difference	(1.0, 4.6)	

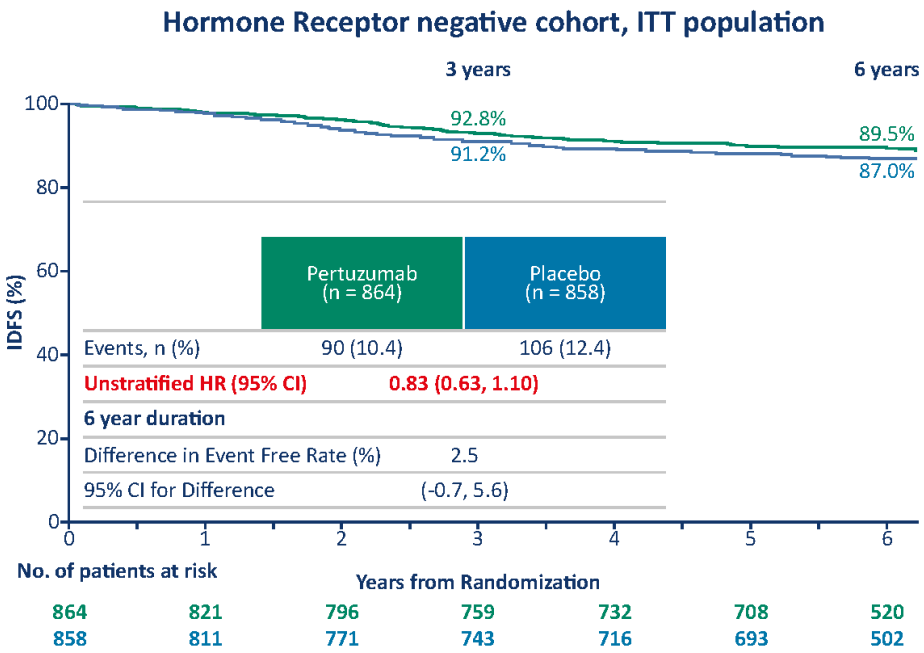
74.1 median follow-up

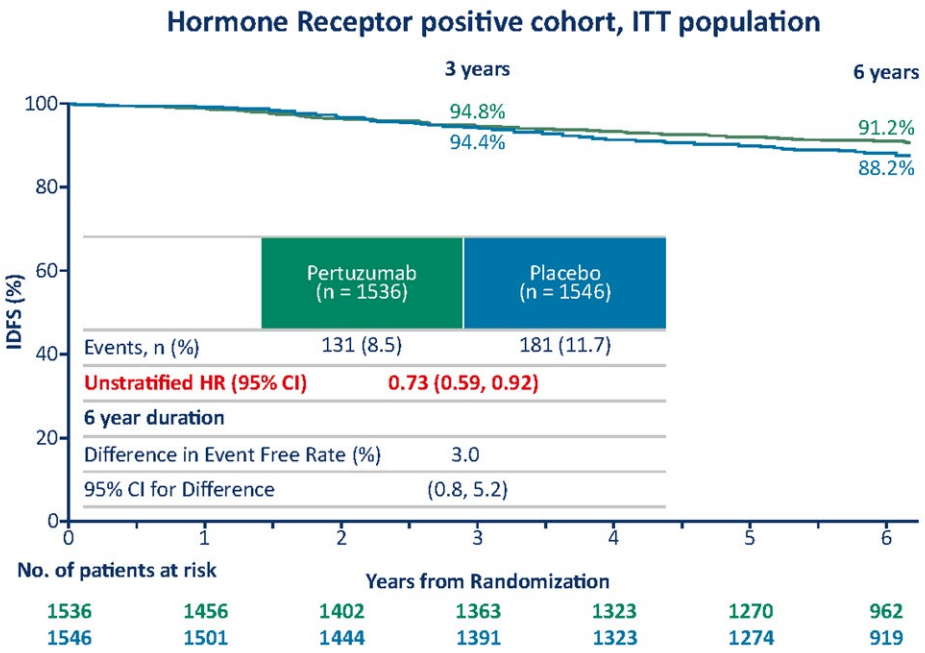
APHINITY Updated Analysis
Time to first IDFS event by treatment regimen and nodal status



74.1 median follow-up

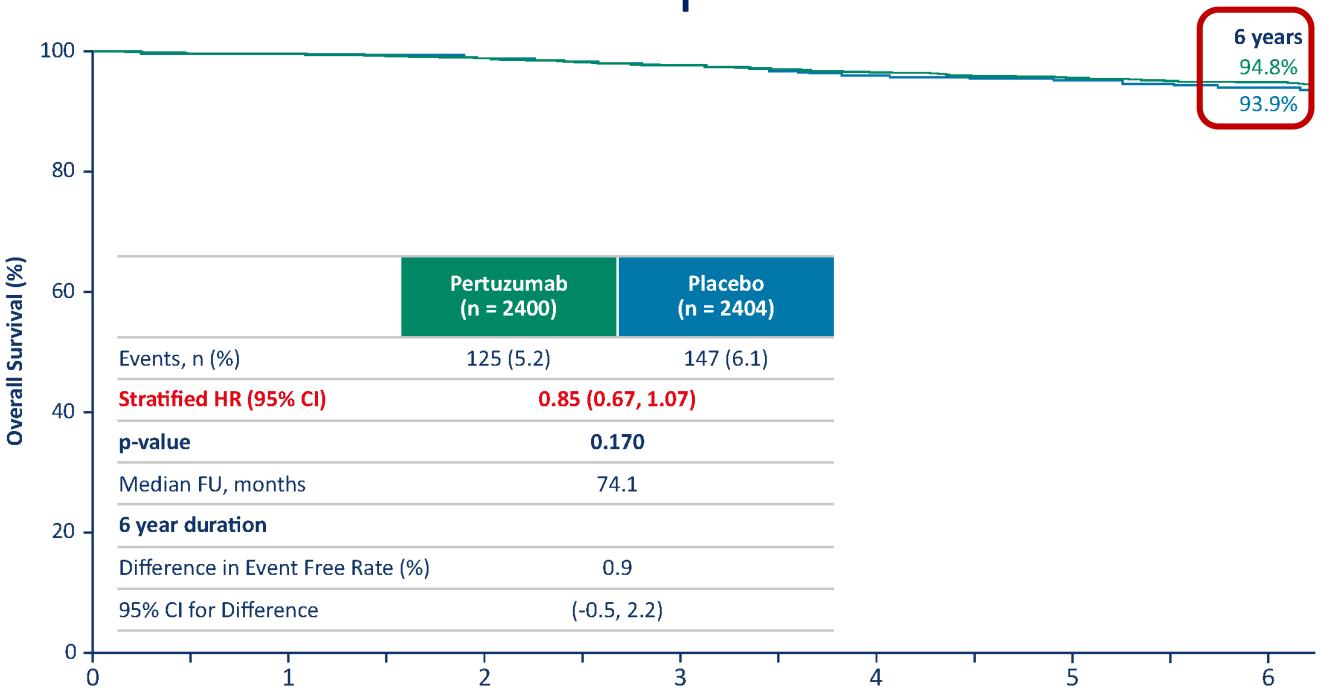
APHINITY Updated Analysis
Time to first IDFS event by treatment regimen and HR status





74.1 median follow-up

APHINITY Interim Overall Survival Analysis
Median follow-up 74.1 months



	Pertuzumab (n = 2400)	Placebo (n = 2404)
Events, n (%)	125 (5.2)	147 (6.1)
Stratified HR (95% CI)	**0.85 (0.67, 1.07)**	
p-value	**0.170**	
Median FU, months	74.1	
6 year duration		
Difference in Event Free Rate (%)	0.9	
95% CI for Difference	(-0.5, 2.2)	

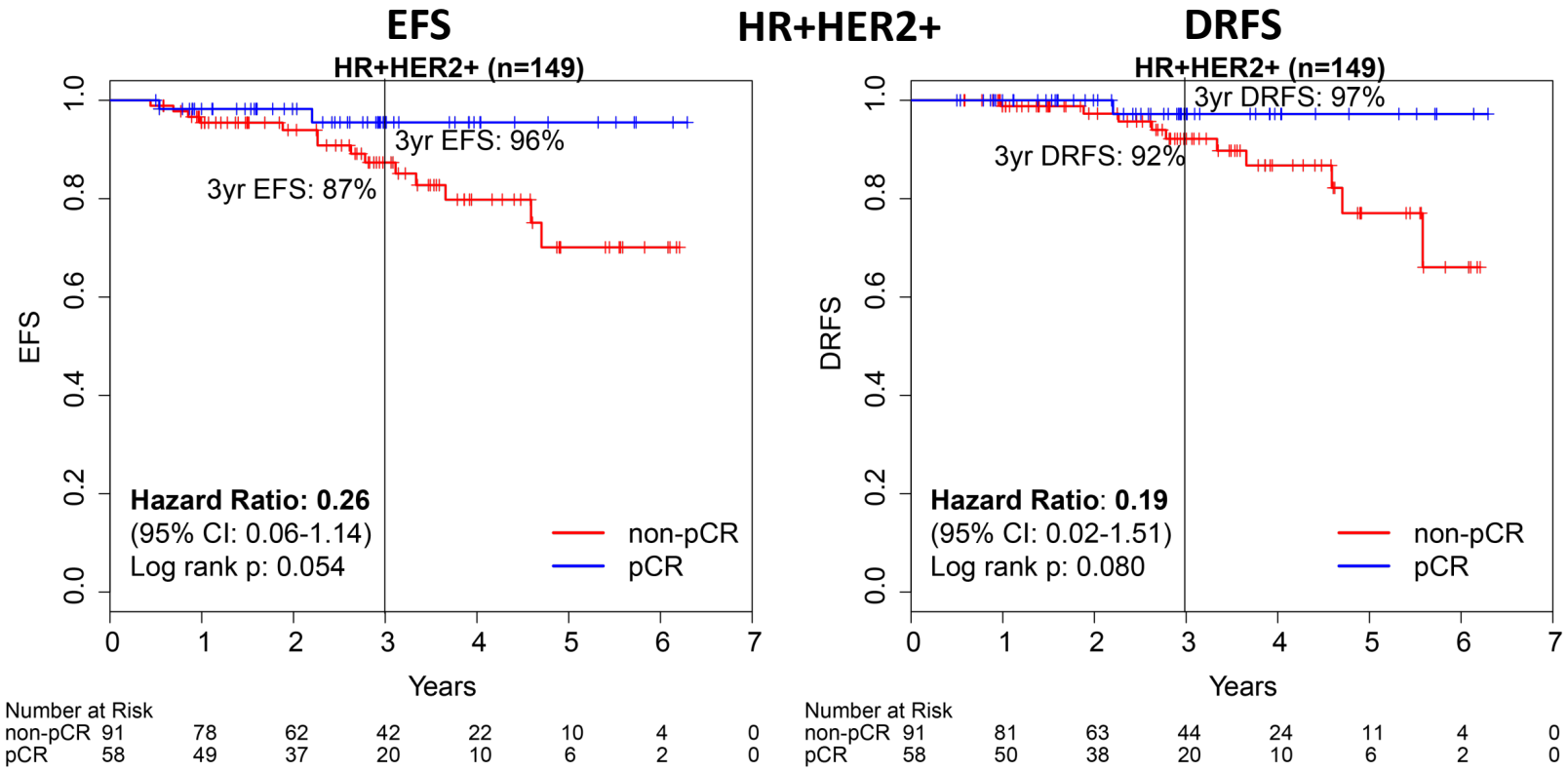
No. of patients at risk

2400	**2304**	**2261**	**2216**	**2161**	**2090**	**1544**
2404	**2339**	**2292**	**2241**	**2165**	**2107**	**1522**

For majority of patients with stage II/III disease, neoadjuvant therapy is the preferred approach

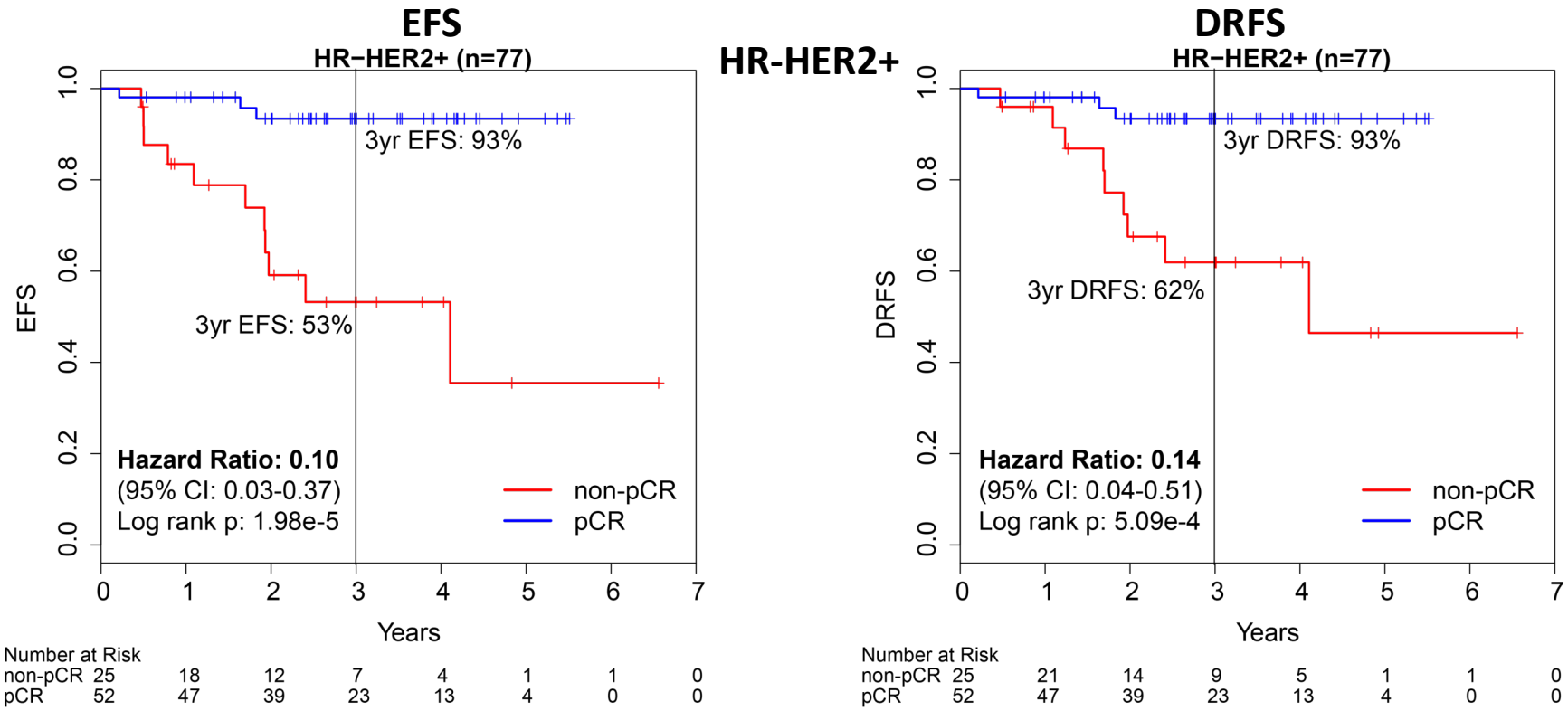
- Increases rate of conservative surgery
- Decreases need for axillary dissection
- Provides option of reducing treatment, at least on trial, in patients with pathologic complete response
- Allows for treatment escalation in those without a pathologic complete response
- In multiple trials, leads to same long term disease free survival
- Outside of a trial, a standard adjuvant regimen can be used in neoadjuvant setting

pCR is predictive of EFS and DRFS in HR+/HER2+



Yee et al, SABCS 2017; JAMA Oncology 2020

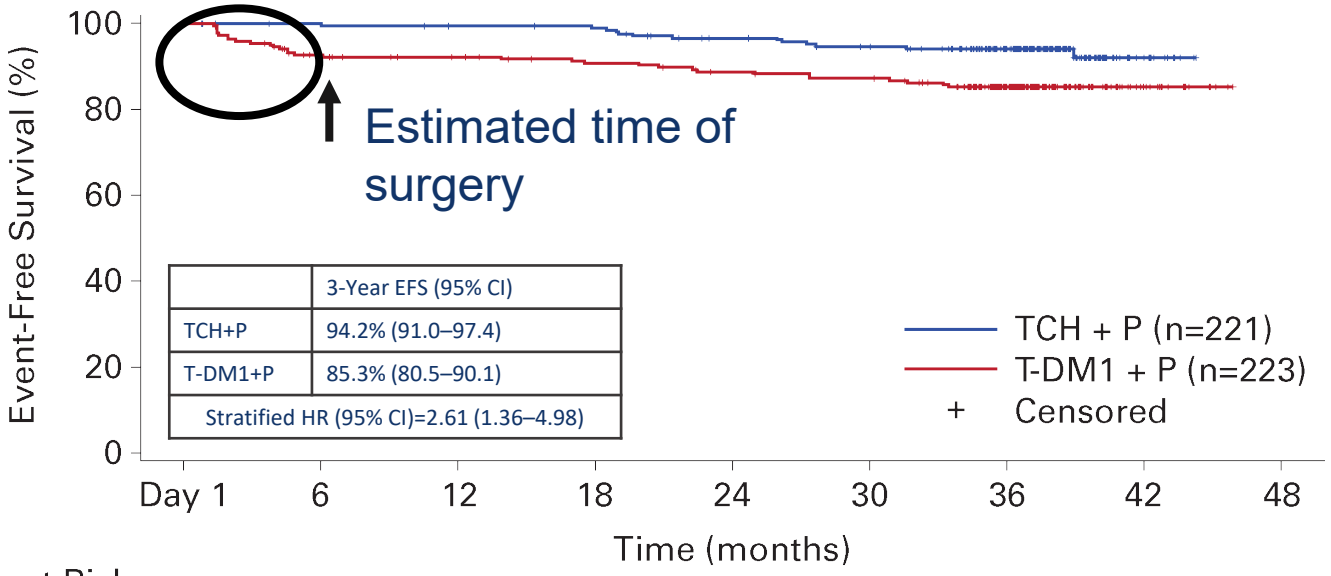
pCR is predictive of EFS and DRFS in HR–/HER2+



Yee et al, SABCS 2017

Kristine Trial: Neoadjuvant TCHP vs T-DM1/P

Early progression seen more (6.7% vs. 0%) on T-DM1/P arm, largely in HER2 2+ pts

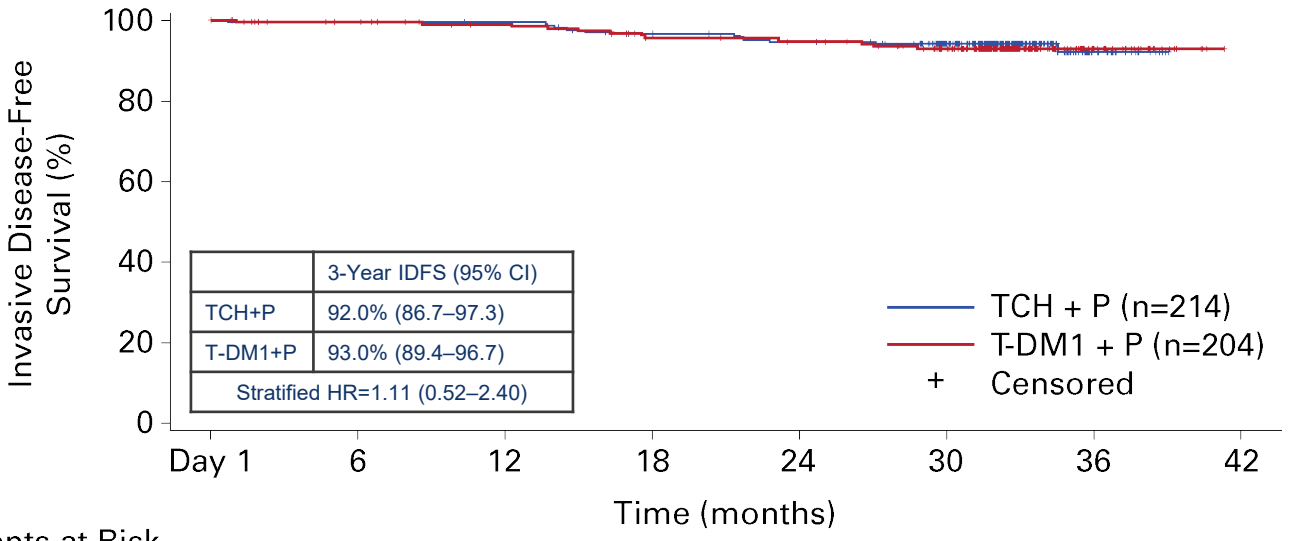


No. of Patients at Risk

TCH + P	221	214	211	209	197	190	140	10
T-DM1 + P	223	199	192	185	177	173	126	16

Hurvitz et al, ASCO 2019 and JCO 2019

Kristine Trial: Invasive Disease-Free Survival



No. of Patients at Risk

TCH + P	214	212	209	198	191	161	17
T-DM1 + P	204	193	187	177	174	156	24

Hurvitz et al, ASCO 2019 and JCO 2019

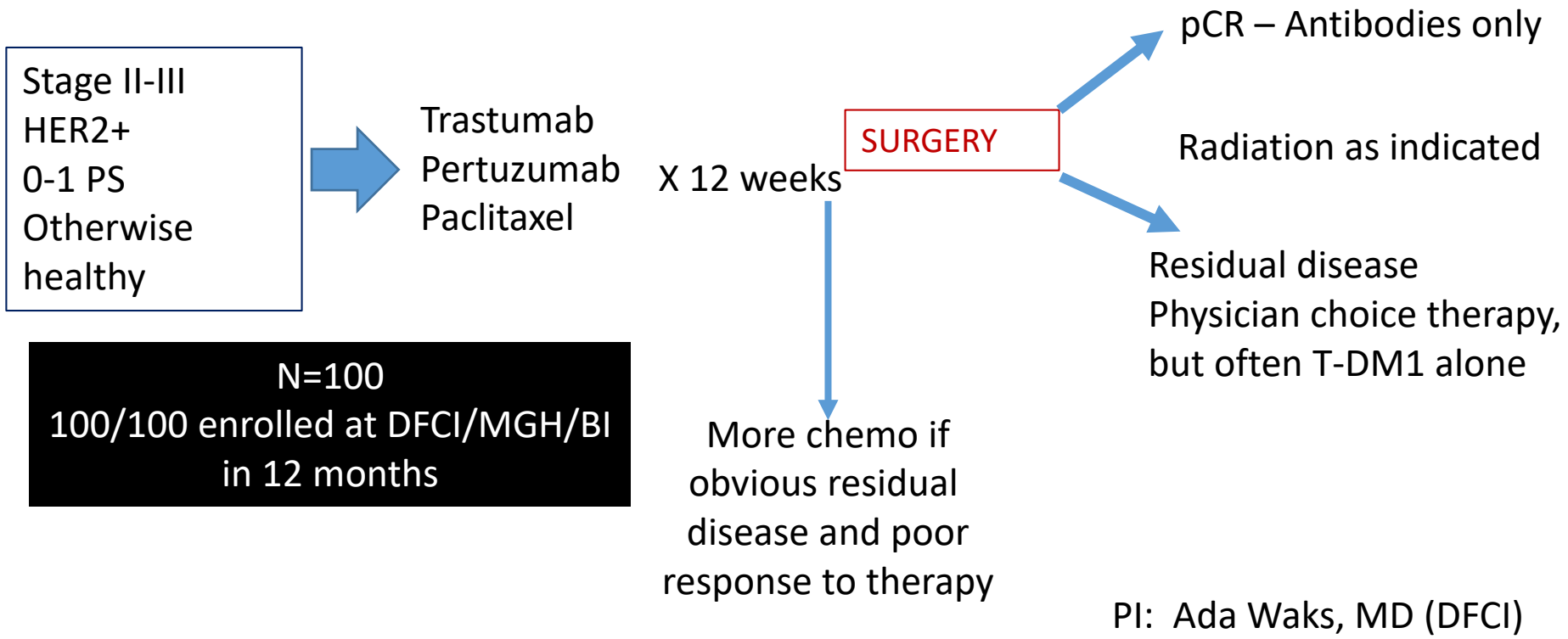
We need to stop praying at the altar of pCR!

E. Winer: St Gallen 2019

Time for a new approach for clinical trials in the neoadjuvant setting

- We should generally put the brakes on looking for “better” regimens/higher pCR rates that to date have not led to clinically significant improvements in EFS/OS
- Instead, we should:
  1. Maximize the biologic insights from neoadjuvant trials
  2. Explore ways in which we can de-escalate therapy by considering the importance of pathologic CR as a powerful biomarker for the individual patients
  3. In the absence of a pCR, we can pursue salvage regimens on and off trial, including
     - Completion of standard therapy
     - Use of new regimens (such as T-DM1)
     - Novel therapies in relatively high risk patients

DAPHNE Trial: Preop THP in Stage II/III HER2+ Disease
A Feasibility Trial



PI: Ada Waks, MD (DFCI)

COMPASS Trial Schema

Eligibility
HER2+ breast ca
Stage 2 or 3a
(T2-3, N0-2)
Newly diagnosed,
no prior therapy

Registration

Part 1 preop
THP x 4 (12 weeks)

pac weekly or doc q3w (T)
PLUS
trastuzumab (H) &
pertuzumab (P) q3w

Research Biopsy

Surgery

Part 1 pCR (~40-45%)
No further chemo

Part 2 RD (~55%)

SOC chemo as deemed necessary

Eligibility
HER2+ RD
Any ER-
ER+ if N+
~ 50% Part 1, 50%
outside enrollees

Registration

R

T-DM1 to 1y

T-DM1 to 1y + tucatinib

Primary Objectives: 3y RFS HER2+

Optimal result from such a study…

- De-escalation of therapy for some
- Escalation of therapy for those with residual disease
- Development of even more effective escalation approaches
- Biologic insights to differentiate patient populations and design tailored treatments

DFCI Study Hypothesis and Rationale

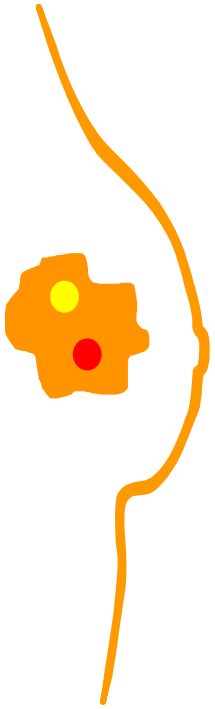
- HER2 heterogeneity is associated with inferior pathologic complete response (pCR) rate to neoadjuvant targeted anti-HER2 thera

Investigating the impact of HER2 heterogeneity on response to therapy is an important step in understanding tumor sensitivity to anti-HER2 agents and potentially to backing off on therapy

Metzger et al, ASCO 2019

Study Design

- Centrally-confirmed HER2+ BC
- Stage II and III (N = 164)
- Two image-guided research biopsies



T-DM1 + Pertuzumab q3w x 6

SURGERY

HER2 Heterogeneity defined as either

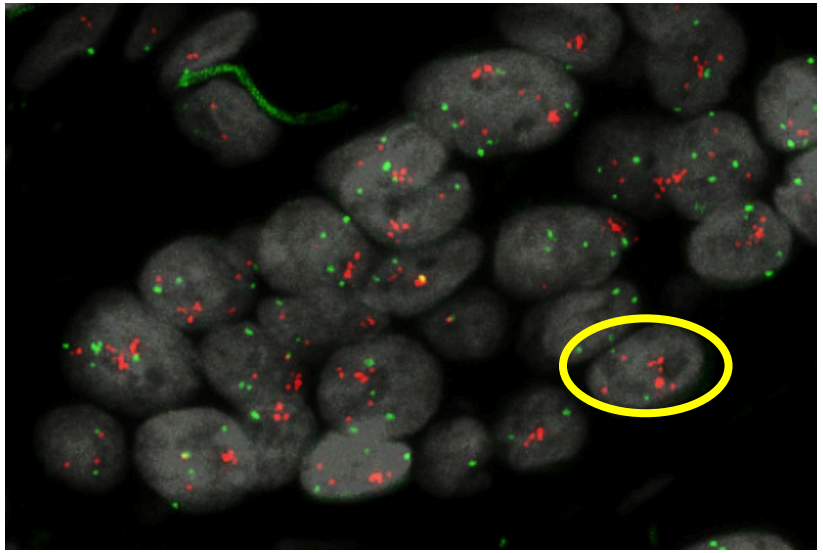
1) HER2 positivity by FISH in > 5% and < 50% of tumor cells (i.e., CAP guideline)

2) An area of tumor that tested HER2 negative

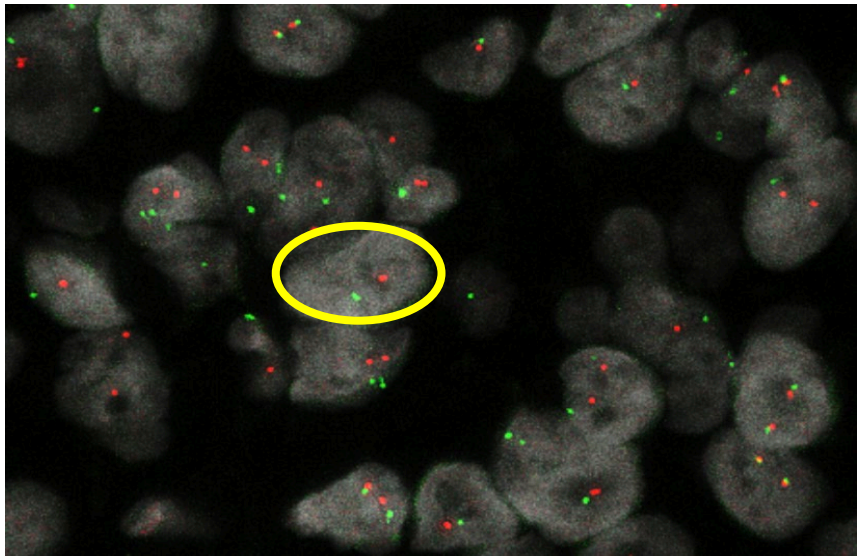
Blinded assessment performed by central laboratory (European Institute of Oncology, Milan)

Prevalence of Heterogeneity

- 16/157(10%) of evaluable cases were classified as HER2 heterogenous
- 13 (81%) hormone receptor positive and 3 (19%) hormone receptor negative

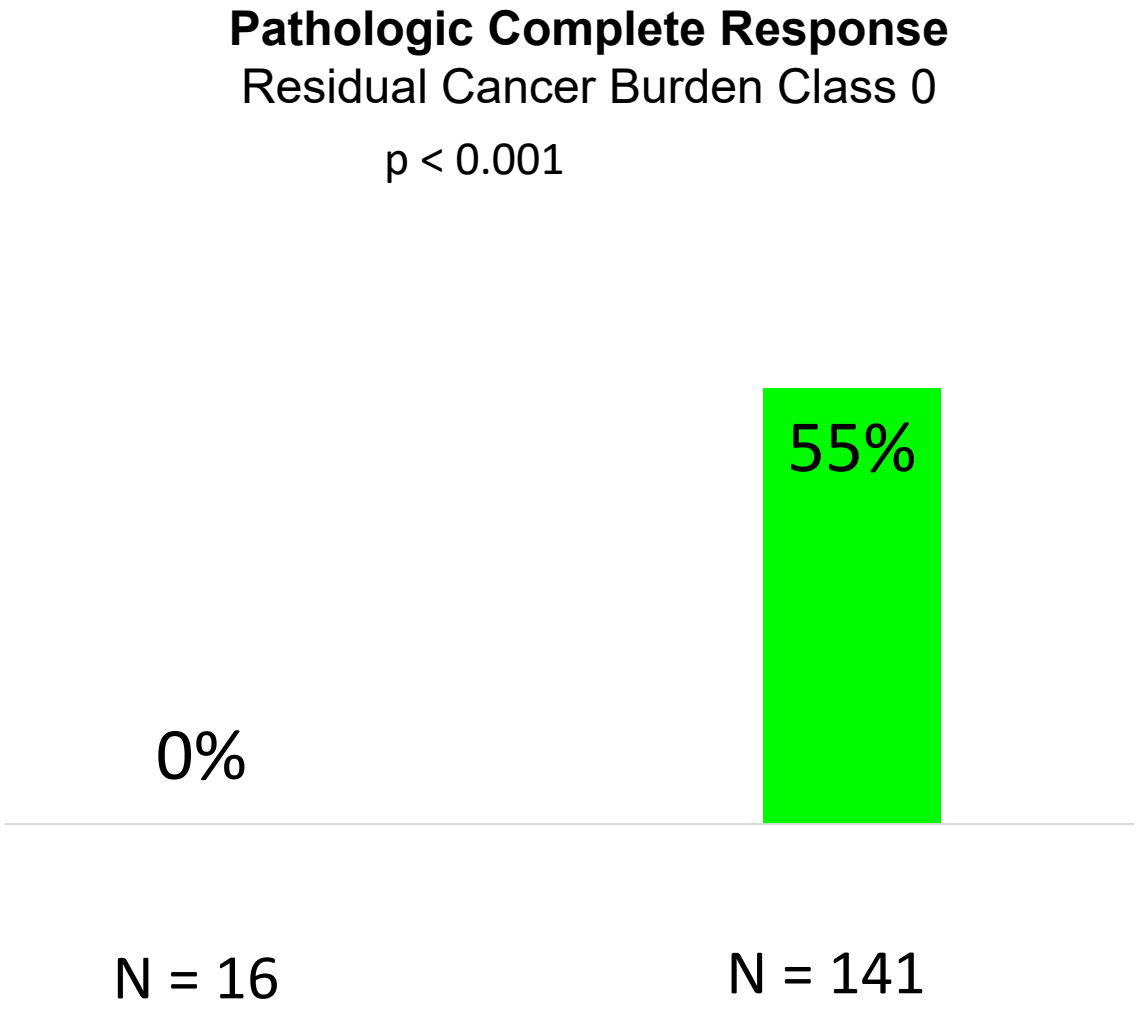


FISH ratio = 3.85



FISH ratio = 1.1

Effect of Heterogeneity on pCR



Pathologic CR by HR Status

HR-	HR+
pCR 65%	pCR 42%

The study met its primary endpoint by demonstrating a significant association between HER2 heterogeneity and pCR adjusted by ER status ($p < 0.001$)

Three comments

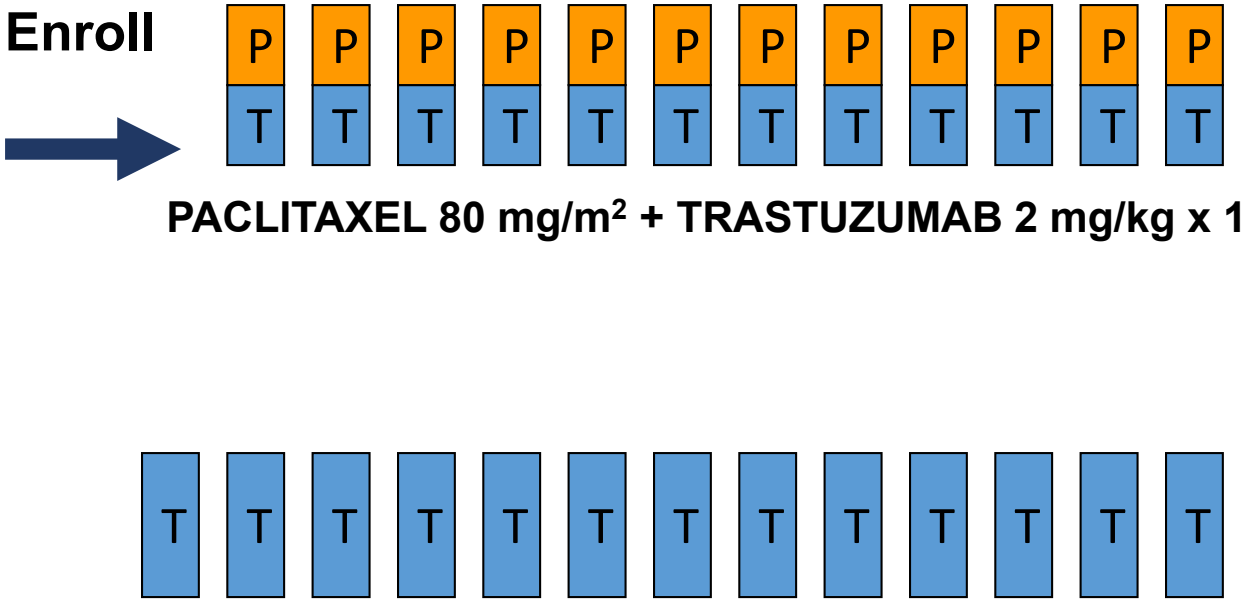
- Heterogeneity may account for drug resistance, leading to either early or late treatment failure
- If clinically significant, treatment strategies may need to include HER2-directed and non-directed therapy
- Heterogeneity probably needs to be distinguished from tumors that are simply HER2 low and are not pathway driven

APT: Study Design

HER2+
ER+ or ER-
Node Negative
≤ 3 cm

N=410

Enroll



PACLITAXEL 80 mg/m^2 + TRASTUZUMAB 2 mg/kg x 12

FOLLOWED BY 13 EVERY 3 WEEK DOSES OF TRASTUZUMAB (6 mg/kg)*

Tolaney et al, NEJM 2015

Disease-Free Survival Events with Median Follow-up of 7 Years

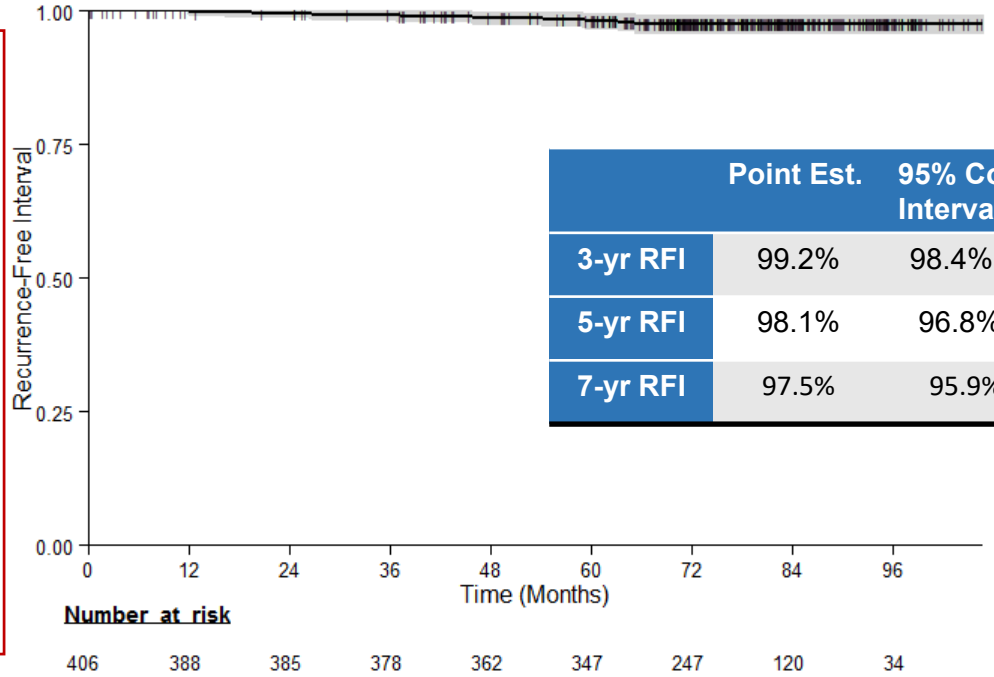
DFS Event	N (%)	Time to event [months; mean(range)]
Any recurrence or death	23 (5.7)	
Local/Regional Recurrence*	5 (1.2)	
Ipsilateral axilla (HER2+)	3	29 (12-54)
Ipsilateral breast (HER2+)	2	51 (37-65)
New Contralateral Primary Breast Cancer	6 (1.5)	
HER2+	1	56
HER2-	3	36 (12-59)
Unknown	2	87 (84-90)
Distant Recurrence	4 (1.0)	49 (27-63)
Death		
Non-breast cancer related	8 (2.0)	58 (13-71)

Tolaney et al, ASCO 2017

APT: Updated Recurrence Free Interval

RFI Events

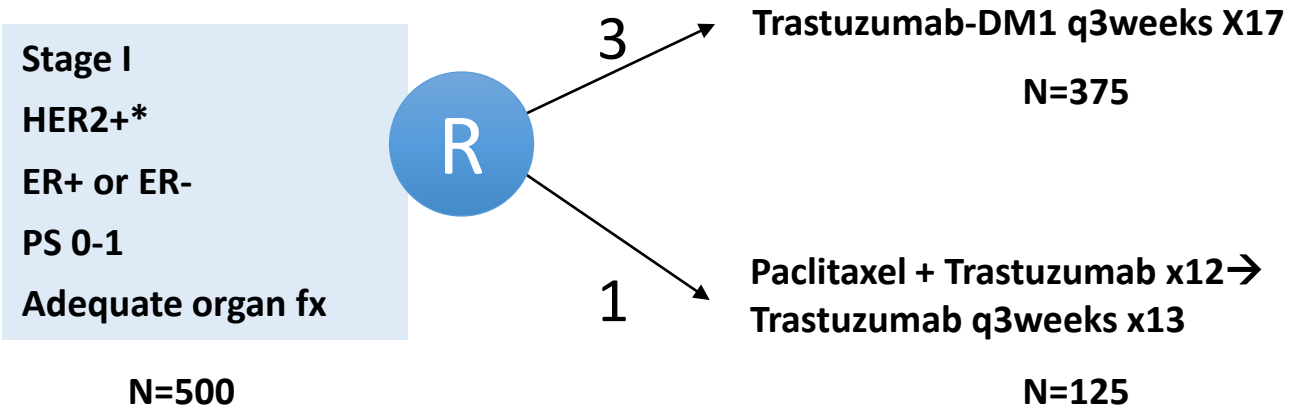
- **Invasive Local/Regional Recurrence**
- **Distant Recurrence**
- **Death from Breast Cancer**



	Point Est.	95% Conf. Interval	No. of events
3-yr RFI	99.2%	98.4% to >99.9%	3
5-yr RFI	98.1%	96.8% to 99.5%	7
7-yr RFI	97.5%	95.9% to 99.1%	9

Tolaney et al, ASCO 2017

ATEMPT Trial Schema



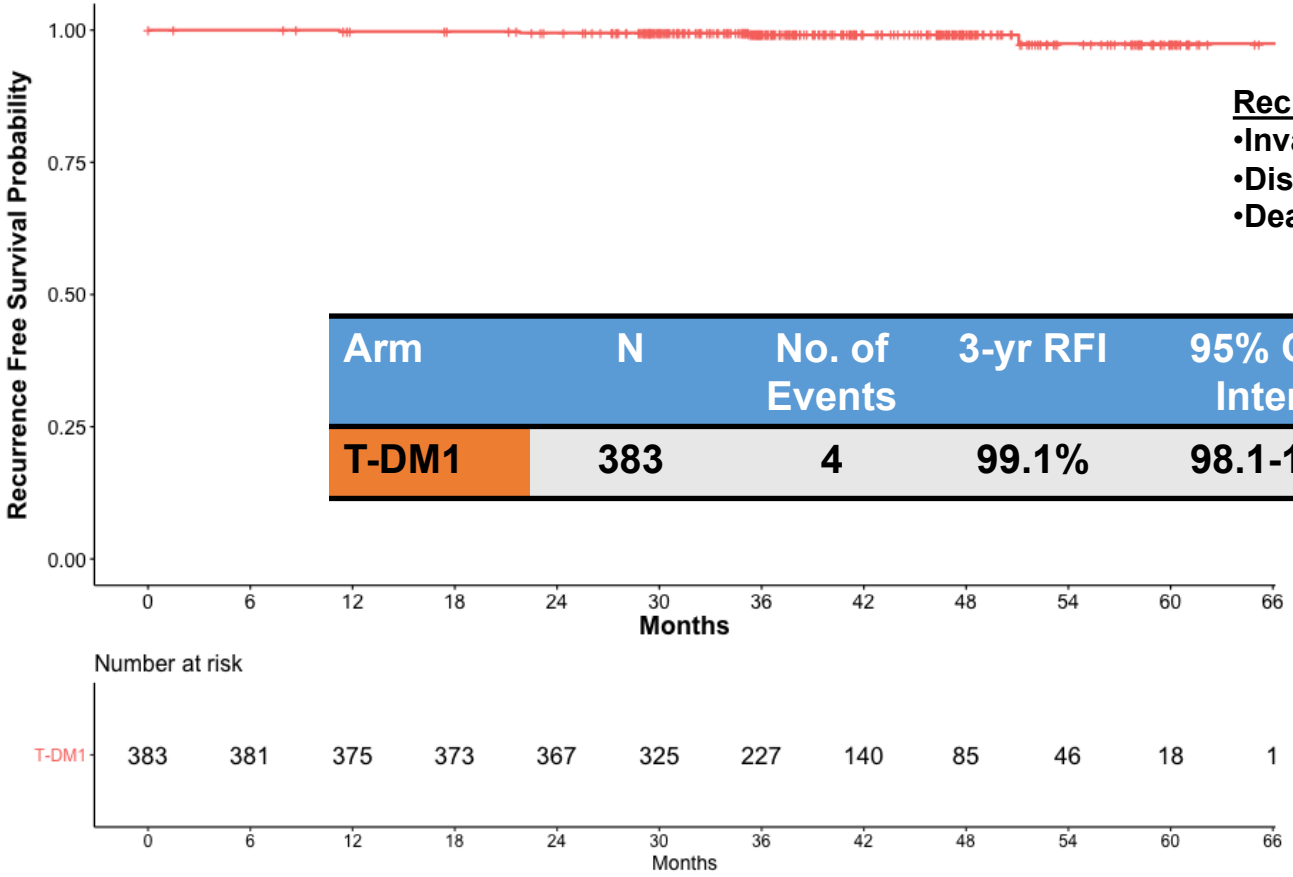
All HER2 testing centrally confirmed

Adjuvant endocrine therapy can be initiated after completion of 12 weeks of therapy

Adjuvant radiation therapy can be administered concurrently with study treatment.

Tolaney et al, SABCS 2019

Recurrence Free Interval: T-DM1



Recurrence Free Interval=
- **Invasive Local/Regional Recurrence**
- **Distant Recurrence**
- **Death from Breast Cancer**

Arm	N	No. of Events	3-yr RFI	95% Conf. Interval
T-DM1	383	4	99.1%	98.1-100%

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Summary

- Treatment of HER2+ metastatic disease has evolved dramatically – individuals with the disease are living longer and longer and far better
- Most adjuvant and neoadjuvant therapy is curative
- We can often reduce the extent of local therapy
- A minority of patients develop recurrent disease, and as noted above, advances are being made
- Brain metastases remain a major challenge
- Health disparities (not discussed) are still huge
- Health care givers and researchers need to work together seamlessly to make additional progress

What is a biomarker?
What makes a good biomarker?

Andrea L. Richardson, MD PhD

November 11, 2020

biomarker noun

bio·mark·er | \ ˈbī-ō-ˌmär-kər \

Definition of *biomarker*

: a distinctive biological or biologically derived indicator (such as a metabolite) of a process, event, or condition (such as aging, disease, or oil formation)

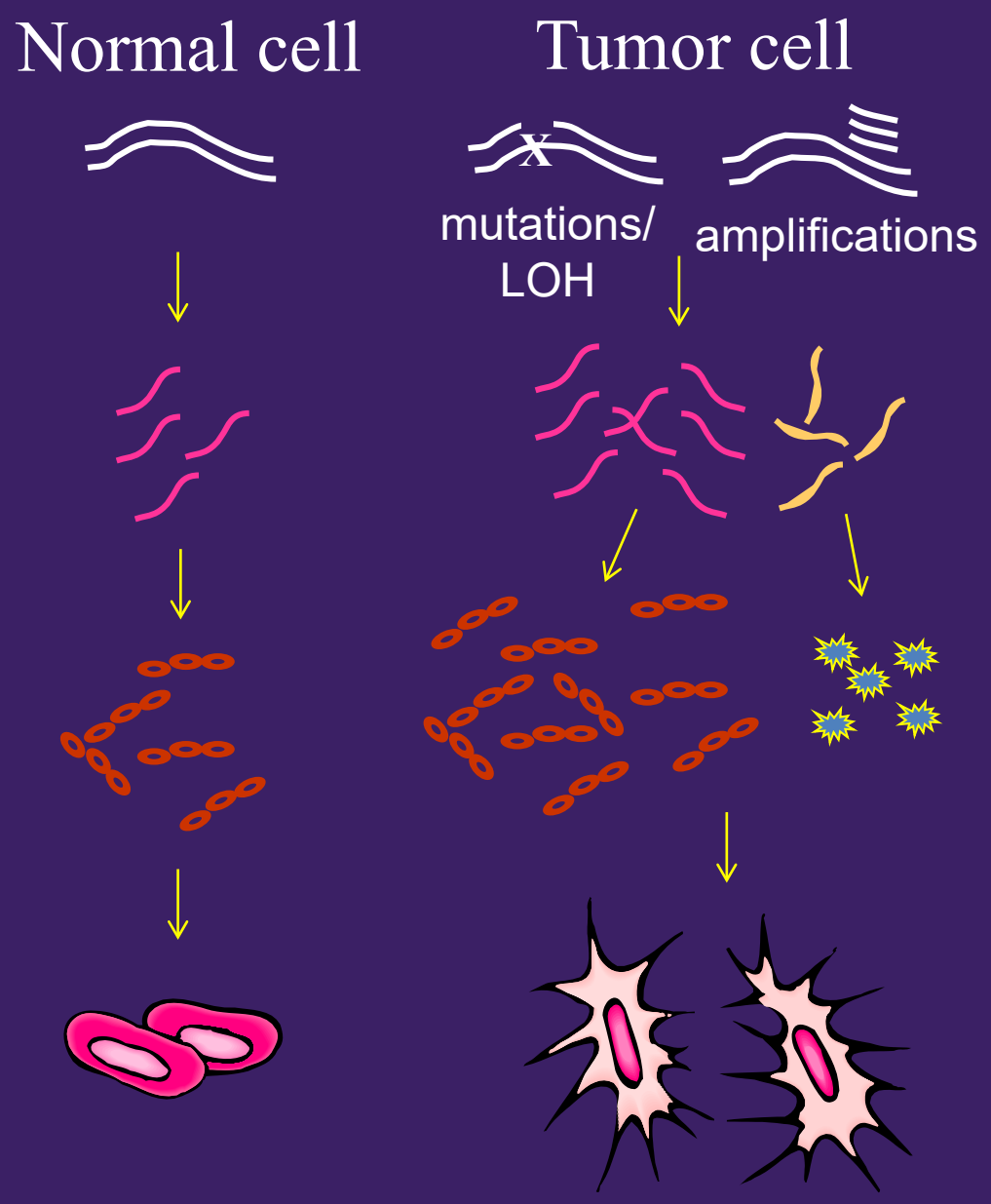
Uses of biomarkers

Table 1 – Intended use contexts for tumor biomarker tests.

- Risk categorization
- Screening for new cancer
- Differential diagnosis
  - Cancer vs. benign
  - Epithelial vs. hematopoietic vs. mesenchymal
  - Organ of origin
- Prognosis
  - Early stage
  - Metastatic
- Prediction of therapy activity
  - Early stage
  - Metastatic
- Monitoring disease status
  - Early stage
  - Metastatic

Biomarker validation and testing. Hayes, D.F. Molecular Oncology, 2015; 9(5): 960-966.

What can be a tumor biomarker?



DNA alterations

↓

Altered RNA levels

↓

Altered proteins

↓

Changes in cell appearance and uncontrolled growth and spread

Assay

- A laboratory procedure or method to detect and measure a biomarker.
- Preanalytic requirements: What material can be used to test, tissue cold ischemia time, tissue fixative, fixation time, quality of DNA or RNA.
- Key ingredients: specific antibody, DNA probe, reagents or solutions, dyes.
- Details of the procedure: How much of each ingredient, incubation times, incubation temperatures
- Method of interpretation: cellular location, how many cells to evaluate, how many sequencing reads, etc.
- Specified cutoffs for defining positive or negative.

Examples of biomarkers: Proteins

Assay: Immunohistochemistry

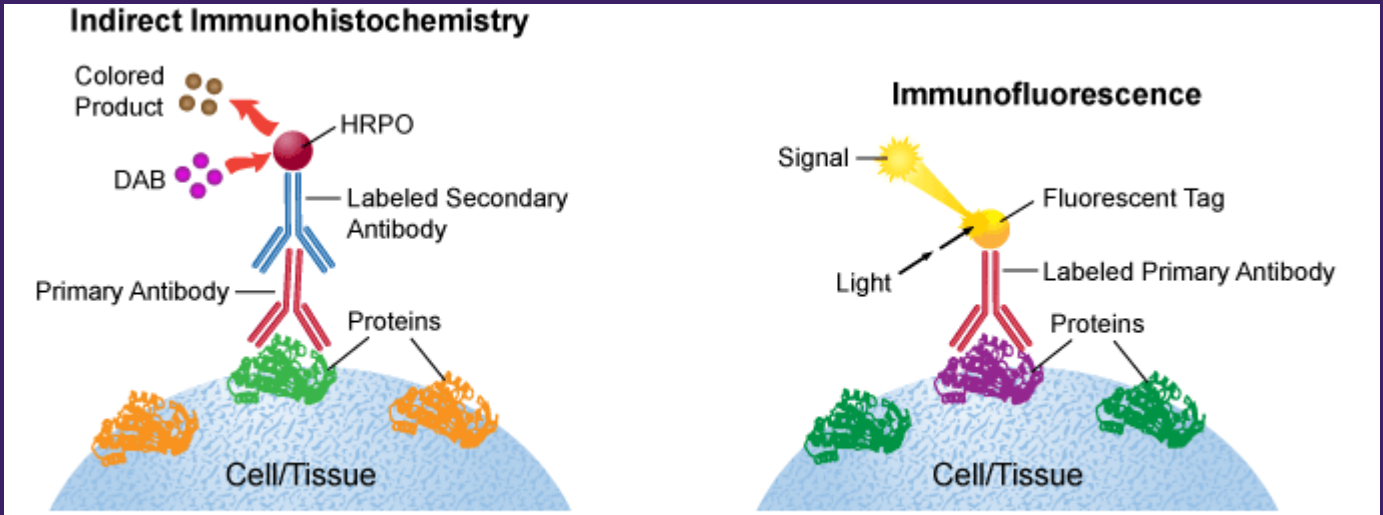
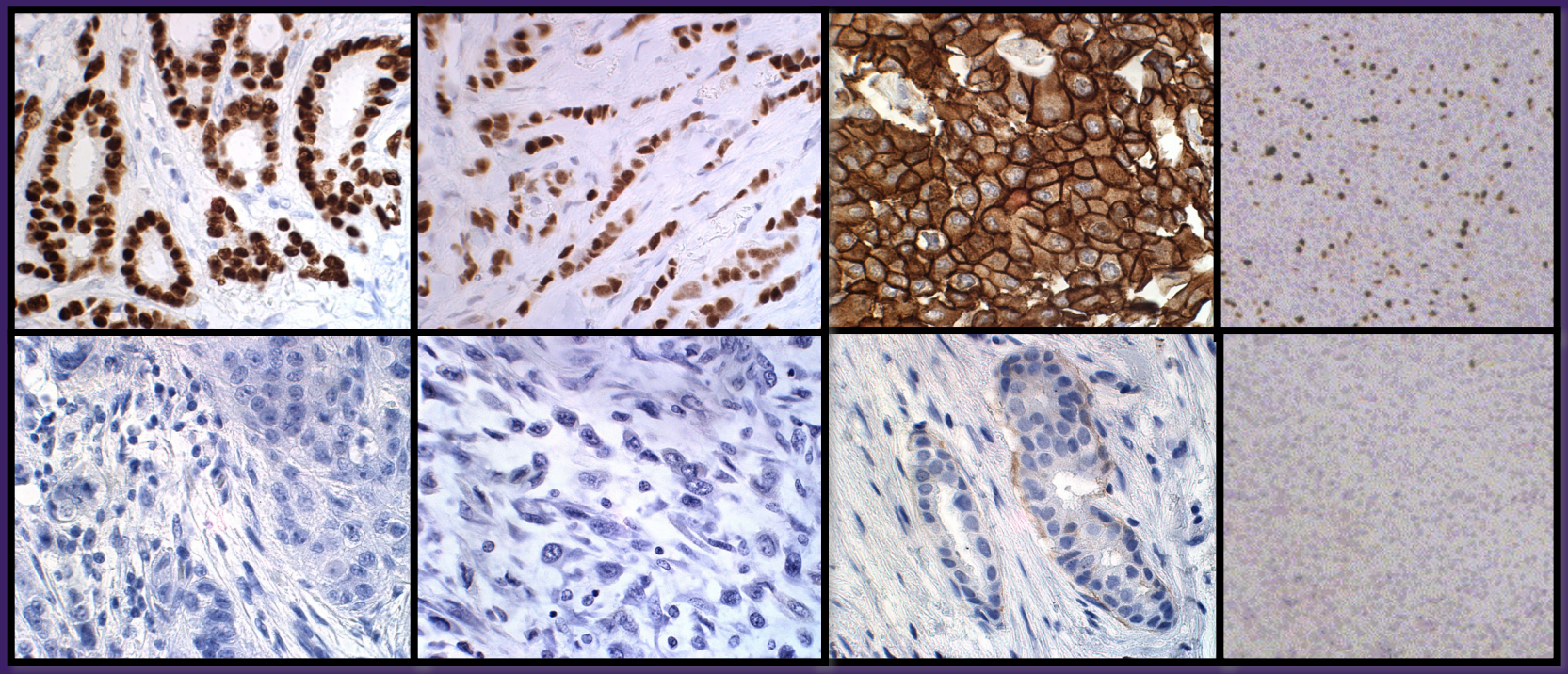


Diagram 1: *Illustration of Indirect Immunohistochemistry and Immunofluorescence methods.*

Can be performed in most pathology laboratories using commercial antibodies and detection kits. Large IHC machines automate the process.

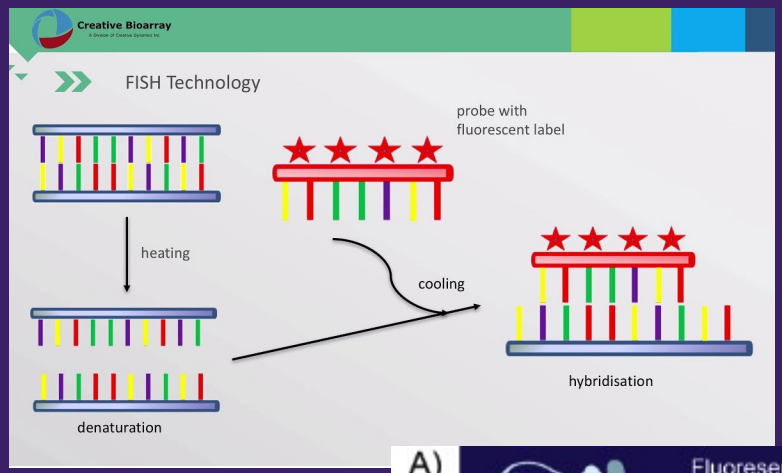
Examples of biomarkers: Proteins

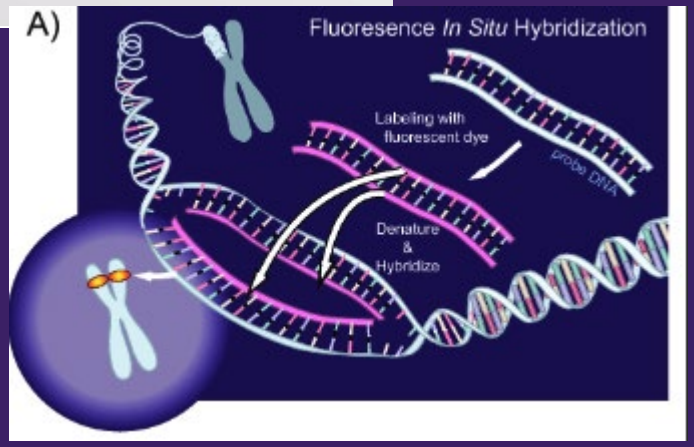
A major advantage is that protein expression specific to invasive carcinoma can be determined and distinguished from expression in stroma, normal cells, or carcinoma in situ.

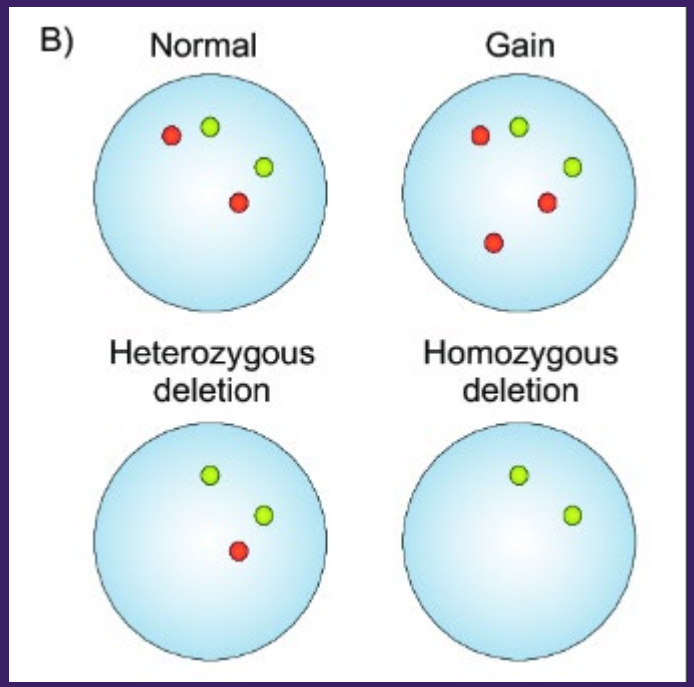


ER	PR	HER2	Ki-67
nuclear	nuclear	membrane	nuclear

Examples of biomarkers: DNA gene amplification Assay: Fluorescence In Situ Hybridization (FISH)



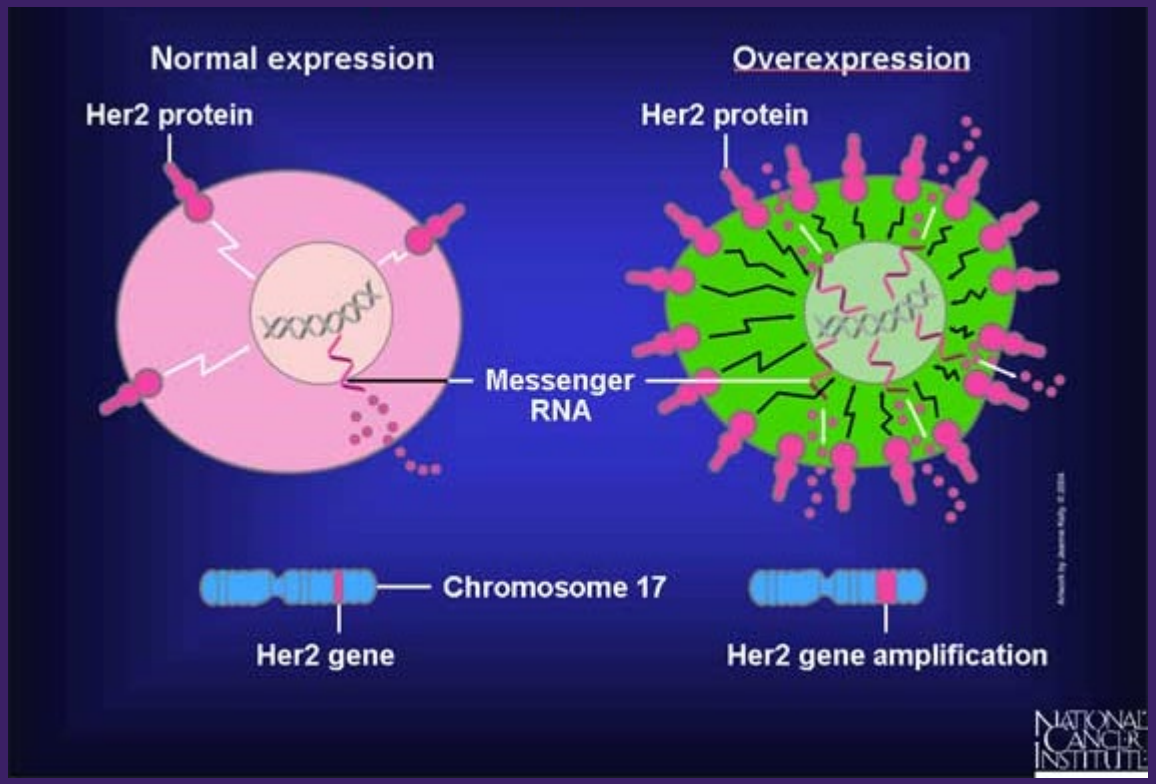


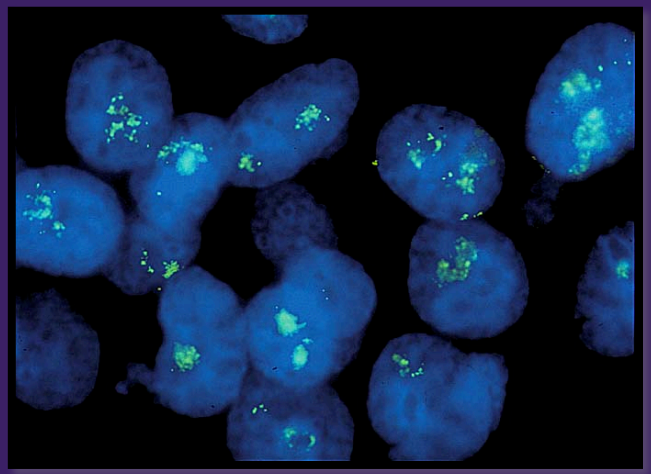


Performed in larger pathology laboratories using commercial probes and detection kits. Scoring/counting is done by cytotechnologists.

Examples of biomarkers: HER2 amplification

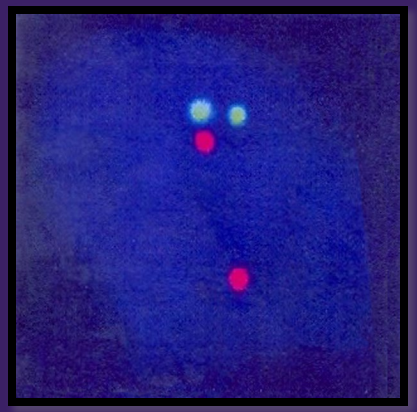
Assay: FISH





Performed in larger pathology laboratories using commercial probes and detection kits. Scoring/counting is done by cytotechnologists.

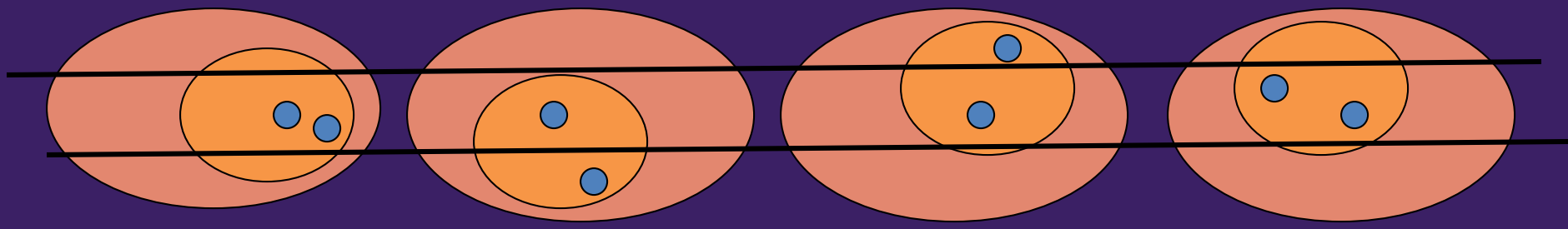
Dual probe HER2 FISH



Only portions of nuclei are present on sections used for FISH.

Normal cells have 2 chromosome 17's and 2 CEP17 sequences. The average number of CEP17 sequences observed in FISH studies for normal cells is 1.61.

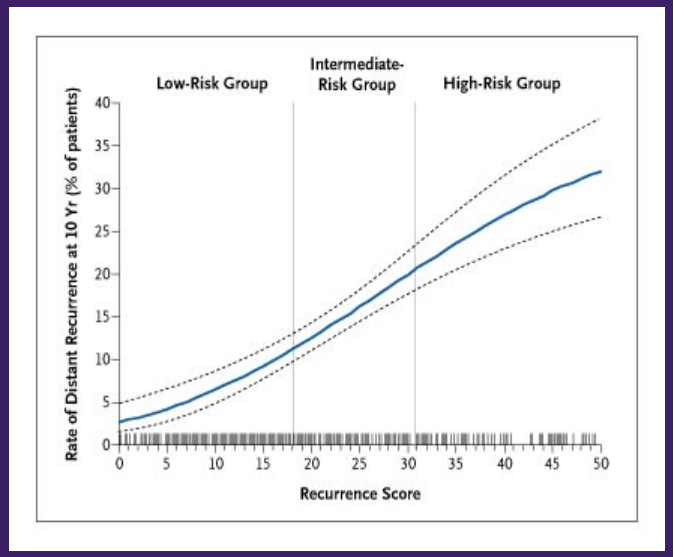
HER2 Ratio: Count of HER2 copies / Count of CEP17 copies



Examples of biomarkers: mRNA expression levels
Assay: RT-PCR (example: recurrence score)

250 candidate genes (literature) → RT-PCR assay on fixed tissue specimens → Tailored to 16 genes → Validation subsets from prospective NSABP datasets N0, ER+ Tamoxifen-treated

+ 1.04	+ 0.47	- 0.34
PROLIFERATION Ki-67 STK15 Survivin Cyclin B1 MYBL2	**HER2** GRB7 HER2	**ESTROGEN** ER PGR Bcl2 SCUBE2
	GSTM1	**REFERENCE** Beta-actin GAPDH RPLPO GUS TFRC
INVASION Stromolysin 3 Cathepsin L2	CD68	
	BAG1	



Paik, NEJM 2004

Paik et al, SABCS 2003

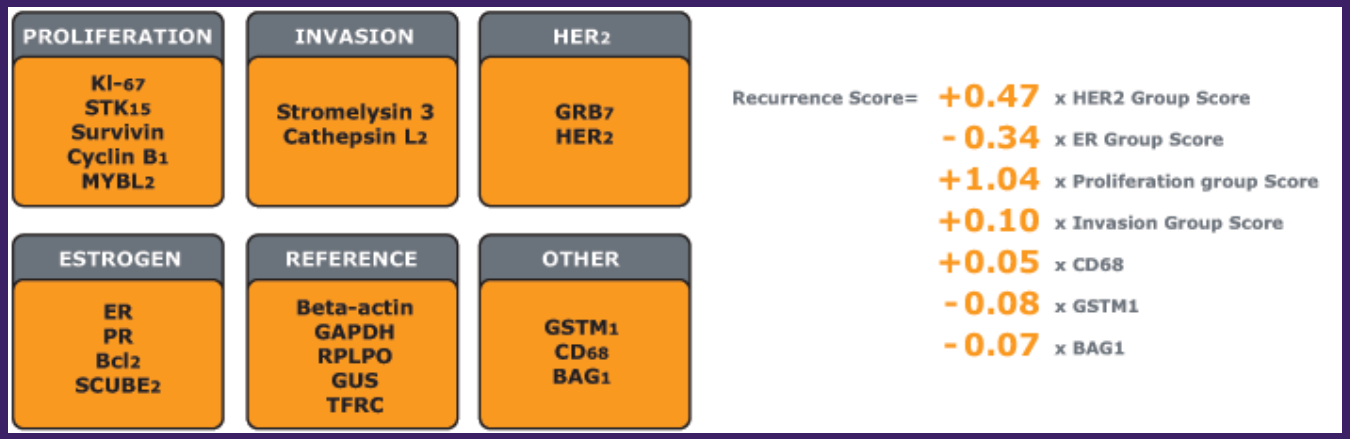
Biomarkers: mRNA or Protein?

Immunohistochemistry detects protein on intact tissue.

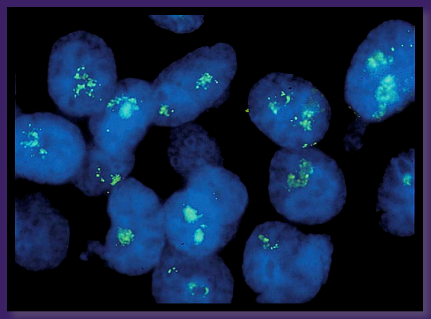
The Onco*type* DX assay detects mRNA with RT-PCR on tissue that's been ground up.

Single gene scores are reported for ER, PR, and HER2.

mRNA levels for these three genes are major determinants of the recurrence score.

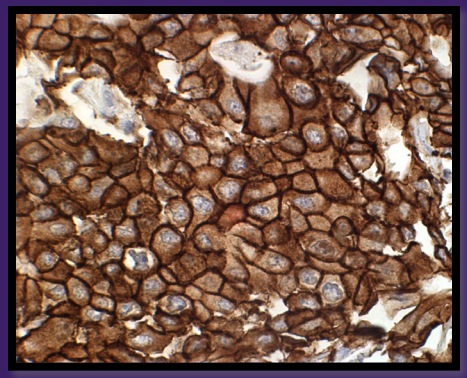


HER2 – DNA, mRNA, or protein?



DNA – in situ hybridization

mRNA – Onco*type* single gene score



Protein - IHC

HER2 – mRNA – Onco*type* DX

36 HER2 positive cases by both IHC and FISH (amplified)

- 10 reported as positive
- 12 reported as equivocal
- 14 reported as negative

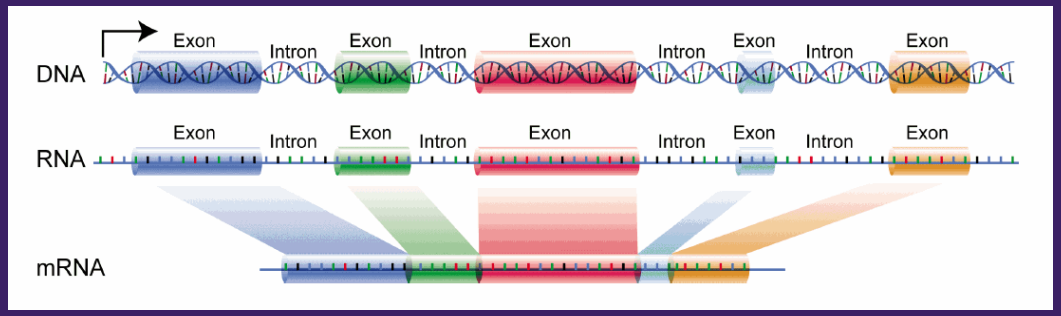
Therefore, there was a 39% (14/36) false negative rate (probably dilution of the total mRNA by non-tumor cells).

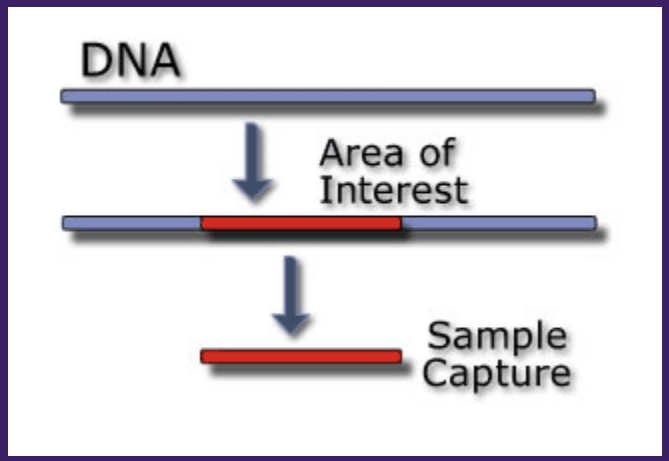
Unfortunately, some patients did not receive HER2 targeted therapy due to the negative Onco*type* DX result.

Dabbs, DJ, et al, High false-negative rate of HER2 quantitative reverse transcription polymerase chain reaction of the Oncotype DX test J Clin Oncol 29:1-7, 2011.

Bartlett, JMS, Starczynski, J, qRT-PCR and the Oncotype DX test for assessment of HER2 status: time to reflect again? J Clin Oncol 29: 2011.

Examples of biomarkers: Gene mutations
Assay: Next generation sequencing of DNA





- Capture and sequence all the exons of a panel of genes of interest
- Will find any type of mutation present anywhere in the coding exons of the genes you “captured” (e.g. PIK3CA, AKT, HER2)

Mutation Drivers

- Errors happen with every cell replication cycle
- Many of these potential mutations are repaired.
- Increased mutation occurs due to mutagenic exposures or to defects in DNA repair.
- "Driver" mutations are those that give a growth/survival advantage and are enriched in tumor cells.
- Specific drugs target specific mutated proteins: example: PIK3CA mutations make a protein that is "on" all the time and drives the tumor cell to proliferate. PIK3CA inhibitors specifically target tumors that have this mutation.

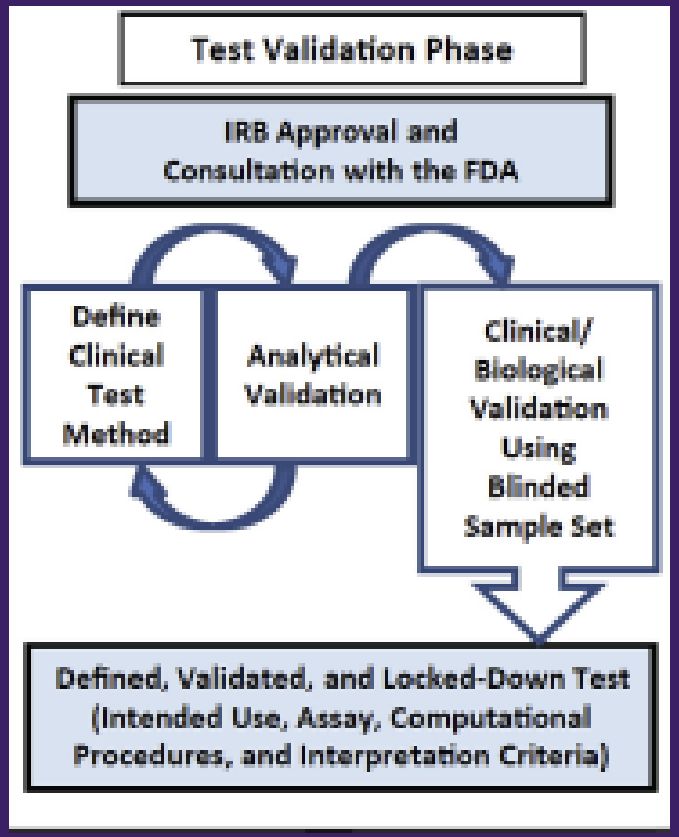
What makes a good biomarker?

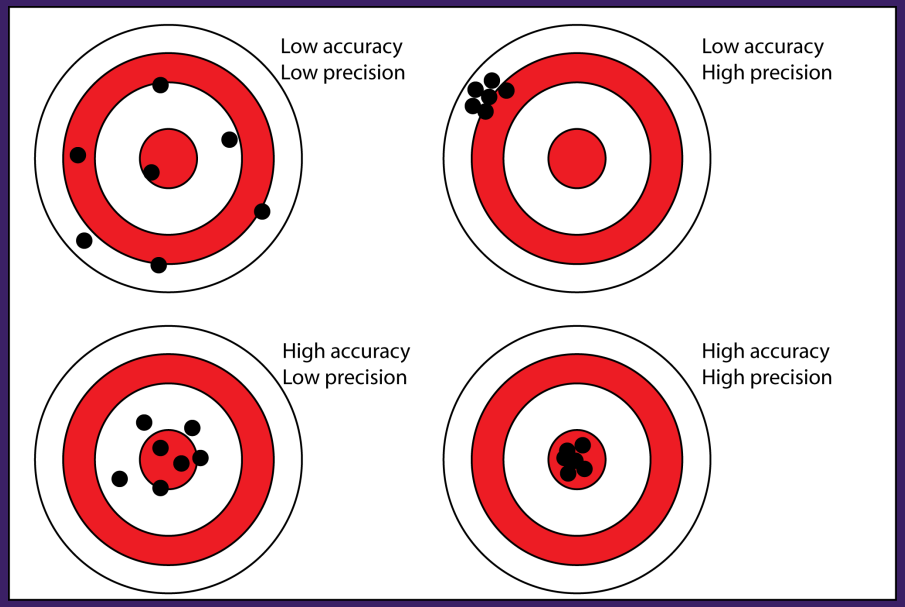
- **Analytical validity:** Is the test accurate and reliable for measuring what is supposed to measure?
- **Clinical validity:** Does the test accurately and reliably identify a clinical disorder or separate one population into two or more relevant groups.
- **Clinical utility:** Does using the biomarker test to guide clinical decisions result in improved outcomes compared to not using the test.

Analytical Validity of the Assay

- **Preanalytic requirements:** What material can be used to test, tissue cold ischemia time, tissue fixative, fixation time, quality of DNA or RNA.
- **Key ingredients:** specific antibody, DNA probe, reagents or solutions, dyes.
- **Details of the procedure:** How much of each ingredient, incubation times, incubation temperatures
- **Method of interpretation:** cellular location, how many cells to evaluate, how many sequencing reads, etc.
- **Specified cutoffs for defining positive or negative.**

Analytic validity





- Test replicates for reproducibility
- Test in the type of specimen to which assay will be applied (FFPE, age of specimen, type of patient)
- Test a blinded sample set and compare to a “gold standard”…do you get the same results?

Biomarker validation and testing. Hayes, D.F. Molecular Oncology, 2015; 9(5): 960-966.

Analytic validity: ASCO/CAP guidelines for ER and PgR testing

- Hammond et al. 2010 JCO 28(16): 2784-2795
- Why develop new guidelines?
  - Review of literature indicated that up to 20% of IHC determinations of ER and PgR testing worldwide were probably inaccurate
- Major issues with testing are due to
  - pre-analytic variables
  - thresholds for positivity
  - interpretation criteria

Pre-analytic variables: delay of fixation

Khoury et al. Delay to formalin fixation effect on breast biomarkers. Mod Path (2009) 22:1457

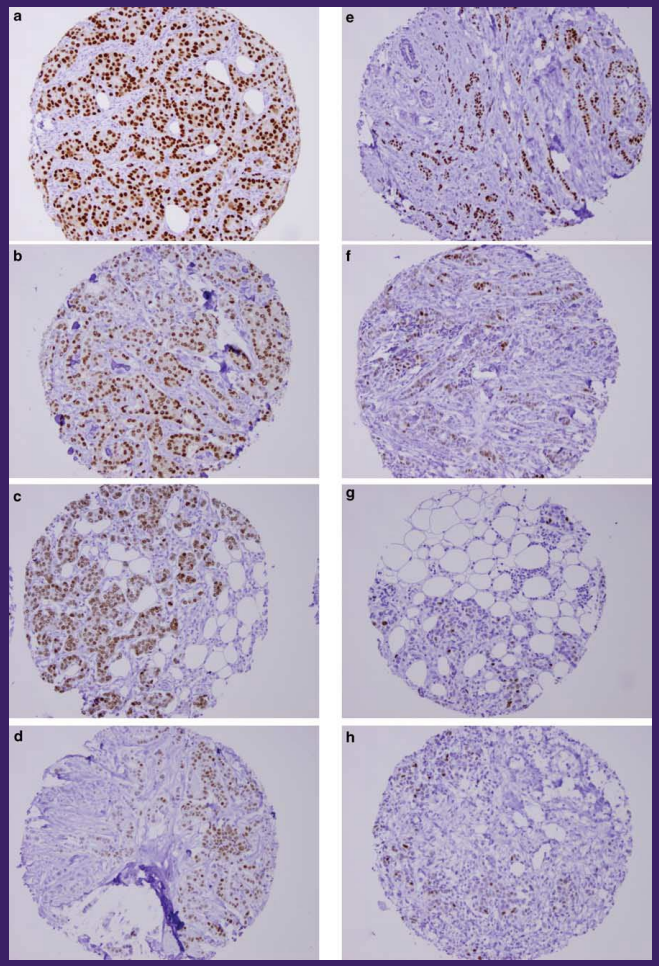
ER PR

0 hr

1 hr

4 hr

8 hr



Mann et al. Reliance on hormone receptor assays of surgical specimens may compromise outcome of patients with breast cancer. J Clin Oncol (2005) 23 (22):5148

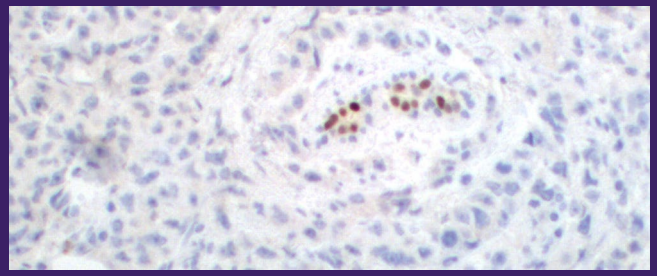
Table 2. ER and PR Status: Comparison Between Core Biopsy and Surgical Specimen

Specimen Type and % Staining	Surgical Specimen		
	0	< 10%	> 10%
ER core biopsy			
0	9	0	0
< 10	0	0	1
≥ 10	13	0	77
PR core biopsy			
0	22	2	1
< 10	3	1	3
≥ 10	8	0	60
ER/PR core biopsy			
Negative	9	0	0
Equivocal	0	0	1
Positive	9	0	81

NOTE. Data are No. of patients.
Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

ASCO/CAP guidelines for ER and PR interpretation

- Optimal algorithm for ER/PR testing
  - Positive: ≥ 1% of tumor cell nuclei are staining
  - Negative: < 1% of tumor cell nuclei are staining with evidence that the sample can express ER or PR (positive intrinsic controls are seen)



  - Uninterpretable: finding that no tumor nuclei are staining and that the internal epithelial elements present in the sample lack any nuclear staining

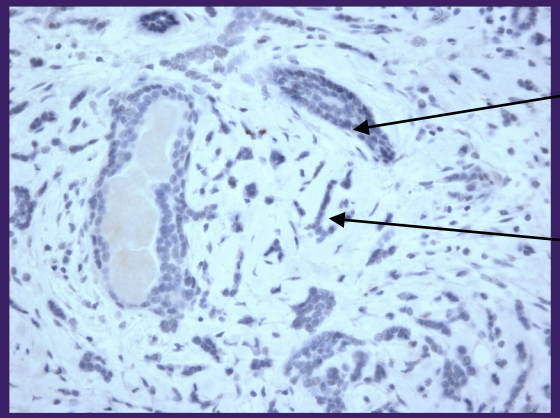
Case 1: Patient comes for recommendations for adjuvant therapy

- 43 year old with mammographically detected mass
- S/P surgical excision of a 1.8 cm invasive ductal carcinoma, grade II, 0/7 positive lymph nodes
- Original receptor studies on excision :
  - Estrogen receptor NEGATIVE
  - Progesterone receptor NEGATIVE
  - C-Erb-B2/HER2 NEGATIVE (0)

A review of pathology was performed.

- **Review results of the receptor studies:**

Estrogen receptor	**Uninterpretable**
Progesterone receptor	**Uninterpretable**



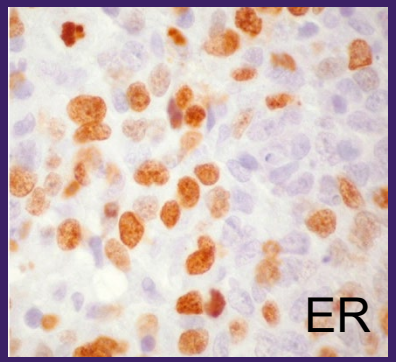
Normal duct cells are also negative

Tumor cells are negative

More Case 1:

- You learn that the surgery had been performed at 4 pm on a Friday afternoon
- A prior core biopsy had been done but no receptor studies had been performed.
- You request that receptor studies be performed on core biopsy

• Estrogen receptor	Positive (50% of cells)
• Progesterone receptor	Positive (40% of cells)



- Surgical excisions and mastectomies are more likely to have delay in time to tumor fixation and reduced tissue penetration than core biopsies, especially if surgery is late on a Friday…..

- Poorly fixed specimens cannot be resurrected. False negative results cannot be resolved by
  - Central pathology review of IHC slides
  - Repeat of IHC by a "better lab" on same specimen
  - mRNA assay such as 21-gene assay or 70-gene assay

Analytic validity: HER2 test interpretation

The results of IHC and FISH are concordant in ~95% of cases.

Discordant cases: protein IHC positive (3+) but no gene amplification (FISH negative)

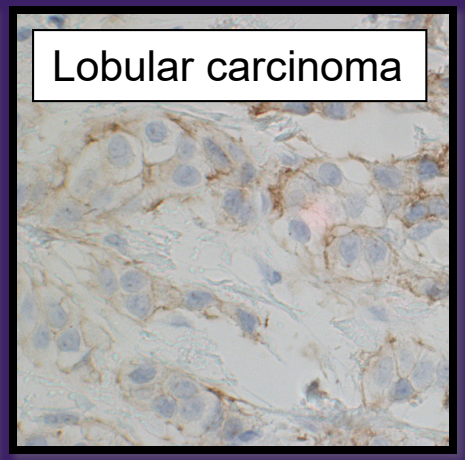
- False positive IHC (majority of cases) –
  - Technical problems with the assay
  - Overinterpretation of results
  - Estimated to be as high as 20%.

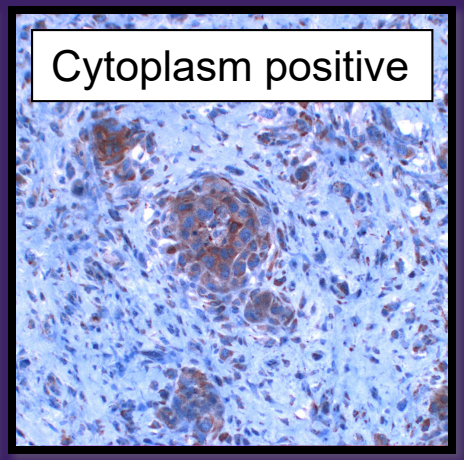
HER2: IHC 3+, No amplification – False positive

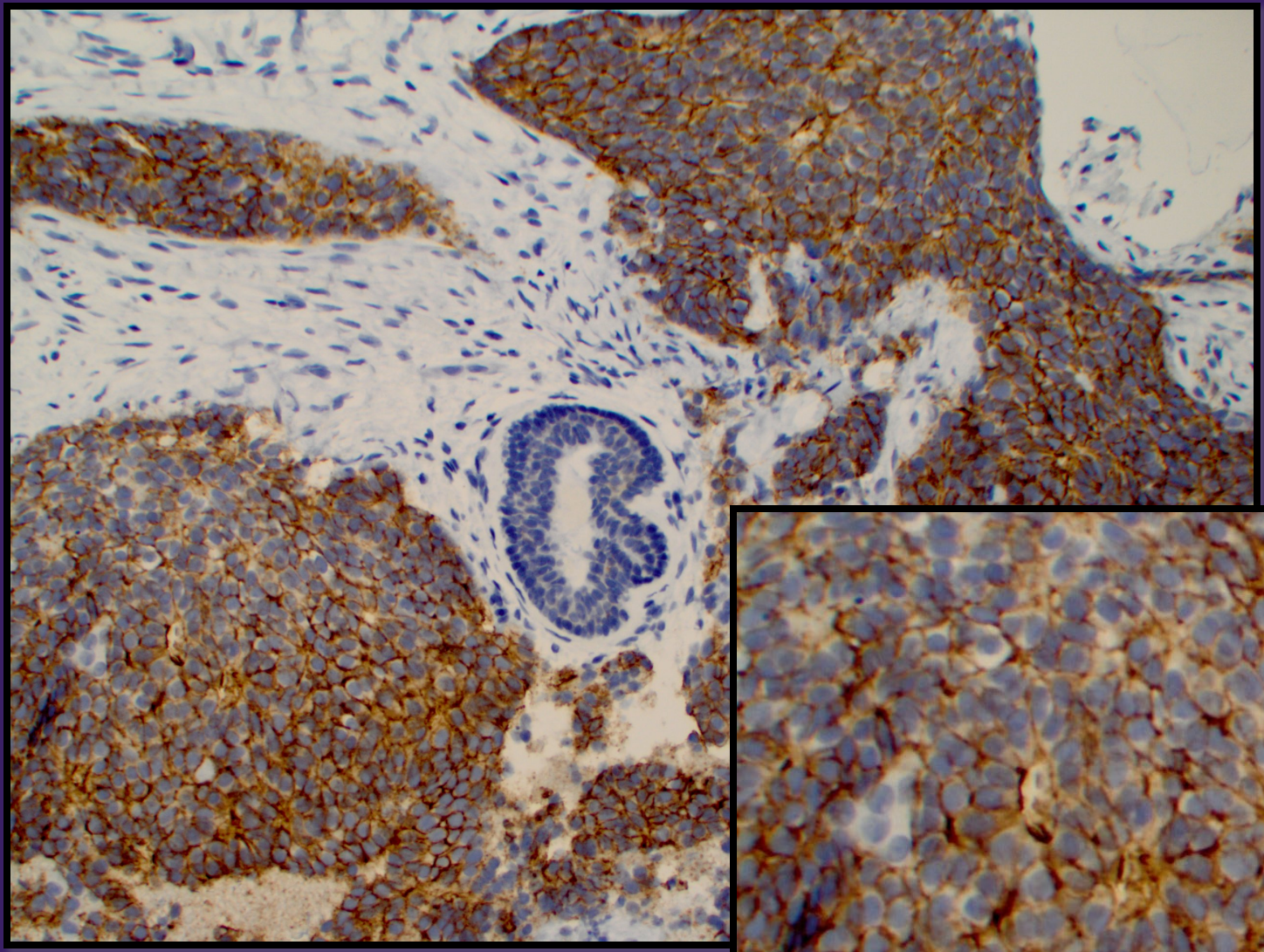
Overstaining – normal breast tissue should be negative

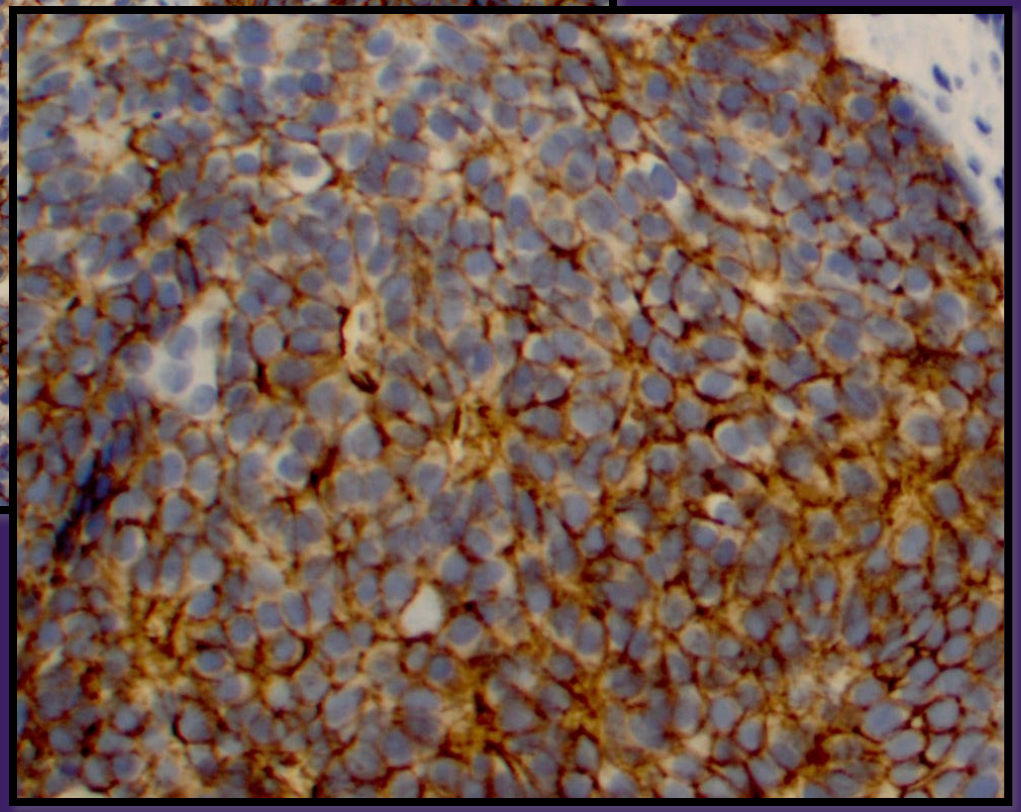
Edge artifact – lobular carcinomas can appear falsely positive in edges or between cells

Cytoplasmic positivity – only membrane positivity should be scored

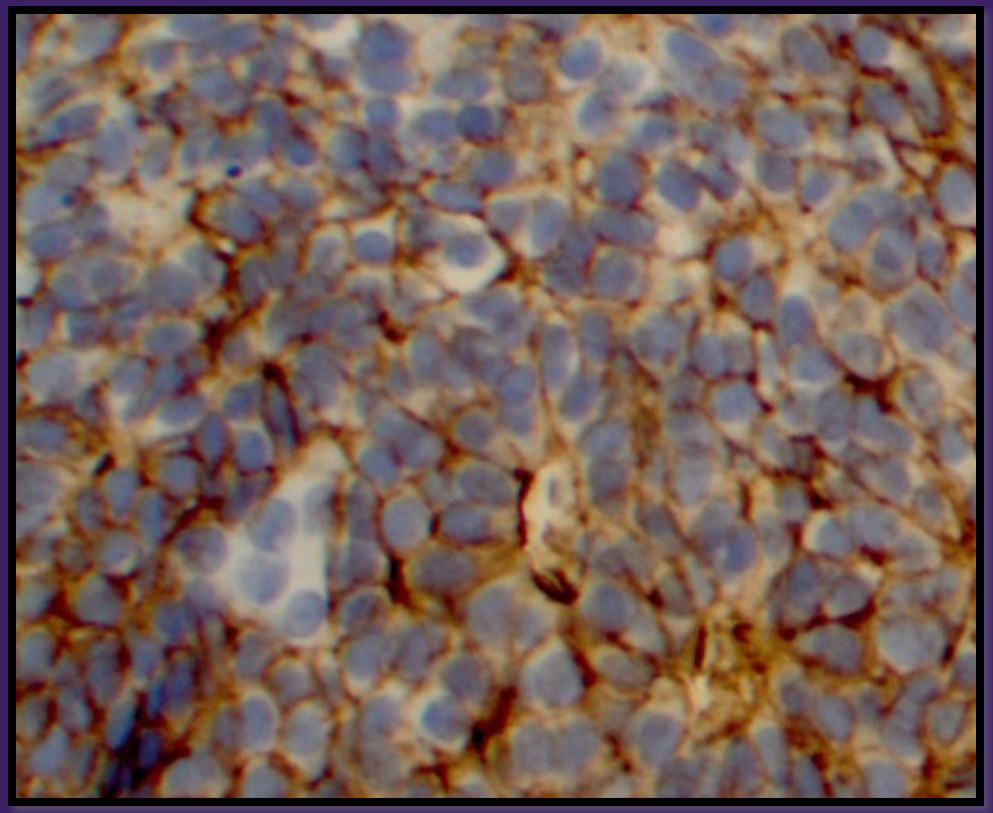




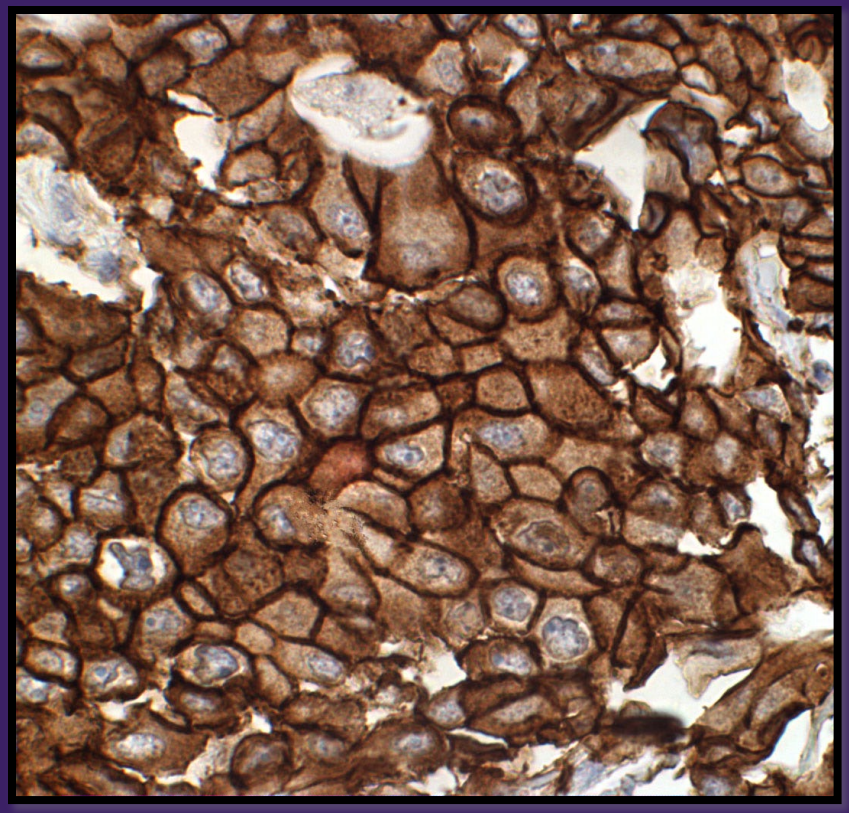




Interpreted as 3+ but FISH not amplified.



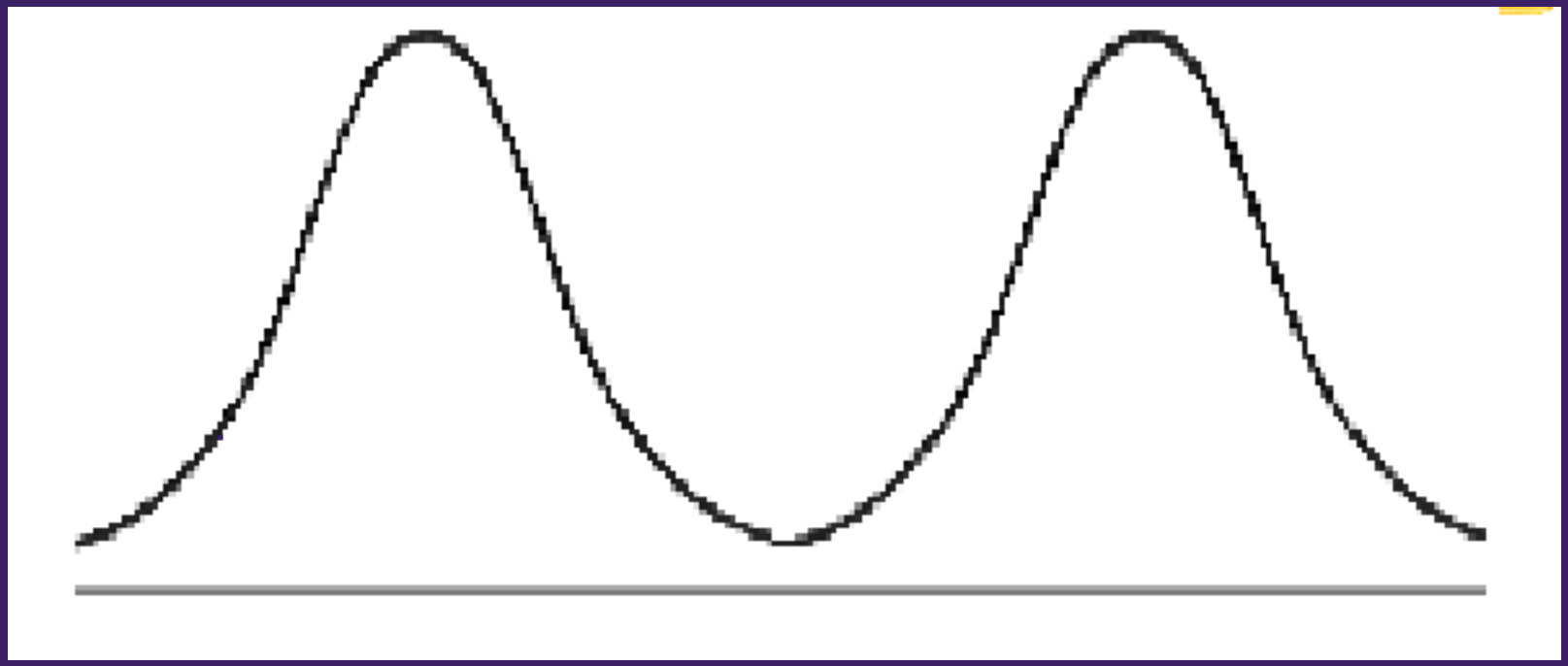
Immunoreactivity appears to be between cells rather than on membrane. Therefore, this should not have been interpreted as 3+.



Compare to this cancer with strong complete membrane positivity (3+).

Clinical validity: Does test reliably separate a population into two relevant groups?

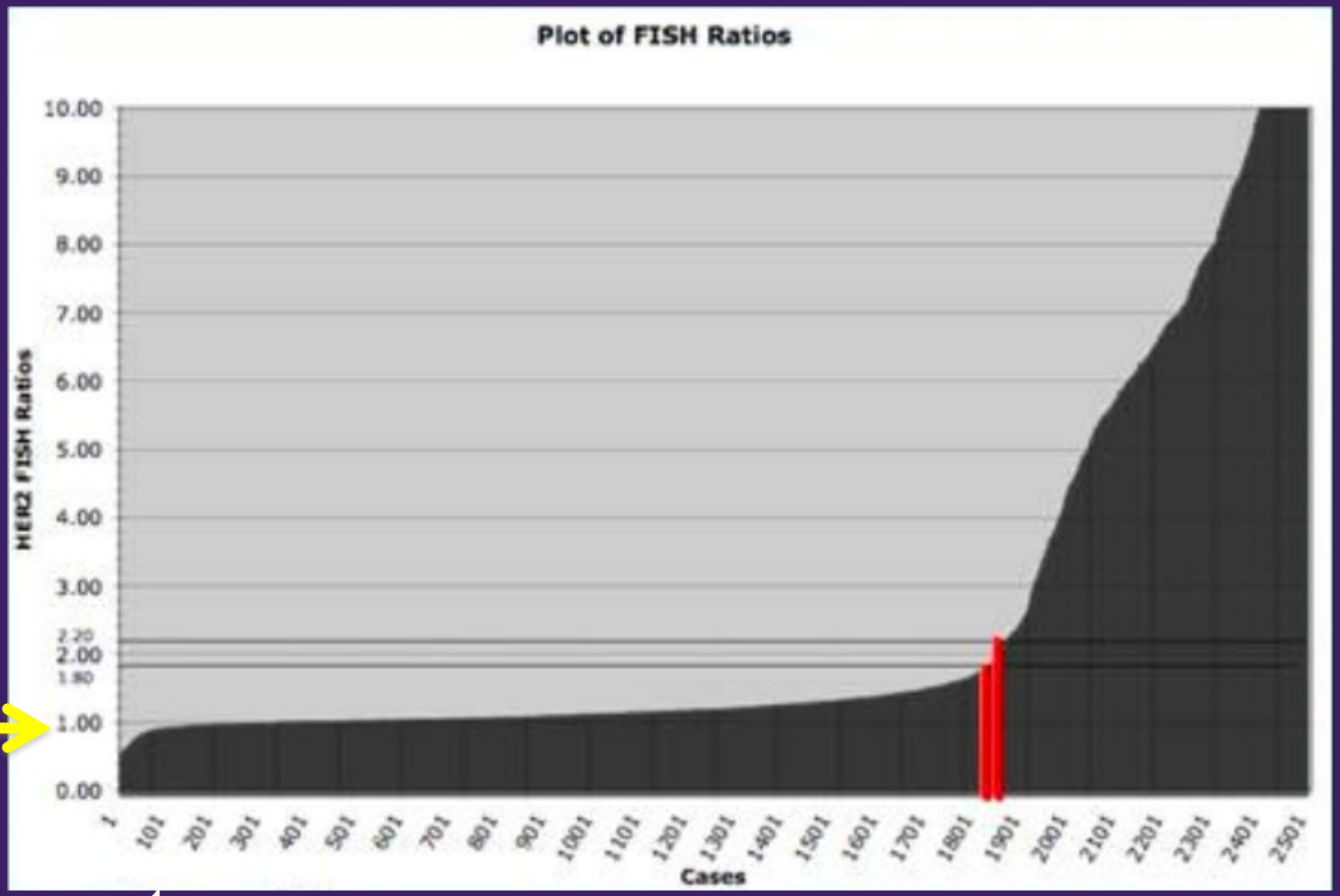
An ideal biomarker would be like this:



Negative
Don't treat

Positive
Treat – improved outcome

Clinical validity: HER2 is a pretty good biomarker! Splits pretty cleanly into 2 groups.

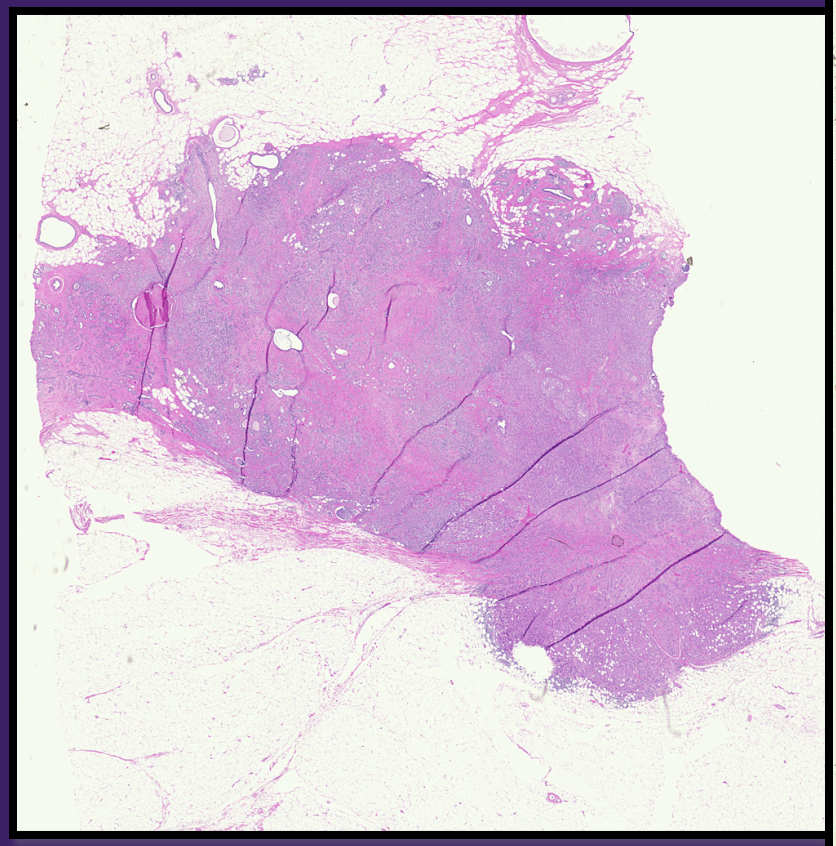


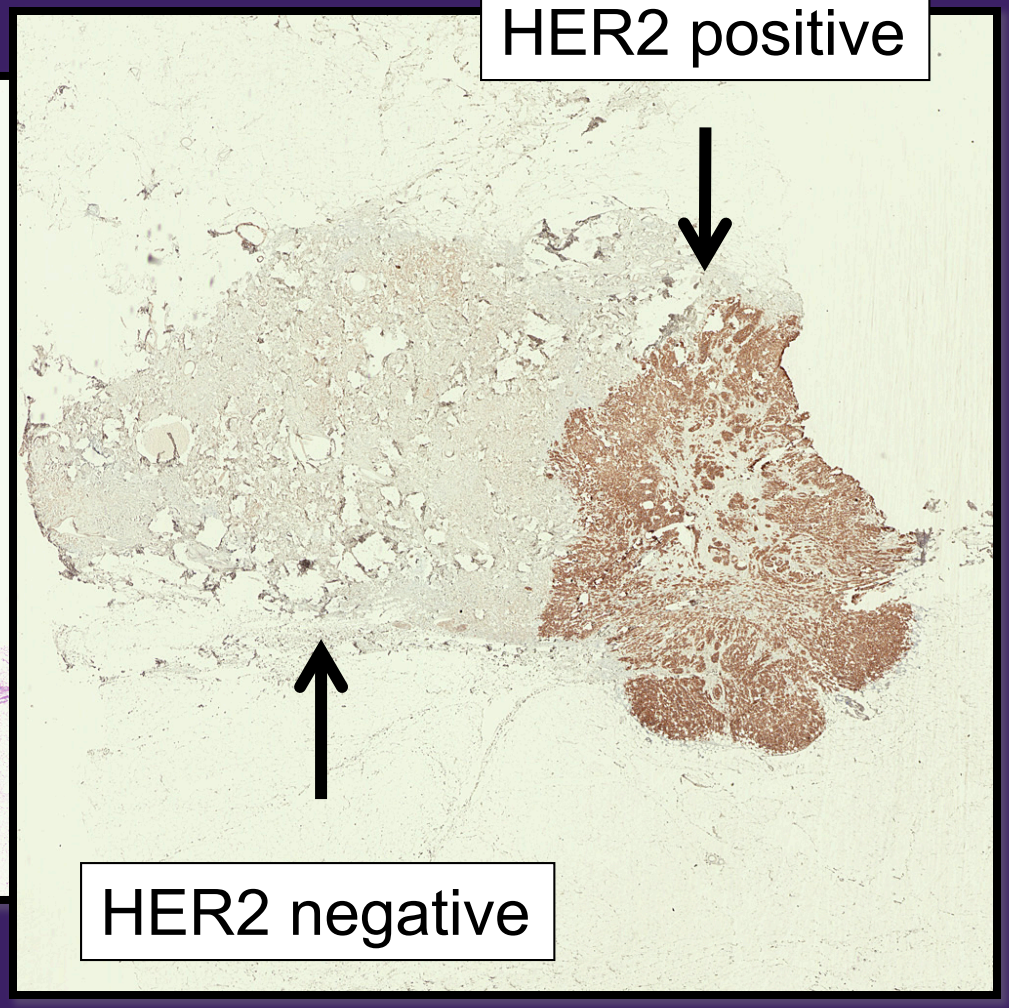
Normal

2 genes/
2 chromosomes = 1

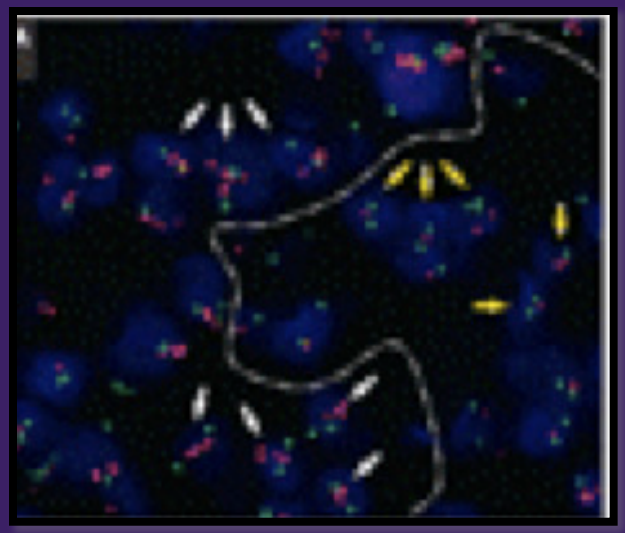
M. Press ASCO 2008

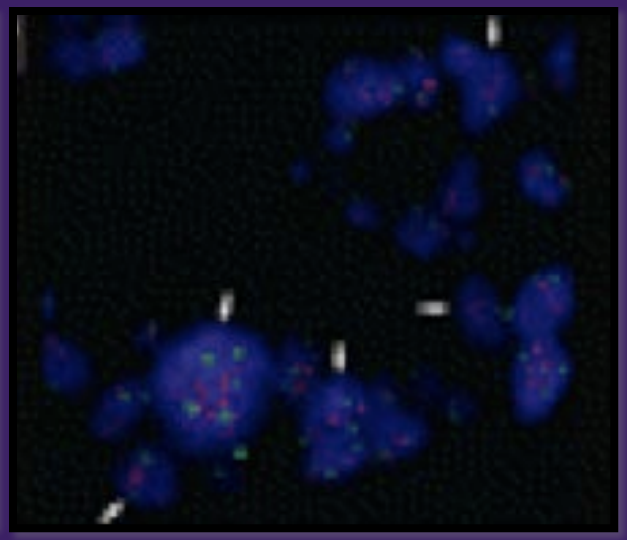
Some cases don't fit though: Heterogeneity

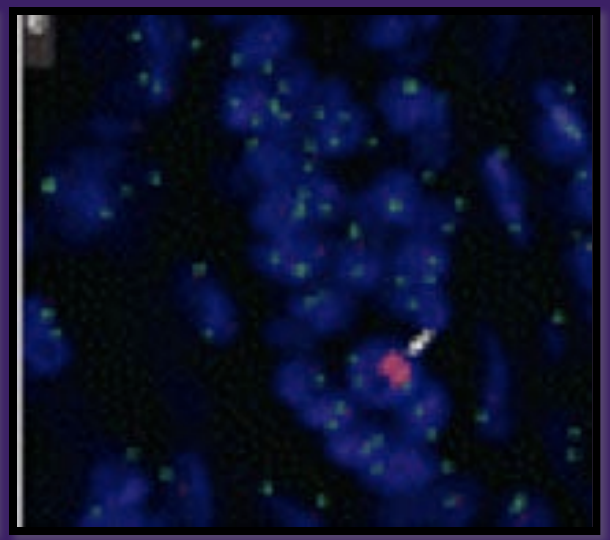




HER2: Heterogeneity - FISH







Two separate populations.

Intermingled cells.

Most common pattern.

Single cells.

Starczynski, J, et al HER2 amplification in breast cancer, Anatomic Pathol 137:595-605, 2012.

HER2: Heterogeneity for HER2

Significance?

Relationship to prognosis and response to HER2 targeted therapy is under investigation.

~1/3 of HER2 positive cancers treated with HER2 targeted therapy are HER2 negative after neoadjuvant therapy.

Likely due to heterogeneity of expression with preferential survival of HER2 negative subset.

Poor prognostic factor.

Ki-67 Labeling Index: Maybe not so good

- Proliferation rate is one of the most important prognostic factors, especially for ER-positive breast cancer.
- But how should it be measured?
  - mRNA expression (e.g. 21-gene assay, 70-gene assay)
  - S-phase fraction
  - Ki-67 immunohistochemistry
- Change in Ki-67 after 2 weeks of neoadjuvant therapy has been suggested as a biomarker for prediction of therapy response in ER+ breast cancer

DOI: 10.1093/jnci/djr393
Advance Access publication on September 29, 2011.

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COMMENTARY

Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group

Mitch Dowsett, Torsten O. Nielsen, Roger A'Hern, John Bartlett, R. Charles Coombes, Jack Cuzick, Matthew Ellis, N. Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

Manuscript received March 14, 2011; revised September 1, 2011; accepted September 2, 2011.

JNCI 2011; 103:1656-64

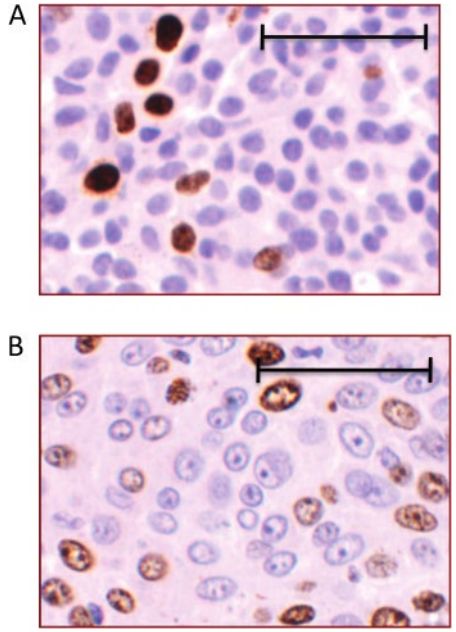
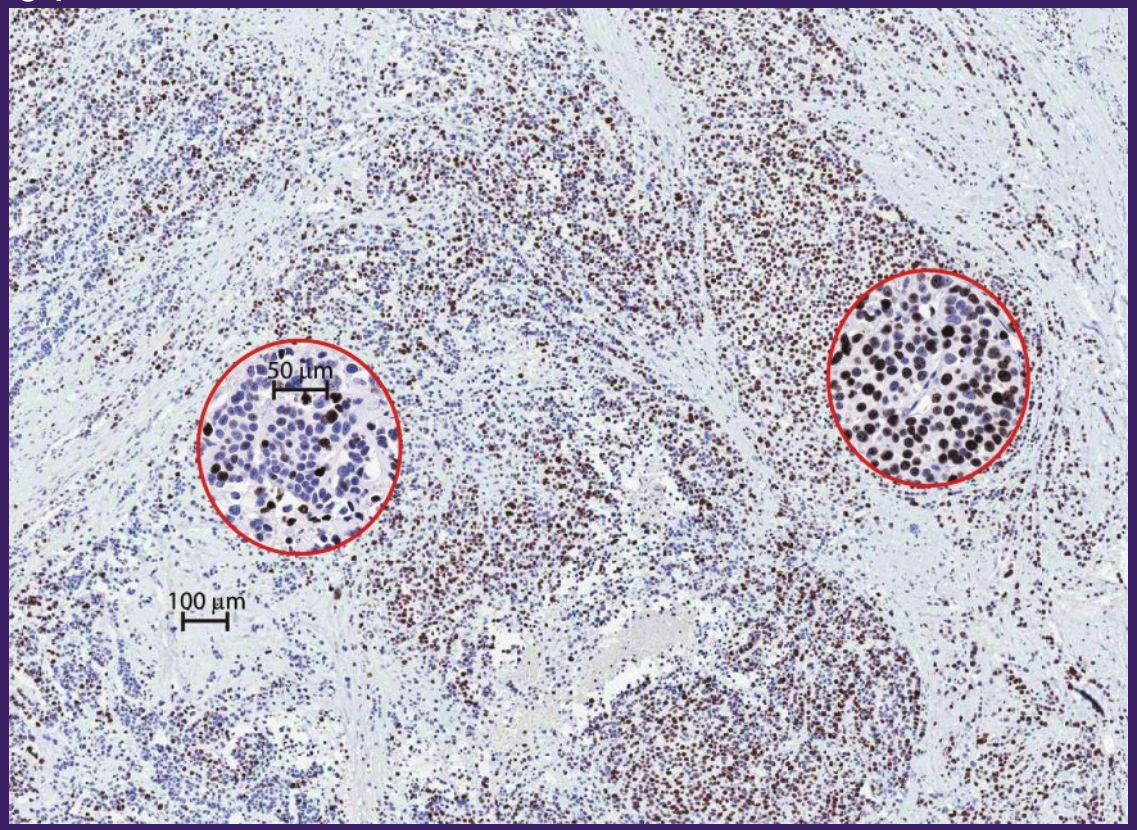


Figure 2. Examples of Ki67 staining in breast cancer. Tumor biopsies were fixed in neutral buffered formalin and sections stained for Ki67 with the MIB1 antibody (**brown stain**) and counterstained with Mayer's hematoxylin (**blue stain**). **A**) Well-fixed specimen. **B**) Poorly fixed specimen. The micrograph was taken using a Leica Microsystems Ariol image analyzer (Leica Microsystems, Gateshead, UK). Scale bar = 50 μm.



Box 1. Recommendations for Ki67 assessment in breast cancer

Preanalytical

- Core-cut biopsies and whole sections from excision biopsies are acceptable specimens; when comparative scores are to be made, it is preferable to use the same type for both samples (eg, in presurgical studies).
- TMAs are acceptable for clinical trial evaluation or epidemiological studies of Ki67.
- Fixation in neutral buffered formalin should follow the same guidelines as published for steroid receptors (39,40).
- Once prepared, tissue sections should not be stored at room temperature for longer than 14 days. Results after longer storage must be viewed with caution.

Analytical

- Known positive and negative controls should be included in all batches; positive nuclei of nonmalignant cells and with mitotic figures provide evidence of the quality of an individual section.
- Antigen retrieval procedures are required. The best evidence supports the use of heat-induced retrieval most frequently by microwave processing.
- The MIB1 antibody is currently endorsed for Ki67.

Interpretation and scoring

- In full sections, at least three high-power (×40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
- For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
- If pharmacodynamic comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
- If there are clear hot spots, data from these should be included in the overall score.
- Only nuclear staining is considered positive. Staining intensity is not relevant.
- Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
- Image analysis methods for Ki67 remain to be proven for use in clinical practice.

Data handling

- The Ki67 score or index should be expressed as the percentage of positively staining cells among the total number of invasive cells in the area scored.
- Statistical analysis should take account of the log-normal distribution generally followed by Ki67 measurement.
- The most appropriate endpoint in comparative studies of treatment efficacy or response is the percentage suppression of Ki67-positive cells.
- The most appropriate endpoint for assessing residual risk of recurrence is the on-treatment proportion of Ki67-positive cells.
- Cut points for prognosis, prediction, and monitoring should only be applied if the results from local practice have been validated against those in studies that have defined the cutoff for the intended use of the Ki67 result.

JNCI 2011; 103:1656-64

OPEN ACCESS Freely available online May 2012 7(5):e37379 PLoS one

How Reliable Is Ki-67 Immunohistochemistry in Grade 2 Breast Carcinomas? A QA Study of the Swiss Working Group of Breast- and Gynecopathologists

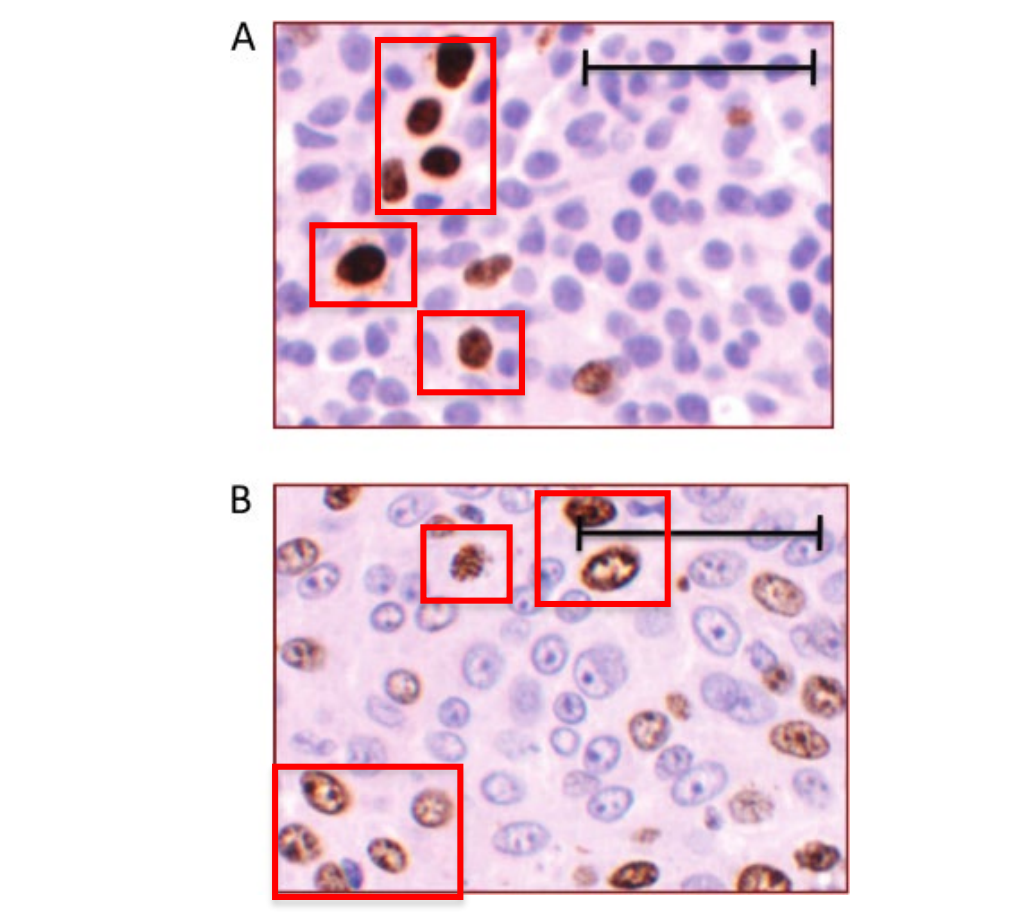
Zsuzsanna Varga[1], Joachim Diebold[2], Corina Dommann-Scherrer[3], Harald Frick[4], Daniela Kaup[5], Aurelia Noske[1], Ellen Obermann[6], Christian Ohlschlegel[7], Barbara Padberg[4], Christiane Rakozy[8], Sara Sancho Oliver[9], Sylviane Schobinger-Clement[9], Heide Schreiber-Facklam[10], Gad Singer[11], Coya Tapia[12], Urs Wagner[13], Mauro G. Mastropasqua[14], Giuseppe Viale[14], Hans-Anton Lehr[15]*

- 5 pathologists evaluated MIB-1-LI (Ki-67) in 10 breast cancers
- The *same tumors* were re-examined by *same pathologists* 4 months later
- For 10 cases evaluated by 5 pathologists
  - overall K=0.56-0.72 (pretty good).
  - Just G2 cancers, Kappa= 0.17-0.49 (poor)
- Not accounted for by central vs local staining, counting vs eyeballing, self selected vs pre-defined areas of interest

- Update in 2017: They are better, but still only agree if Ki67 is <5 or >30. Its no good in the middle where we really need it.

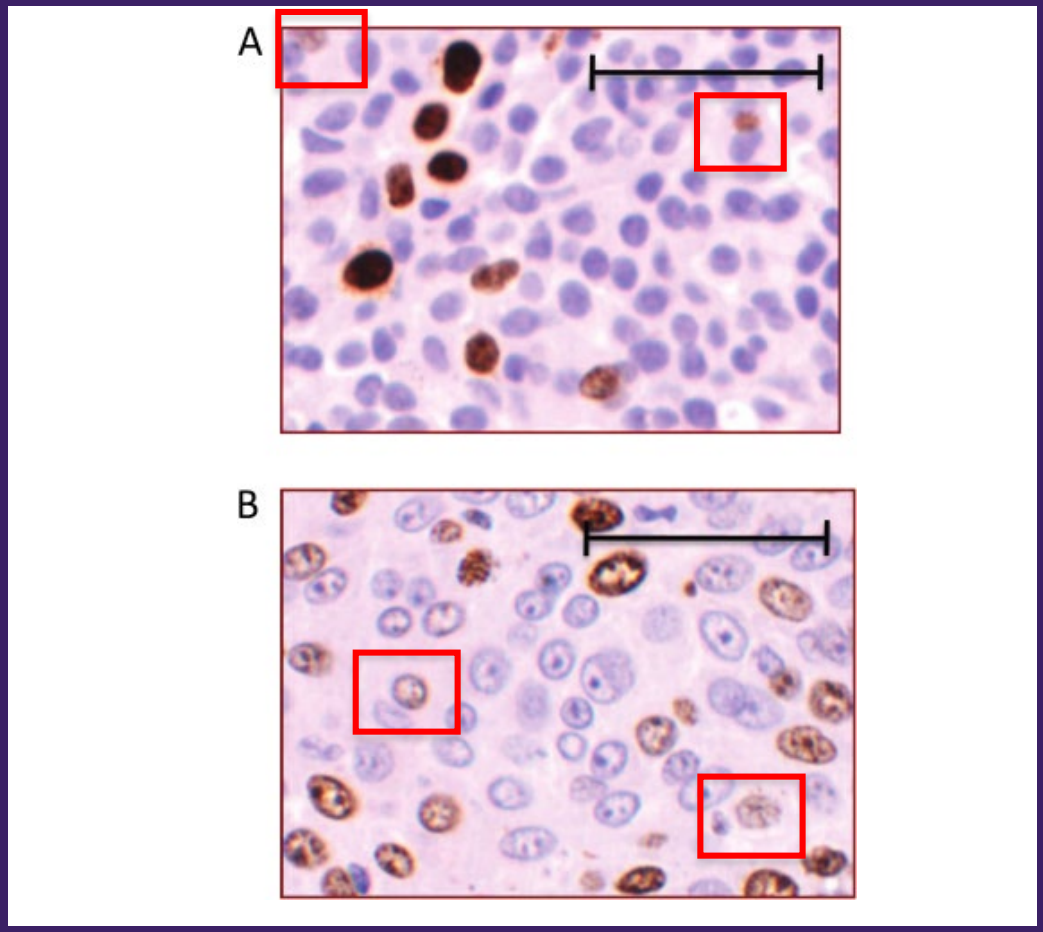
This suggests Ki-67 IHC may not be ready for prime-time!!

Some nuclei are indisputably positive



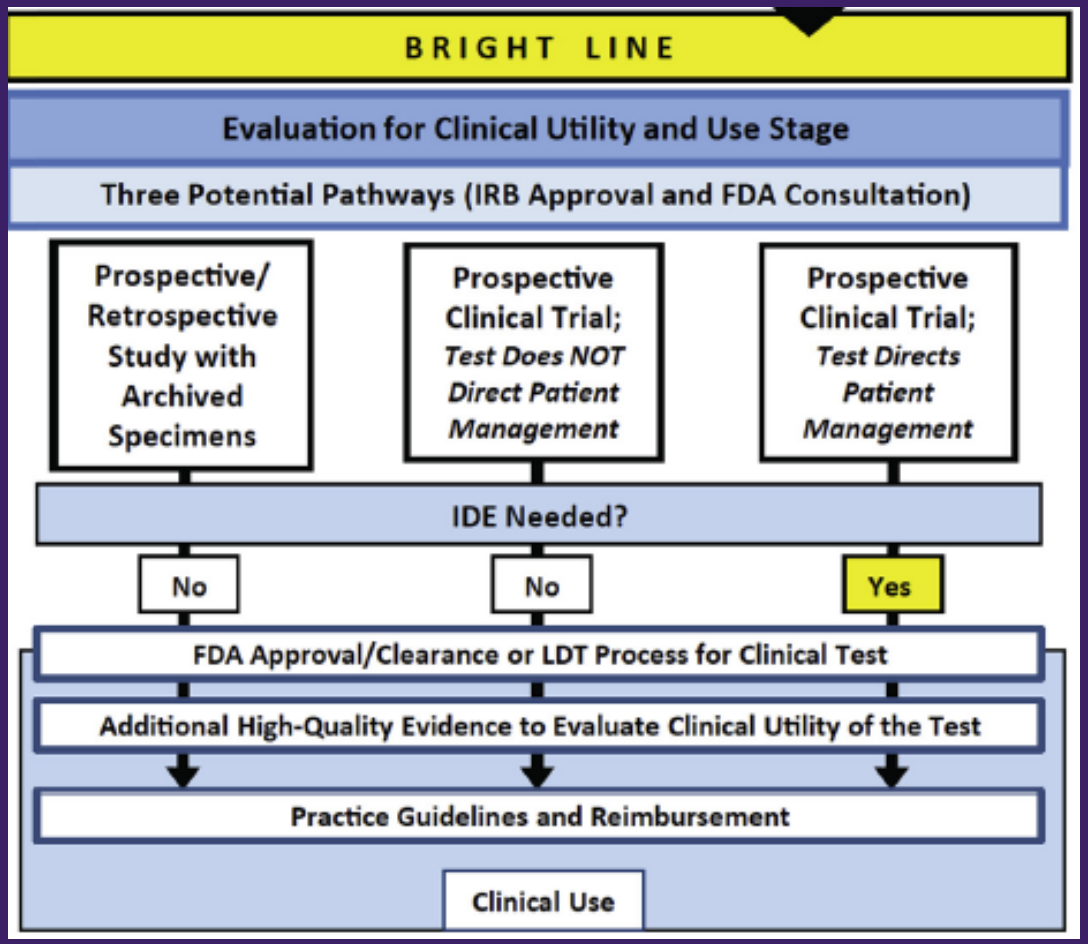
JNCI 2011; 103:1656-64

Other faintly staining nuclei are less clear and likely variably called positive or negative



JNCI 2011; 103:1656-64

Clinical Utility



Biomarker validation and testing. Hayes, D.F. Molecular Oncology, 2015; 9(5): 960-966.

Clinical Utility

- Does using the test to guide clinical decisions improve patient outcomes or outcomes are same with lower toxicity.

- ER for endocrine therapy– Yes
- HER2 for HER2 targeted therapy – Yes
- Oncotype RS for giving chemotherapy or not – Yes
- PD-L1 for immunotherapy – not clear
- PIK3CA mutation – maybe

Summary

- Biomarkers can be proteins, mRNA, or DNA molecules that indicate a biologic state
- Assays must be standardized and validated
- Best biomarkers are bi-modal, splitting cleanly into two groups
- Guidelines are important for minimizing pre-analytic variables, interpretation mistakes, or classification variability (what is called “positive”).
- Clinical utility of a biomarker test must be demonstrated by high levels of evidence in clinical trials.

Thank You!

MAYO CLINIC
Cancer Center

Breast Cancer Vaccines for HER2+ and TNBC

Breast Cancer SPORE Advocate Workshop
November 11, 2020

Keith L. Knutson, PhD
Professor
Department of Immunology
Department of Cancer Biology

Conflicts of Interest

- **Marker Therapeutics, Inc.**
  - Cancer Vaccines and T Cell Therapy – Houston Tx
  - Scientific Advisory Board (unpaid)
  - Several Patent Licensing Agreements (Mayo)
- **Kiromic, Inc.**
  - Cancer Vaccines/CAR T cell therapy – Lubbock, TX
  - Scientific Advisory Board
  - Stock owner
- **Antigen Express, Inc.**
  - Cancer Vaccines – Cambridge, MA
  - Scientific Advisory Board (Paid)
- **AffyImmune**
  - CAR T cell therapy – Cambridge, MA
  - Scientific Advisory Board (Paid)
- **Macrogenics, Inc.**
  - Biologics – Bethesda, MD
  - Grant funding
- **Leidos**
  - Biologics – Reston, VA
  - Collaborations
- **Bolt Therapeutics**
  - Biologics – Redwood, CA
  - Grant Funding

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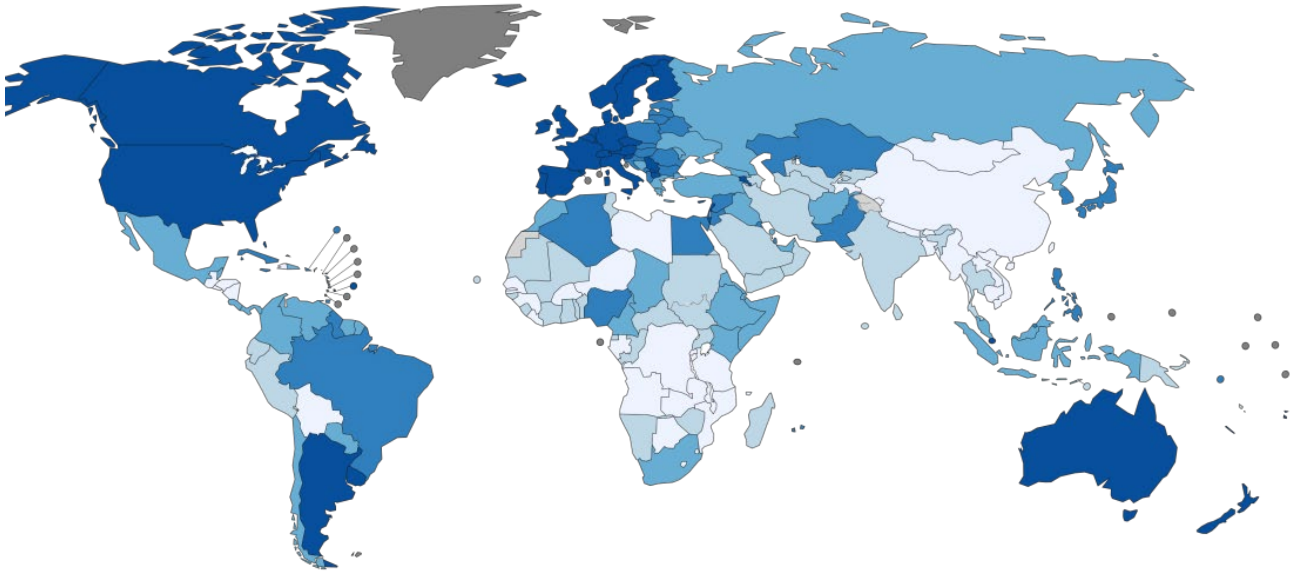
Breast cancer burden

Worldwide: 2,000,000 cases/year, 500,000 deaths/year

USA: 240K cases/year, 40K deaths/year



Estimated USA Breast Cancer Costs: $ 180,000,000,000

0.2% of the GDP

Eur J Health Econ. 2007

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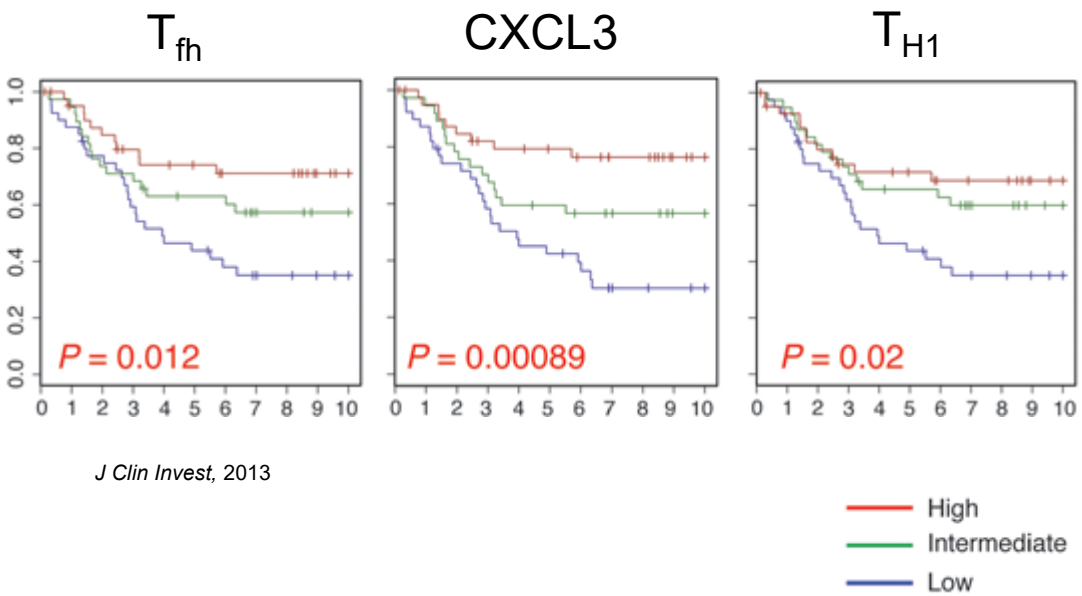
The immune system naturally responds to breast cancer

HER-2 Breast Cancer – 10 Year Survival Analysis



J Clin Invest, 2013

Patients with Breast Cancer Demonstrate Elevated T cell and Antibody Immunity to Several Tumor Antigens

Disis et al., 2000, *Breast Cancer Research and Treatment*

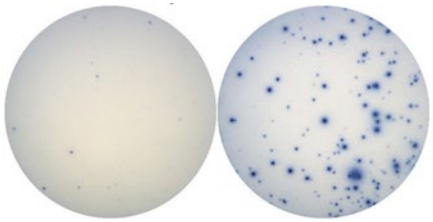
Kalli et al., 2008, *Cancer Research*

Karyampudi et al., 2013, *Plos One*

Krempski, et al. 2011, *Journal of Immunology*

Karyampudi, et al., 2014, *Cancer Research*

Knutson, et al., *2006, Journal of Clinical Oncology*



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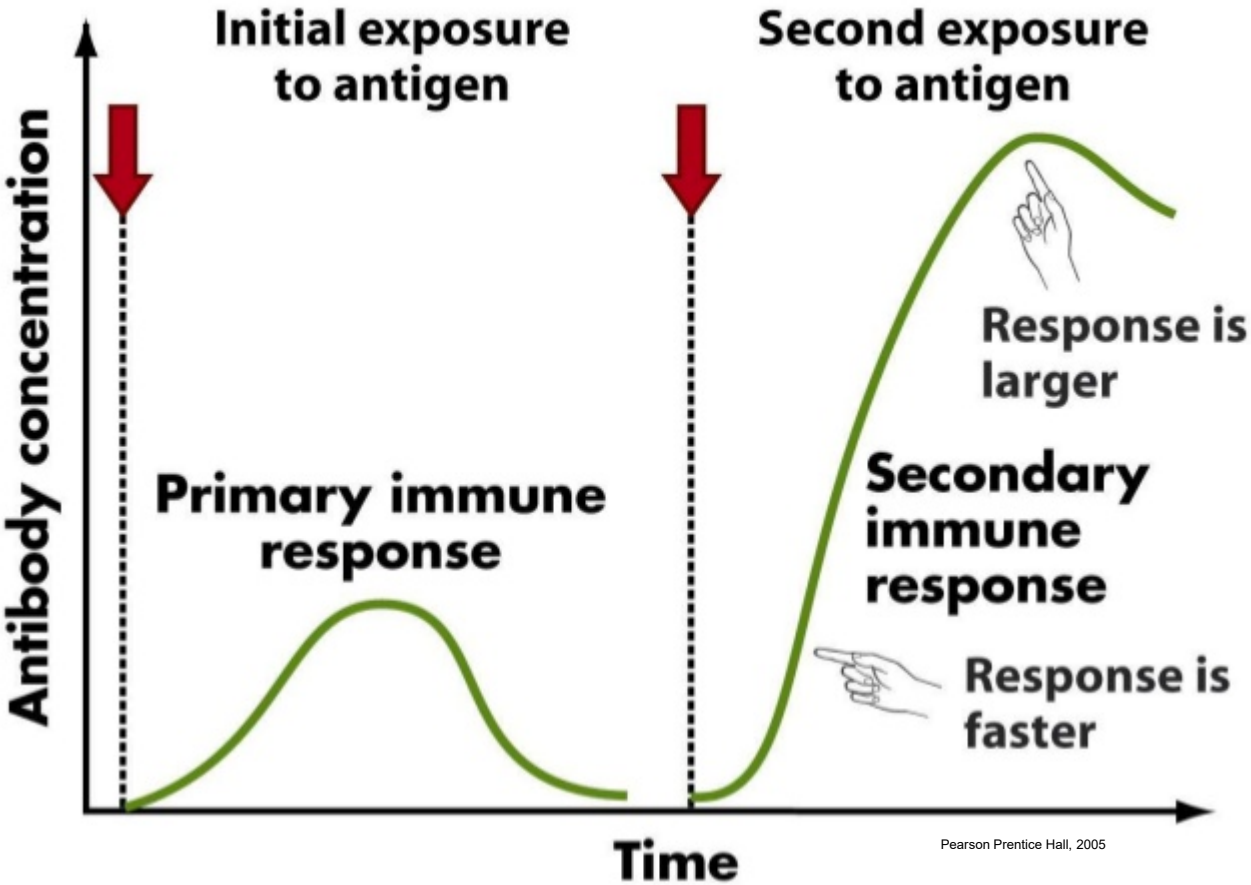
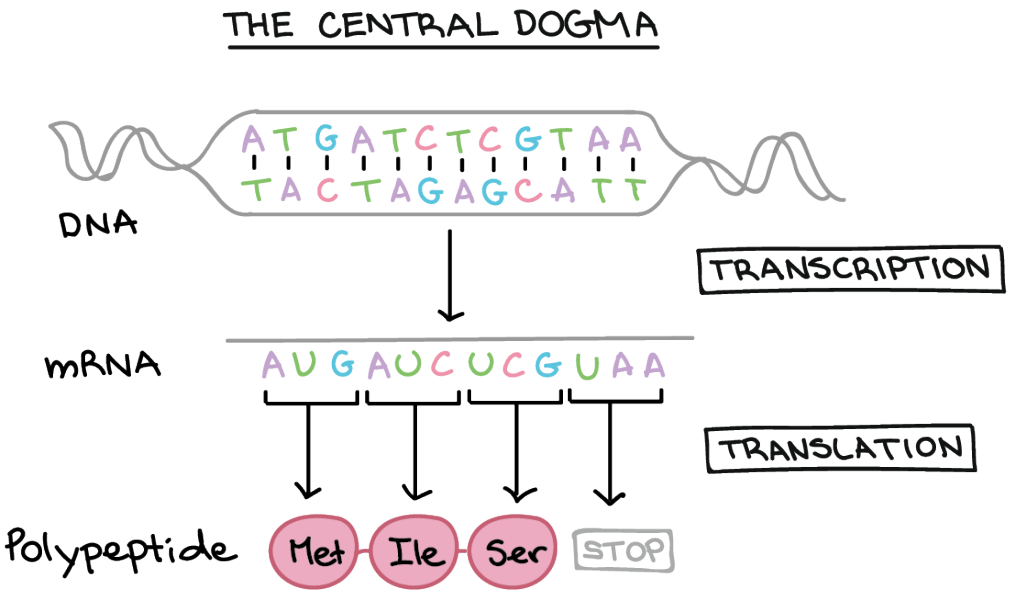
Principles of vaccination



Pearson Prentice Hall, 2005

Target antigen choices for a cancer vaccine



Khan Academy

- **Viral antigens**
- **Mutation derived antigens**
- **Aberrantly expressed self protein antigens**

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The adaptive immune system is the body's drug making machinery

CD4 "helper" T cells

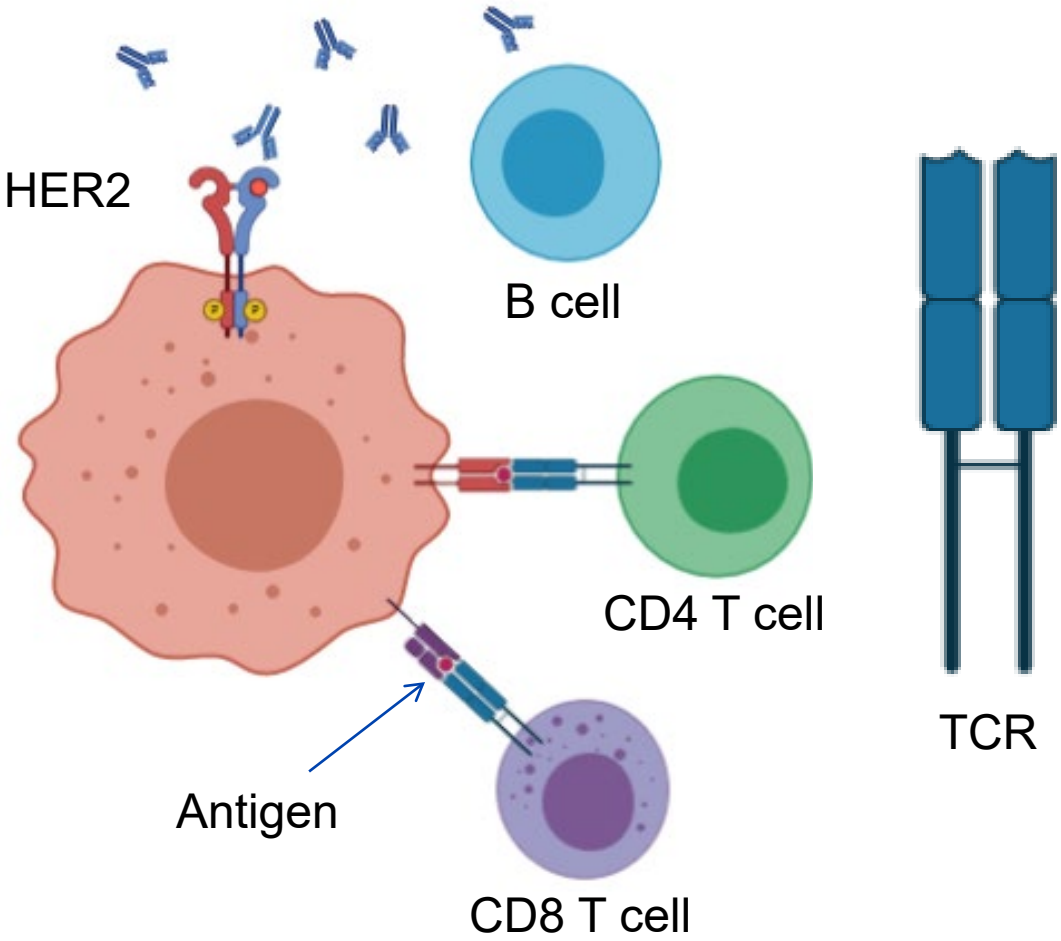
- Inflammation (macrophages and neutrophils)
- Antibodies
- Activate cytotoxic T cells
- Immune-surveillance
- Epitope-spreading

CD8 "cytolytic" T cells

- Tumor lysis

B cells

- Antibodies
- Signaling
- ADCC
- Complement



Biorender

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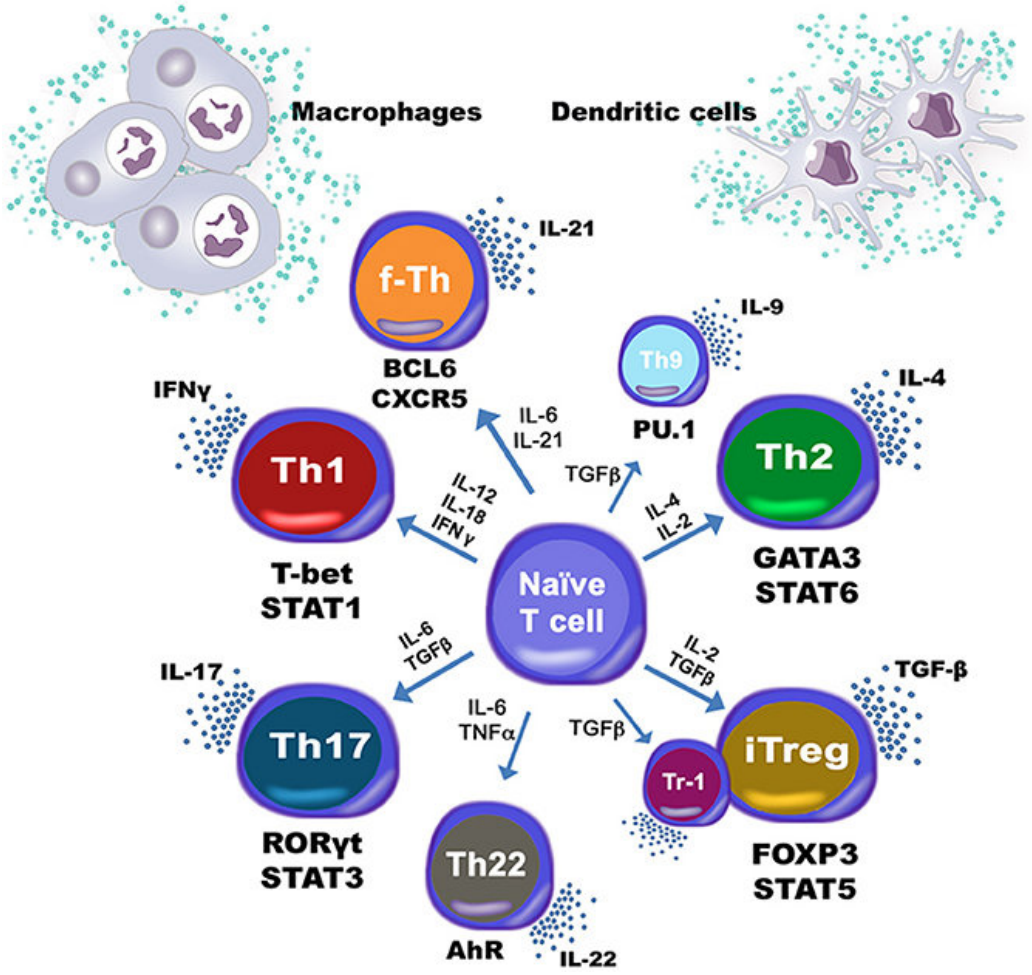
Differentiation of the adaptive immune response



Front Cell Develop Biol, 2014

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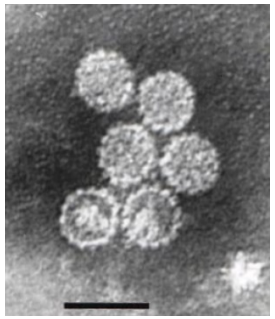
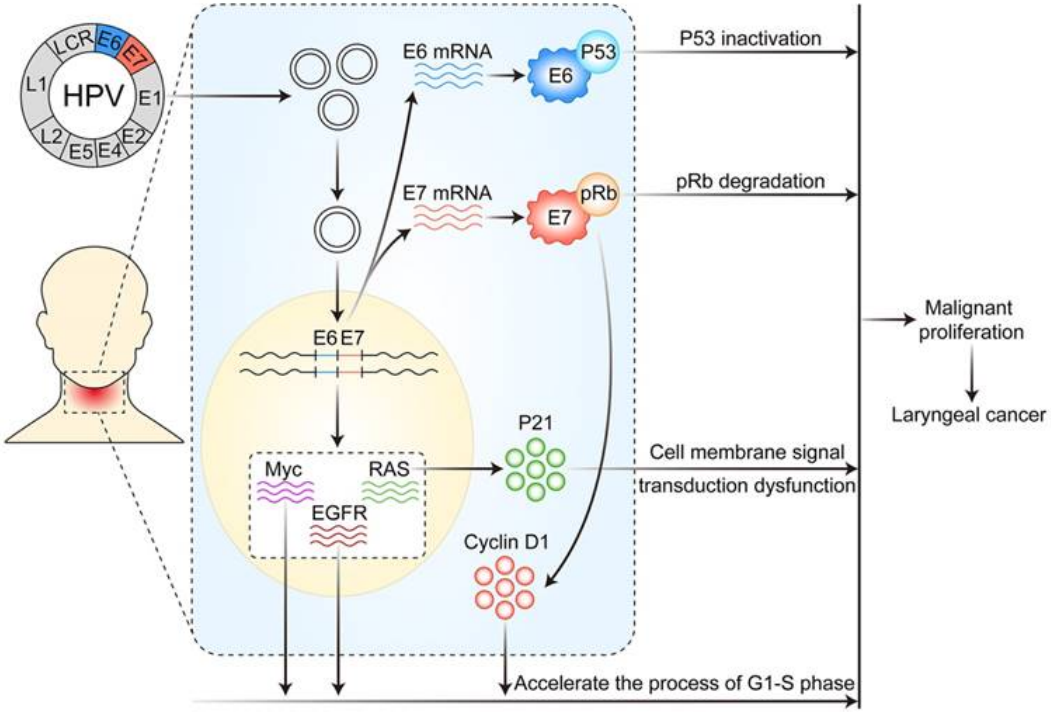
Prevailing data suggest that viruses do not cause breast cancer

- **Human papilloma virus**
- **Epstein-Barr virus**
- **Mouse mammary tumor virus**
- **Bovine Leukemia virus**



https://commons.wikimedia.org/wiki/File:Papillomavirus.jpg



J Cancer 2019; 10(19):4455-4462. doi:10.7150/jca.34016

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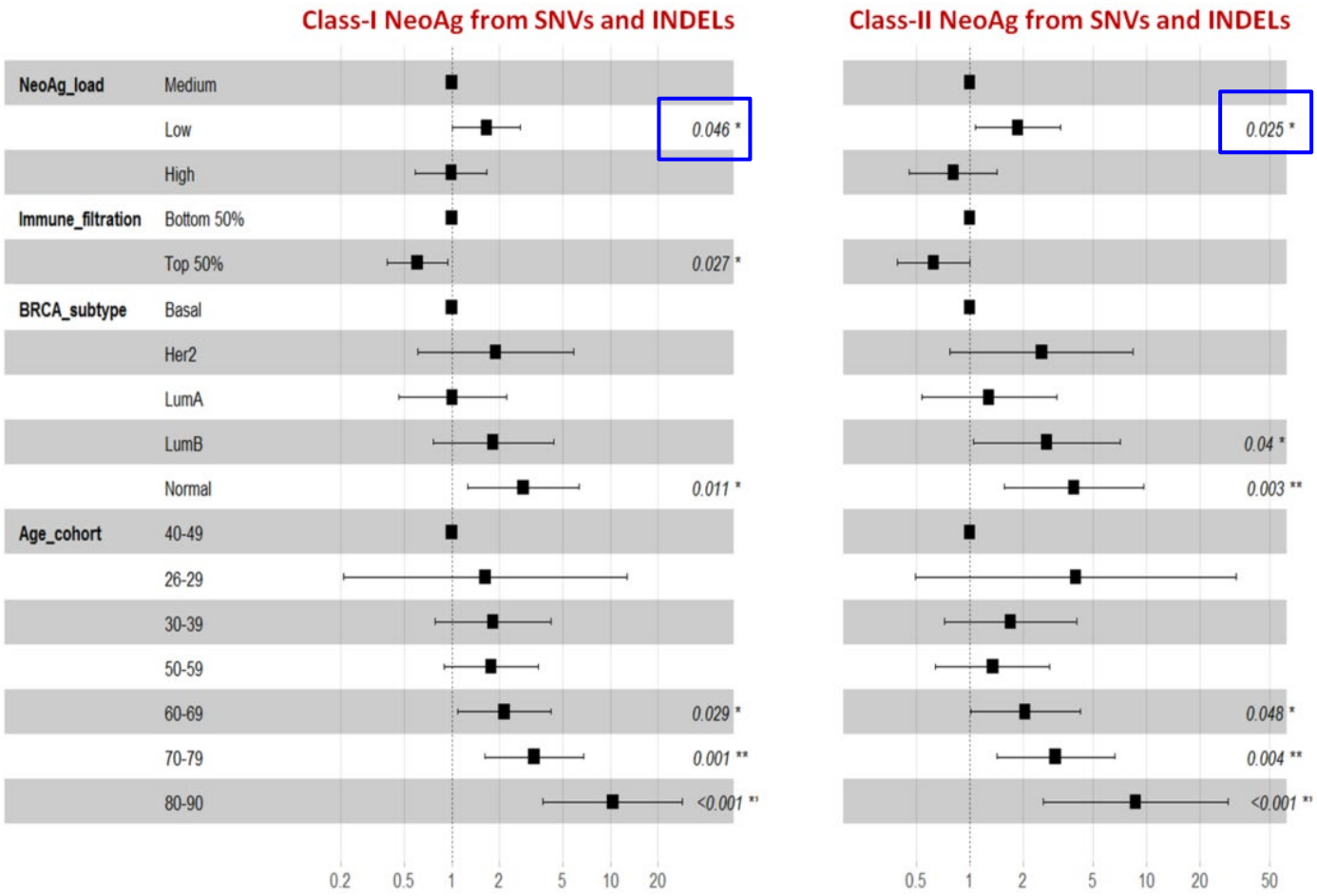
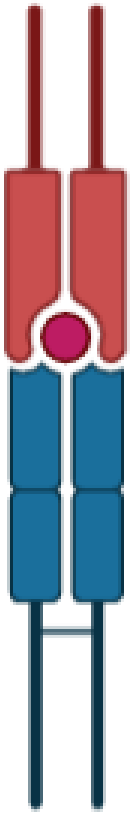
Lower mutation load correlates with worse overall survival

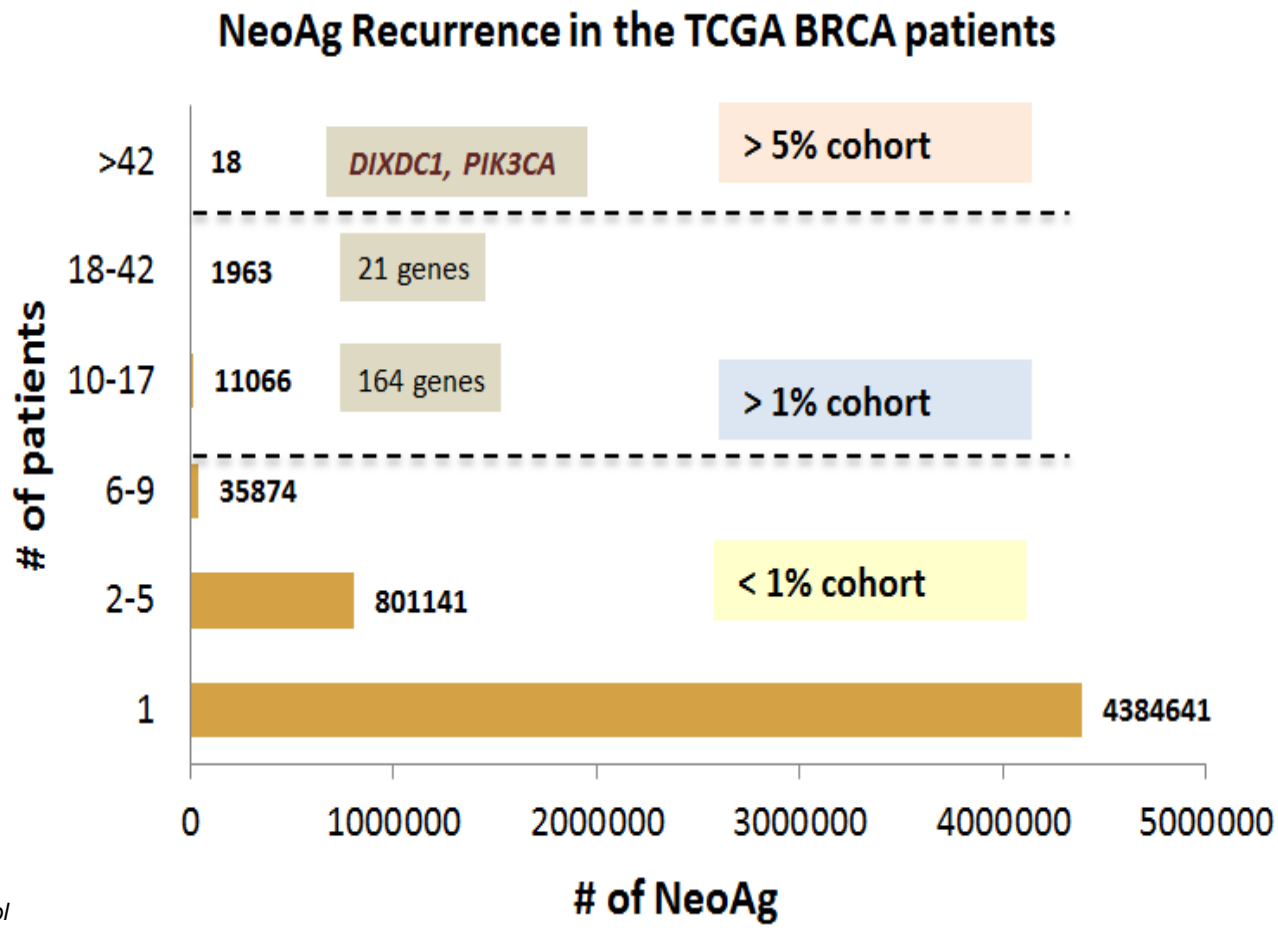


Cox Proportional Hazard model: adjusted for TIL, Breast Cancer Subtypes, Age, and Mutation Burden

©2017 MFMER

Neoantigens are largely private making every vaccine different





Ren et al., 2020, *Oncoimmunol*

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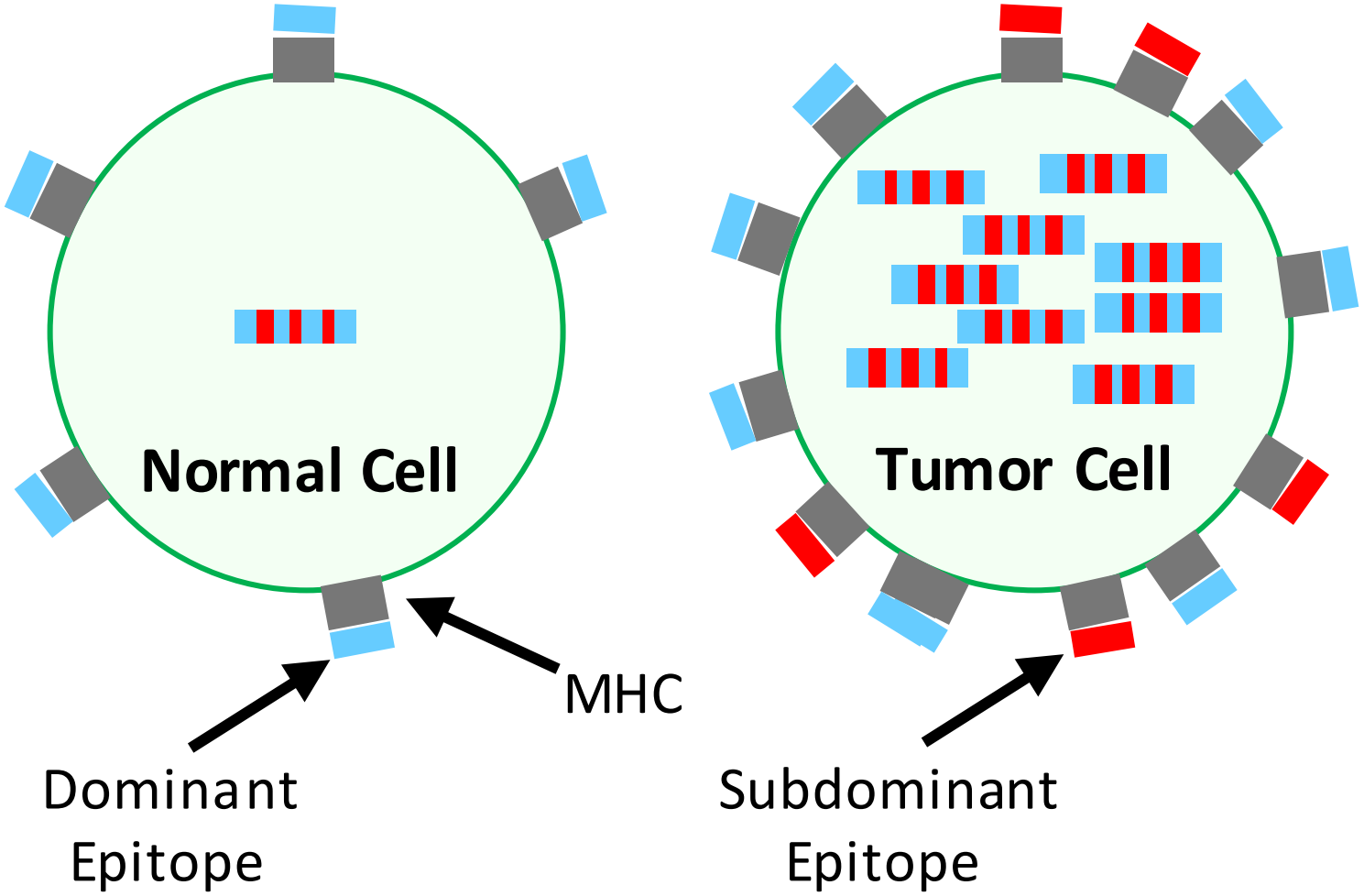
Overexpressed self proteins as a source of tumor neoantigens



Cibotti, R., *Proc Natl Acad Sci U S A*, 1992. **89**(1): p. 416-20

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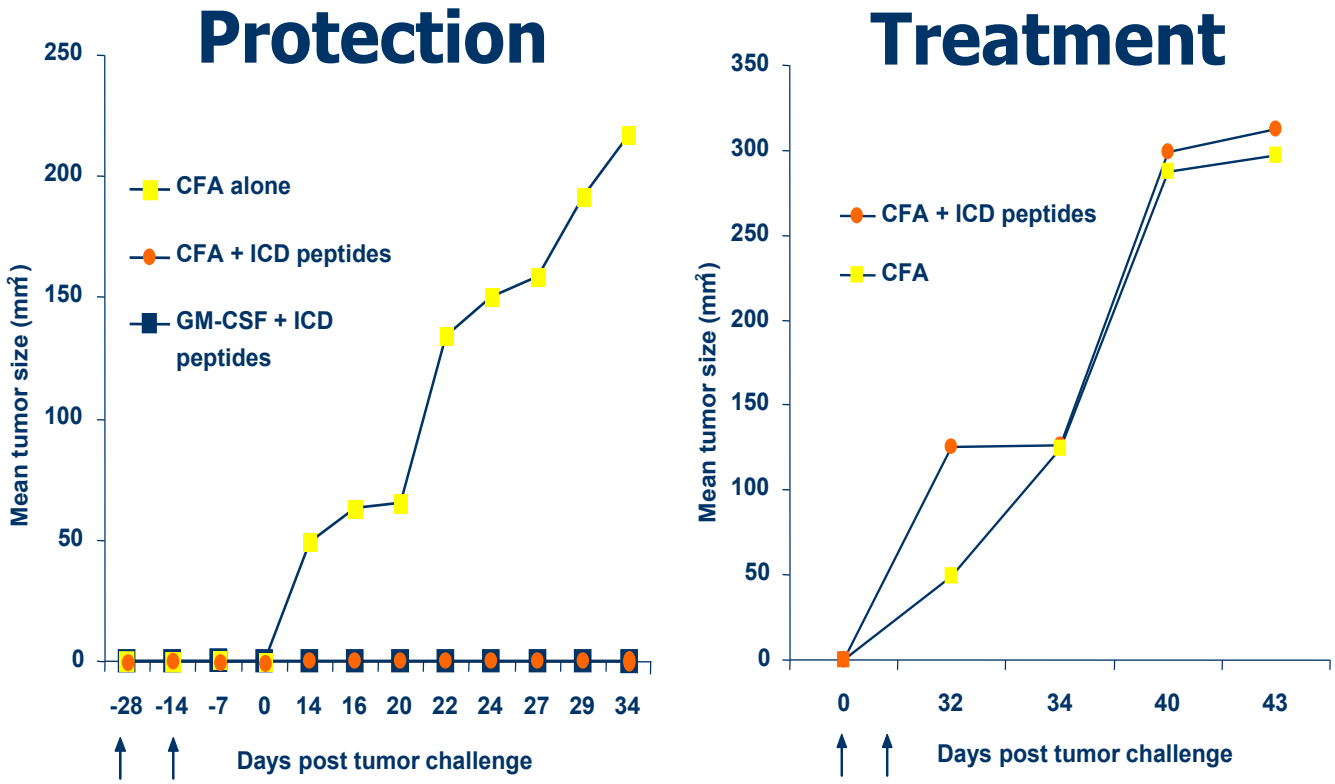
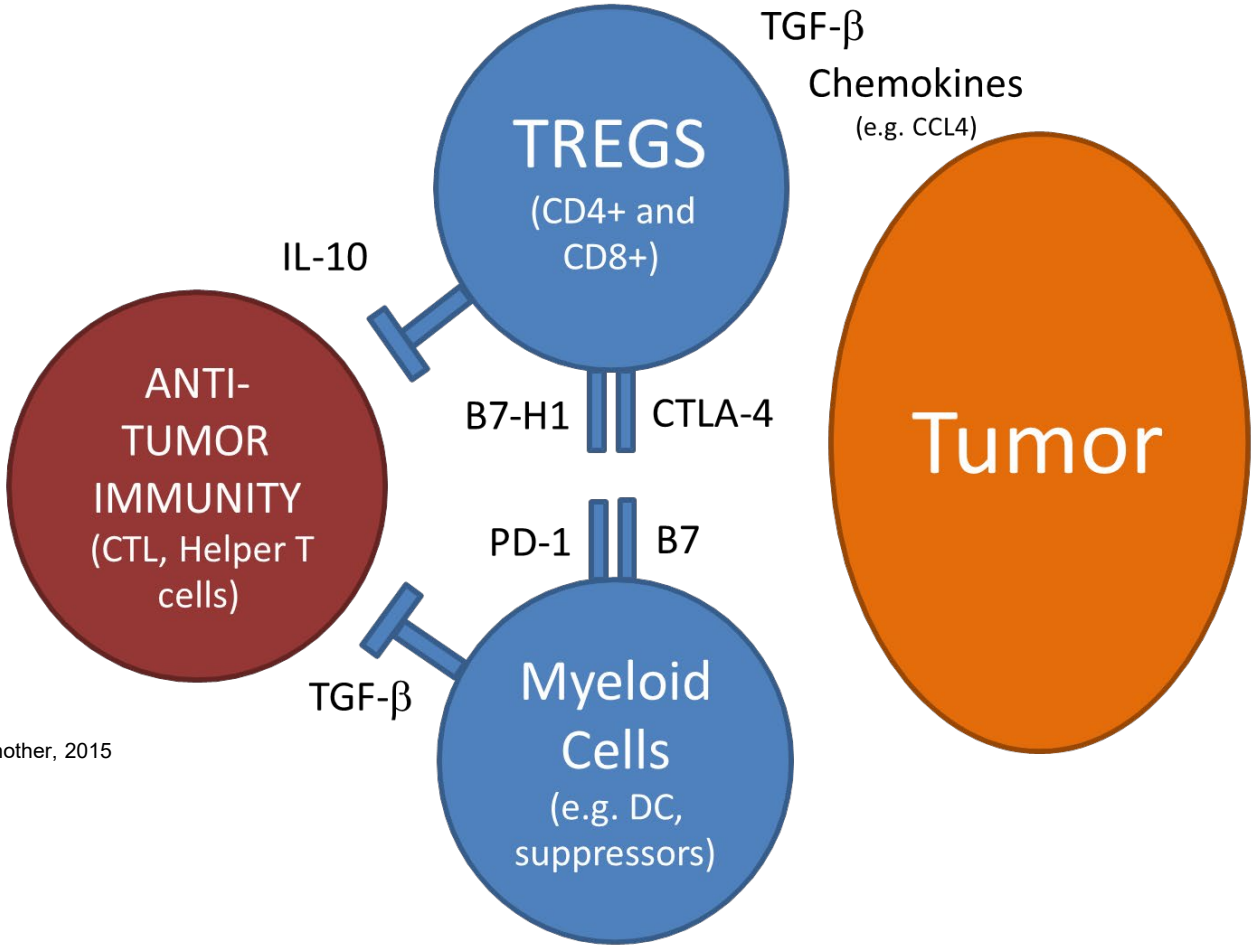
Early murine HER2 vaccine development informed on best use of vaccines



Knutson et al., *Clin Breast Cancer,* 2001

Cancers quickly suppress the immune system to prevent their destruction



J Immunother Cancer, 2020
Cancer Res, 2017
Cancer Res, 2016
Cancer Immunol and Immunother, 2015
Cancer Immunol Res, 2014
Plos One, 2013
Plos One, 2011
J Immunol 2013
J Immunol 2009
J Immunol 2006
Nature Med 2004
Nature Med 2003

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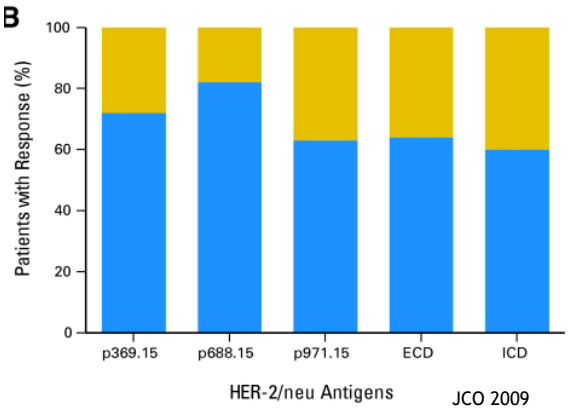
Early HER2 vaccines demonstrated feasibility but were less than optimal in coverage

- ECD Vaccine
- ICD Vaccine
- HLA-A2 Vaccine
- E75 Vaccine



JCO 2009

E75 is useful in 40% and requires frequent boosting

Vaccine Prolongs Remission in Triple-Negative Breast Cancer

SAN FRANCISCO -- Treatment with a novel peptide vaccine appeared to delay disease recurrence in triple-negative breast cancer (TNBC) patients with low HER2 expression, a subgroup analysis of a phase II trial found.

At a median follow-up of 26.1 months, disease recurrence occurred in 7.5% of TNBC patients who received nelipepimut-S (NeuVax) compared with 26.7% in the control arm (HR 0.26, 95% CI 0.08-0.81, *P*=0.01), reported Guy T. Clifton, MD, of San Antonio Military Medical Center in Texas.

"We think the results are intriguing in light of what we now understand as far as triple-negative breast cancer being a more immunogenic subtype of breast cancer that's more responsive to immunotherapy," he said during his presentation here at the ASCO-SITC Clinical Immuno-Oncology Symposium.

In the NeuVax and control arms, respectively, rates of disease-free survival (DFS) among the 97 TNBC patients were:

- 92.6% versus 70.2% at 24 months
- 82.3% versus 70.2% at 36 months

Knutson KL, et al., JCI 2001
Disis ML, et al., JCO, 2002
Knutson KL, et al., Clin Cancer Res, 2002

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Binding of predicted HER2 neoantigens to purified HLA-DR

			IC$_{50}$ nM to purified HLA														
Sequence	Peptide Name	Position[¶]	DRB1 *0101	DRB1 *0301	DRB1 *0401	DRB1 *0404	DRB1 *0405	DRB1 *0701	DRB1 *0802	DRB1 *0901	DRB1 *1101	DRB1 *1201	DRB1 *1302	DRB1 *1501	DRB3 *0101	DRB4 *0101	DRB5 *0101
NLELTYLPTNASLSF	**HER-2/neu.59**	**59**	**4.9**	**7356**	**6.2**	**2.7**	**38**	**7.2**	**94**	**3055**	**30**	**141**	**105**	**23**	**ND**	**29**	**189**
LTYLPTNASLSFLQD	HER-2/neu.62	62	9.7	3364	19	16	80	15	426	4081	213	150	47	132	141	1633	173
IQEVQGYVLIAHNQV	HER-2/neu.77	77	57	7763	111	178	102	35	213	302	165	3438	103	75	13,508	546	1361
YVLIAHNQVRQVPLQ	HER-2/neu.83	83	28	454	53	104	1185	92	300	358	208	302	1.9	679	649	124	18
HNQVRQVPLQRLRIV	**HER-2/neu.88**	**88**	**950**	**971**	**840**	**78**	**1303**	**80**	**85**	**6644**	**21**	**42**	**270**	**340**	**ND**	**18**	**173**
MEHLREVRAVTSANI	HER-2/neu.347	347	9.6	2970	533	12	200	9.7	95	4345	262	221	23	86	ND	81	216
LREVRAVTSANIQEF	HER-2/neu.350	350	17	3913	43	8.2	50	12	456	5187	661	161	1.5	27	ND	163	94
LSVFQNLQVIRGRIL	**HER-2/neu.422**	**422**	**1.3**	**345**	**6.3**	**33**	**26**	**7.1**	**148**	**859**	**9.6**	**486**	**80**	**33**	**ND**	**67**	**17**
RGRILHNGAYSLTLQ	HER-2/neu.432	432	2.4	710	480	129	2845	5.6	5077	430	773	40	1.3	5.4	358	562	82
LRSLRELGSGLALIH	HER-2/neu.455	455	7.1	ND	896	14	603	142	1075	594	309	498	16	24	16,142	549	726
VLGVVFGILIKRRQQ	HER-2/neu.666	666	67	2449	177	335	101	17	35	ND	12	268	17	185	ND	958	38
SRLLGICLTSTVQLV	HER-2/neu.783	783	80	2923	85	13	90	9.0	634	137	80	446	4.7	39	3567	481	392
PIKWMALESILRRRF	**HER-2/neu.885**	**885**	**12**	**30**	**14**	**250**	**161**	**664**	**312**	**3620**	**133**	**66**	**349**	**3.3**	**ND**	**62**	**3.4**
IKWMALESILRRRFT	HER-2/neu.886	886	16	10	37	1075	435	1795	515	9282	136	241	1118	11	ND	340	3.3
FSRMARDPQRFVVIQ	HER-2/neu.976	976	29	35	512	2224	855	1423	798	1481	49	6867	240	1408	901	227	45

[¶]Position of N-terminal amino acid; ND=not determined; Peptides that constitute degenerate pool are in bold

Karyampudi, *Cancer Res*, 2010

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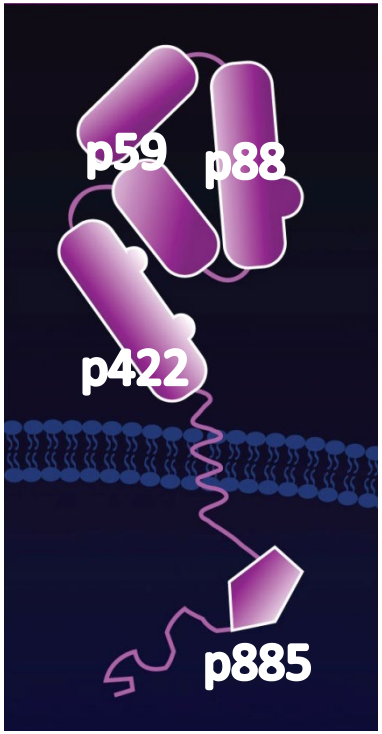
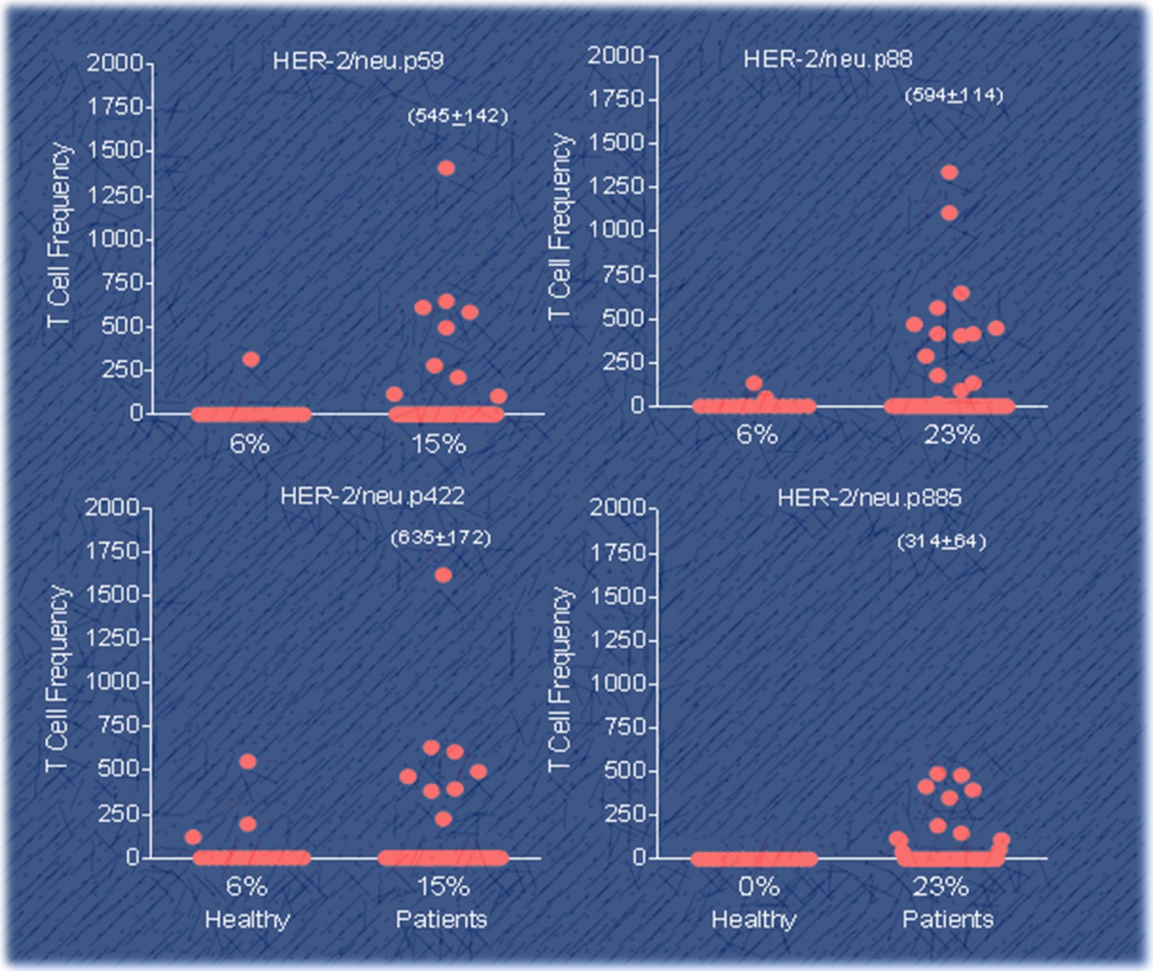
Detection of pre-existent immunity



DRB1*0101, DRB1*0301
DRB1*0401, DRB1*0404
DRB1*0405, DRB1*0701
DRB1*0802, DRB1*0901
DRB1*1101, DRB1*1201
DRB1*1302, DRB1*1501
DRB3*0101, DRB4*0101
DRB5*0101



Karyampudi et. al., Clin Cancer Res. 2010
Knutson KL and Ishioka G, 2007, HLA DR binding peptides and their uses. Patented 12/740,562.

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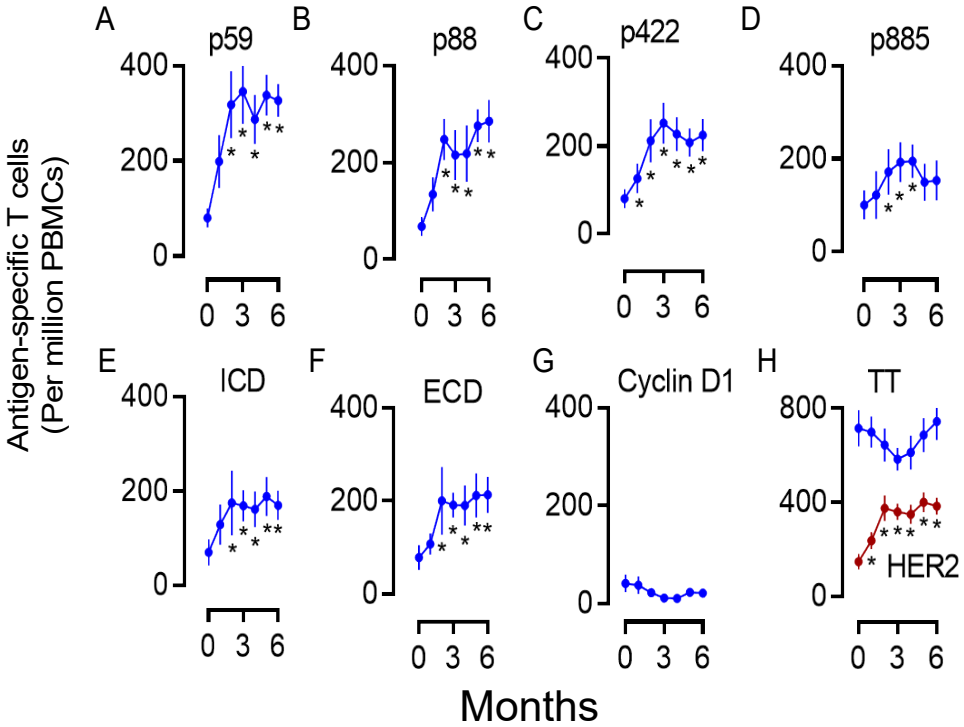
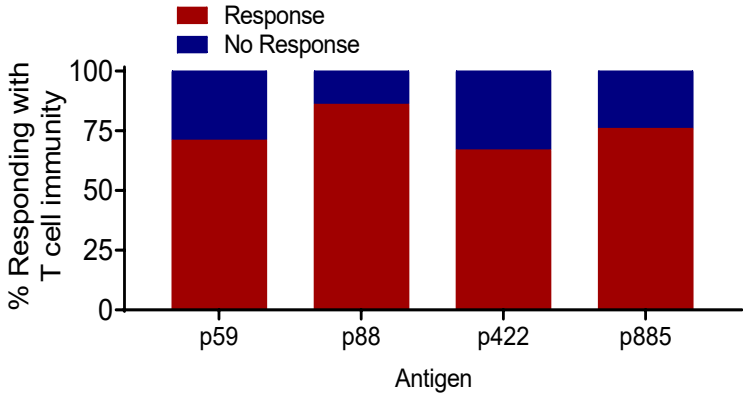
Vaccine induces immunity to naturally processed antigens



100% of patients demonstrated immunity following vaccination

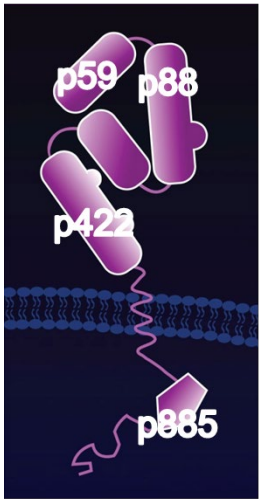


Stages II-III, 22 patients, only 1 relapse over 7 years

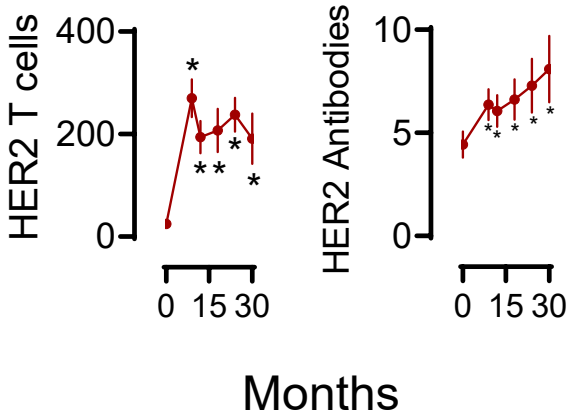
Amy Degnim

Knutson et. al., 2020 Clinica Cancer Research

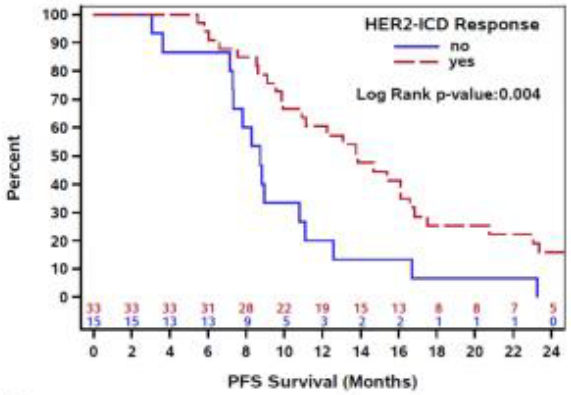
Generation of durable HER2-specific T cells in majority of patients with resected HER2 breast cancer

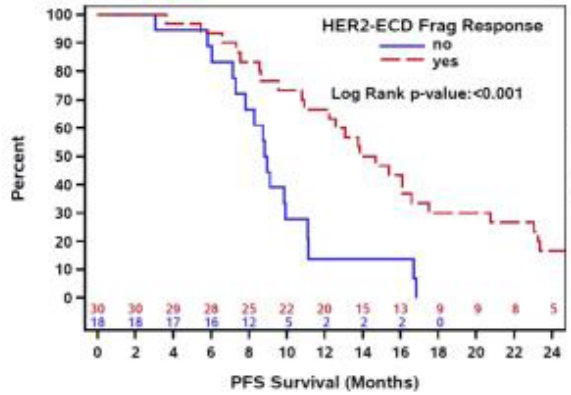


DRB1*0101, DRB1*0301
DRB1*0401, DRB1*0404
DRB1*0405, DRB1*0701
DRB1*0802, DRB1*0901
DRB1*1101, DRB1*1201
DRB1*1302, DRB1*1501
DRB3*0101, DRB4*0101
DRB5*0101



Knutson et. al., 2020 Clinical Cancer Research





Norton, *Breast Cancer Res Treat,* 2018
Knutson, *Cancer Res* 2016
Taylor, *Clin Cancer Res*, 2007

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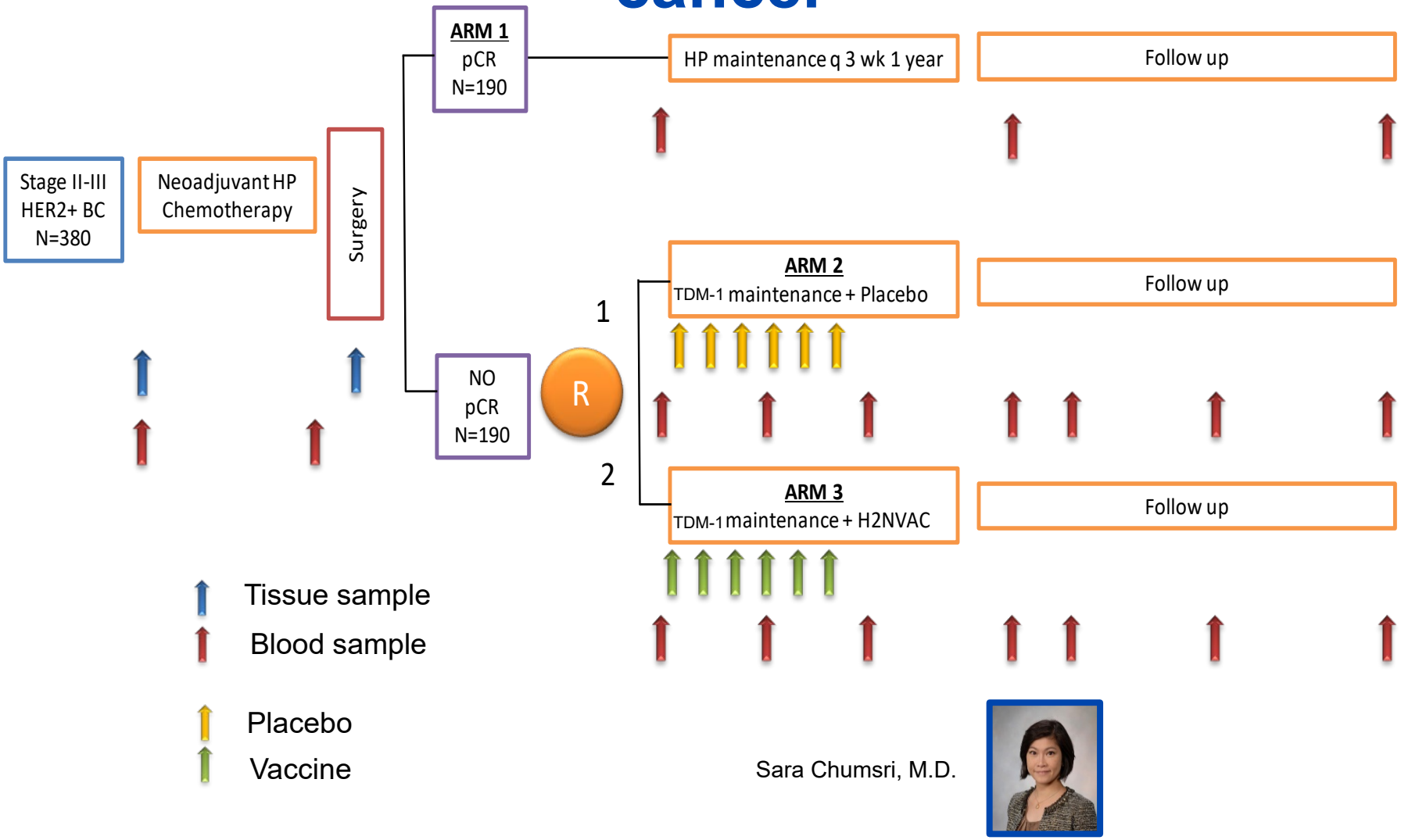
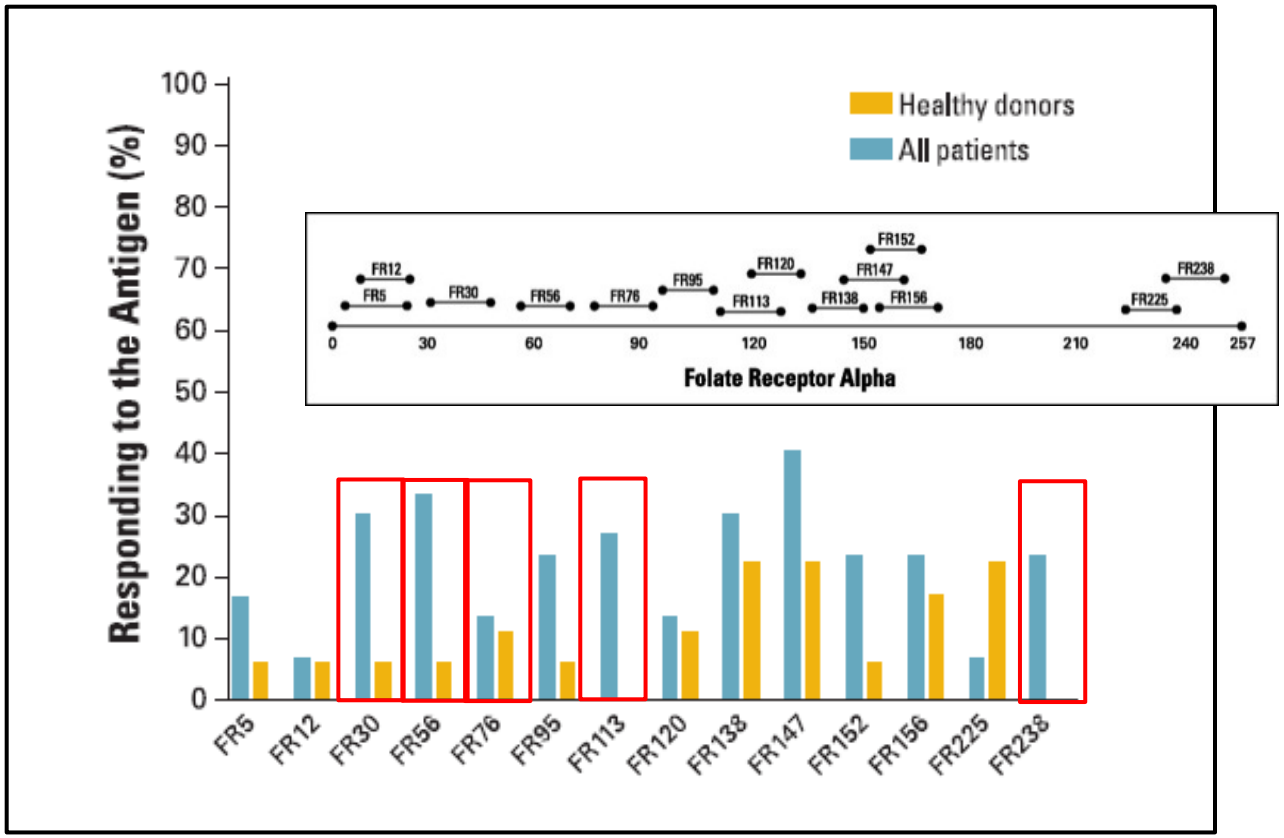
BC170530: Phase II resected HER2+ breast cancer

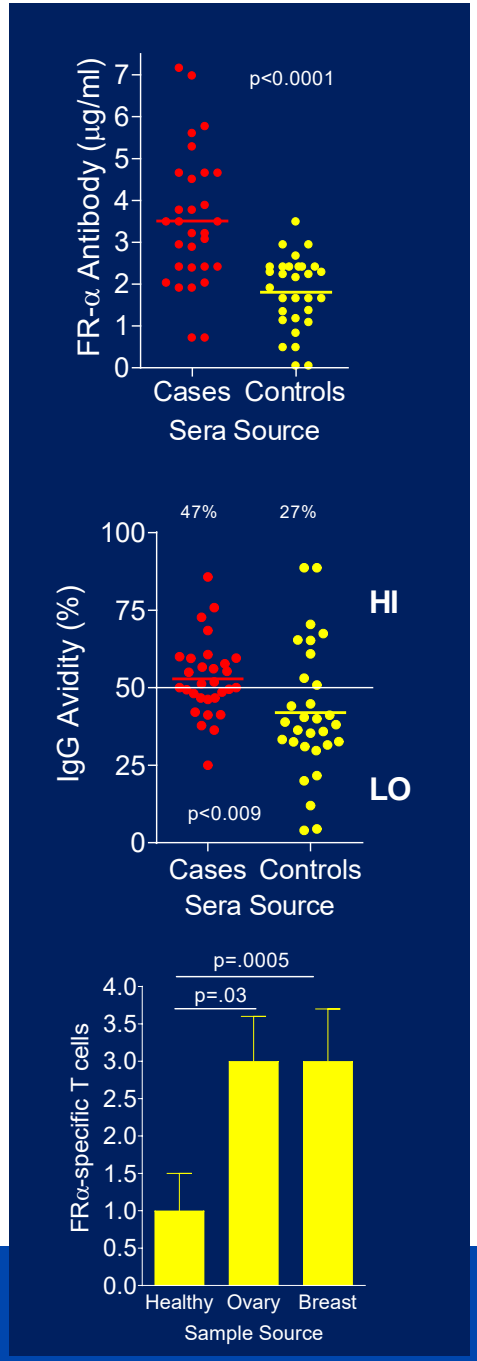


Sara Chumsri, M.D.

Spontaneous immunity to the folate receptor alpha in cancer patients

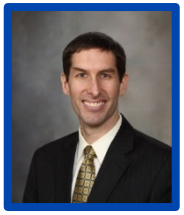


Knutson, K. L. et al. JCO; 24:4254-4261 2006



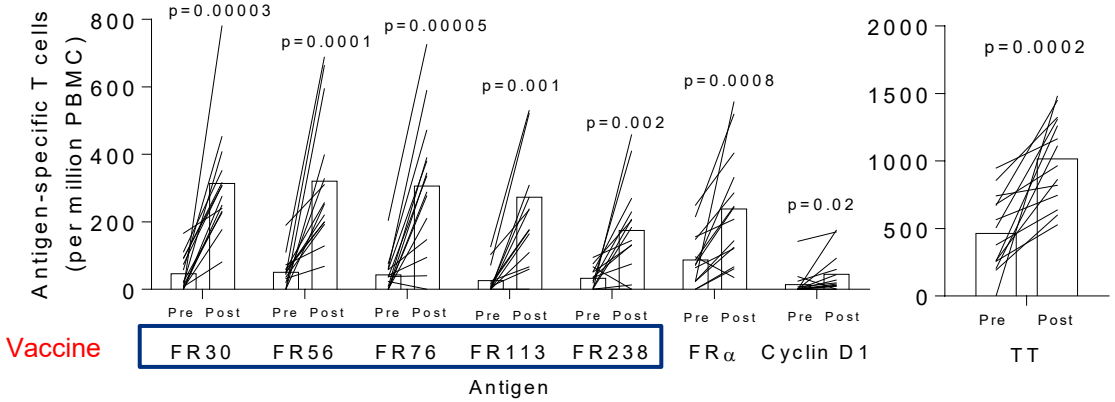
FRα peptide vaccine generates immunity in breast and ovarian cancer patients

Kim Kalli, Ph.D. (decd)

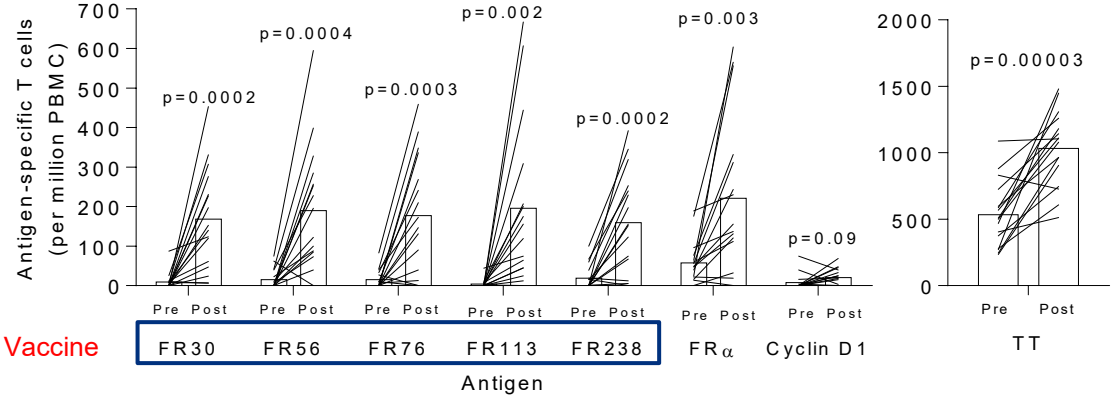


Matt Block, M.D., Ph.D.

Vaccine period 0-6 months



Follow up period 12-18 months



Kalli, Block *Clin Cancer Res*, 2018

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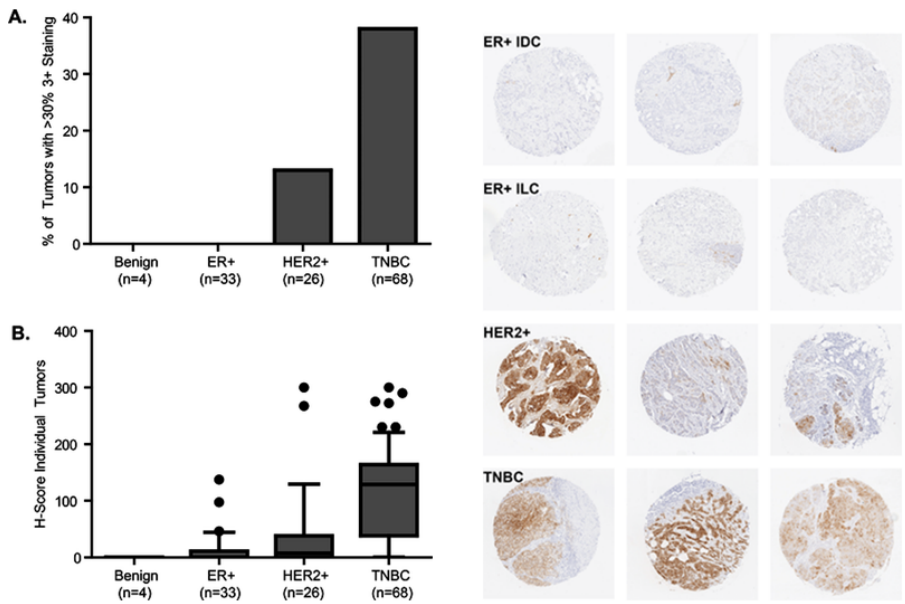
BC141410: FRα Vaccination to Prevent Progression of Triple Negative Breast Cancer

FRα is preferentially expressed in TNBC



Necela BM, PLoS ONE 10(3): e0122209. doi:10.1371/journal.pone.0122209

Stages IIb/III TNBC

Convention al Therapy

Placebo N=93

Vaccine N=187

Edith Perez

Kathryn Ruddy

Multicenter Phase II Trial to Test Whether Vaccine Prevents Recurrence in Patients Diagnosed and Treated for TNBC

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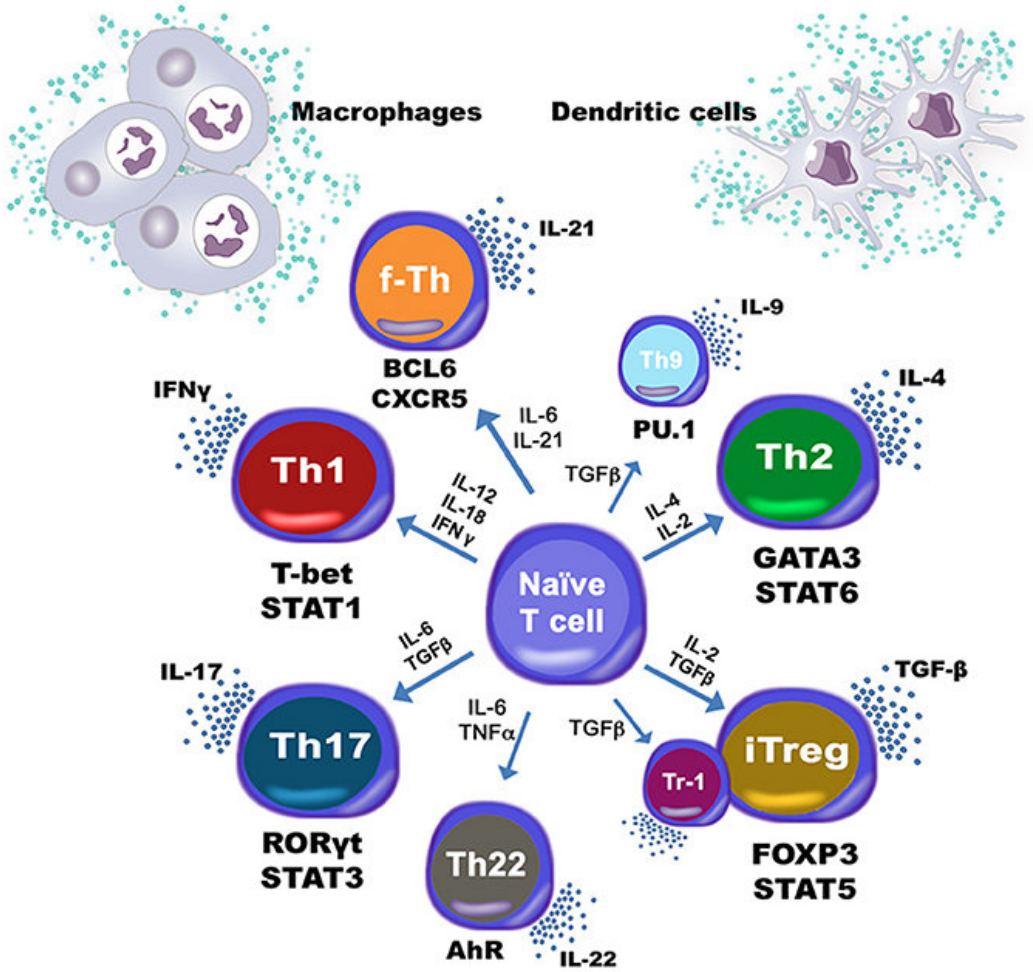
Differentiation of the adaptive immune response



Front Cell Develop Biol, 2014

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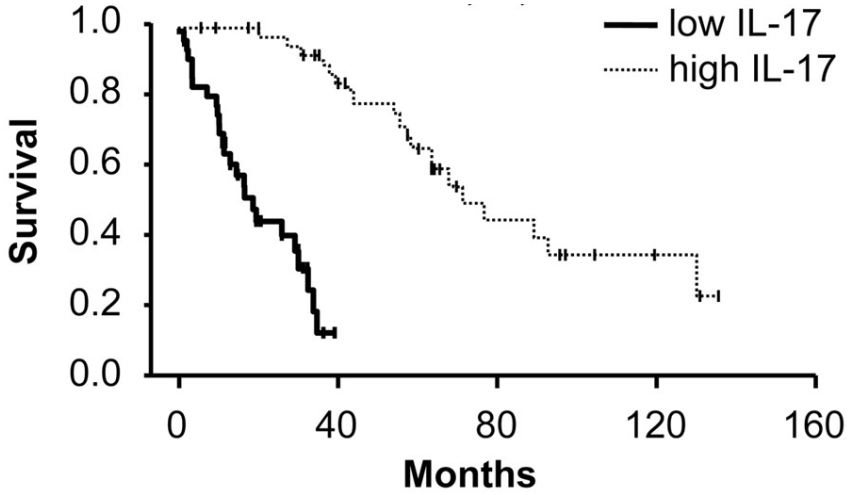
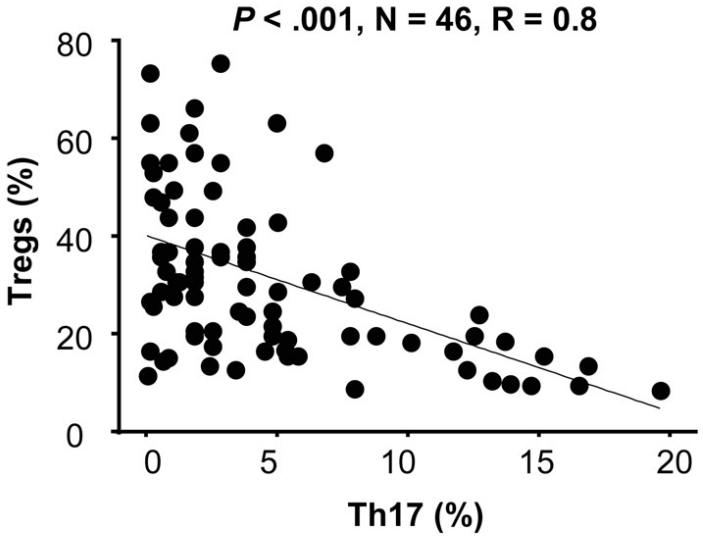
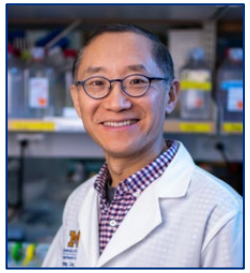
2011: IL-17 association with improved survival in ovarian cancer







Kryczek *et al.*, J Immunol 2011

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The combination of IL-15 and p38 MAPK inhibition in DCs leads enables induction of IL-17 producing T cells

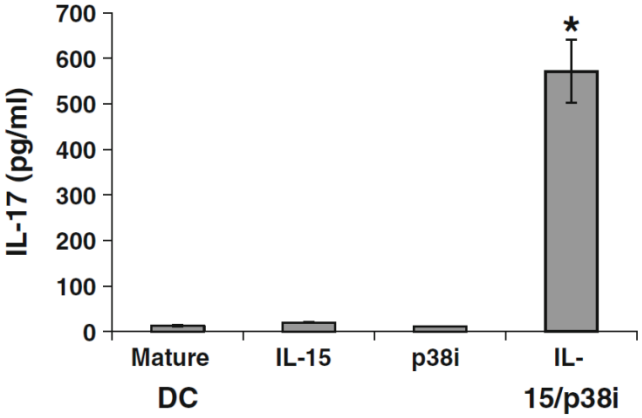
Martin Cannon, Ph.D.

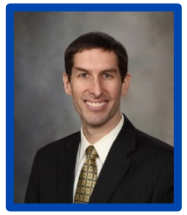
Cancer Immunol Immunother (2013) 62:839–849
DOI 10.1007/s00262-013-1391-0

ORIGINAL ARTICLE

Modulation of p38 MAPK signaling enhances dendritic cell activation of human CD4^{+} Th17 responses to ovarian tumor antigen

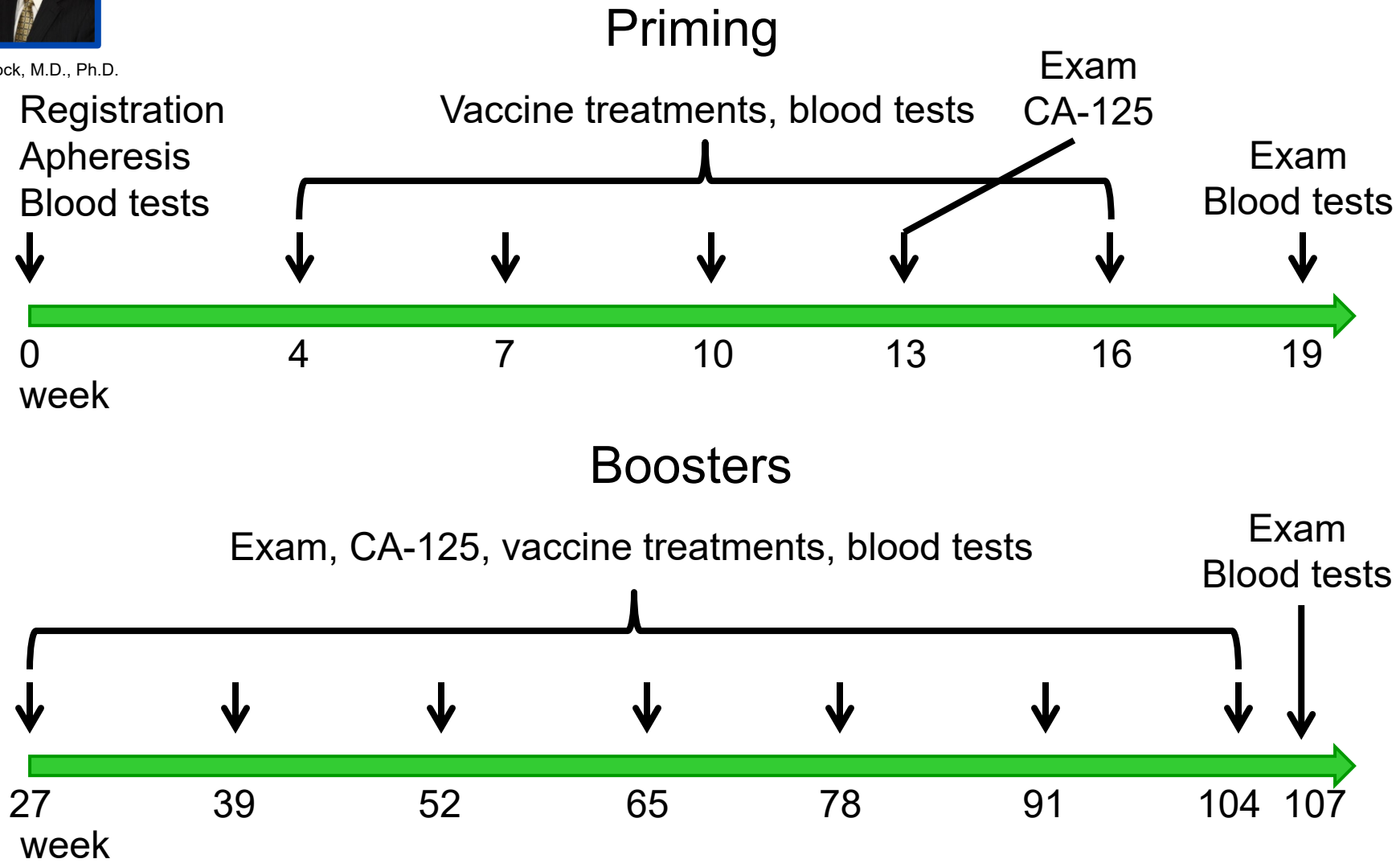
Martin J. Cannon · Hannah E. Goyne · Pamela J. B. Stone · Laura J. MacDonald · Lindsey E. James · Everardo Cobos · Maurizio Chiriva-Internati





Matt Block, M.D., Ph.D.

MC1361: Phase I Trial Schema



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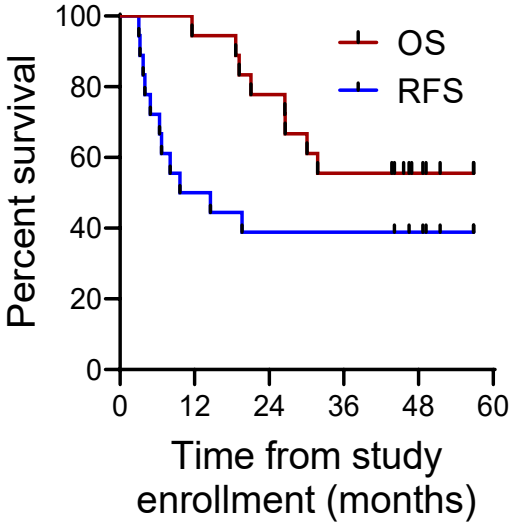
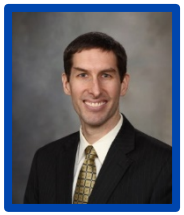
Results of phase I trial of ovarian cancer patients treated with Th17-inducing DC vaccine

Patients appear to be protected from disease progression

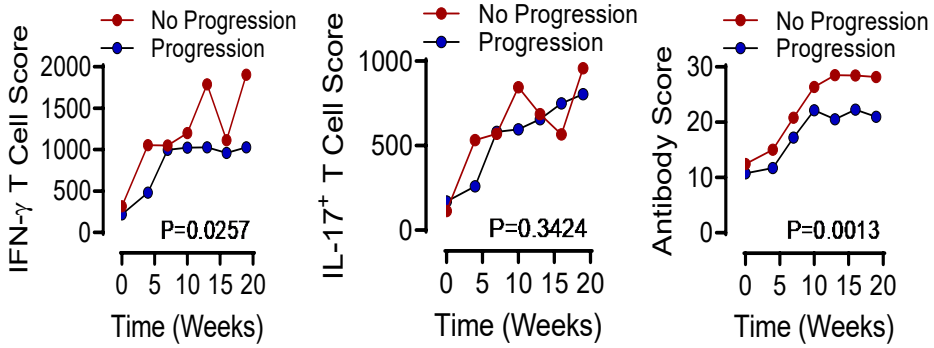




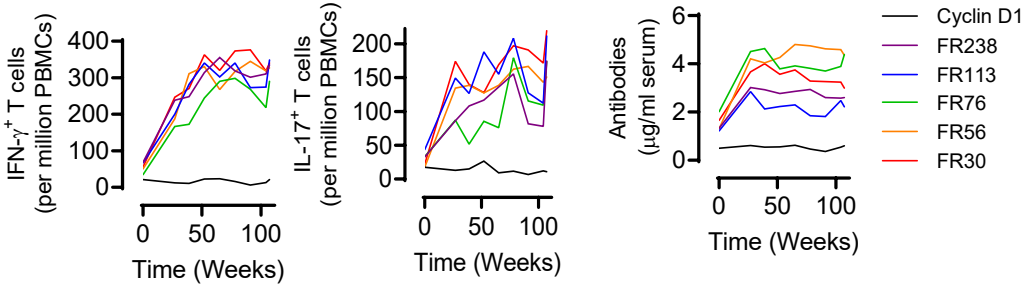
Matt Block, M.D., Ph.D.

Michael Gustafson, Ph.D.

Immunity correlates with outcome



Immunity is persistent



Block et al., 2020 Nature Comm

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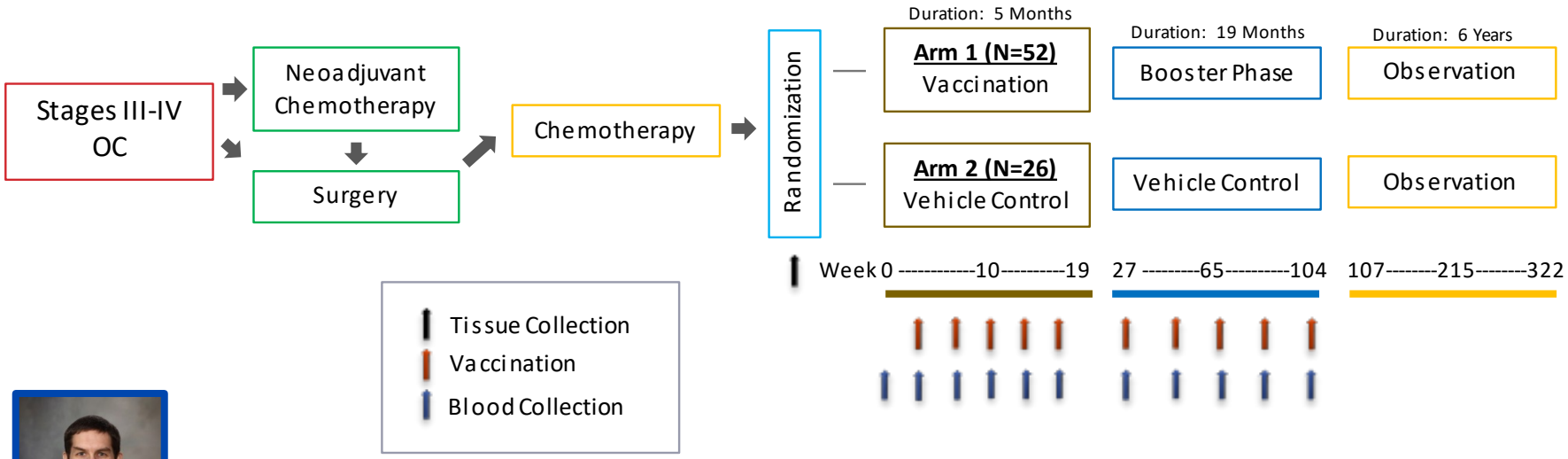
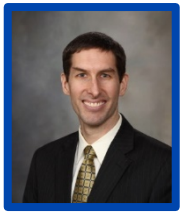
Phase II trial design MC1963





Matt Block, M.D., Ph.D.

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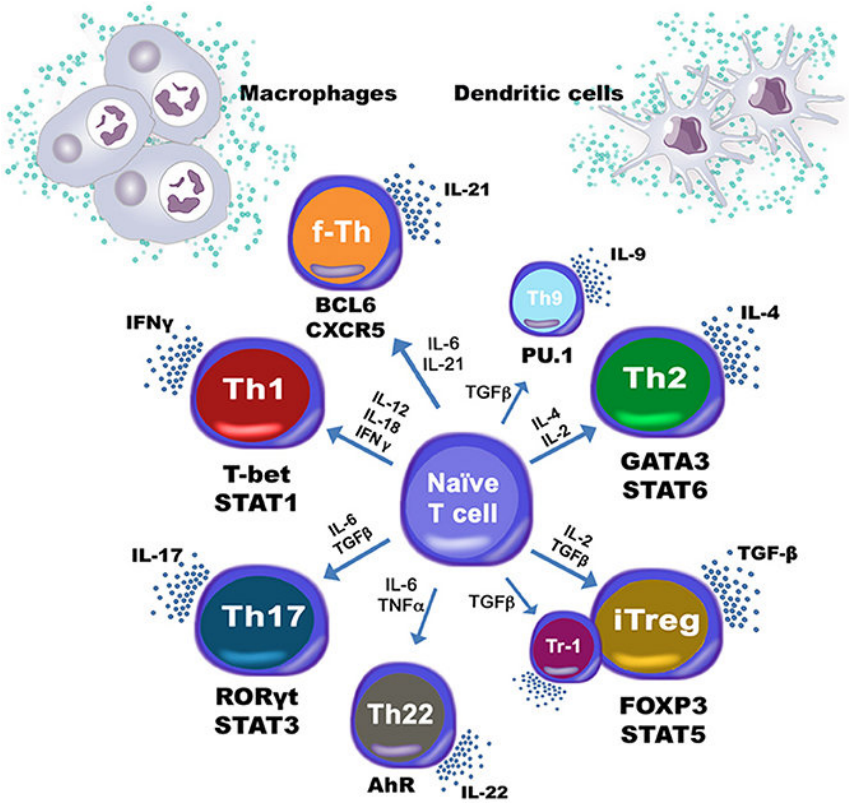
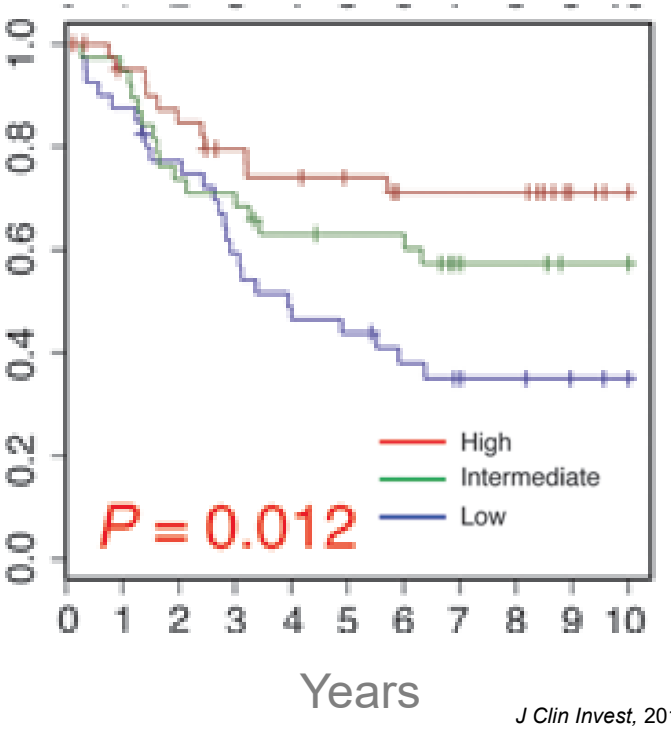
Can the natural immune response inform on the what type of vaccine



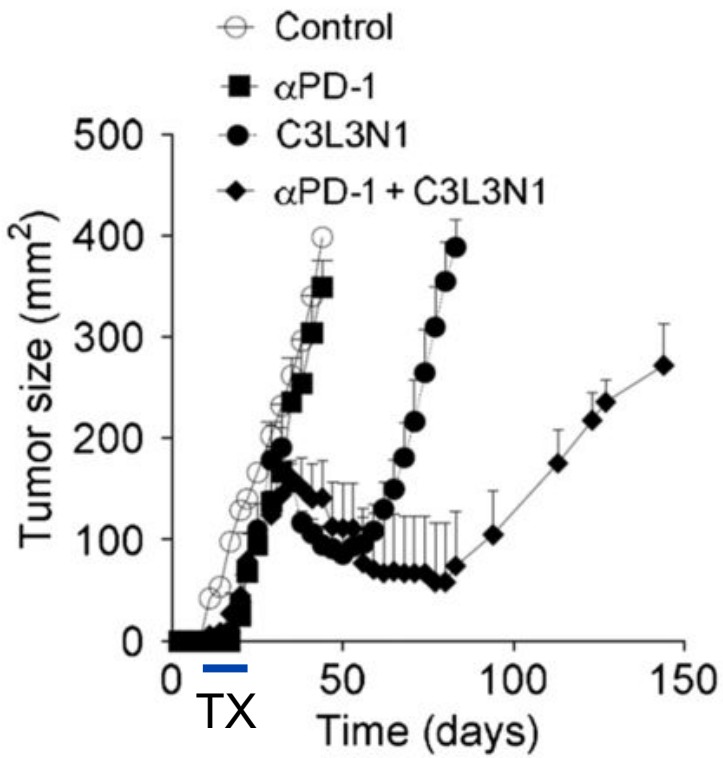
Front Cell Develop Biol, 2014

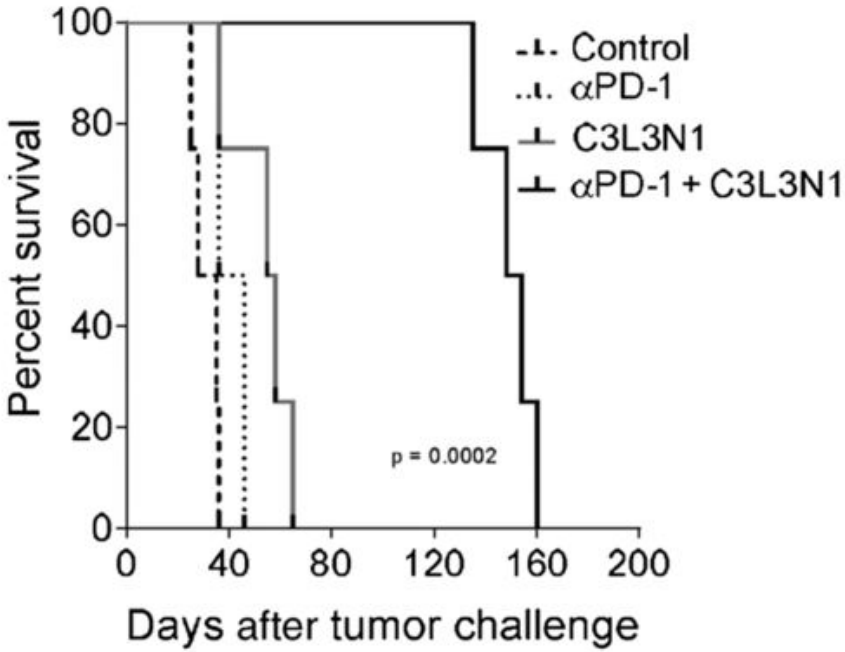
F-Th immunity improves survival in HER2



J Clin Invest, 2013

Combination vaccine and ICB therapy results in complete regression and sustained progression free survival





~75% Complete Regression Rate

Karyampudi, et al. *Cancer Res*. 2014

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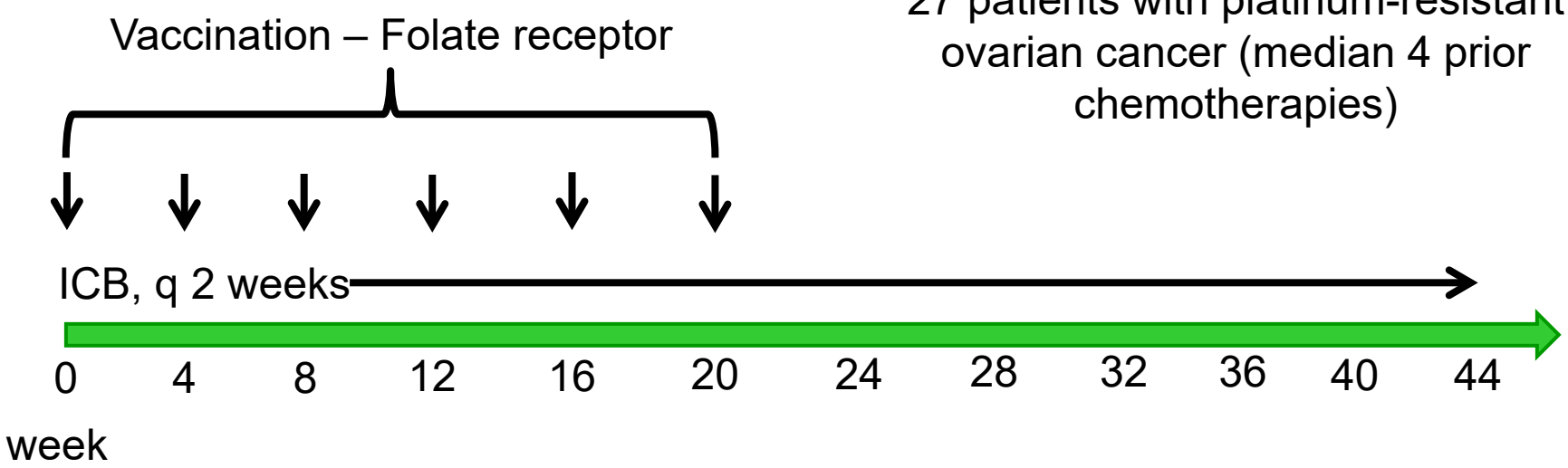
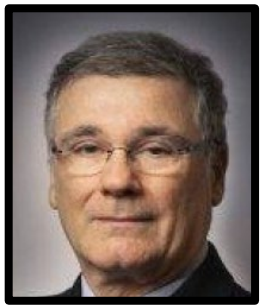
Phase I Trial Schema
Combination FRα vaccine + Durvalumab

27 patients with platinum-resistant ovarian cancer (median 4 prior chemotherapies)





Patrick Yeramian, M.D. (Amylyx)

Jason Konner, M.D. (MSKCC)

ICB = anti-PD-L1 (Durvalumab)

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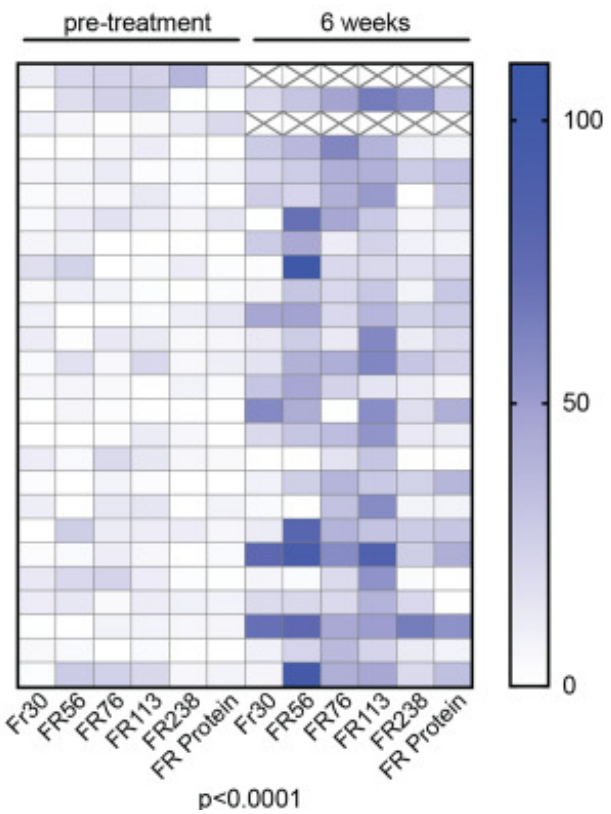
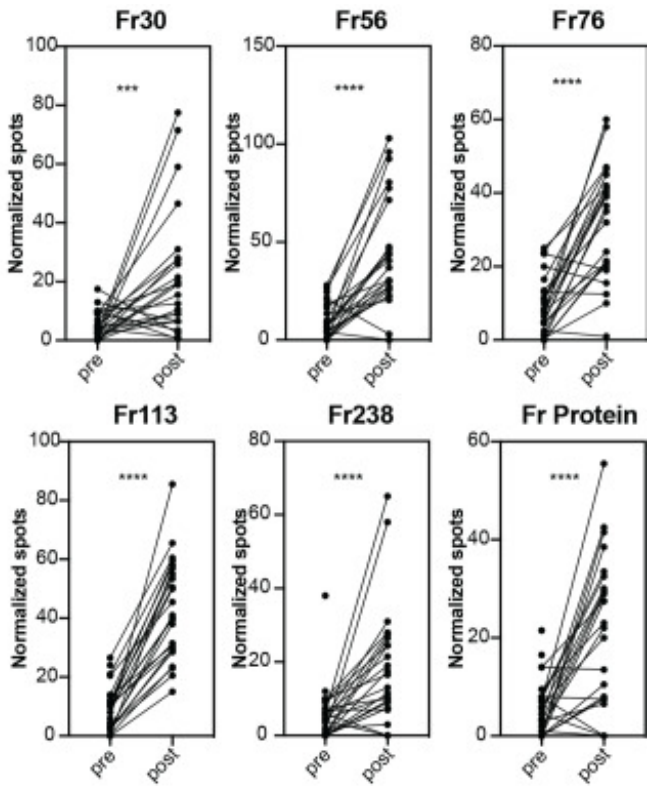
Combination FRα vaccine + Durvalumab induces immunity to FRα





Courtney Erskine

Zamarin et al., 2020, *J. Immuno Ther Cancer*

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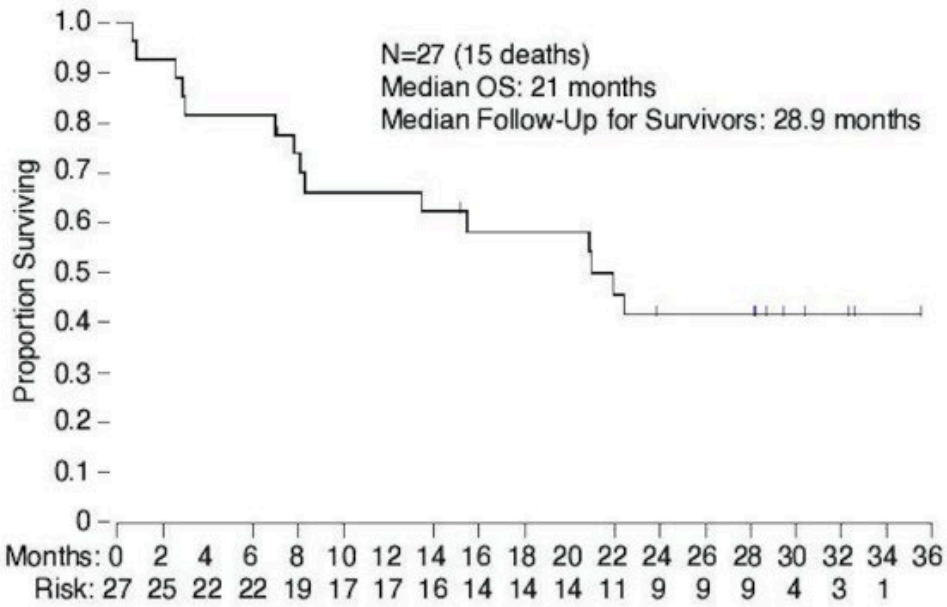
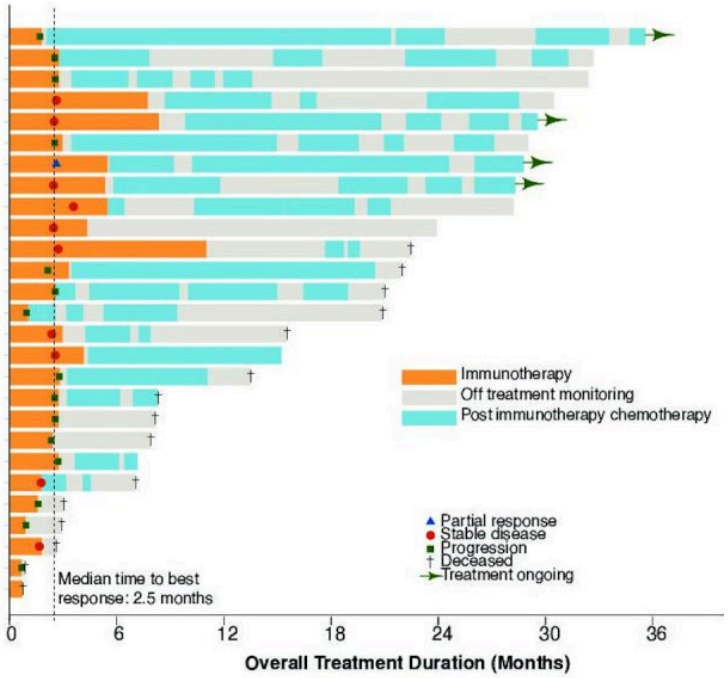
Combination FRα vaccine + Durvalumab results in improved OS?

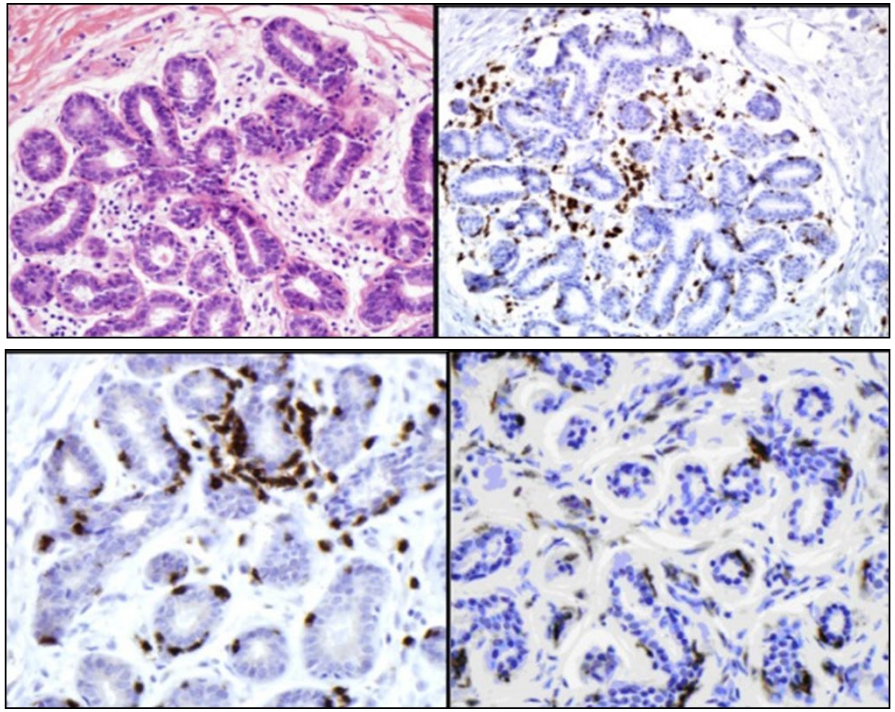




Zamarin et al., 2020, *J. Immuno Ther Cancer*

A prevention vaccine for breast cancer

- To develop a vaccine that targets all three major subsets of breast cancer
- To develop a vaccine that reduces the incidence of breast cancer
- To develop a vaccine that prevents death from breast cancer
- To develop a safe and cost-effective vaccine



NBCC
National Breast Cancer Coalition

NIH
NATIONAL CANCER INSTITUTE
Division of Cancer Prevention

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Antigen selection

- **HER2/neu**
  - Expressed in majority of breast cancers and amplified in 20%.
- **MAGE3**
  - Expressed in ~50% of breast cancers.
- **MUC1**
  - Overexpressed in 90% of breast cancer.
- **Survivin**
  - Overexpressed in more than 90% of breast cancer.
- **Mammaglobin A**
  - Expressed 10 fold-higher in 40-80% of breast cancers.
- **hTERT**
  - Overexpressed in more than 90% of breast cancer.

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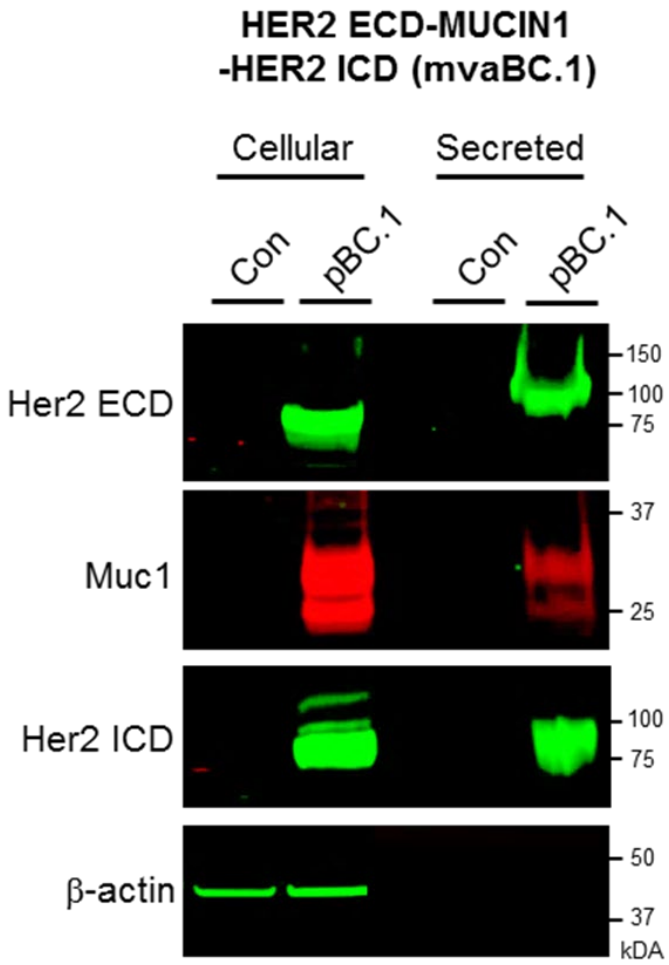
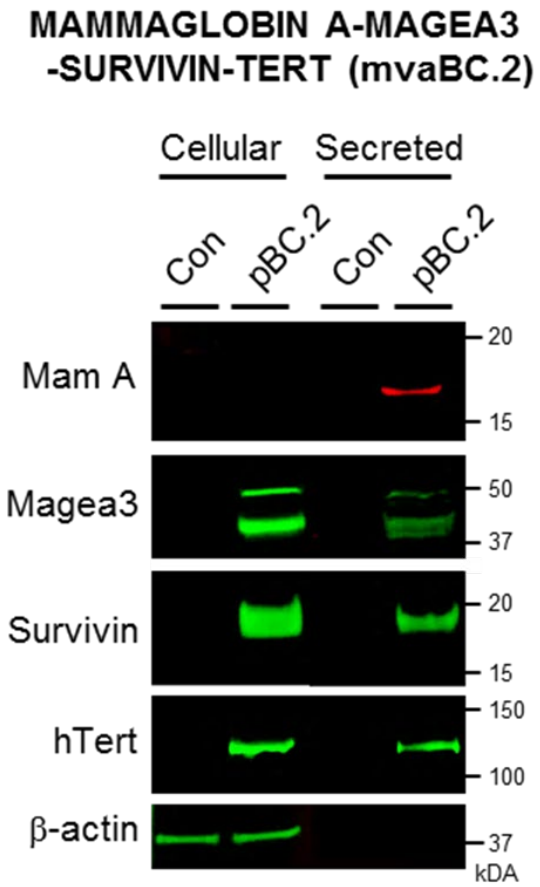
Vaccines are made and ready for production





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Phase I trial design-Prevention vaccine

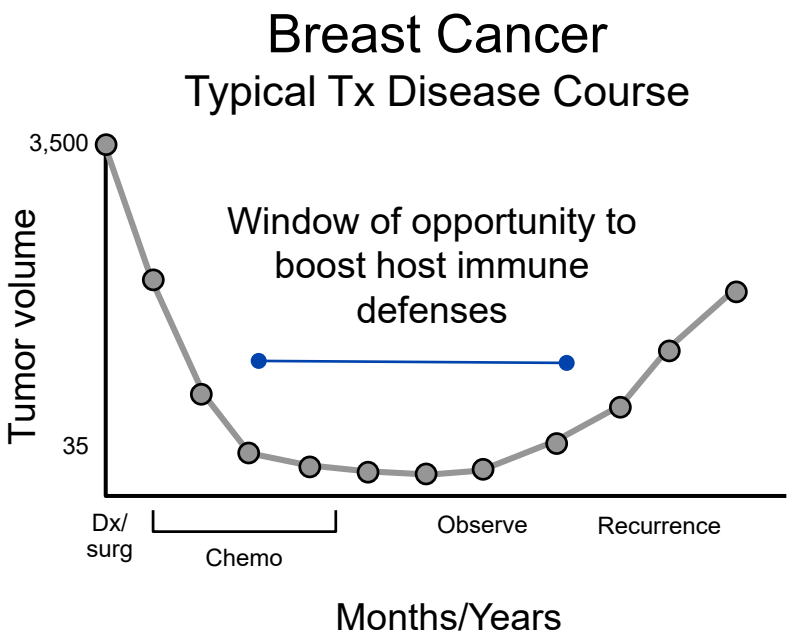
Sara Chumsri, M.D.

Metastatic Patients	Metastatic Patients	NED Stage III Patients
Registration	Registration	Registration
Mandatory biopsy – normal breast tissue	Mandatory biopsy – normal breast tissue	Mandatory biopsy – normal breast tissue
Treatment Cycle 1: Artemis 1.P1 + hGM-CSF followed by Cycle 2: Artemis 1.V1 30 ± 3 days	Treatment Cycle 1: Artemis 1.P1 + Artemis 1.P2 + hGM-CSF followed by Cycle 2: Artemis 1.V1 + Artemis 1.V2 30 ± 3 days	Treatment Cycle 1: Artemis 1.P1 + Artemis 1.P2 + hGM-CSF followed by Cycle 2: Artemis 1.V1 + Artemis 1.V2 30 ± 3 days
Clinical follow-up 30 ± 3 days after last vaccination	Clinical follow-up 30 ± 3 days after last vaccination	Clinical follow-up 30 ± 3 days after last vaccination
Mandatory biopsy – normal breast tissue	Mandatory biopsy – normal breast tissue	Mandatory biopsy – normal breast tissue
Survival follow-up monitoring	Survival follow-up monitoring	Survival follow-up monitoring

NBCC National Breast Cancer Coalition

NIH NATIONAL CANCER INSTITUTE Division of Cancer Prevention

Conclusions



- More needs to be done in the disease free period to boost host immunity against cancers at high risk for relapse
- Vaccines can be developed that target aberrantly expressed and mutated proteins. Useful for preventing disease recurrence?
- Repolarizing immune response may improve outcomes using modifying signaling pathways in autologous DCs.
- Immunizing with antigen vaccines may improve outcomes in patients treated with ICB.
- Can we prevent cancers with vaccines?

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Acknowledgements

Mayo

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Mayo Breast Cancer SPORE
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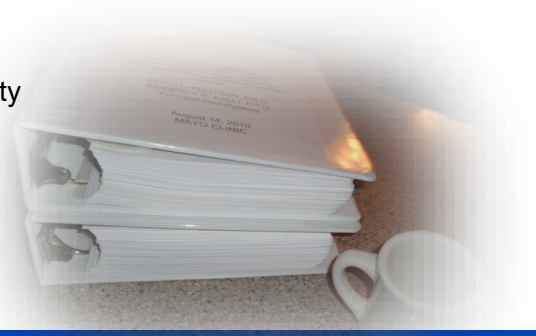
MOCA
VaxOnco
TapImmune
Andersen Foundation
Cancurables
National Breast Cancer Coalition
Department of Defense BCRP
Department of Defense OCRP



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Skill Builders

A COMMUNICATIONS COURSE FOR RESEARCH ADVOCATES

MAYO CLINIC

Communications Skill Builders

Research Advocacy Network
AdvocateInstitute

Critical Essence Model

Examine your notes so far:

- How might you categorize the information in a way that makes sense to audiences that weren't here?
  - Combining "conference" information for a cumulative report
  - Separating by session
  - Treatment, trials, experts, etc.

Look over your critical essence paragraphs:

- Do you see any themes, concepts or values that are within?

Step 1

Pick the most important audience that you want to share conference information with.

This should be a specific person, group, organization, etc.

Research Advocacy Network
AdvocateInstitute

Step 2

Identify:

1. (Hook) What do they know that they want to know about information from this conference?

2. (Lede) What additional information should they want to know from this conference?

3. (Influential Conclusion) What do they not know- that they should know?

Step 3

Choose the best communication channel to deliver the messaging to the intended audience.

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Critical Essence Model

Tonight and tomorrow:

- How might you categorize the information in your note?
  - This can inform your notetaking for tomorrow!
- Do you see any overarching themes, concepts or values that are within?
- Who is your #1 priority audience to share this information with?

Research Advocacy Network
AdvocateInstitute

Breast Cancer SPORE Advocate Workshop

Day 3 - Slides

November 12, 2020

Specialized Programs of Research Excellence (SPORE):

Translational Science, NCI Perspective

Translational Research Program (TRP)
Division of Cancer Treatment and Diagnosis (DCTD)
National Cancer Institute (NCI)
National Institutes of Health (NIH)

9609 Medical Center Drive, Room 3W110, MSC 9726
Rockville, MD 20850-9726
Tel: 240-276-5730; Fax: 240-276-7881
Website: trp.cancer.gov

JoyAnn NP Rohan, PhD

NIH NATIONAL CANCER INSTITUTE

The Birth of Specialized Programs of Research Excellence

Excerpt from 1992 Senate Report

The **Specialized Programs of Research Excellence** (**SPORE**) is a *translational research program* established in 1992 by the National Cancer Institute in response to the Congressional mandate to integrate laboratory and clinical investigations for the rapid translation of basic scientific discoveries into clinical application.

The Committee believes that breast cancer research must be a top priority for the National Cancer Institute. Of the $40,000,000 the Committee has provided for research on cancers affecting women, the Committee directs that not less than $30,000,000 be available for breast cancer research. Of this total, $20,000,000 is to be available for basic breast cancer research to understand the cause and find a cure for breast cancer, and $10,000,000 is to be used to establish up to six specialized breast cancer research centers. The Committee urges that the centers be established through the P-50 grant mechanism that links laboratory studies with clinical research to rapidly translate basic scientific discoveries in breast cancer into clinical medicine. The grantee institution receiving the award must make a major commitment to support a comprehensive, interdisciplinary approach to breast cancer research that will integrate basic research, etiology, diagnosis, prevention, clinical applications, training, and quality-of-life issues. The Committee also urges NCI to ensure that the centers encourage cutting edge and innovative research; provide training for young researchers; attract qualified scientists who previously have not been given the opportunity to concentrate their talents on breast cancer research; and expedite the translation of research advances from the laboratory to the patient.

The Committee urges the establishment of up to six specialized programs of research excellence [SPORE] through the P50 grant mechanism that links laboratory studies with clinical research to rapidly translate basic scientific discoveries in breast cancer into clinical medicine. The grantee institution receiving the award must make a major commitment to support pilot interdisciplinary collaborative projects in addition to career development.

By its participation in the trans-NIH healthy women's study, the NCI will explore whether reducing fat intake lowers the risk of developing breast, colorectal, and other cancers in addition to cardiovascular disease. The NCI will begin a 3-year feasibility study to determine effective ways to promote dietary change among minority and less educated women by restricting their fat intake. The tumor suppressive effects of tamoxifen will be assessed in a randomized clinical trial that will enroll women at high risk for devel-

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SPORE Program (1992-2011)

	1992-95	1996-98	1999	2000	2001	2002	2003-7	2008	2009	2010	2011
Breast	4	6	6	9	9	9	9	10	10	8	6
Prostate	2	3	3	4	8	11	11	7	9	9	8
Lung	2	3	3	3	6	6	7	6	7	7	7
Gastrointestinal	1	2	2	2	2	4	4	5	5	6	7
Ovary			4	4	4	4	4	4	4	4	4
Bladder					1	1	1	1	1	1	0
Skin					1	2	2	5	3	4	3
Brain						2	2	4	2	2	4
Head & Neck						3	3	5	4	5	5
Lymphoma						2	2	4	4	4	5
Endometrial							1	0	1	2	2
Cervical							1	0	1	1	1
Kidney							1	0	1	1	1
Leukemia							1	1	2	2	2
Myeloma							1	1	1	2	2
Pancreas							3	2	2	3	3
Sarcoma										1	1
Thyroid											
Neuroendocrine											
Pediatrics/RAS											
Liver											
Total Programs*	9	14	18	22	31	44	53	55	57	62	61
Annual Budget	20M	30M	39M	48M	68M	112M	126M	123M	128M	134M	122M

SPORE Program (1992-2011)

	1992-95	1996-98	1999	2000	2001	2002	2003-7	2008	2009	2010	2011
Breast	4	6	6	9	9	9	9	10	10	8	6
Prostate	2									9	8
Lung	2									7	7
Gastrointestinal	1									6	7
Ovary										4	4
Bladder										1	0
Skin										4	3
Brain										2	4
Head & Neck						3	3	5	4	5	5
Lymphoma						2	2	4	4	4	5
Endometrial							1	0	1	2	2
Cervical							1	0	1	1	1
Kidney							1	0	1	1	1
Leukemia							1	1	2	2	2
Myeloma							1	1	1	2	2
Pancreas							3	2	2	3	3
Sarcoma										1	1
Thyroid											
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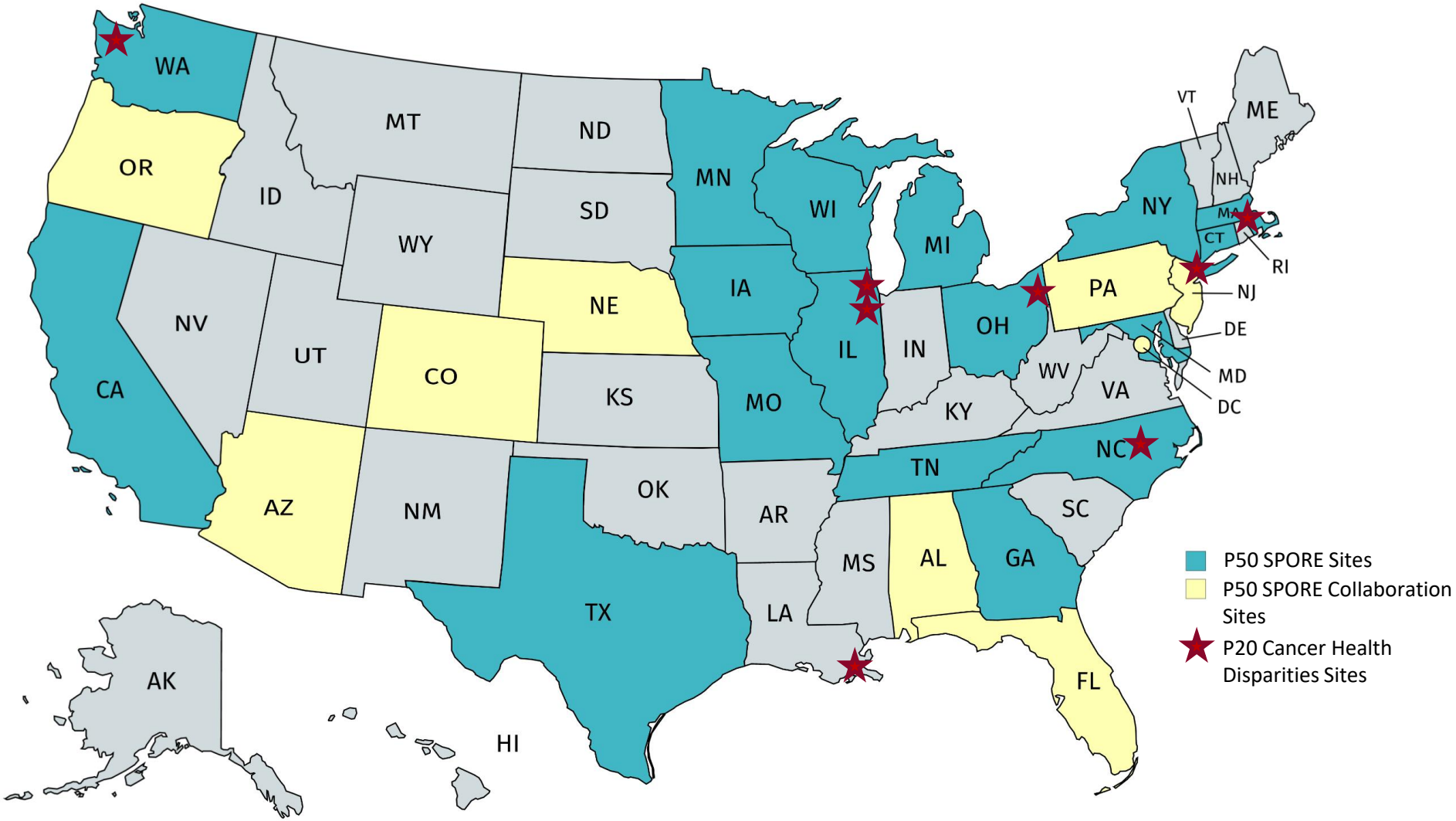
Joe W Gray – UCSF
James Inglehart – Duke
Edison Liu (Chuck Perou/Shelley Earp) – UNC
Kent Osborne (Matthew Ellis) - BCM

SPORE Program: Last 10 Years

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Breast	6	6	5	6	5	5	4	4	4	6
Prostate	8	6	7	6	7	8	9	8	7	7
Lung	7	6	3	3	4	3	3	3	4	4
Gastrointestinal	7	5	5	5	4	5	3	2	5	5
Ovarian	4	4	5	3	4	2	3	4	3	3
Bladder	0	1	1	1	1	1	0	2	1	1
Skin/Melanoma	4	4	4	5	4	4	4	2	3	3
Brain	4	4	5	5	5	4	5	4	6	6
H&N/Thyroid	5	4	4	4	4	5	4	3	2	2
Lymphoma	5	4	3	3	3	3	4	4	4	4
Endometrial	2	1	1	1	0	1	1	1	1	1
Cervical	1	1	1	1	1	1	1	1	1	1
Kidney	1	1	1	0	1	2	2	2	2	2
Leukemia	2	2	3	2	2	2	3	3	3	3
Myeloma	2	2	2	2	2	2	2	1	2	1
Pancreatic	3	3	2	3	2	3	3	3	1	1
Sarcoma	1	2	2	2	1	1	0	1	1	1
Neuroendocrine					1	1	1	1	1	1
Pediatrics/RAS					1	1	1	1	1	0
Liver								1	2	2
Total SPOREs	62	56	56	52	52	54	53	51	54	54
Annual Budget	$123.3M	$115.8M	$106.0M	$106.0M	$106.0M	$106.0M	$106.0M	$109.2M	$109.39M	$112.20M

SPORE Sites in FY 2020



Distribution of SPORE Advocates

ORGAN SITE	Number of SPORE Advocates	ORGAN SITE	Number of SPORE Advocates
bladder	1	lung	10
brain	10	lymphoma	5
breast	41	myeloma	7
cervical	2	neuroendocrine	2
endometrial	1	ovarian	6
gastrointestinal	7	pancreas	4
kidney	4	prostate	23
leukemia	8	skin	7
liver	9	**Total**	**147**

Roles of SPORE Advocates

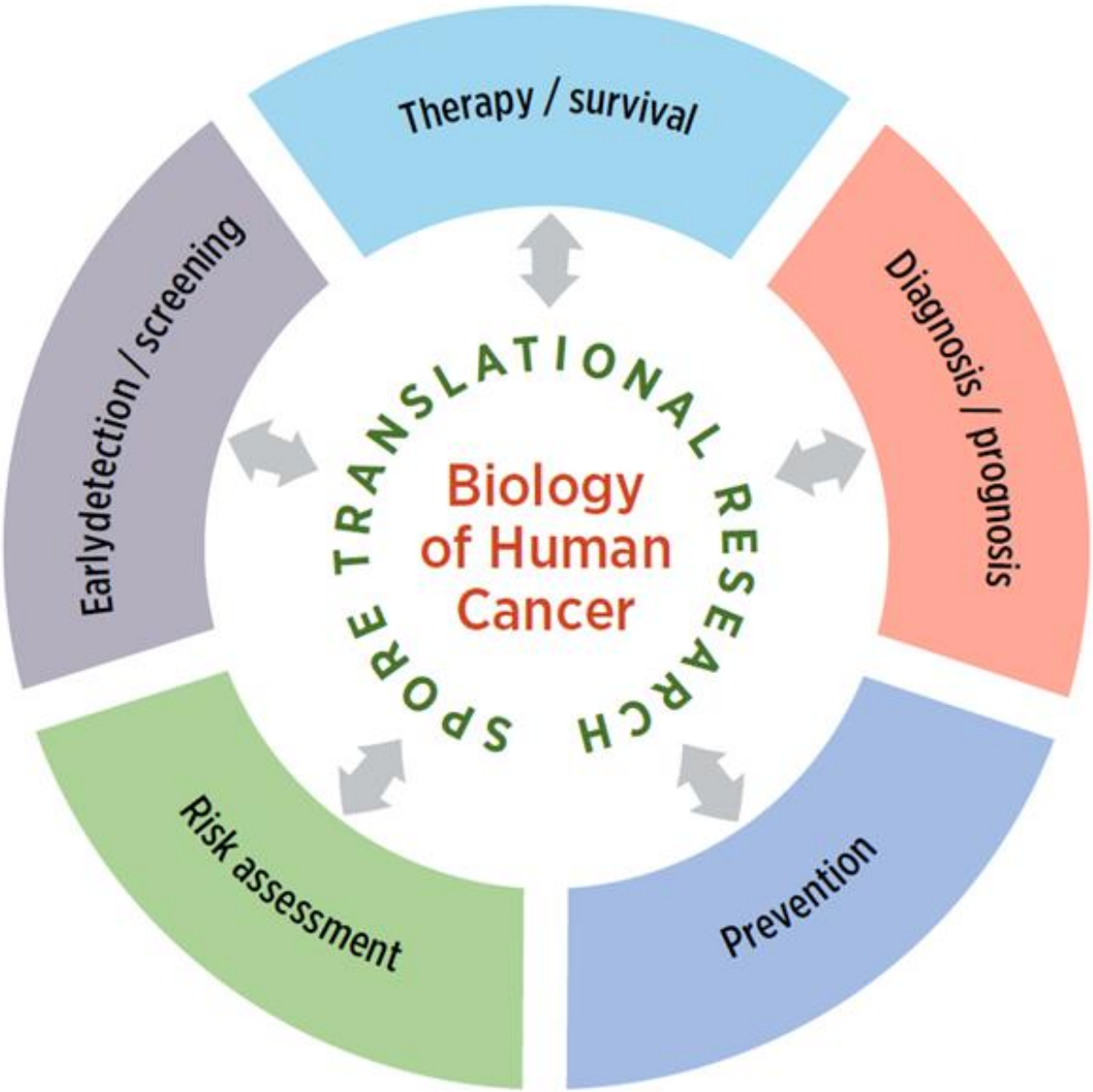
- Serve and support
  - EAB
  - Clinical trial consultants/patient navigators
  - DRP/CEP project committee members
  - Partnerships with Core and Project Leaders
  - IAB
  - Community Outreach
  - Patient Advocacy Council
  - Science Symposium
  - Attend SPORE Workshops
- Participate in SPORE Peer Review (via Special Emphasis Panel)

Translational Research in SPORE (PAR-20-305)

Translational research uses knowledge of human biology to develop and test the feasibility of cancer-relevant interventions* in humans *AND/OR* determines the biological basis for observations made in individuals with cancer or in populations at risk for cancer

** The term "interventions" is used in its broadest sense to include molecular assays, imaging techniques, drugs, biological agents, and/or other methodologies applicable to the prevention, early detection, diagnosis, prognosis, and/or treatment of cancer.*

Bi-Directional Translational Research



Individual Research Projects

- Test novel translational ideas
- Minimum of 3 projects required
  - Option: Renewal applications may propose two large-scale, multi-institutional continuation projects
- Project co-leaders:
  - Basic and clinical/applied scientists (minimum 2)
  - Each having minimum effort of 0.6 person months
- Interaction of projects with cores
- Human application/endpoint within the project period
- Clinical Trial Requirement: At least one project must conduct, as a specific aim, a SPORE investigator-initiated clinical trial as defined by the NIH. A population science study is acceptable as a clinical trial if it meets the NIH definition. The SPORE mechanism does not support large, randomized Phase II clinical trials.

Breast SPORE Projects

- Endocrine Resistance
- TNBC
- HER2+
- Metastatic Breast Cancer
- Genomic Instability
- Vaccines/Immunology
- Biomarker Development
- Early Detection, Prevention, Population Sciences (EPPS)
- 27 clinical trials related to funded SPOREs

What is a Human Endpoint?

- **At least one of the following six human endpoints should be proposed in each SPORE research project:**

  1) **Early phase clinical trials* of new investigational drugs (INDs) and biologics, experimental procedures, medical devices, or combinations thereof**

  2) **Early phase clinical trials* of new combinations or new uses of the FDA-approved agents and devices**

  3) **Discovery and development of biomarkers, only when measurements are made in human biospecimens, or directly in human subjects**

*To qualify as a human endpoint, the clinical trial protocol must be developed by the project leader/SPORE investigators.

What is a Human Endpoint?

- **SPORE human endpoints cont'd:**

  4) **Laboratory studies using clinical materials that lead to new clinical hypotheses (reverse translation)**

  5) **IND-directed toxicological studies* conducted following a pre-IND meeting† with the FDA in which the plan proposed by the investigators is acceptable to the FDA.**

  6) **Population, behavioral, or psychosocial studies, when these studies address mechanistic aspects of the biology of the disease**

- **Cell lines, organoids, xenografts, or patient-derived xenografts (PDX) using primary human tumors are not sufficient as human endpoints**

*Although IND-directed toxicology studies do not involve human beings, these studies are the last steps before clinical trials begin, and are therefore are considered programmatically appropriate as a human endpoint for SPORE translational projects.
†A pre-IND meeting is not required prior to submission, but plans for the meeting should be discussed in the application.

Biospecimen/Pathology Core

- Support scientific projects of the SPORE
  - Integration with cancer center biospecimen core and clinical laboratory
- Pathological, clinical, family history information and linkage to databases, etc.
- Pre-analytical (parameters of collection and preservation) and analytical considerations
- Priority plan to share biospecimens with others in the scientific community
- Technology development that supports research projects may be included
- Data management plan

Career Enhancement Program (CEP)

- **Encourage investigators to develop careers in translational cancer research**
  - **Not a training program: pre- or post-doctoral, pre-clinical or clinical fellows are not eligible.**
  - **Investigators with faculty appointments within one year are eligible.**
- **Provide a plan for solicitation, review, and award with special emphasis on recruitment of women, individuals from underrepresented racial and ethnic groups, as well as individuals with disabilities**
- **Provide a short description of the types of potential candidates, the names and research activities of translational science mentors/advisors, and the process for monitoring progress of the candidates**
- **Minimum of $50,000 direct cost per year from the NCI for awardees, often matched by institutions**
- **CEP Director should have minimum effort of 0.3 person months**
- **Potential for promotion to full SPORE project**

Developmental Research Program (DRP)

- Explore innovative ideas
  - Pilot projects
  - High-risk/high-payoff projects
  - Collaborations
- Human endpoint not required
- Provide a plan for solicitation, review, and award
- Minimum of $50,000 direct cost per year from the NCI for awardees, often matched by institutions
- $25K direct cost/year additional funds can be requested for a DRP Project led by an investigator from an underrepresented racial/ethnic group (per NIH Definition, Section A)
- Funds should not be used for purchase of large equipment
- DRP Director should have minimum effort of 0.3 person months
- Potential for promotion to full SPORE project

SPOREs Unique Features

Scientific focus: organ-specific; group of related cancers; cancers related by common pathway alteration or cross-cutting theme

Team approach; at least one basic and one clinical/applied science co-leader must head each project

Flexibility to terminate projects and to replace projects within funding period. This allows the PI to move rapidly to refocus research based upon new knowledge and opportunities in the field.

Career Enhancement Program: not a training program. Allows basic and clinical scientists to become involved in translational research.

Involve input from **patient advocates**

All scientific projects must be **translational** and have a **human endpoint within 5 years.**

Developmental Research Program for cutting-edge pilot studies, high risk/high payoff studies, and initiation of collaborations.

Biospecimens/Pathology CORE is required: a source of research specimen and analytic services. SPOREs must share specimens among other SPOREs and with the general scientific community, when appropriate. Many R01s depend upon biospecimen resources in SPOREs.

SPOREs must **collaborate**.

SPORE Requirement Summary

- Minimum of three translational research projects with a human endpoint
  - Renewals may submit two large-scale continuation projects
  - A clinical trial in at least one project
- May include one or more EPPS projects
- Scientific Collaboration
  - Included in Overall (Program Overview)
- Shared Resource Cores:
  - Biospecimen/pathology: required
  - Stats, clinical, animal, etc.: optional
- Administrative Core
- Developmental Research Program (DRP)
- Career Enhancement Program (CEP)
- External Advisory Board Members
- Commitment to attend and participate in SPORE-relevant meetings/workshops
- Minimum Time Commitment:
  - SPORE Director(s): ≥ 2.4 person months (or ≥ 1.8 person months for 3 or more PD/PIs)
  - Project Co-Leader(s): ≥ 0.6 person months
  - Core Director(s): ≥ 0.6 person months
  - DRP/CEP Director(s): ≥ 0.3 person months

Funding Policy

- Scientific assessment of SPORE applications is conducted through a peer-review process. A Patient Advocate serves on the peer-review panel.
- Overall Impact Score is assigned to each grant.
- All applications compete for the same pool of available funds.
- Program establishes and NCI leaders approve a funding plan for all SPOREs competing in a given fiscal year.
- Funding decisions are based on overall impact score and availability of funds. However, programmatic priorities may also play a role
  - Any Project that does not score well may be removed (including its budget)
  - Any required SPORE Component that does not score well may jeopardize funding of the entire grant
  - Scientific, budgetary, and commitment overlap are not permitted

SPORE Review

- Conducted by a Special Emphasis Panel (SEP) and not by a standing Study Section

- In the review, TRP request that reviewers
  - place emphasis on the quality of translational science,
  - determine the potential impact on patient care, which should be the primary determinants of the overall impact score, and
  - note that the overall organizational context and procedural elements of the proposal should only have a minimal influence.

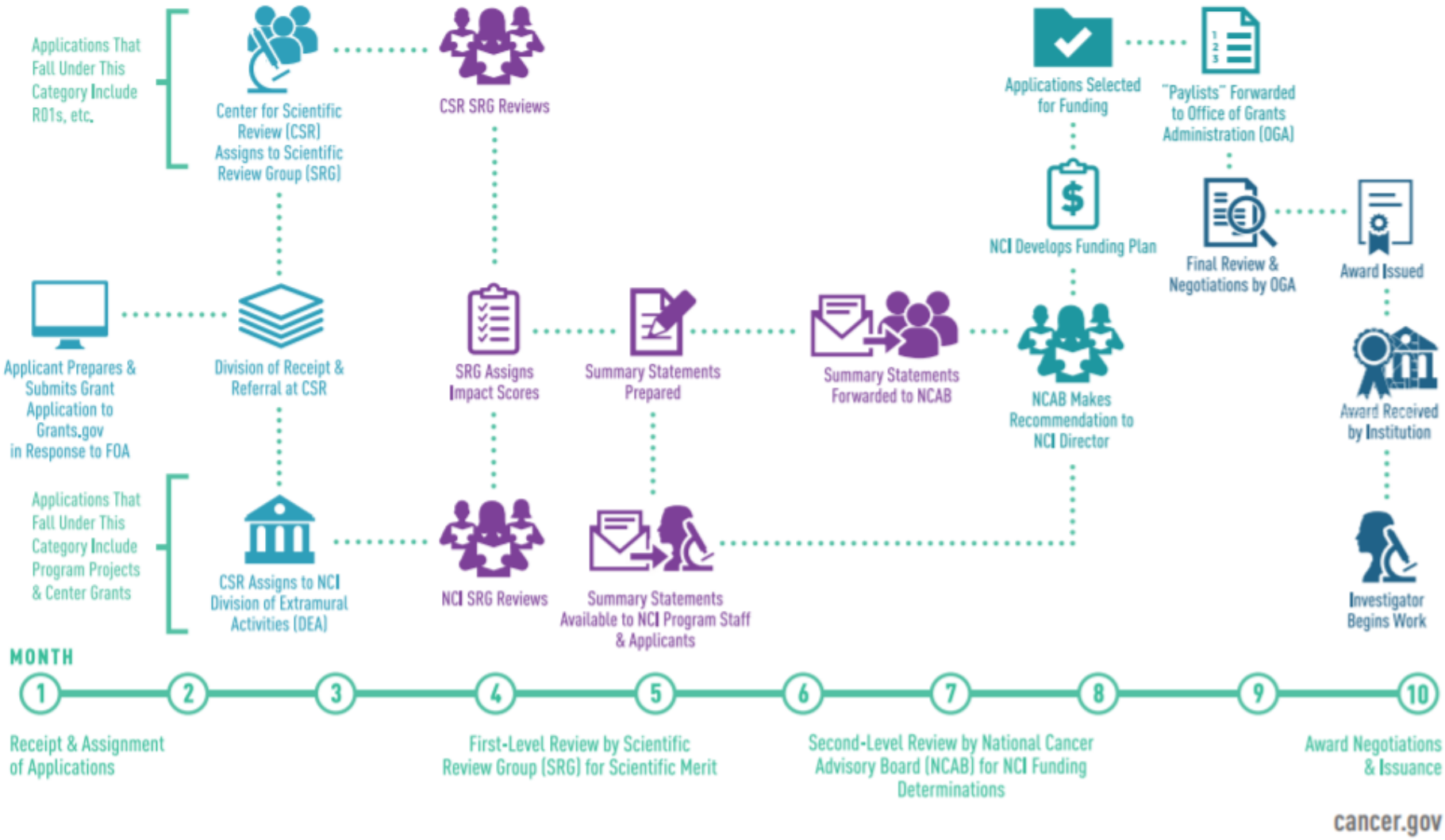
- The sum may be equal, less, or greater than its parts.

PROGRAM CHARACTERISTICS	OVERALL IMPACT	SCORE RANGE
Projects uniformly superb - essentially no weaknesses; strong supporting cores; and outstanding progress for competing renewals • Sustained powerful impact of all projects on the human disease • High likelihood that all translational research objectives will be achieved during the project period • Exceptional research approaches and high innovation • Highly qualified investigators; strong collaboration between basic and applied co-leaders **Programmatic components superb** • Uniformly strong developmental programs • Superb overall program organization and capabilities, administration, and horizontal and vertical scientific collaborations	**High**	**1**
Projects uniformly strong - only a few weaknesses; strong supporting cores; and excellent progress for competing renewals • High impact of all projects on the human disease • Likely that most translational research objectives will be achieved during the project period • Strong research approaches; innovation may vary • Highly qualified investigators; strong collaboration between basic and applied co-leaders **Programmatic components uniformly strong** • Uniformly strong developmental programs • Strong overall program organization and capabilities, administration, and horizontal and vertical scientific collaborations		**2 OR 3**
Generally strong projects - moderate to significant weaknesses, but strengths prevail; generally strong supporting cores; and good progress overall for competing renewals • High likelihood that most projects will impact the human disease • Likely that some of proposed translational research objectives will be achieved during project period • Generally well designed research approaches, but some deficiencies; innovation may vary • Highly qualified investigators; evidence of collaboration between basic and applied co-leaders **Programmatic components generally strong** • Generally strong developmental programs • Generally strong overall program organization and capabilities, administration, and horizontal and vertical scientific collaborations	**Moderate**	**4 OR 5**
Uneven quality of projects - significant weaknesses in several projects; quality of cores may be uneven; and uneven progress for competing renewals • Some projects not likely to have impact on the human disease • Unlikely to achieve some of proposed translational research objectives during the project period • Uneven quality of research approaches, but some substantial concerns; innovation may vary • Qualified investigators; evidence of collaboration between basic and applied co-leaders **Programmatic components uneven** • Quality of developmental programs may be high or low • Quality of overall program organization and capabilities, administration, and horizontal and vertical scientific collaborations uneven	**Moderate to Low**	**6 OR 7**
Serious weaknesses in most projects– weaknesses prevail; quality of cores may be uneven; and limited progress for competing renewals • Most projects unlikely to have impact on the human disease • Unlikely to achieve most of proposed translational research objectives during the project period • Many problems in research approaches, even if ideas are excellent; innovation may vary • Qualified investigators; limited evidence of collaboration between basic and applied co-leaders **Programmatic components uneven** • Quality of developmental programs may be high or low • Quality of overall program organization and capabilities and administration may be high or low, and serious weaknesses in horizontal and/or vertical scientific collaborations	**Low**	**8 OR 9**

Lifecycle of a Grant

- Funding Opportunity Announcement
- Apply
- Review
- Funding Decisions
- Award
- Post-Award
- Close-out

Lifecycle of a Grant



The Breast SPORE Community

- Bi-monthly Teleconferences
  - SPORE PIs & Team
  - P20 PIs & Team
  - Adhoc Meetings
- Breast SPORE Workshops
- TBCRC
- SABCS

Important Websites

- **TRP/SPORE Program:**
  http://trp.cancer.gov
- **SPORE Program Advances:**
  https://trp.cancer.gov/spore_advances/spore_advances.htm
- **Division of Cancer Treatment & Diagnosis (DCTD):**
  http://dctd.cancer.gov/
- **SPORE Program Announcement:**
  http://grants.nih.gov/grants/guide/pa-files/PAR-20-305.html
- **NCI's National Clinical Trials Network:**
  http://www.cancer.gov/research/areas/clinical-trials/nctn
- **NCI Disease-Specific Steering Committees:**
  http://www.cancer.gov/aboutnci/organization/ccct/steering-committees
- **Office of Extramural Research (OER):**
  http://grants.nih.gov/grants/oer.htm

TRP Contact Information

Toby T. Hecht, Ph.D.
Associate Director
Translational Research Program
toby.hecht2@nih.gov

Peter Ujhazy, M.D., Ph.D.
Deputy Associate Director, TRP
Lung, Myeloma, Sarcoma SPOREs
pu5s@nih.gov

Julia T. Arnold, Ph.D.
Program Director
Prostate, Bladder SPOREs
ja146x@nih.gov

Leah Hubbard, Ph.D.
Program Director
Head & Neck/Thyroid, Brain, Skin SPOREs
leah.hubbard@nih.gov

Igor A. Kuzmin, Ph.D.
Program Director
Leukemia, Lymphoma, Kidney SPOREs
igor.kuzmin@nih.gov

Steven F. Nothwehr, Ph.D.
Program Director
GI, Pancreatic, Neuroendocrine, RAS SPOREs
steve.nothwehr@nih.gov

JoyAnn Phillips Rohan, Ph.D.
Program Director
Breast, Ovarian, Cervical, Endometrial SPOREs
joyann.rohan@nih.gov

Terese Trent, B.A.
Program Support
Translational Research Program
tt21x@nih.gov

Tamara Walton, M.P.A., M.H.A.
Program Coordinator
Translational Research Program
tamara.walton@nih.gov

General Contact Information
Tel: 240-276-5730
Fax: 240-276-7881
trp.cancer.gov

Thank you!

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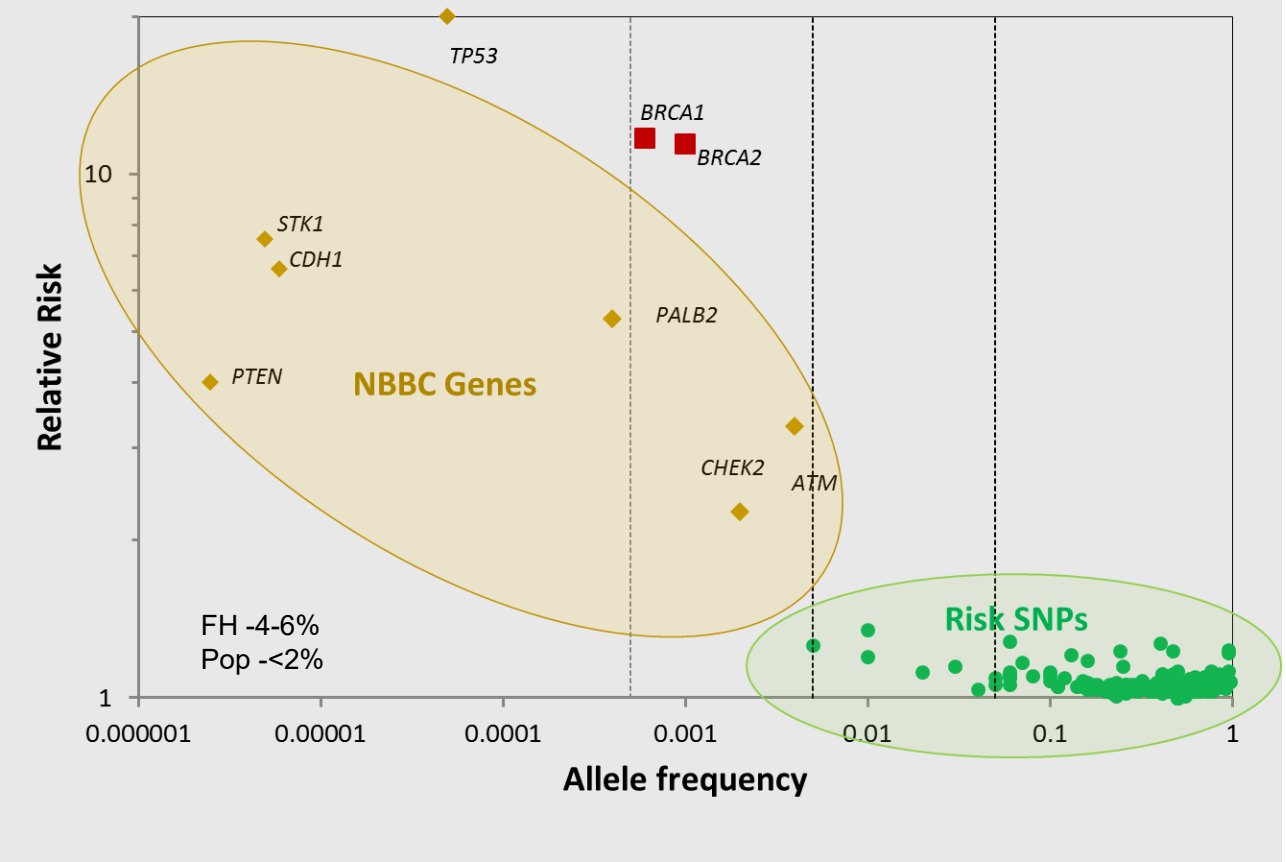
New insights into the Genetic Basis for TNBC Susceptibility

Fergus J. Couch, Ph.D.
Mayo Clinic

Disclosures/ Conflict of Interest

Research support -	GRAIL Inc. QIAgen
Consultant -	AstraZeneca

Breast cancer genetics – rare and common



Germline hereditary cancer genetic testing

- NCCN guidelines recommend clinical genetic testing for pathogenic variants (PV) among women at increased risk of carrying a PV based on:
- Family history of cancer (breast, male breast, ovarian, pancreatic, prostate)
- Young age at diagnosis
- Multiple primary cancers
- ASBrS suggests testing of all women with breast cancer
- USPSTF recommends testing of unaffected women based on risk stratifying models
- Risks of breast cancer caused by PVs in cancer predisposition genes are not fully established for high-risk or population-based women with breast cancer

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Breast cancer risk estimates by panel gene

Gene	Relative Risk	
	Literature (Easton et al 2015)	Clinical testing (Couch et al 2017)
ATM	2.8 (90% C.I. 2.2-3.7)	2.8 (95%C.I. 2.2-3.6)
BARD1	Insufficient data	2.2 (95%C.I. 1.3-3.6)
BRIP1	No evidence of association	1.6 (95%C.I. 1.1-2.4)
CHEK2 (truncating)	3.0 (90% C.I. 2.6-3.5)	2.3 (95%C.I. 1.9-2.9)
CHEK2 (missense)	1.58 (95% C.I. 1.42-1.75) for I157T	1.5 (95%C.I. 1.3-1.7)
MRE11A	Insufficient data	0.9 (95%C.I. 0.5-1.6)
NBN	2.7 (90% C.I. 1.9-3.7) for c.657del5	1.1 (95%C.I. 0.7-1.8)
PALB2	5.3 (90% C.I. 3.0-9.4)	7.5 (95%C.I. 5.1-11.2)
RAD50	Insufficient data	0.8 (95%C.I. 0.5-1.6)
RAD51C	No evidence of association	0.8 (95%C.I. 0.5-1.4)
RAD51D	No evidence of association	3.1 (95%C.I. 1.2-7.9)

Case-control association study (Ambry Genetics clinical testing cohort (n=90,000) vs. gnomAD (n=50,000))

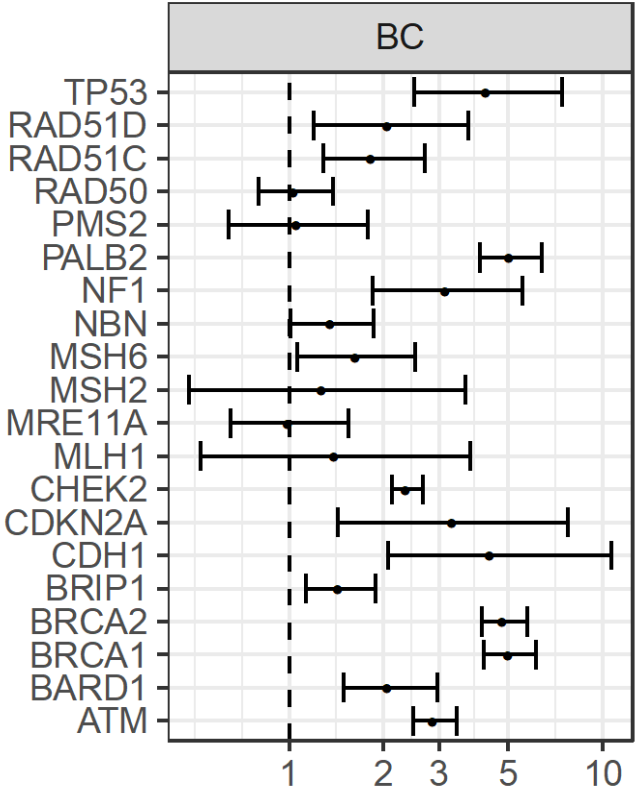
Multigene Panel Testing – High/moderate risk genes

Gene	OR	95% CI	p-value	CaseAC	CaseAN	ControlAC	ControlAN
PTEN	8.79	2.66-34.38	1.69E-05	24	100726	3	110691
PALB2	5.1	4.06-6.4	1.89E-57	367	83862	96	111508
BRCA1	5.04	4.16-6.12	2.98E-82	586	98560	132	111419
BRCA2	4.86	4.11-5.74	6.63E-100	741	98560	172	110427
CDH1	4.43	2.07-10.64	4.16E-05	32	98660	8	109193
TP53*	4.29	2.5-7.38	3.17E-09	66	100932	17	111505
NF1	3.19	1.84-5.53	1.02E-05	41	71446	20	111100
ATM	2.91	2.48-3.42	9.53E-44	481	79912	231	111313
CHEK2	2.38	2.12-2.67	3.66E-52	804	79836	467	109773
BARD1	2.09	1.49-2.96	1.78E-05	81	75818	56	109604
RAD51D	2.09	1.2-3.72	0.00765	31	71590	23	111248

Multigene Panel Testing Genes – Low risk

Gene	OR	95% CI	p-value	CaseAC	CaseAN	ControlAC	ControlAN
RAD51C	1.84	1.28-2.71	0.00117	64	75964	51	111480
MSH6	1.65	1.06-2.52	0.0202	36	42868	56	110120
BRIP1	1.45	1.13-1.88	0.00434	118	75964	119	111395
MLH1	1.41	0.52-3.78	0.588	6	42868	11	110958
NBN	1.37	1.01-1.86	0.0491	81	75818	87	111166
MSH2	1.29	0.48-3.65	0.604	6	42868	12	110699
PMS2	1.07	0.64-1.78	0.796	22	42868	52	108839
MRE11A	1	0.65-1.54	1	36	75818	53	111326

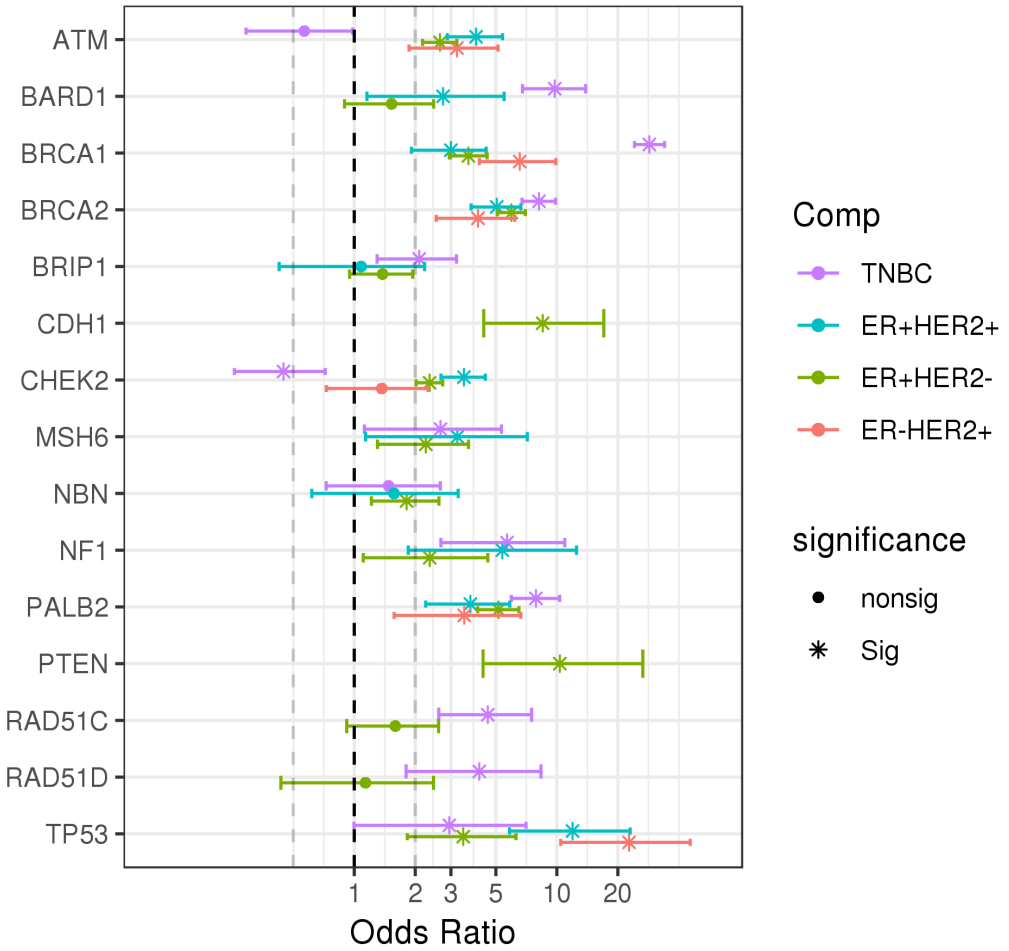
Risks for Breast Cancer



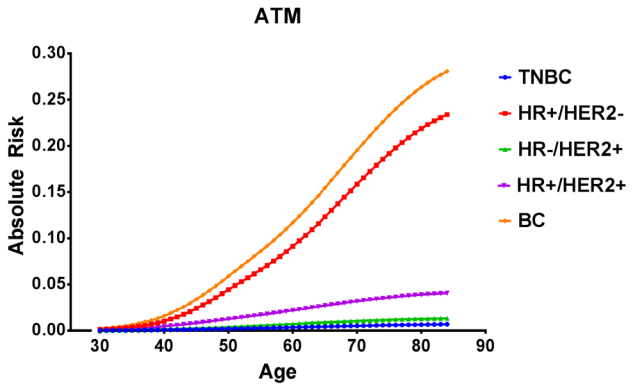
Characteristics of 55,000 breast cancer patients by tumor subtype

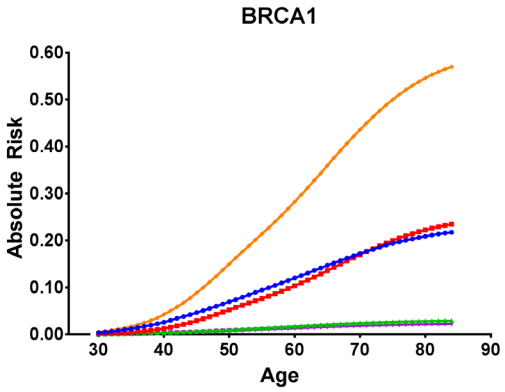
Patient Characteristic	ER+/HER2- (N=26620)	ER+/HER2+ (N=5979)	ER-/HER2+ (N=2701)	TNBC (N=10292)	p value
mean age (SD)	51.0 (11.5)	46.8 (11.2)	47.1 (11.2)	49.8 (11.2)	< 0.001
personal Ca Hx					
BC (age at diagnosis)					< 0.001
18-36	2307 (8.7%)	1125 (18.9%)	496 (18.4%)	1353 (13.2%)	
37-45	6828 (25.7%)	1816 (30.5%)	788 (29.3%)	2286 (22.3%)	
46-50	5345 (20.1%)	1018 (17.1%)	473 (17.6%)	1700 (16.6%)	
51-60	6322 (23.8%)	1256 (21.1%)	603 (22.4%)	3291 (32.1%)	
>60	5727 (21.6%)	747 (12.5%)	334 (12.4%)	1629 (15.9%)	
Multiple BC	3758 (14.1%)	649 (10.9%)	287 (10.6%)	1161 (11.3%)	< 0.001
Ovarian Ca	321 (1.2%)	54 (0.9%)	21 (0.8%)	118 (1.1%)	0.067
family Hx (1st & 2nd degree)					
Breast cancer	16658 (65.2%)	3374 (59.6%)	1481 (57.4%)	5234 (54.2%)	< 0.001
Ovarian cancer	3331 (13.0%)	664 (11.7%)	322 (12.5%)	1059 (11.0%)	< 0.001

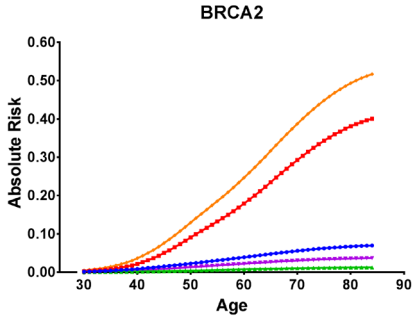
Genes associated with breast cancer subtypes (n=55,000)

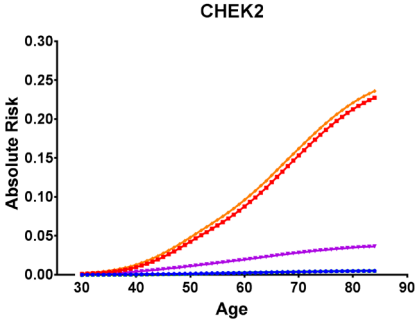


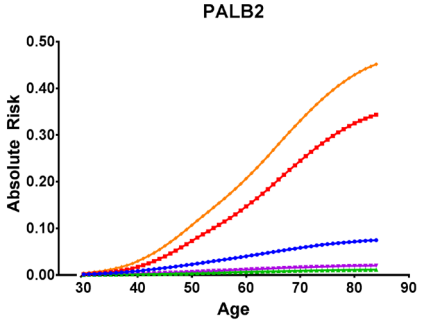
Lifetime risks for breast cancer by subtype











Germline hereditary cancer genetic testing

- Breast cancer risk estimates for predisposition gene mutations apply to high-risk patients qualifying for clinical genetic testing

- Risk estimates for women in the general population remain to be defined

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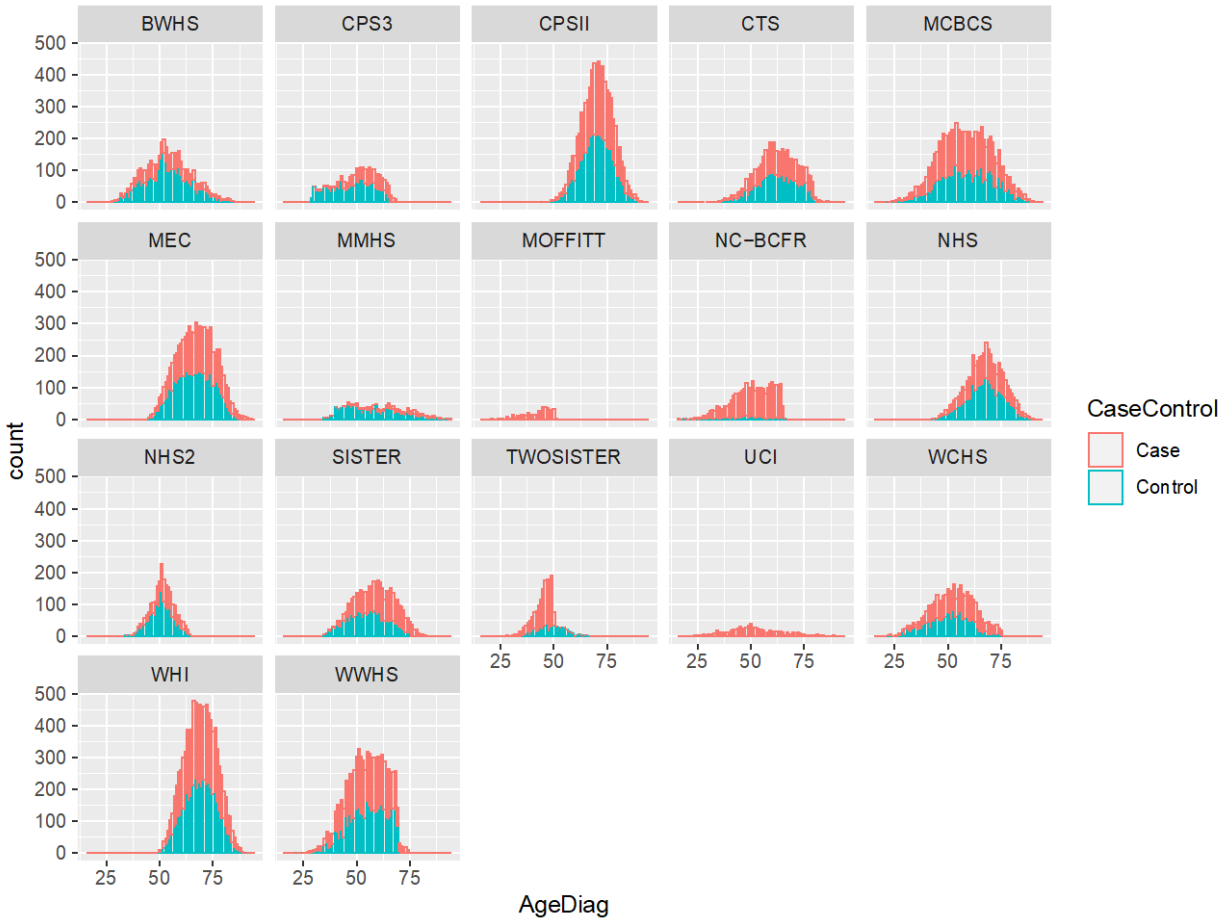
Population-based studies

- Developed the CAnceR RIsk Estimates Related to Susceptibility “CARRIERS” study
- Define the population-based frequencies of pathogenic mutations in cancer predisposition genes
- Estimate age-related and lifetime risks of breast cancer in the general population

Phenotypic characteristics of CARRIERS participants

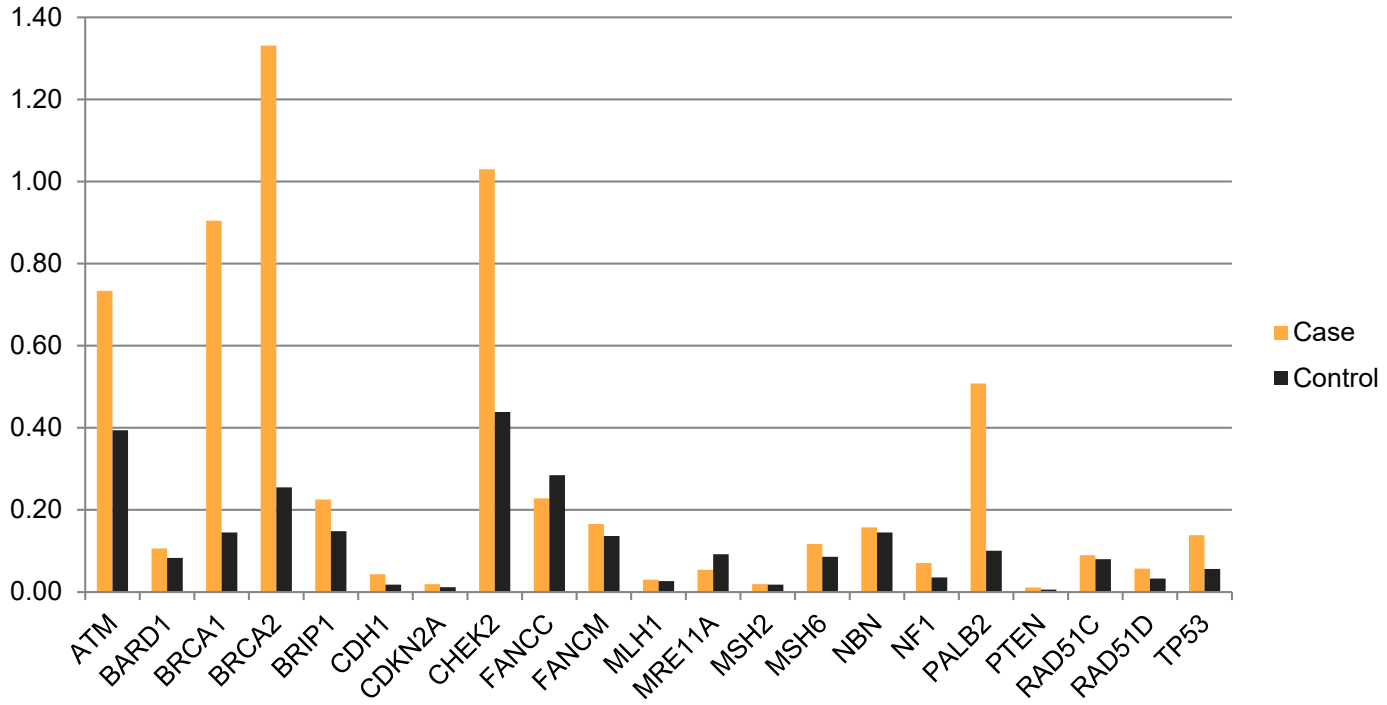
Phenotype	Control (35867)	Case (n=39553)
AgeDiag		
Mean (SD)	60.7 (11.8)	60.3 (11.9)
Range	19.0 - 94.0	16.0 - 94.0
Case Status		
invasive	0 (0.0%)	33039 (85.8%)
in situ	0 (0.0%)	5467 (14.2%)
Race/ethnicity		
African American	5222 (14.6%)	5287 (13.4%)
Asian	1387 (3.9%)	1936 (4.9%)
White	27151 (75.8%)	29250 (74.3%)
Hispanic	1450 (4.1%)	2208 (5.6%)
Unknown	66 (0.2%)	190 (0.5%)

Age distribution by study



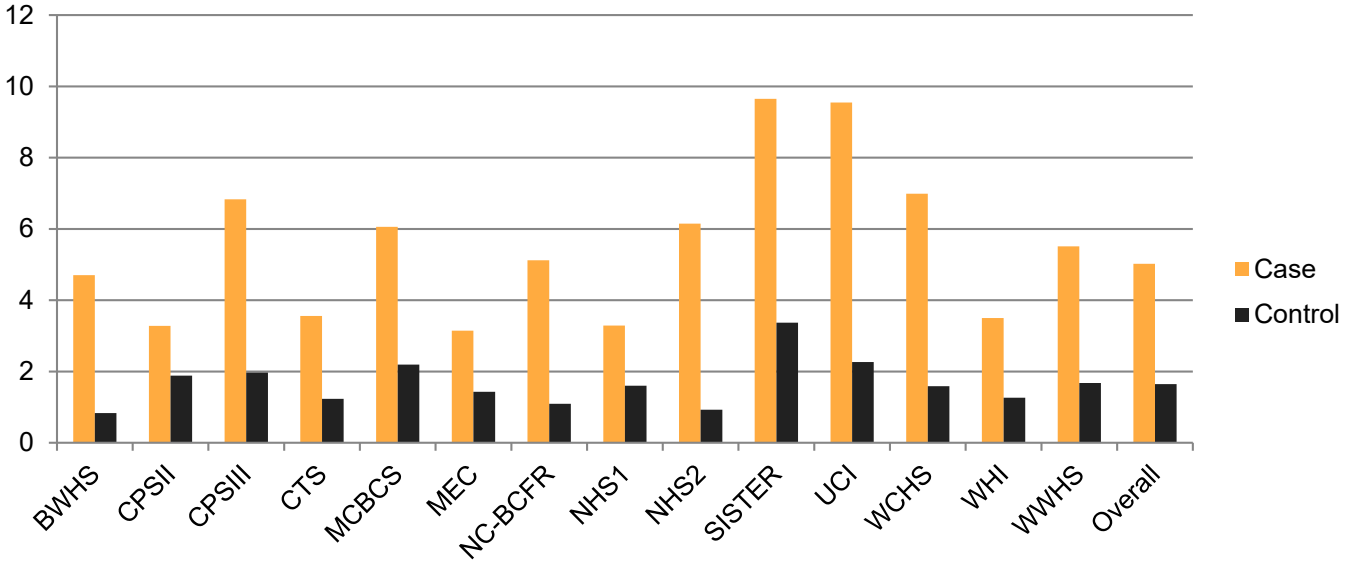
Family history by study

Mutation frequency (%) in 21 cancer predisposition genes in overall CARRIERS study



Total mutation frequency (%) in 12 known breast cancer risk genes by study

(*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, TP53*)



Associations between gene mutations and breast cancer

	OR	95% CI	95% CI	p.value
ATM	1.815	1.461	2.266	< 0.001
BARD1	1.366	0.872	2.160	0.177
BRCA1	7.349	5.165	10.811	< 0.001
BRCA2	5.280	4.129	6.845	< 0.001
BRIP1	1.349	0.927	1.976	0.120
CDH1	2.500	1.009	7.073	0.060
CHEK2	2.364	1.940	2.894	< 0.001
MSH6	1.126	0.697	1.834	0.628
NBN	1.049	0.705	1.564	0.812
NF1	1.125	0.651	1.954	0.672
PALB2	3.660	2.575	5.331	< 0.001
RAD51C	1.203	0.754	1.930	0.438
RAD51D	1.723	0.884	3.512	0.119

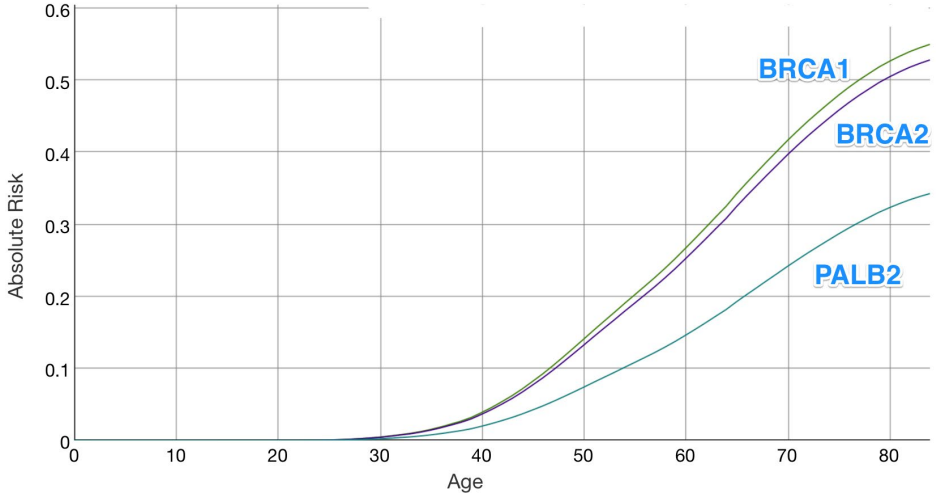
Associations between genes and ER negative breast cancer

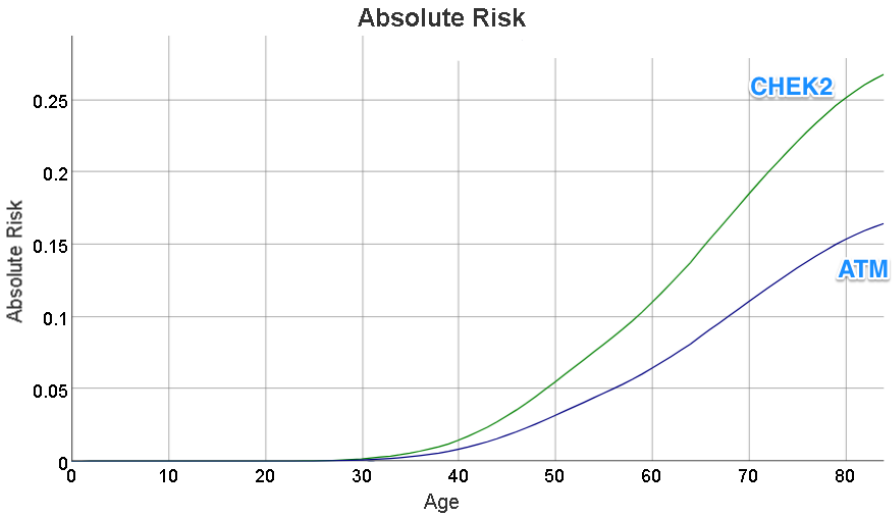
	OR	95%CI	95%CI	p.value
ATM	1.038	0.585	1.722	0.892
BARD1	2.517	1.180	5.002	0.011
BRCA1	25.404	16.762	39.780	< 0.001
BRCA2	8.964	6.394	12.604	< 0.001
CDH1	3.298	0.646	14.218	0.116
CHEK2	1.320	0.783	2.108	0.269
MSH6	1.842	0.729	4.072	0.157
PALB2	8.903	5.461	14.646	< 0.001
RAD51C	2.189	0.967	4.494	0.043
RAD51D	3.933	1.400	10.289	0.006

Associations between genes and ER positive breast cancer

	OR	95%CI	95%CI	p.value
ATM	1.955	1.515	2.530	< 0.001
BRCA1	3.278	2.107	5.234	< 0.001
BRCA2	4.744	3.583	6.354	< 0.001
CDH1	3.368	1.238	10.718	0.024
CHEK2	2.556	2.028	3.233	< 0.001
NF1	0.985	0.495	1.900	0.964
PALB2	3.036	1.966	4.789	< 0.001
RAD51C	0.834	0.437	1.536	0.568
RAD51D	1.611	0.709	3.700	0.253

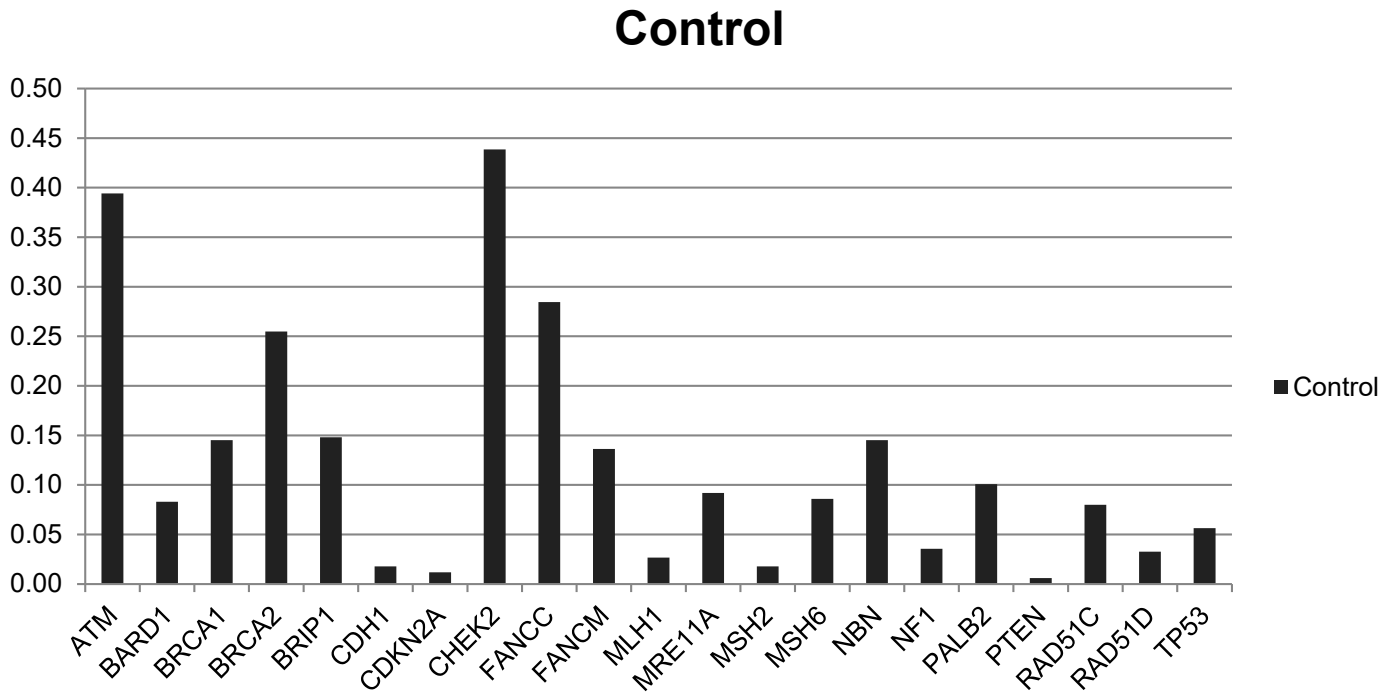
Lifetime risk estimates for overall breast cancer





Overall CARRIERS odds ratio extrapolated to SEER incidence rates

PV frequency (1.8%) in 21 cancer predisposition genes in unaffected population-based women in CARRIERS



African-American population-based breast cancer risks

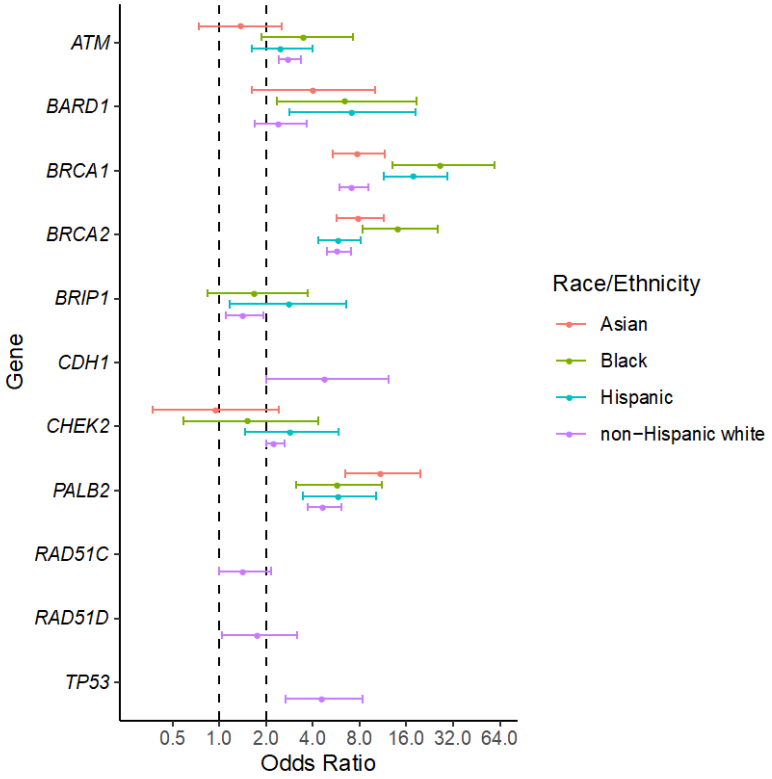
	Affected (N=3916)		Unaffected (N=4925)			
Gene	Mutated alleles No.	Mutation frequency %	Mutated alleles No.	Mutation frequency %	Odds ratio† (95% CI)	p value
ATM	28	0.72	16	0.33	1.81 (0.97-3.48)	0.067
BARD1	7	0.18	8	0.16	0.98 (0.34-2.80)	0.97
BRCA1	41	1.05	1	0.02	42.79 (9.24- >100)	< 0.001
BRCA2	72	1.84	12	0.24	7.31 (4.08-14.29)	< 0.001
BRIP1	6	0.15	6	0.12	1.14 (0.34-3.79)	0.83
CHEK2	15	0.38	6	0.12	3.17 (1.26-9.06)	0.020
NBN	4	0.10	9	0.18	0.51 (0.13-1.63)	0.28
PALB2	39	1.00	5	0.10	8.37 (3.56-24.57)	< 0.001
RAD51C	7	0.18	3	0.06	2.95 (0.80-13.72)	0.12
RAD51D	6	0.15	2	0.04	3.06 (0.67-21.50)	0.18
Total	277	7.07	103	2.09		

Palmer et al., JNCI 2020

Pathogenic mutations and triple negative breast cancer risk in the African American population

	Women with triple negative breast cancer (N=654)			
Gene	Mutated alleles	Mutation frequency %	OR (95% CI)	p value
ATM	0	-	-	-
BARD1	3	0.45		
BRCA1	43	6.57	180 (37.9 - 3238)	<0.001
BRCA2	13	1.99	6.23 (2.65-14.84)	<0.001
BRIP1	2	0.22		
CDH1	1	0.15		
CHEK2	1	0.15		
PALB2	14	2.14	23.5 (8.35-76.71)	<0.001

Cancer risk for each gene in common racial/ethnic populations tested by Ambry Genetics



ER negative breast cancer risk for pathogenic variants in cancer predisposition genes by race and ethnicity

Gene	Non-Hispanic White		Black		Hispanic		Asian	
	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
ATM	1.25	0.84-1.84	ND	ND	ND	ND	ND	ND
BARD1	6.55	3.99-10.51	12.97	4.26-39.33	16.12	5.22-49.15	18.61	6.56-52.84
BRCA1	21.82	17.39-27.42	56.68	26.3-124.11	43.56	26.15-74.91	20.64	12.48-34.02
BRCA2	6.53	5.16-8.23	13.94	7.54-26.44	5.33	3.05-8.97	7.28	3.6-14.48
CHEK2*	0.87	0.6-1.27	ND	ND	ND	ND	ND	ND
NBN	1.78	0.94-3.34	ND	ND	ND	ND	ND	ND
PALB2	6.73	4.85-9.32	4.76	2.04-10.95	9.74	4.38-20.31	17.13	7.04-40.29
RAD51C	1.95	0.98-3.75	ND	ND	ND	ND	ND	ND
RAD51D	4.11	1.84-8.65	ND	ND	ND	ND	ND	ND
TP53	5	2.26-10.82	22.17	3.1-516.63	ND	ND	ND	ND

Implications for Medical Management

Consider/Recommend Breast MRI	Discuss Option of RRM/ Consider based on family history	Unknown or insufficient evidence for BC risk	No increased BC Risk
ATM	*ATM*	*MLH1*	*BRIP1*
BRCA1	*BRCA1*	*MSH2*	
BRCA2	*BRCA2*	*MRE11A*	
CDH1	*CDH1*	*MSH6*	
CHEK2	*CHEK2*	*PMS2*	
NBN	*NBN*	*RAD50*	
NF1	*NF1*	*RAD51C*	
PALB2	*PALB2*	*RAD51D*	
PTEN	*PTEN*		
STK11	*TP53*		
TP53			
BARD1			

Summary for Panel Testing

- Testing of BRCA1, BRCA2, and moderate risk predisposition genes can result in improved clinical management of patients
- Accurate population-based and family history-based risk estimates for each gene are now available for non-Hispanic whites
    - still needed for other races and ethnicities
- Age-related risks must be defined for improved medical management of women with PVs
- Should testing be restricted to women qualifying for testing based on risk stratifying criteria or should all breast cancers be tested?
- Should testing be adopted based on specific criteria (e.g. Ashkenazi Jewish ancestry) in the general population ??

Research Advocacy Network

Skill Builders

A COMMUNICATIONS COURSE FOR RESEARCH ADVOCATES

MAYO CLINIC

Communications Skill Builders

Research Advocacy Network
AdvocateInstitute

Critical Essence Model

Examine your notes so far:

- How might you categorize the information in a way that makes sense to audiences that weren't here?
  - Combining "conference" information for a cumulative report
  - Separating by session
  - Treatment, trials, experts, etc.

Look over your critical essence paragraphs:

- Do you see any themes, concepts or values that are within?

Step 1

Pick the most important audience that you want to share conference information with.

This should be a specific person, group, organization, etc.

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Step 1

Visualize your specific audience…

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Step 2

Identify:

1. (Hook) What do they know that they want to know about information from this conference?

What will get their attention?

Step 2

Identify

2. (Lede) What additional information should they want to know from this conference?

Summarize the most important aspect.

Step 2

Identify

3. (Influential Conclusion) What do they not know- that they should know?

What will pique their interest enough to keep them coming back/paying attention to you as an influencer?

Step 2

Discussion:

- Who is your audience
- Hook
- Lede
- Conclusion

Step 3

GOAL:
Choose the best communication channel to deliver the messaging to the intended audience.

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Step 3

Let's list as many types of communication mediums as we can…

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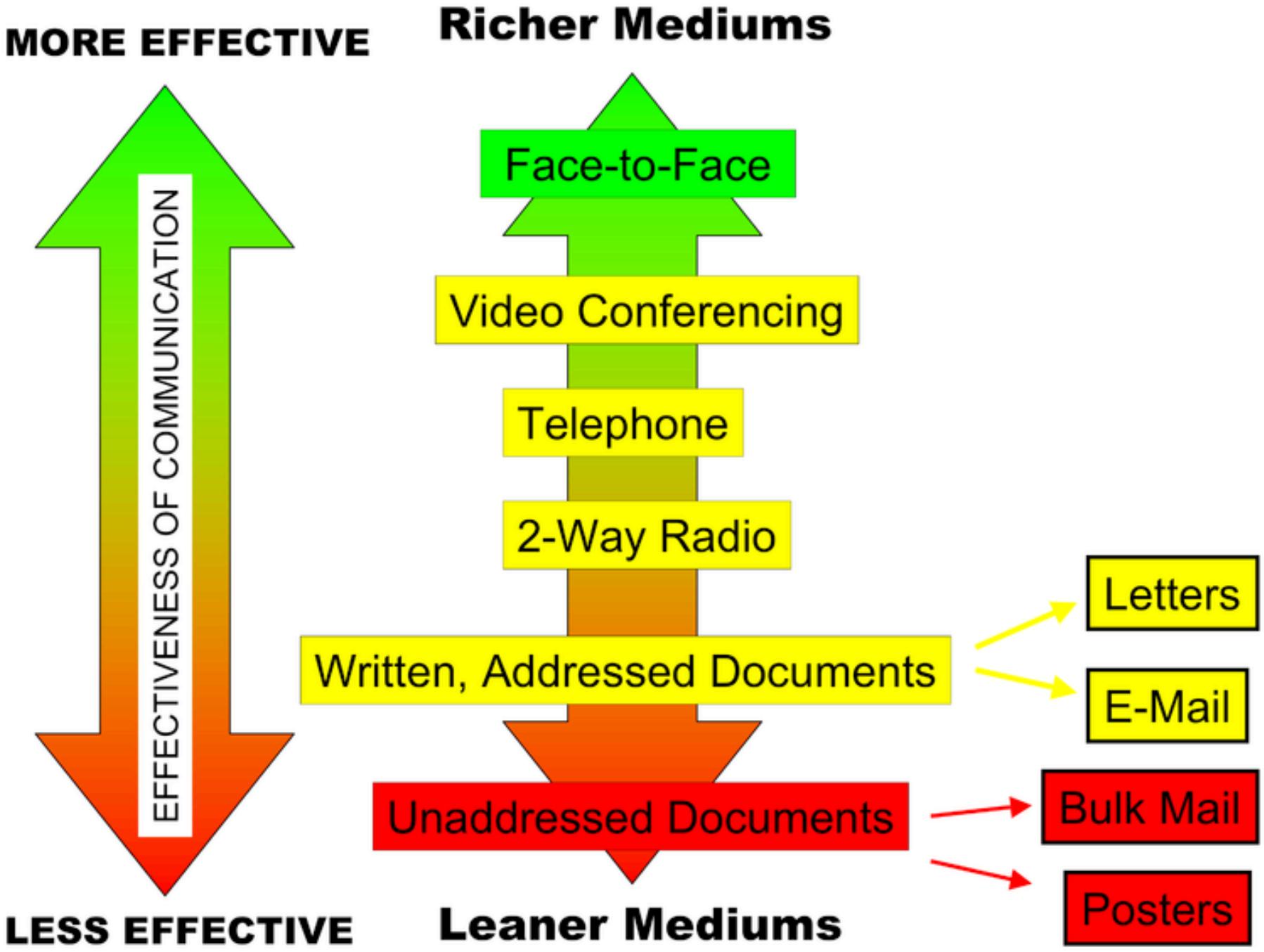
Group List of Communication Mediums

- Face to face
- Follow up minutes
- Email
- E blasts
- Social media (public) – fb, LI, twitter,
- Social media (private) – groups
- Hyperlinks references
- Newsletter
- Text messages
- Webinar
- Blogs
- Websites
- Videos
- Youtube
- Newspaper, magazines
- Op Eds
- Slack, Teams, other internal channel
- Phonecall
- Whatsapp, Google Duo
- Fact Sheets

Rich vs. Lean Communication Mediums



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Step 3

Rank the order you believe the audience you've identified would consume this information.

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Step 3

Small Group Discussion:

What communication medium(s) do you think your chosen audience would best respond to and why?

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Communication Plan

Information: Mayo Spore Conference Date: November 2020
Audience_____

Date	Topic	Channel	Assessment
Mon, 11/16	Hook, lede, conclusion	text	
Wed, 11/18	More info on lede	phone	
Mon, 11/23	Curated links to videos of the actual talks and articles (note the time of the talk, etc.) + invite to talk next week on zoom	Email	
Mon, 11/30	Fb group post reminding of zoom talk	Fb group post	
Tues, 12/1	Conference summary ppt	zoom	
12/1 – 12/15	tidbits	Social media	

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Critical Essence Model

Developing the Message:

Credibility + Trustworthiness

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Critical Essence Model

Credibility

- Your credentials
- Affiliations
- Experience

Trustworthiness

- Personal connection
- Common ground
- Authenticity

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Thank you!

Amy J. Hauenstein, PhD
Learning Designs, LLC

Connect with me:

www.learningdesignsllc.com

amy@learningdesignsllc.com

LinkedIn + Twitter: @amyhauenstein

Immune cells in Breast Cancer: is it a big deal or not at all?

Bringing TILs into daily practice

Roberto Salgado

Scientific Collaborator of the Immuno-Task Force of the Breast International Group (BIG)

Honorary Research Associate at the Division of Research at the Peter Mac Callum Cancer, Melbourne, Australia

Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium

Chair of International Immuno-Oncology Biomarkers Working Group

www.tilsinbreastcancer.org

Disclosures

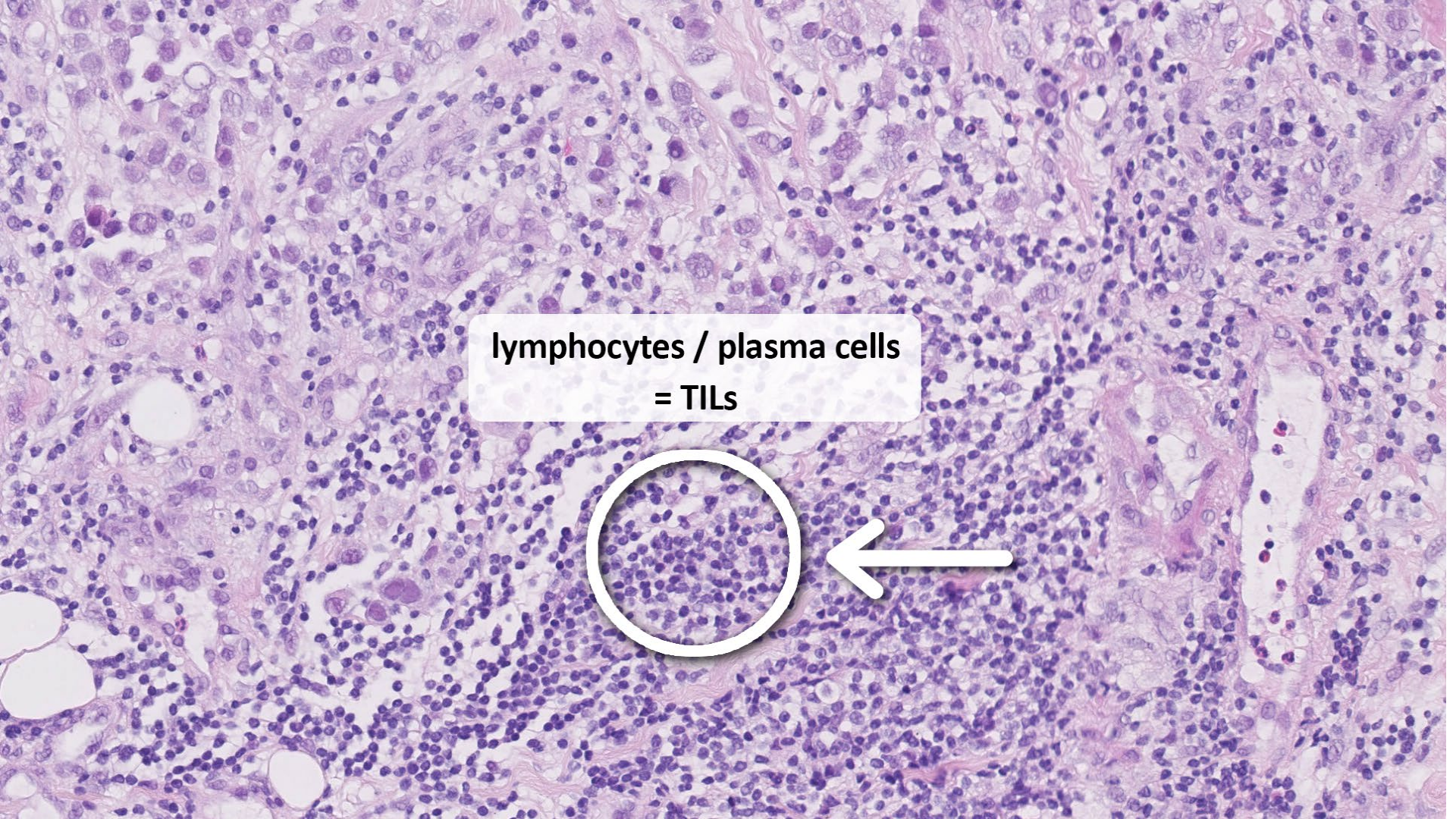
- Advisory Board role for BMS, Roche.
- Research funding by Roche, Puma, Merck.
- Member of an advisory group to the Belgian Government on reimbursement of assays in genomics and pathology.
- **I have no financial conflicts of interest related to TILs.**
- **I have no financial conflicts of interests related to any of the current work presented.**
- **Chair of The International Immuno-Oncology Biomarker Working Group (www.tilsinbreastcancer.org)**

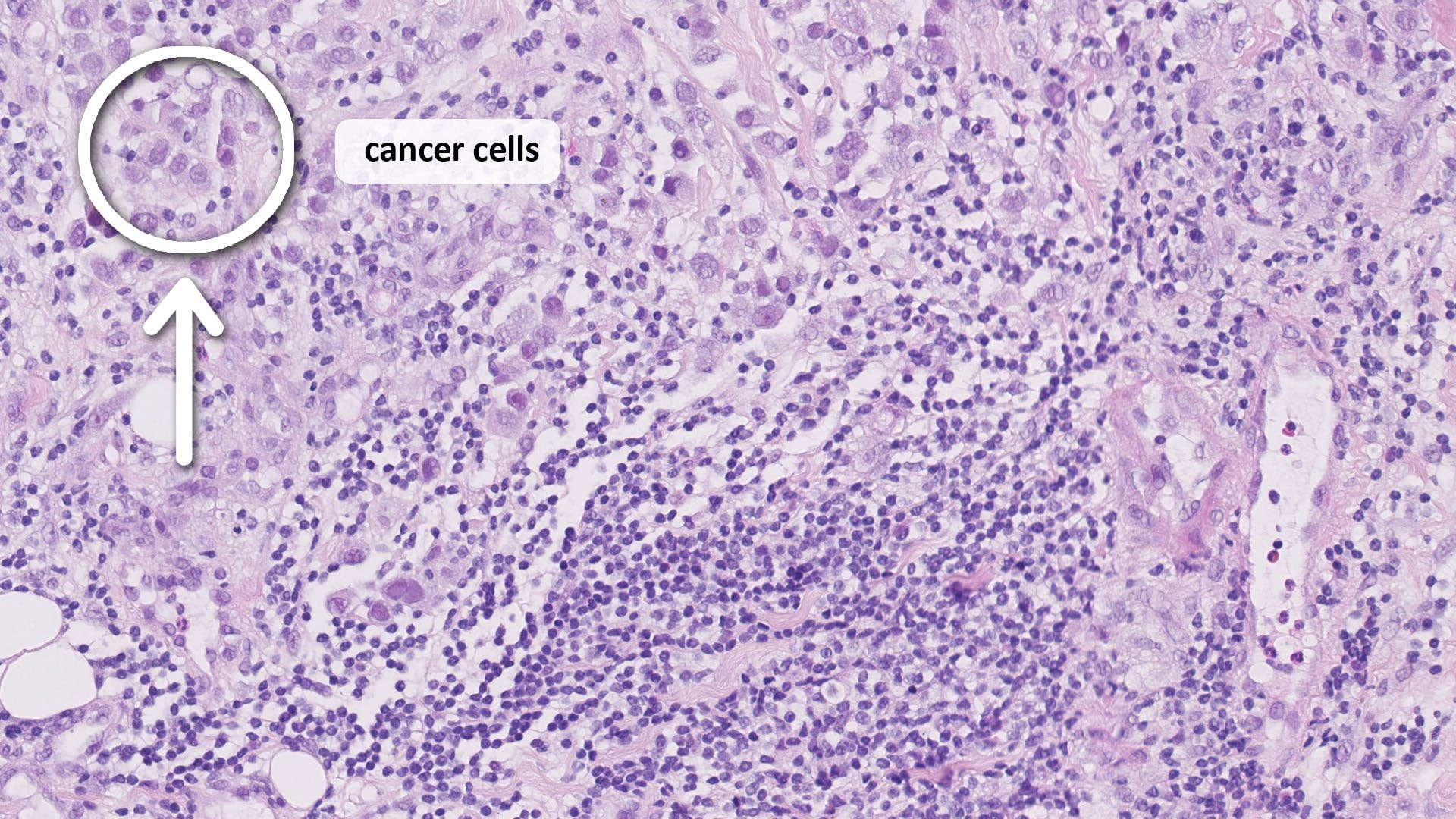
What you see is all there is

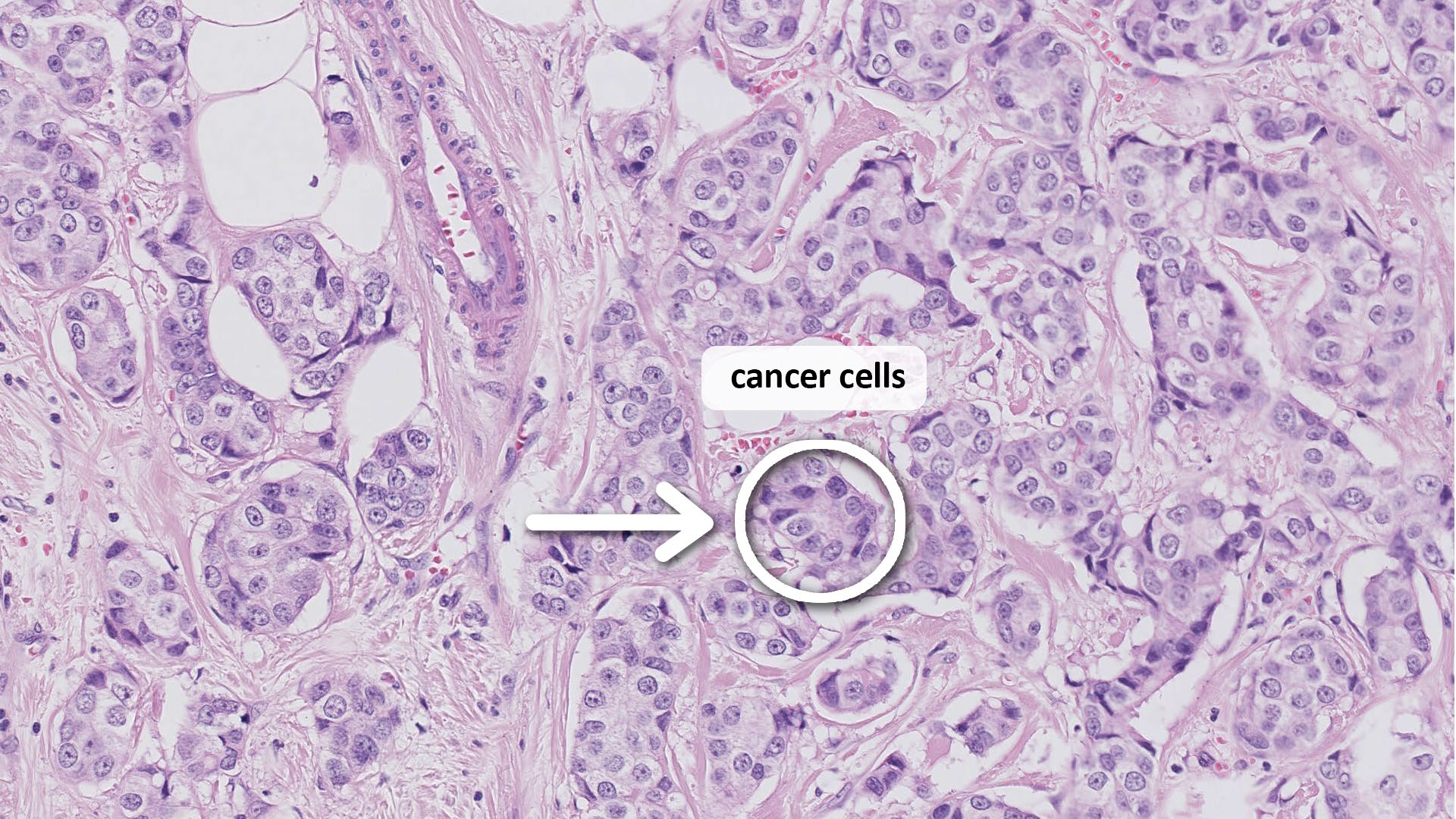
Daniel Kahneman

What are TILs?

Tumor Infiltrating Lymphocytes


lymphocytes / plasma cells
= TILs


cancer cells


cancer cells

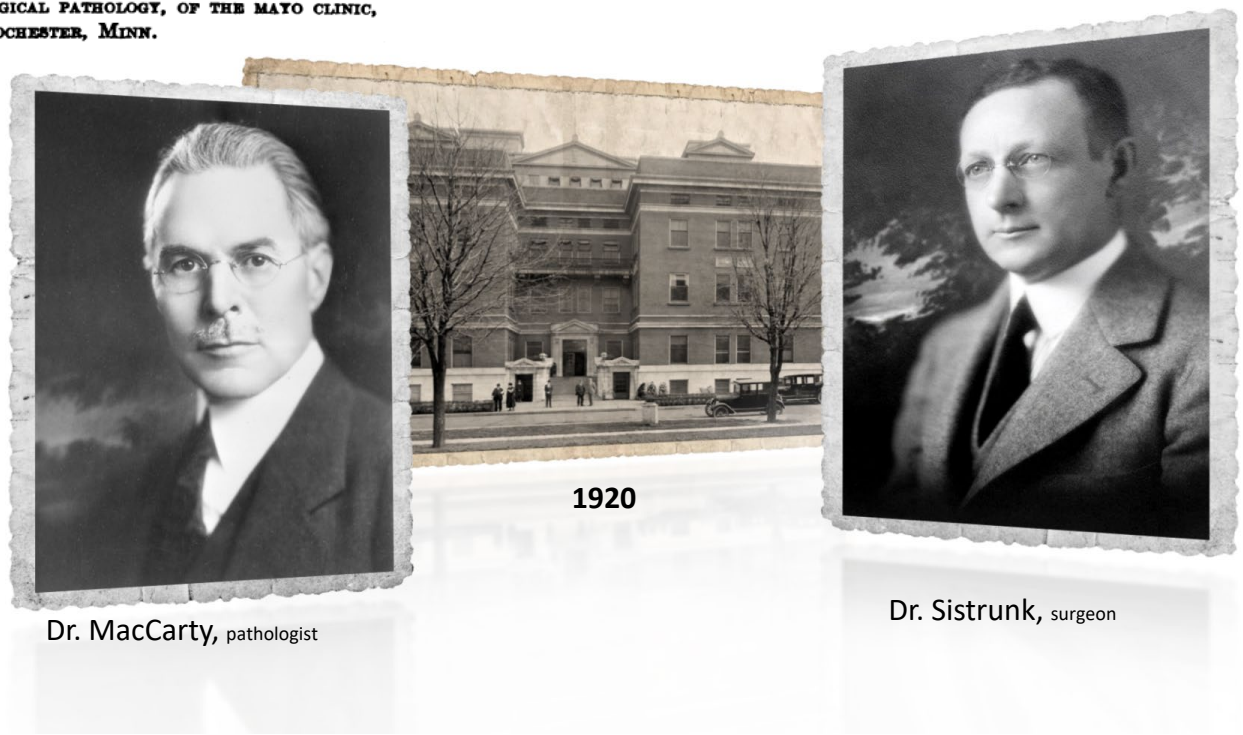
2020

The **Centenary Anniversary** of **TILs**

(Tumor Infiltrating Lymphocytes)

LIFE EXPECTANCY FOLLOWING RADICAL AMPUTATION FOR CARCINOMA OF THE BREAST: A CLINICAL AND PATHOLOGIC STUDY OF 218 CASES*

By Walter E. Sistrunk, M.D.
of the section on surgery
and
William C. MacCarty, M.D.
of the section on surgical pathology, of the Mayo Clinic,
Rochester, Minn.



* Presented before the Southern Surgical Association, December, 1920.

Courtesy of Mayo Clinic

"...patients with glandular and with local lymphocytic infiltration ***lived 146 per cent longer*** *than patients with glandular involvement without lymphocytic infiltration. As general facts, they give some clue to the* ***defensive mechanism of the body against malignant neoplasms****."*

Lymphocyte Infiltrates as a Prognostic Variable in Female Breast Cancer

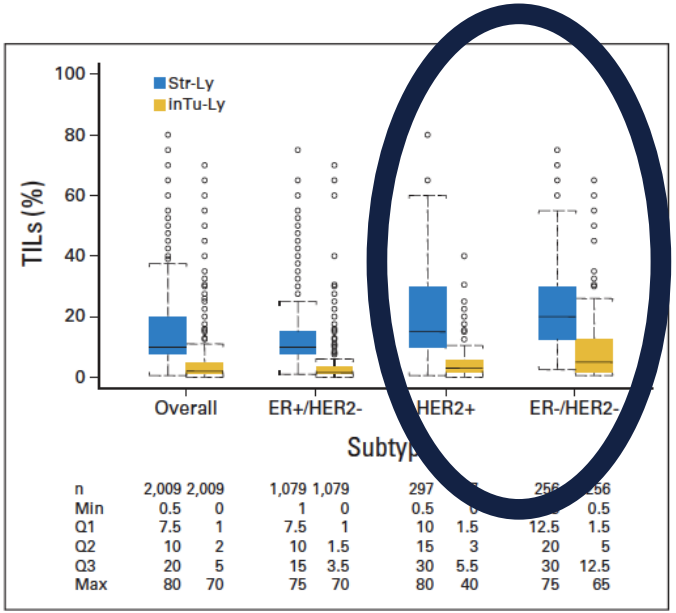
S. Aaltomaa, P. Lipponen, M. Eskelinen, V.-M. Kosma, S. Marin, E. Alhava and K. Syrjänen

The predictive value of lymphocyte infiltrates (LI) was studied in 489 patients with breast cancer followed-up for over 10 years. LI were positively correlated to axillary lymph-node status, tumour diameter and histological and morphometric variables ($P < 0.001$). In a multivariate analysis LI were independently related to axillary lymph-node status. LI predicted recurrence-free survival (RFS) in rapidly proliferating tumours ($P = 0.0269$). LI predicted RFS ($P = 0.08$) and breast cancer related survival (BS) ($P = 0.0168$) in rapidly proliferating, axillary lymph-node negative tumours. In a multivariate analysis LI independently predicted BS ($P = 0.08$) in rapidly proliferating tumours. LI independently predicted BS in rapidly ($P = 0.025$) and slowly ($P = 0.09$) proliferating, axillary lymph-node negative tumours. If the tumours were not categorised according to proliferation rate, LI and outcome were not significantly related. The results clearly confirm the presence of efficient immunological antitumour defence mechanisms in human breast cancer. Consequently tumour–host interactions are subject to further studies particularly in axillary lymph-node negative breast cancer.

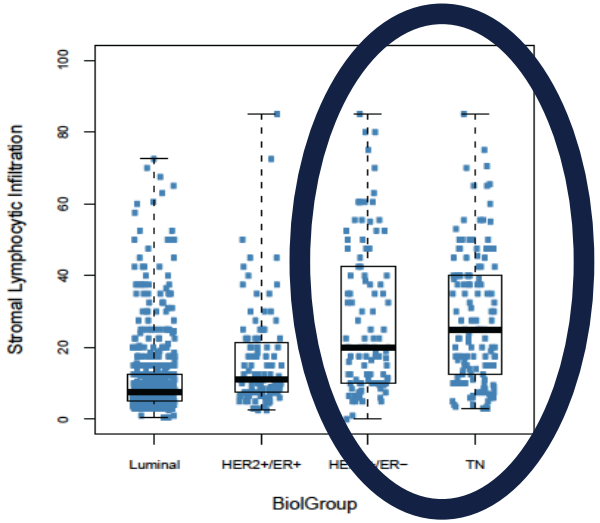
Eur J Cancer, Vol. 28A, No. 4/5, pp. 859–864, 1992.

12. The average length of postoperative life of patients without lymphocytic infiltration, hyalinization, and fibrosis was 42 per cent. less than the average length of postoperative life of patients with lymphocytic infiltration, hyalinization, and fibrosis.

Higher levels in HER2+ and TNBC



	Overall		ER+/HER2-		HER2+		ER-/HER2-	
n	2,009	2,009	1,079	1,079	297	[illegible]	256	256
Min	0.5	0	1	0	0.5	0	[illegible]	0.5
Q1	7.5	1	7.5	1	10	1.5	12.5	1.5
Q2	10	2	10	1.5	15	3	20	5
Q3	20	5	15	3.5	30	5.5	30	12.5
Max	80	70	75	70	80	40	75	65



	Luminal	HER2+/ER+	HER2+/ER−	TN
n	591	103	106	134
Min	0.5	2.5	0	3
Q1	5	7.5	10	12.5
Q2	7.5	11	20	25
Q3	12.5	21.2	42.5	40
Max	72.5	85	85	85

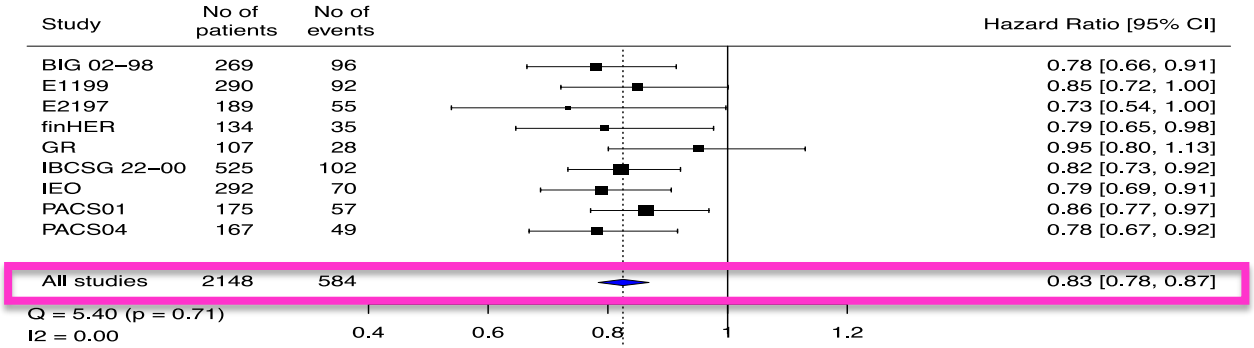
Loi et al, JCO 2013; Ann Oncol 2014

We are doing nothing new

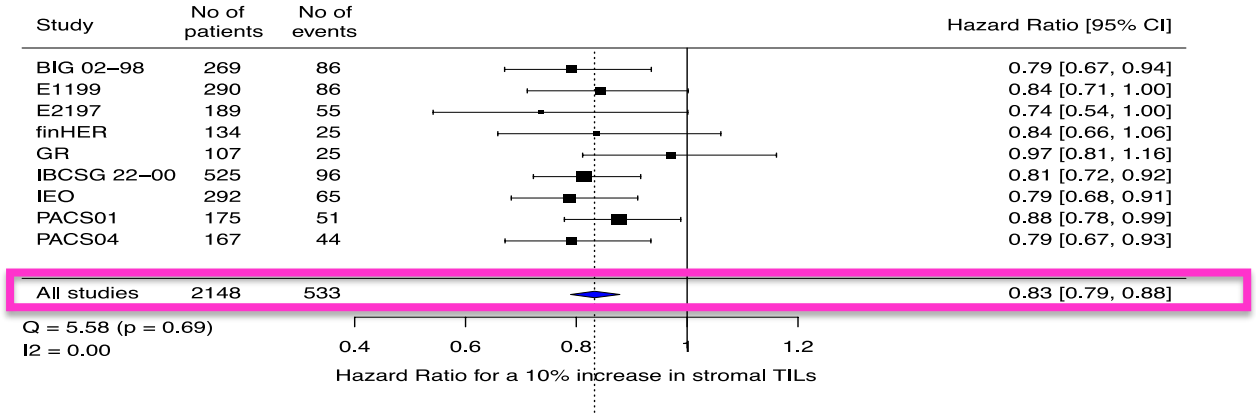
TNBC, TILs AND PROGNOSIS

Pooled individual patient data analysis from 2148 early-stage TNBC treated with anthracycline-based adjuvant CT showed significant predictive value of sTILs for iDFS, dDFS, and OS

dDFS



OS

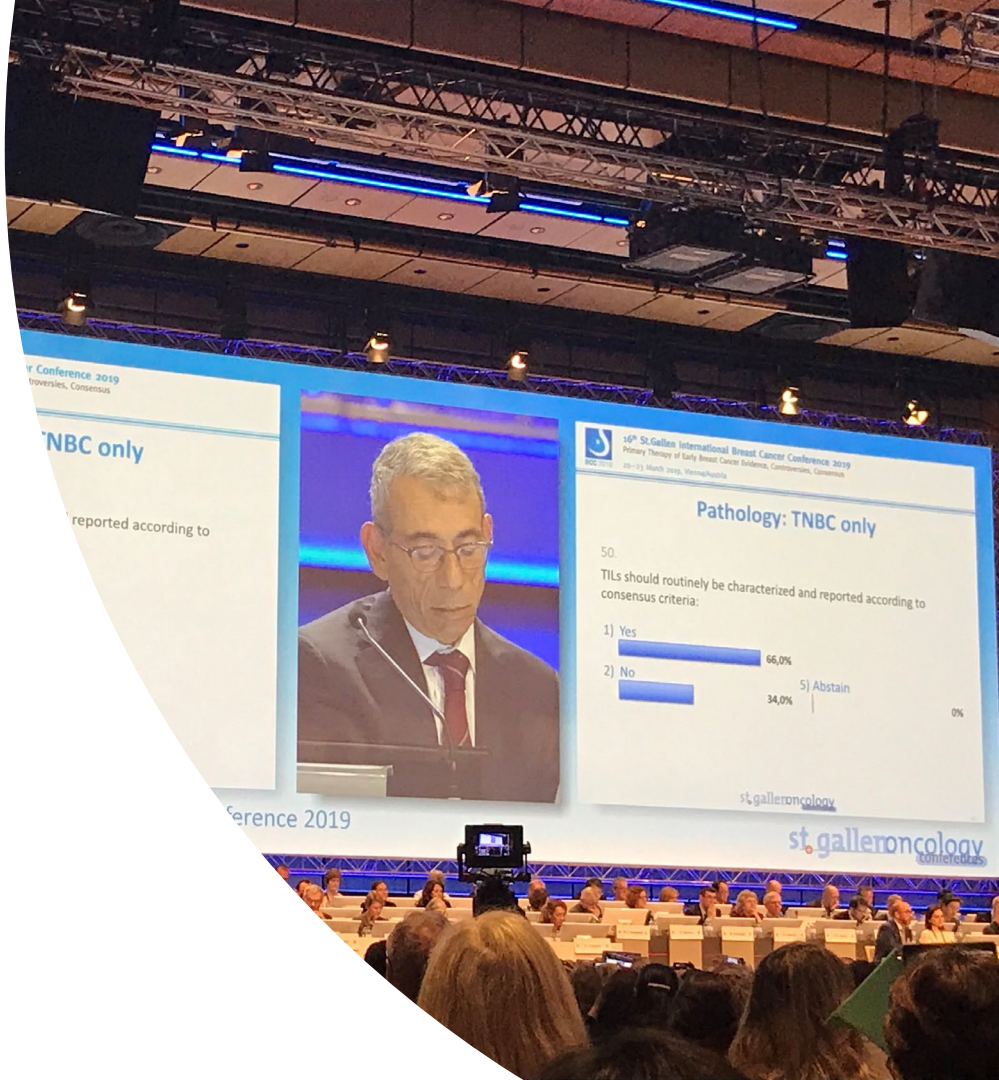


Loi S, et al. JCO 2019

Clinical Guidelines

- St. Gallen 2019: The Panel recommended that TILs be routinely characterized in TNBC because of its prognostic value. However, data are inadequate to recommend TILs as a test to guide neo-adjuvant treatment choices in TNBC, as treatments are largely governed by stage.

  = similar in ESMO 2019 BC Guidelines



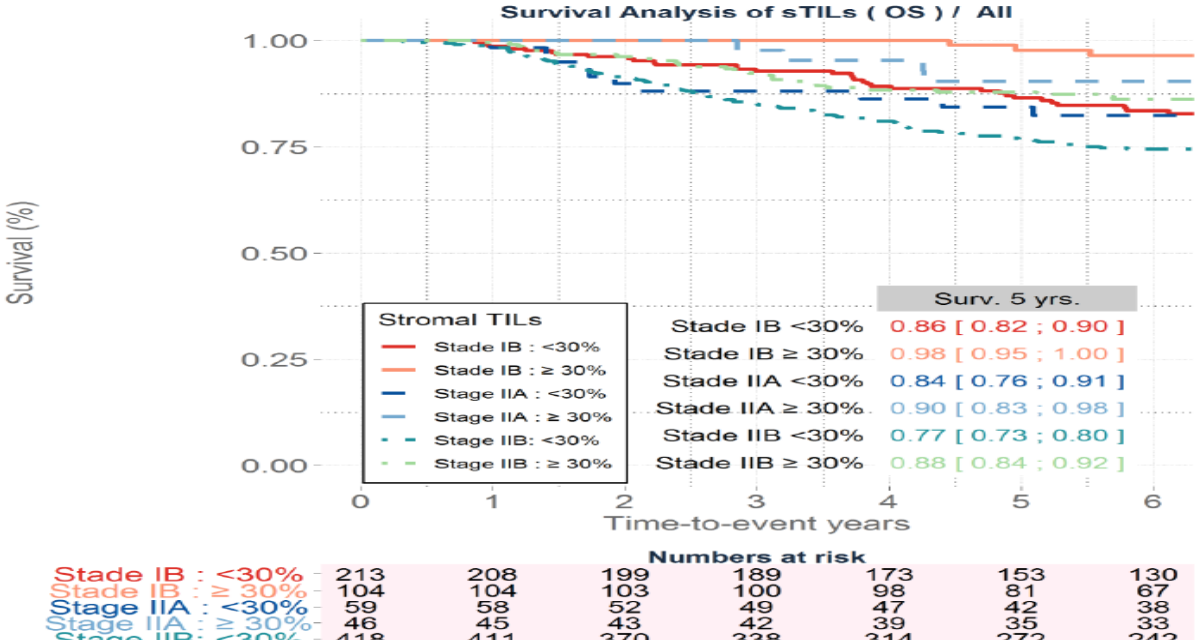
How to use TILs as a prognostic factor in TNBC? = actually determining TILs at the moment of diagnosis

- Examples:
  - T1a,b,c- high vs low TIL- how well do they do **WITHOUT** chemotherapy? Can we withhold chemo in high TIL TNBC T1a,b N0?
  - High TIL Node negative and N1-3 – can we get away with **LESS** chemo- 4 cycles AC for example vs 6 cycles

How do TILs affect stage?

	Stade IA	Stade IB	Stade IIA	Stade IIB
AJCC8	-	T1N0G1 T1N0G2 T1N0G3	T0N1G1 T1N1G1 T2N0G1	T0N1G2 T1N1G2 T2N0G2 T0N1G3 T1N1G3 T2N0G3

AJCC8-OS-All Treatments



AJCC8

Stade IA	Stade IB	Stade IIA	Stade IIB
-	T1N0G1	T0N1G1	T0N1G2
	T1N0G2	T1N1G1	T1N1G2
	T1N0G3	T2N0G1	T2N0G2
			T0N1G3
			T1N1G3
			T2N0G3

Manuscript in preparation

Intrinsic prognostic value of tumor infiltrating lymphocytes (TILs) in early-stage triple negative breast cancer (TNBC) not treated with adjuvant chemotherapy

A pooled analysis of 4 individual cohorts

Ji Hyun Park †, Sarah Flora Jonas†, Guillaume Bataillon†, Carmen Criscitiello†,
Roberto Salgado, Sherene Loi, Giuseppe Viale, Hee Jin Lee, Maria Vittoria Dieci, Sung-Bae Kim,
Anne Vincent-Salomon, Giuseppe Curigliano °, Fabrice Andre °, Stefan Michiels°*.

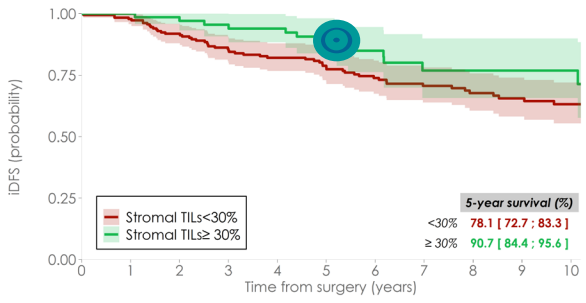
Annals of Oncology, 2019

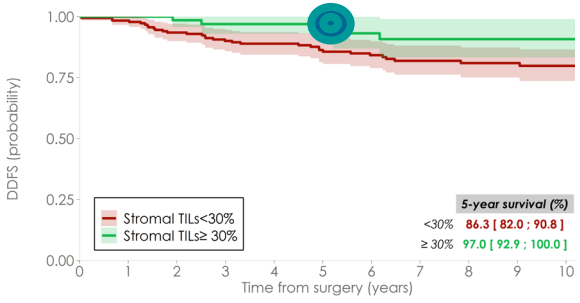
Excellent Outcomes In pStage I tumors

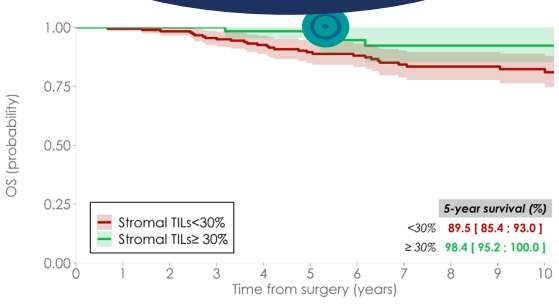
5Y: 91%	3Y: 93%
84-96	89-96

5Y: 97%	3Y: 97%
95-100	95-99

5Y: 98%	3Y: 99%
95-100	97-100







Numbers at risk

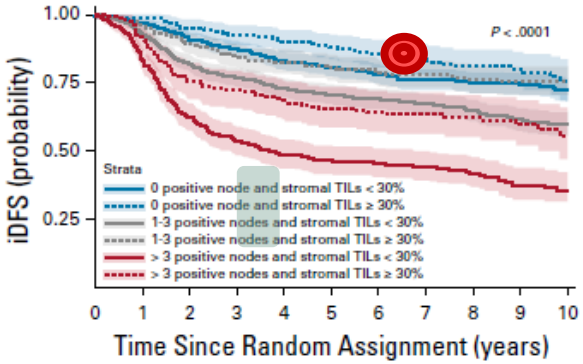
Stromal TILs<30%	189	180	165	149	136	113	97	82	66	58	45
Stromal TILs≥ 30%	74	71	65	61	59	49	36	24	19	15	14

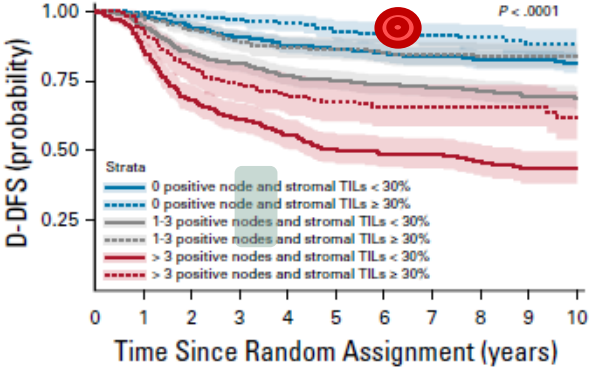
Numbers at risk

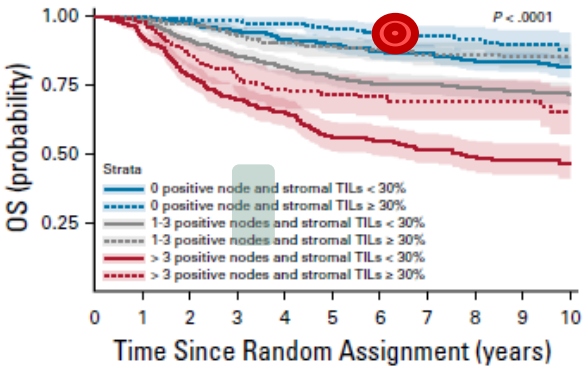
Stromal TILs<30%	189	181	168	157	148	127	113	98	82	73	59
Stromal TILs≥ 30%	74	71	66	62	61	52	40	30	25	20	18

Numbers at risk

Stromal TILs<30%	189	183	176	166	154	132	119	102	85	76	62
Stromal TILs≥ 30%	74	71	67	64	62	53	41	31	26	21	19

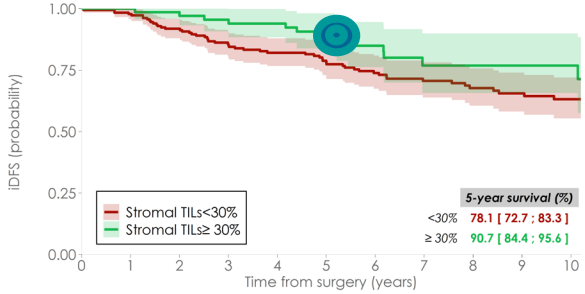




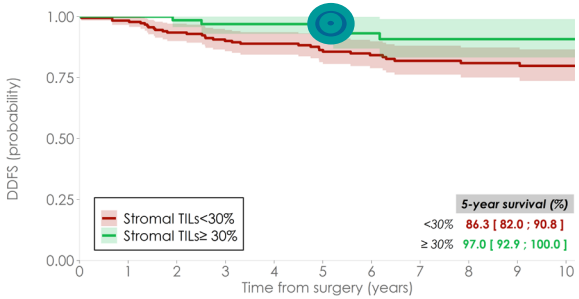


Excellent Outcomes In pStage I tumors

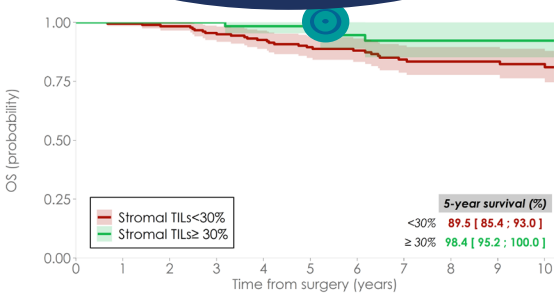
5Y: 91%	3Y: 93%
84-96	89-96



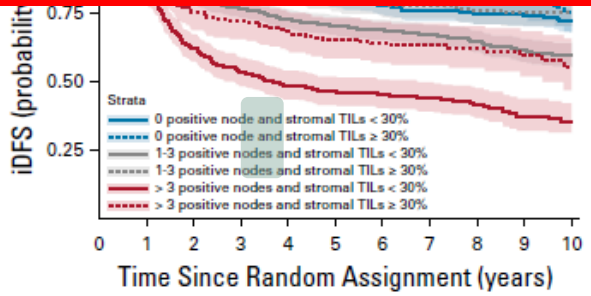
5Y: 97%	3Y: 97%
95-100	95-99

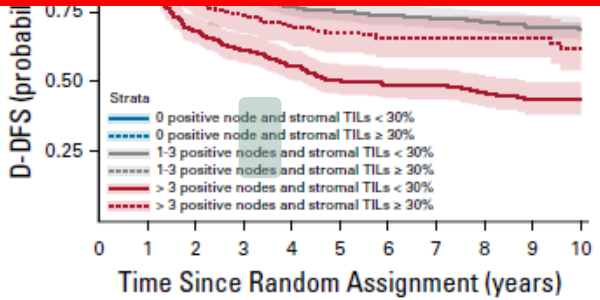


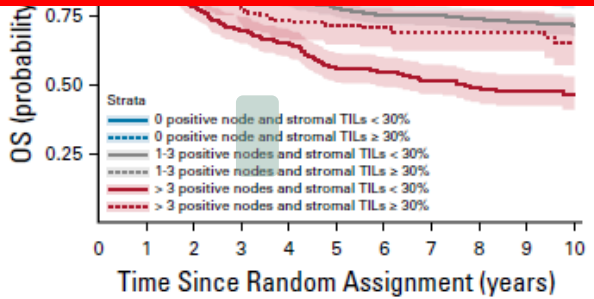
5Y: 98%	3Y: 99%
95-100	97-100



Absolute benefit of chemo could be *minimal* in this group!







VIRTUAL 2020 ESMO congress

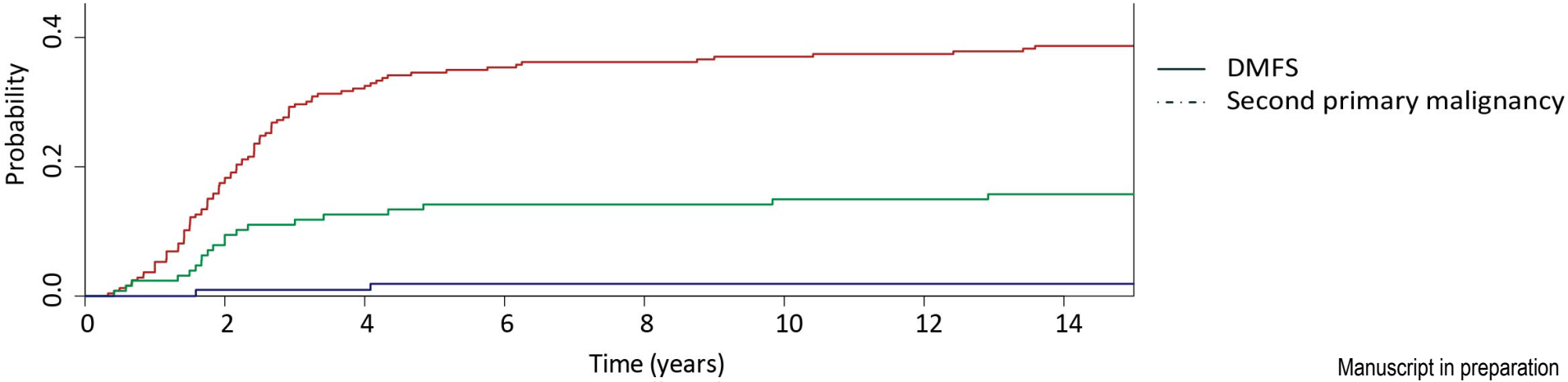
Prognostic value of tumor infiltrating lymphocytes in young triple negative breast cancer patients who did not receive adjuvant systemic treatment

V. de Jong, Wang Y , M. Opdam, N. ter Hoeve, K. Jozwiak, M. Hauptmann, N. Stathonikos, H. Horlings, A. Broeks, S. Michiels, E. van der Wall, P. van Diest, S. Siesling, M.K. Schmidt, S. Loi, M. Kok, S. Linn, G. Dackus*, R. Salgado*

Corresponding author: Sabine Linn; s.linn@nki.nl

Low incidence of DMFS in high sTILs group

	DMFS 15 years
sTILs < 30%	39% (36-42)
sTILs 30%-75%	16% (12-19)
sTILs ≥ 75%	1.9% (0-3.4)



Manuscript in preparation

Coming in 2021

In partnership with dr. M. Goetz and dr. J. Carter

Pooled analysis evaluating the prognostic impact of TILs in >1100 TNBC patients that NEVER

received chemotherapy.

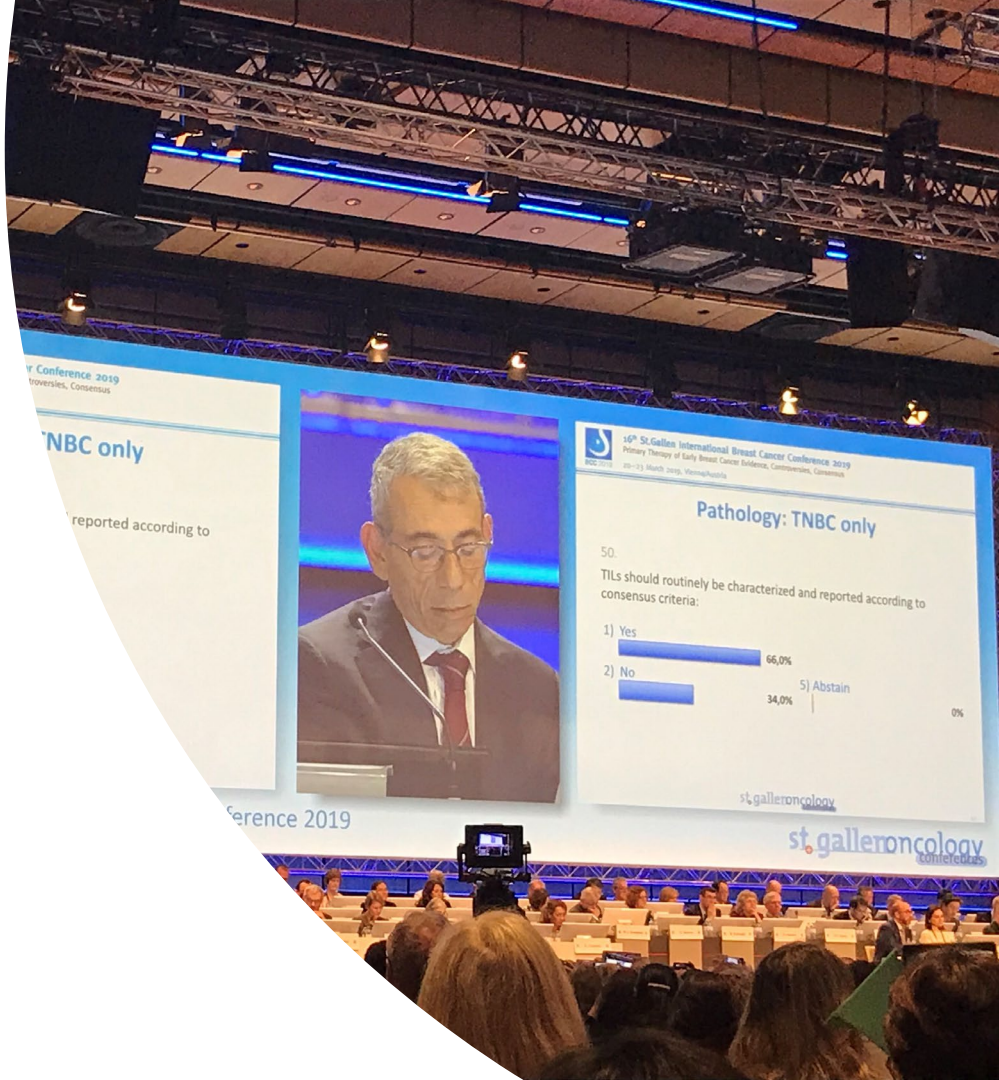
Results expected mid-2021

(before the Mayo Heritage days)

Clinical Guidelines

- St. Gallen 2019: The Panel recommended that TILs be routinely characterized in TNBC because of its prognostic value. However, data are inadequate to recommend TILs as a test to guide neo-adjuvant treatment choices in TNBC, as treatments are largely governed by stage.

  = similar in ESMO 2019 BC Guidelines



The Narrative to get TILs in daily practice

Why should oncologists and patients need to know?

- TILs can be assessed in a reproducible manner by Pathologists.
- Pathologists only need a microscope and a HE and can be trained using a freely available training-tool (www.tilsinbreastcancer.org).
- The scoring can be done at the moment of making a diagnosis.
- Stage I TNBC with high TILs have excellent 5-year survival, irrespective of treatment.
- So, if TILs are one day used for prediction for ICI, the pathologist has already scored them for prognostic reasons in the primary sample. This can help in PDL1-assessment.

TNBC, TILs, PDL1 AND PREDICTION

KEYNOTE-119: Phase 3 Study of Pembrolizumab versus Single-Agent Chemotherapy for Metastatic Triple-Negative Breast Cancer (mTNBC)

Javier Cortes[1], Oleg Lipatov[2], Seock-Ah Im[3], Anthony Goncalves[4], Keun Seok Lee[5], Peter Schmid[6], Kenji Tamura[7], Laura Testa[8], Isabell Witzel[9], Shoichiro Ohtani[10], Stefania Zambelli[11], Nadia Harbeck[12], Fabrice Andre[13], Rebecca Dent[14], Xuan Zhou[15], Vassiliki Karantza[15], Jaime Mejia[15], Eric P. Winer[16]

1. IOB Institute of Oncology, Quiron Group; Vall d'Hebron Institute of Oncology (VHIO), Madrid & Barcelona, Spain; 2. Republican Clinical Oncology Dispensary, Republic of Bashkortostan, Russian Federation; 3. Seoul National University, Seoul, Republic of Korea; 4. Institut Paoli-Calmettes, Marseille, France; 5. National Cancer Center, Goyang, Republic of Korea; 6. Barts Cancer Institute, Queen Mary University London, London, UK; 7. National Cancer Center Hospital, Tokyo, Japan; 8. Instituto do Câncer do Estado de São Paulo - Faculdade de Medicina da Universidade do Estado do São Paulo, São Paulo, Brazil; 9. University Medical Center Hamburg, Hamburg, Germany; 10. Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan; 11. IRCCS Ospedale San Raffaele, Milan, Italy; 12. Breast Center, University of Munich (LMU), Munich, Germany; 13. Faculté de Medicine Paris-Sud XI, Gustave Roussy, Villejuif, France; 14. University of Toronto, Toronto, Ontario, Canada; 15. Merck & Co., Inc., Kenilworth, NJ, USA; 16. Dana-Farber Cancer Institute, Boston, MA, USA

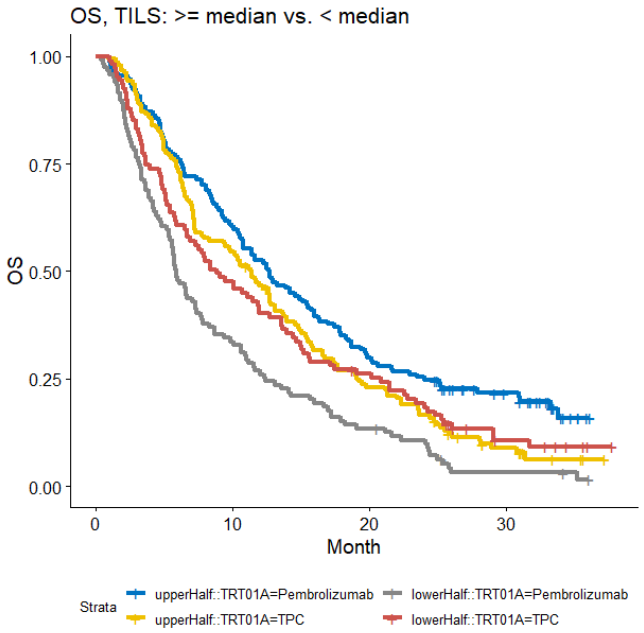
Relationship Between Tumor-Infiltrating Lymphocytes and Outcomes in the KEYNOTE-119 Study of Pembrolizumab Versus Chemotherapy for Previously Treated, Metastatic Triple-Negative Breast Cancer

S. Loi[1]; E. Winer[2]; O. Lipatov[3]; S.-A. Im[4]; A. Gonçalves[5]; J. Cortes[6]; K. S. Lee[7]; P. Schmid[8]; L. Testa[9]; I. Witzel[10]; S. Ohtani[11]; N. Turner[12]; S. Zambelli[13]; N. Harbeck[14]; F. Andre[15]; R. Dent[16]; L. Huang[17]; J. Mejia[17]; V. Karantza[17]; R. Salgado[1]

[1]Peter MacCallum Cancer Centre and University of Melbourne, Melbourne, VIC, Australia; [2]Dana-Farber Cancer Institute, Boston, MA, USA; [3]Republican Clinical Oncology Dispensary, Republic of Bashkortostan, Russian Federation; [4]Seoul National University, Seoul, South Korea; [5]Institut Paoli-Calmettes, Marseille, France; [6]IOB Institute of Oncology, Quiron Group, Madrid, and Vall d'Hebron Institute of Oncology, Barcelona, Spain; [7]National Cancer Center, Goyang, South Korea; [8]Barts Cancer Institute, Centre for Experimental Cancer Medicine, London, United Kingdom; [9]Instituto do Câncer do Estado de São Paulo – Faculdade de Medicina da Universidade do Estado do São Paulo, São Paulo, Brazil; [10]University Medical Center Hamburg, Hamburg, Germany; [11]Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan; [12]The Royal Marsden NHS Foundation Trust, London, United Kingdom; [13]San Raffaele Scientific Institute, Milan, Italy; [14]Breast Center, University of Munich, Munich, Germany; [15]Université Paris-Sud, Orsay, and Gustave Roussy, Villejuif, France; [16]University of Toronto, Toronto, ON, Canada; [17]Merck & Co., Inc., Kenilworth, NJ, USA

OS in subpopulation defined by TILs median cutpoint (TILs ≥ 5% vs. < 5%)

- For TPC arm, the yellow and red curves represent the TILs≥5% and TILs<5%, with little difference observed
- For Pembro arm, there is separation according to the median TILS cut-off consistent with testing as a continuous measure



Loi et al., SABC2019

IMpassion130 phase III study design

Key IMpassion130 eligibility criteria[a]:

- Metastatic or inoperable locally advanced TNBC
  - Histologically documented[b]
- No prior therapy for advanced TNBC
  - Prior chemo in the curative setting, including taxanes, allowed if TFI ≥ 12 mo
- ECOG PS 0-1

Stratification factors:

- Prior taxane use (yes vs no)
- Liver metastases (yes vs no)
- PD-L1 status on IC (positive [≥ 1%] vs negative [< 1%])[c]

R 1:1

Atezo + nab-P arm:

Atezolizumab 840 mg IV
- On days 1 and 15 of 28-day cycle

+ *Nab*-paclitaxel 100 mg/m^2 IV
- On days 1, 8 and 15 of 28-day cycle

Double blind; no crossover permitted

Plac + nab-P arm:

Placebo IV
- On days 1 and 15 of 28-day cycle

+ *Nab*-paclitaxel 100 mg/m^2 IV
- On days 1, 8 and 15 of 28-day cycle

RECIST v1.1
PD or toxicity

- Co-primary endpoints were PFS and OS in the ITT and PD-L1+ populations[d]
  - Key secondary efficacy endpoints (ORR and DOR) and safety were also evaluated

IC, tumour-infiltrating immune cell; TFI, treatment-free interval. [a] ClinicalTrials.gov: NCT02425891. [b] Locally evaluated per ASCO–College of American Pathologists (CAP) guidelines. [c] Centrally evaluated per VENTANA SP142 IHC assay (double blinded for PD-L1 status). [d] Radiological endpoints were investigator assessed (per RECIST v1.1).

Schmid P, et al. IMpassion130. ESMO 2018 (abstract 2056).

LBA15 - Primary results from IMpassion131, a double-blind placebo-controlled randomised phase III trial of first-line paclitaxel (PAC) ± atezolizumab (atezo) for unresectable locally advanced/metastatic triple-negative breast cancer (mTNBC)

Date

19 Sep 2020

Session

Proffered Paper - Breast cancer, metastatic 1

Presenters

David Miles

Citation

Annals of Oncology (2020) 31 (suppl_4): S1142-S1215. 10.1016/annonc/annonc325

Authors

D.W. Miles[1], J. Gligorov[2], F. André[3], D. Cameron[4], A. Schneeweiss[5], C.H. Barrios[6], B. Xu[7], A.M. Wardley[8], D. Kaen[9], L. Andrade[10], V. Semiglazov[11], M. Reinisch[12], M. Patre[13], L. Morales[13], K. Russell[13], M. Donica[14], J. O'Shaughnessy[15]

Author affiliations

More

ESMO2020

ESMO BREAST CANCER
VIRTUAL MEETING

PERFORMANCE OF PD-L1 IMMUNOHISTOCHEMISTRY ASSAYS IN UNRESECTABLE LOCALLY ADVANCED OR METASTATIC TRIPLE-NEGATIVE BREAST CANCER: POST HOC ANALYSIS OF IMPASSION130

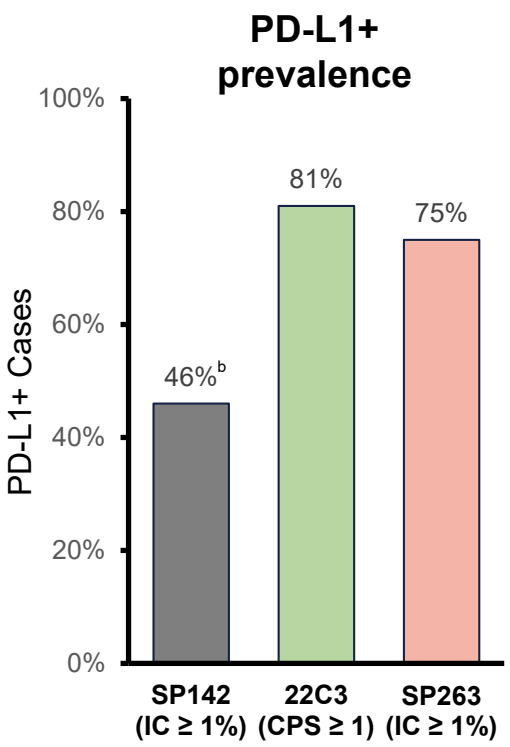
Hope S. Rugo,[1] Sherene Loi,[2] Sylvia Adams,[3] Peter Schmid,[4] Andreas Schneeweiss,[5] Carlos H. Barrios,[6] Hiroji Iwata,[7] Véronique Diéras,[8] Eric P. Winer,[9] Mark M. Kockx,[10] Dieter Peeters,[10] Stephen Y. Chui,[11] Jennifer C. Lin,[11] Anh Nguyen Duc,[11] Giuseppe Viale,[12] Luciana Molinero,[11] Leisha A. Emens[13]

[1]University of California San Francisco Comprehensive Cancer Center, San Francisco, CA, USA; [2]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [3]NYU Langone Medical Center, New York, NY, USA; [4]Barts Cancer Institute, Queen Mary University London, London, UK; [5]University Hospital and German Cancer Research Center Heidelberg, Heidelberg, Germany; [6]Centro de Pesquisa Clínica, HSL, PUCRS, Porto Alegre, Brazil; [7]Aichi Cancer Center Hospital, Nagoya, Japan; [8]Department of Medical Oncology, Centre Eugène Marquis, Rennes, France; [9]Dana-Farber Cancer Institute, Boston, MA, USA; [10]HistoGeneX NV, Antwerp, Belgium; [11]Genentech, Inc., South San Francisco, CA, USA; [12]University of Milan, European Institute of Oncology IRCCS, Milan, Italy; [13]University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, USA

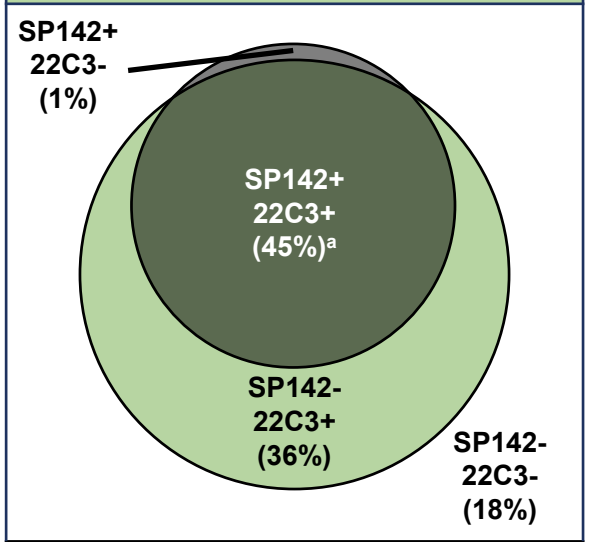
ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Presented at ESMO2019

PD-L1 IHC assays: prevalence and analytical concordance

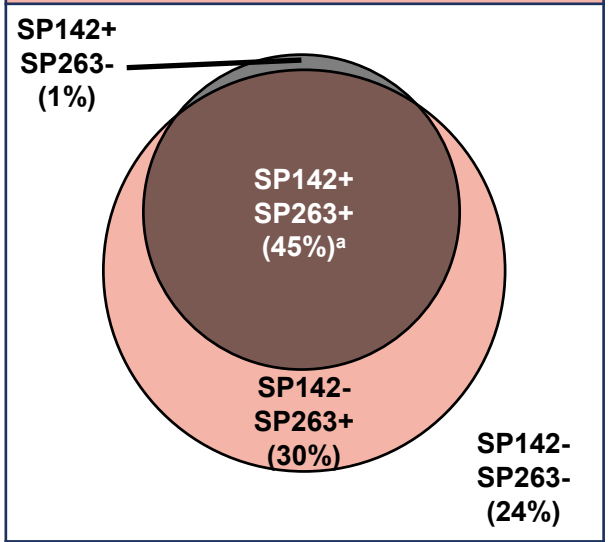


SP142 (IC 1%) and 22C3 (CPS 1)



OPA[c]	64%
PPA	98%
NPA	34%

SP142 (IC 1%) and SP263 (IC 1%)



OPA[c]	69%
PPA	98%
NPA	45%

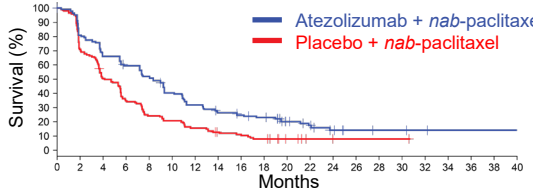
NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement.

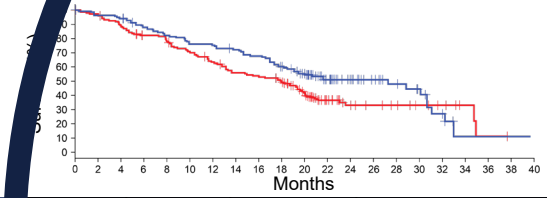
[a] > 97% of SP142+ samples included in 22C3+ or SP263+ samples. [b] Compared with 41% in ITT (Schmid, *New Engl J Med* 2018).

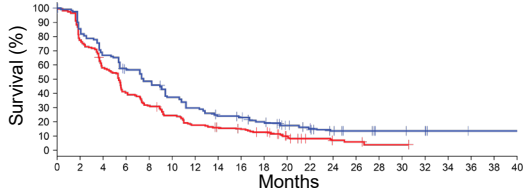
[c] ≥ 90% OPA, PPA ***and*** NPA required for analytical concordance.

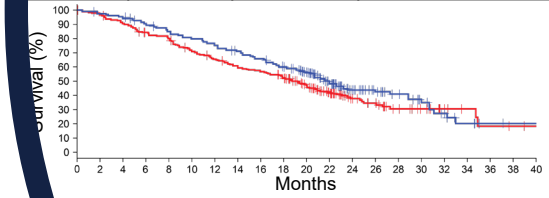
Clinical outcomes in PD-L1+ populations per SP142 (IC 1%), 22C3 (CPS 1) and SP263 (IC 1%)

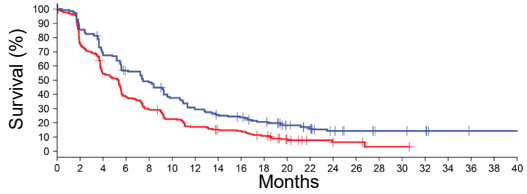
Population	PFS				OS			
	Median PFS, mo			HR (95% CI)	Median OS, mo			HR (95% CI)
	A + nP	P + nP	Δ		A + nP	P + nP	Δ	
SP142 IC ≥ 1%: 46% (285/614)	8.3	4.1	4.2	0.60 (0.47, 0.78)	27.3	17.9	9.4	0.74 (0.54, 1.01)
22C3 CPS ≥ 1: 81% (497/614)	7.5	5.4	2.1	0.68 (0.56, 0.82)	21.6	19.2	2.4	0.78 (0.62, 0.99)
SP263 IC ≥ 1%: 75% (460/614)	7.5	5.3	2.2	0.64 (0.53, 0.79)	22.0	18.7	3.3	0.75 (0.59, 0.96)

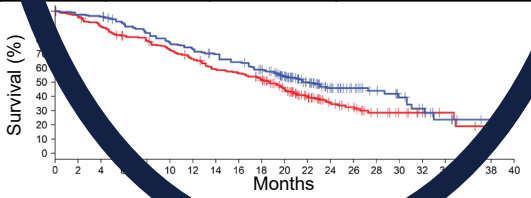












HR adjusted for prior taxanes, presence of liver metastases, age and ECOG PS.

TILs correlate with response to immune checkpoint-inhibition.

PDL1 correlates with TILs.

PDL1 correlates -almost- always with response to immune checkpoint-inhibition, in TNBC.

The Narrative to get TILs in daily practice

Why should oncologists and patients need to know?

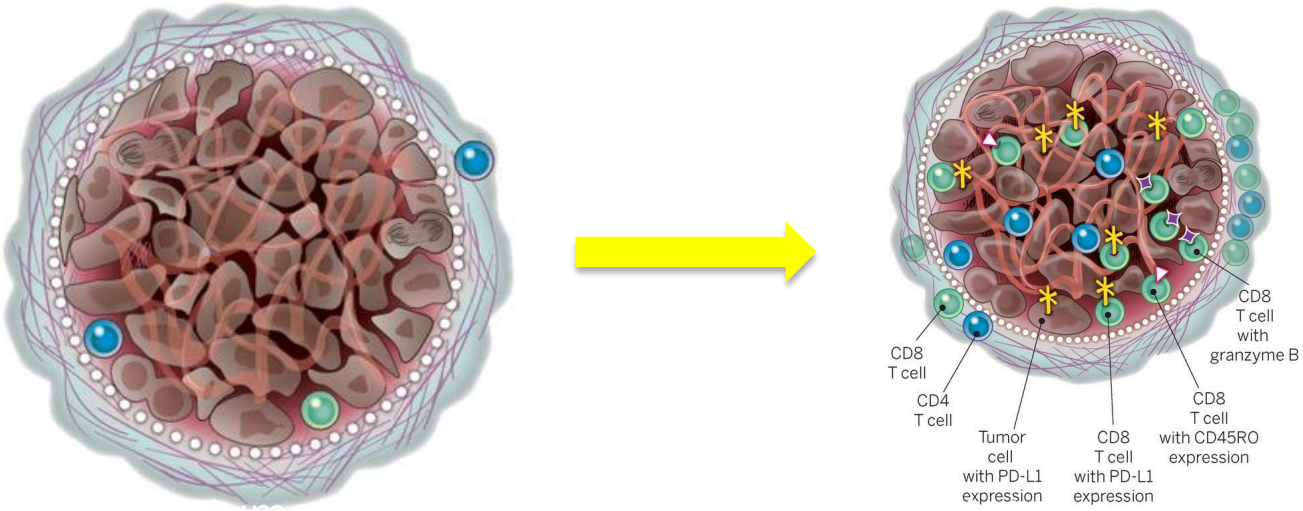
Considering that:

- TILs can be assessed in a reproducible manner by Pathologists.
- Pathologists only need a microscope and a HE and can be trained using a freely available training-tool (www.tilsinbreastcancer.org).
- The scoring can be done at the moment of making a diagnosis.
- Stage I TNBC with high TILs have excellent 5-year survival, irrespective of treatment.
- TILs are associated with prediction.
- PDL1 is associated with prediction.
- If pathologists score TILs already in their daily practice, for prognostic purposes, this information is already present in the report if needed for selection for ICI, in a combination with PDL1, **at term**.
- If the patients develop metastasis, I believe that pathologists can use any PDL1-antibody, as long as it is well validated, and is used in conjunction with TILs, as if there are no TILs, PDL1 IC will be negative, and if there are many TILs, probably it may not matter that much which assay is used, as long as it is validated.

Quo Vadis TILs?

Prognosis High TILs versus Prediction Low TILs

Turning ‘cold’ into ‘hot’ tumor in TNBC
Can we do this?



from: Sharma & Allison. Science 2015

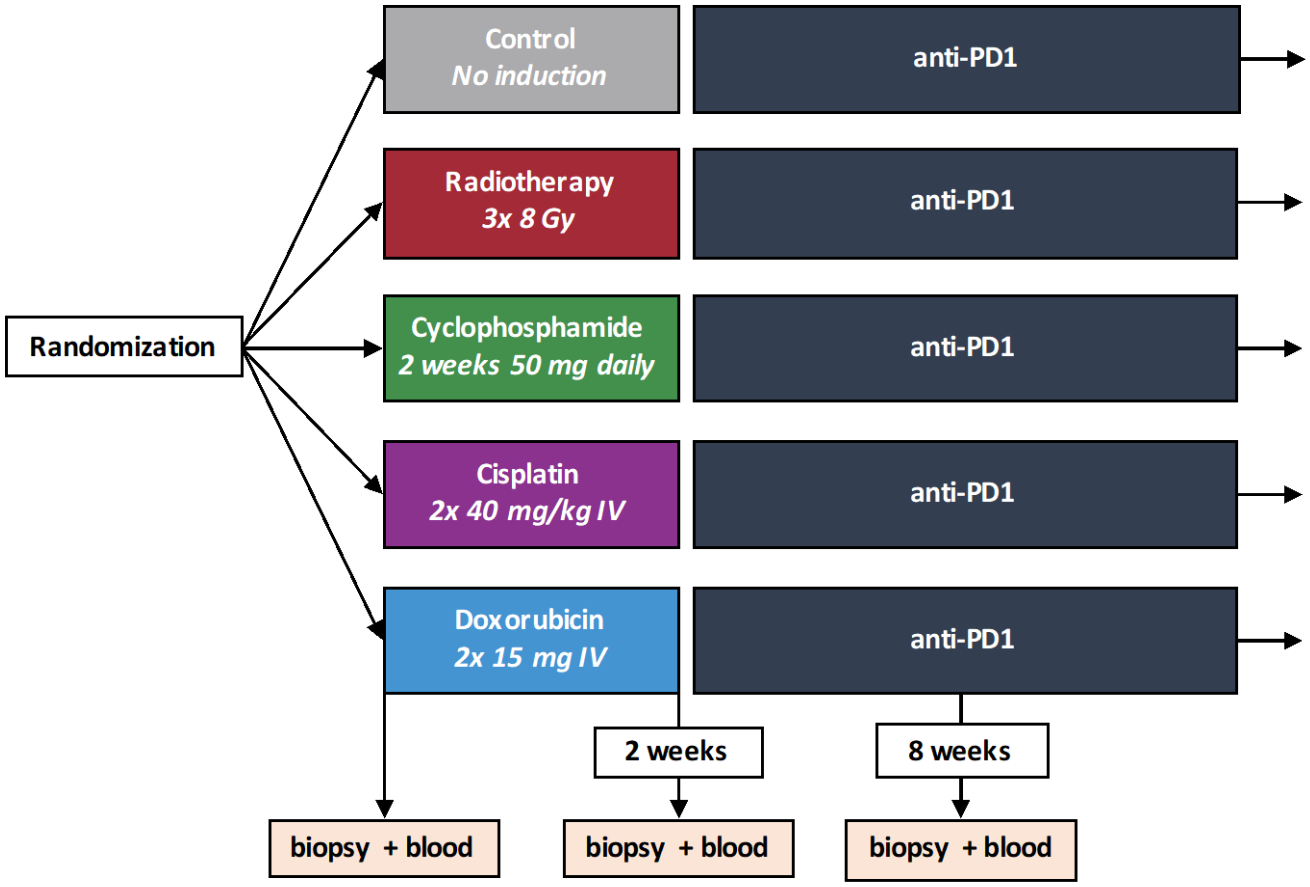
Adaptive phase II randomized trial with nivolumab after induction treatment in TNBC - TONIC trial -

Final results stage I and first translational data

Marleen Kok[1], Leonie Voorwerk[1], Hugo Horlings[1], Maarten Slagter[1], Karolina Sikorska[1], Koen van de Vijver[2], Sarah Warren[3], SuFey Ong[3], Terry Wiersma[1], Nicola Russell[1], Ferry Lalezari[1], Michiel de Maaker[1], Inge Kemper[1], Ingrid Mandjes[1], Myriam Chalabi[1], Lodewyk Wessels[1], Gabe Sonke[1], Roberto Salgado[4], Sabine Linn[1], Ton Schumacher[1] and Christian Blank[1]

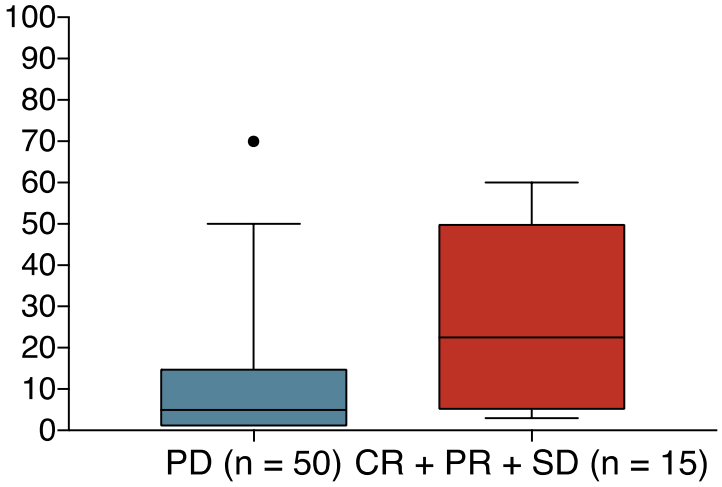
1. Netherlands Cancer Institute, Amsterdam, the Netherlands. 2. University Hospital Gent, Belgium, 3. NanoString technologies, Seattle, USA, 4. GZA Antwerp, Belgium

Voorwerk, Slagter et al. Nature Medicine, 2019



Voorwerk, Slagter et al. Nature Medicine, 2019

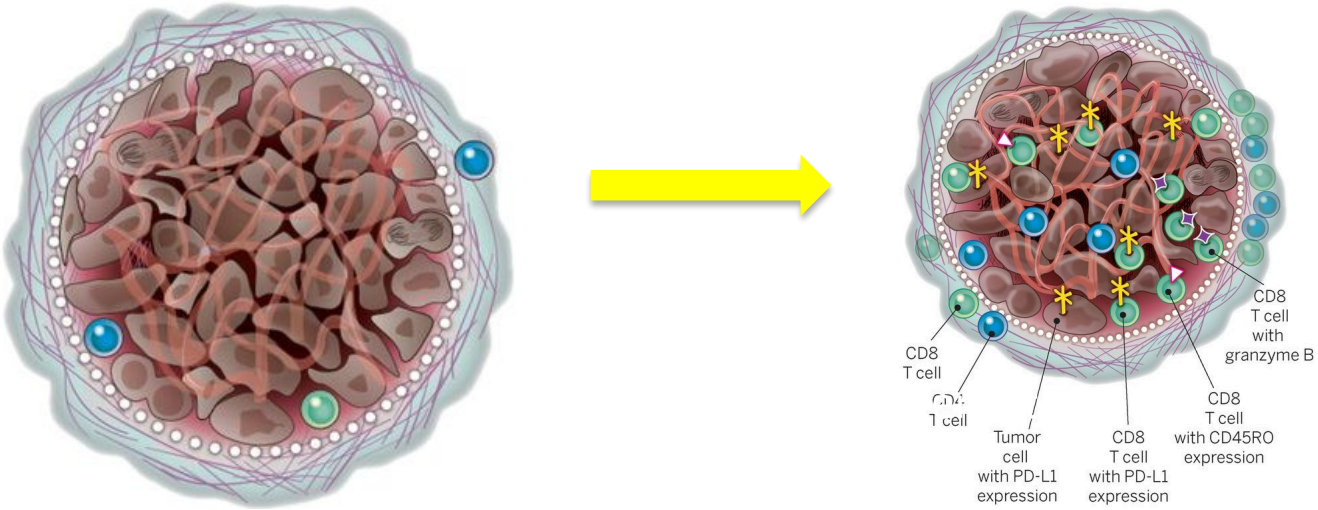
Stromal TIL associated with response



Mann-Whitney
** P < 0,01

Voorwerk, Slagter et al. Nature Medicine, 2019

Turning ‘cold’ into ‘hot’ tumor in TNBC
Can we do this?
Yes We Can



from: Sharma & Allison. Science 2015

The Dynamic Nature of the Immune System

- TNBC outcome is determined by systemic immunity, not cancer cell characteristics.
- That's why Grade is not helpful in predicting outcome in TNBC.
- High Grade and High proliferation is intrinsic to the subtype but does not help predicting outcome in patients.
- (Changes in) Systemic immunity does, that's why Immune therapy works in this subtype.
- On-treatment biopsies may be very useful.

IN TNBC, PROGNOSTIC BIOLOGY DETERMINES PREDICTIVE BEHAVIOUR TO IMMUNE THERAPY (and probably also in HER2+)

- In luminal HR+ disease the cancer cell characteristics (grade + tumor cell proliferation) determine outcome.
- Systemic immunity does not, that's why Immune therapy does not work in this subtype (yet).

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

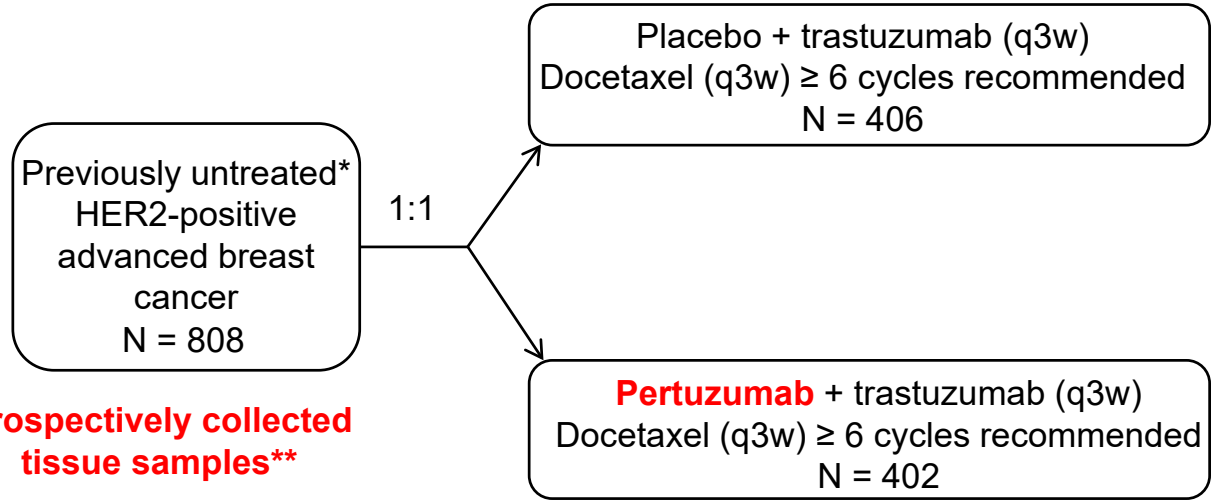
The Dynamic Nature of the Immune System

Prognostic associations of tumor-infiltrating lymphocytes (TILs) in advanced HER2-positive breast cancer treated with pertuzumab and trastuzumab: *a secondary analysis of the CLEOPATRA study*

Stephen J Luen*, Roberto Salgado, Stephen Fox, Peter Savas, Jennifer Eng-Wong, Emma Clark, Astrid Kiermaier, Sandra Swain, Jose Baselga, Stefan Michiels, Sherene Loi

*SABCS clinical scholar award 2016

CLEOPATRA clinical trial



Prospectively collected tissue samples**

After a median of follow up of 50 months:

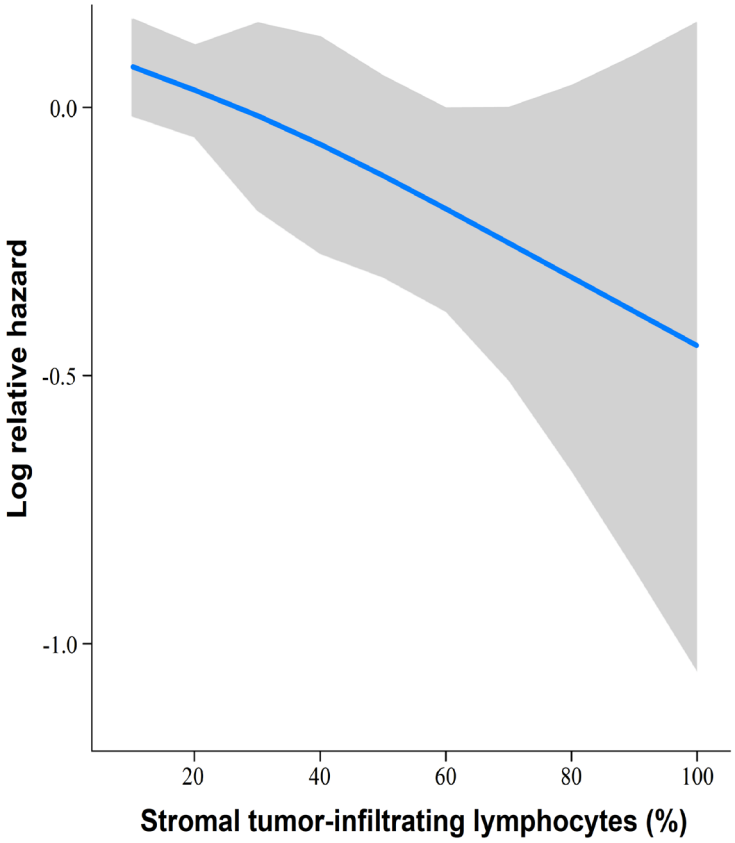
	Improvement in median survival	Hazard ratio (95% confidence interval)	*P* value
PFS	**6.3 months**	**0.68 (0.58 – 0.80)**	**< 0.001**
OS	**15.7 months**	**0.68 (0.56 – 0.84)**	**< 0.001**

* Prior neo(adjuvant) chemotherapy and/or trastuzumab allowed. Prior endocrine therapy allowed.
** Optional metastatic tumor tissue collection included in analyses

Swain et al, NEJM 2015

TIL effect is linearly related to survival

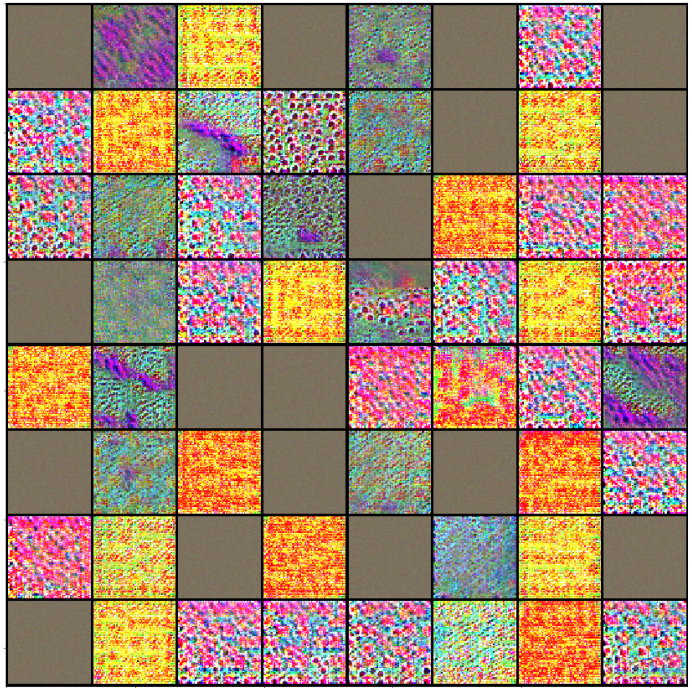
- **Plot demonstrating the log-relative HR for death vs stromal TIL per 10% increment**



Cubic smoothing spline for log relative hazard for death.
95% confidence interval shown in grey.

What may (not does) the future bring?

Artificial Intelligence and TILs



Visualisation of third layer features

The limits of my language means the limits of my world

Ludwig Wittgenstein

I do not think we need to be precise.
It is not always a good idea to be too precise.

The Uncertainty of Science
Richard Feynman

Nearing the final

What you see is all there is

What you see is all there is

We are doing nothing new

What you see is all there is

We are doing nothing new

The Narrative: Why should oncologists and patients need to know?

What you see is all there is

We are doing nothing new

The Narrative: Why should oncologists and patients need to know?

The limits of my language means the limits of my world

What you see is all there is

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The Narrative: Why should oncologists and patients need to know?

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I do not think we need to be precise.
It is not always a good idea to be too precise.

Are TILs a big deal?

If TILs are the answer, what is the question?

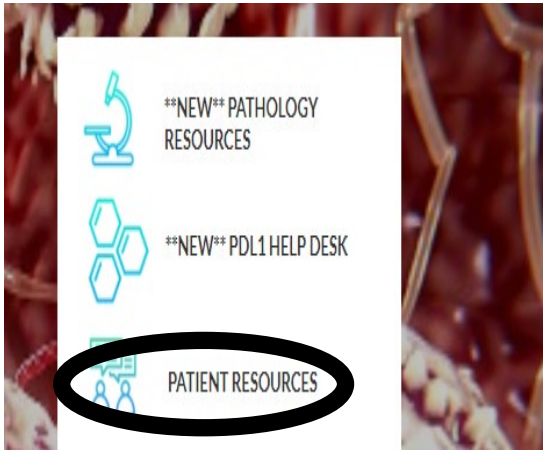
For patients

Submitted last week.
If you want to read it, let me know

The tale of TILs in breast cancer: A report from The International Immuno-Oncology Biomarker Working Group*

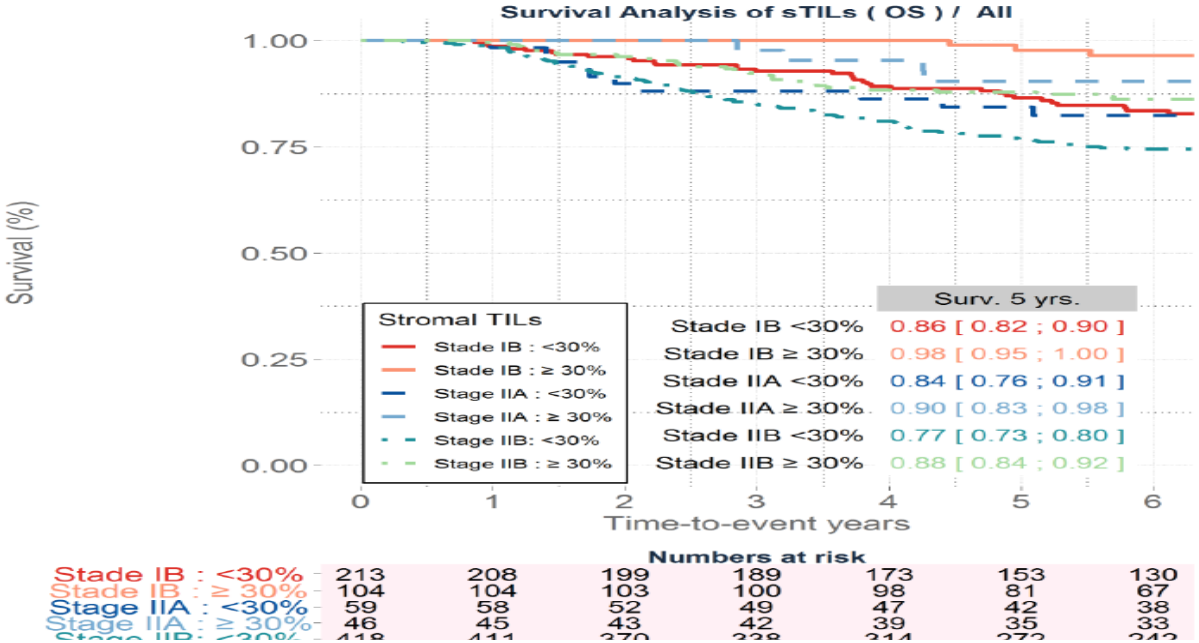
- *"Shouldn't we be seeking an easier and cheaper marker (e.g. TILs) to understand the stroma and direct treatment? I still feel very lucky that my TILs were scored in my pathology report. They've given me a more positive outlook, and this alone is a reason for scoring them."*

www.tilsinbreastcancer.org



For Clinicians

AJCC8-OS-All Treatments



AJCC8

Stade IA	Stade IB	Stade IIA	Stade IIB
-	T1N0G1	T0N1G1	T0N1G2
	T1N0G2	T1N1G1	T1N1G2
	T1N0G3	T2N0G1	T2N0G2
			T0N1G3
			T1N1G3
			T2N0G3

Manuscript in preparation

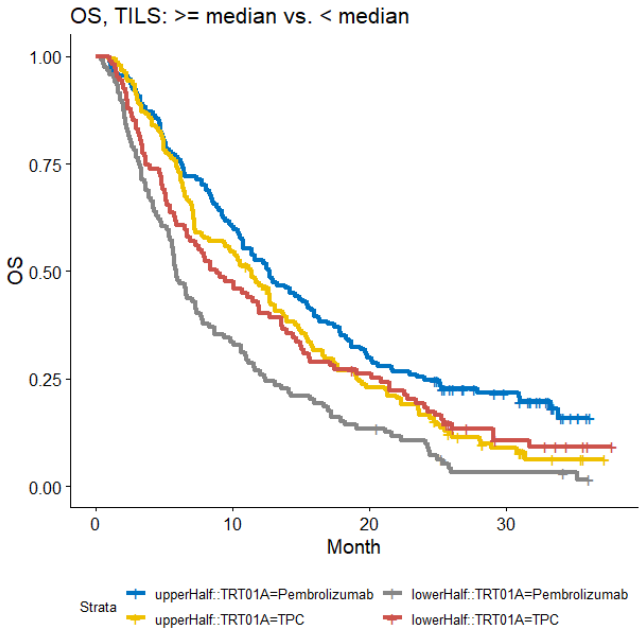
Relationship Between Tumor-Infiltrating Lymphocytes and Outcomes in the KEYNOTE-119 Study of Pembrolizumab Versus Chemotherapy for Previously Treated, Metastatic Triple-Negative Breast Cancer

S. Loi[1]; E. Winer[2]; O. Lipatov[3]; S.-A. Im[4]; A. Gonçalves[5]; J. Cortes[6]; K. S. Lee[7]; P. Schmid[8]; L. Testa[9]; I. Witzel[10]; S. Ohtani[11]; N. Turner[12]; S. Zambelli[13]; N. Harbeck[14]; F. Andre[15]; R. Dent[16]; L. Huang[17]; J. Mejia[17]; V. Karantza[17]; R. Salgado[1]

[1]Peter MacCallum Cancer Centre and University of Melbourne, Melbourne, VIC, Australia; [2]Dana-Farber Cancer Institute, Boston, MA, USA; [3]Republican Clinical Oncology Dispensary, Republic of Bashkortostan, Russian Federation; [4]Seoul National University, Seoul, South Korea; [5]Institut Paoli-Calmettes, Marseille, France; [6]IOB Institute of Oncology, Quiron Group, Madrid, and Vall d'Hebron Institute of Oncology, Barcelona, Spain; [7]National Cancer Center, Goyang, South Korea; [8]Barts Cancer Institute, Centre for Experimental Cancer Medicine, London, United Kingdom; [9]Instituto do Câncer do Estado de São Paulo – Faculdade de Medicina da Universidade do Estado do São Paulo, São Paulo, Brazil; [10]University Medical Center Hamburg, Hamburg, Germany; [11]Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan; [12]The Royal Marsden NHS Foundation Trust, London, United Kingdom; [13]San Raffaele Scientific Institute, Milan, Italy; [14]Breast Center, University of Munich, Munich, Germany; [15]Université Paris-Sud, Orsay, and Gustave Roussy, Villejuif, France; [16]University of Toronto, Toronto, ON, Canada; [17]Merck & Co., Inc., Kenilworth, NJ, USA

OS in subpopulation defined by TILs median cutpoint (TILs ≥ 5% vs. < 5%)

- For TPC arm, the yellow and red curves represent the TILs≥5% and TILs<5%, with little difference observed
- For Pembro arm, there is separation according to the median TILS cut-off consistent with testing as a continuous measure



Loi et al., SABC2019

For pathologists

	FFPE	Easy	Standardized	Reproducible	Well-documented
Histological (sub)type	V	V	V	+/-	V
Grade	V	V	V	+/-	V
Size + extent	V	V	V	V	V
Proliferation	V	V	V	+/-	V
Perineural Invasion	V	V	+/-	+/-	V
Lymphovascular Invasion	V	V	+/-	+/-	V
Necrosis	V	V	+/-	+/-	V
Pharmacodiagnostic biomarker	V	V	V	+/-	V
TILs	V	V	V	V	V

npj Breast Cancer

www.nature.com/npjbcancer

ARTICLE OPEN

Check for updates

Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer

Zuzana Kos et al.[#]

Table 1. Comparison of intraclass correlation coefficient and pair-wise observer concordance rate for 3 ring studies.

	Ring study 1	Ring study 2	Ring study 3
ICC	0.7 (0.62–0.78)	0.89 (0.85–0.92)	0.76 (0.69–0.83)
Concordance rates[a]			
TILs <1 vs ≥1%	0.94 (±0.08)	0.94 (±0.04)	0.91 (±0.06)
TILs <5 vs ≥5%	0.83 (±0.09)	0.89 (±0.05)	0.84 (±0.1)
TILs <10 vs ≥10%	0.77 (±0.08)	0.86 (±0.05)	0.79 (±0.06)
TILs <30 vs ≥30%	0.81 (±0.08)	0.93 (±0.03)	0.87 (±0.04)
TILs <75 vs ≥75%	0.90 (±0.06)	0.92 (±0.03)	0.94 (±0.03)

ICC intraclass correlation coefficient, TILs tumor-infiltrating lymphocytes.
[a]The concordance of all pairs of pathologists was calculated for five different TIL-groups. The values in the table are the sample mean and sample standard deviation of these concordance rates for all pairs of pathologists in each study.

Danish Breast Cancer Group
www.dbcg.dk
Danish guidelines for breast cancer treatment

dbcg.dk/vaerktoejer/retningslinjer-vejledninger/

DBCG

Værktøjer Udvalg Kvalitetsdatabasen Aktuelt Om DBCG

- Pathology procedures and molecular-biomarker analyses in breast cancer.
- Registration of TILs is included in the updated 2020 Danish pathology guidelines for especially ER – and HER2 negative as well as HER2 positive breast cancer.
- Registration of TILs is optional.
- **Allthough TILs is not included in the Danish oncology guidelines (yet) the pathologists found it important to start registration in order to train and get familiar with the analysis of this important biomarker.**

Retningslinjer

1. **DBCG** 38 KB (Indholdet sidst opdateret 15. juni 2011)
2. **Diagnose** 116 KB (Indholdet sidst opdateret 24. maj 2013)
3. **Patologiprocedurer og molekylærpatologiske analyser ved brystkræft, link til DMCG** (Indholdet sidst opdateret 31. April 2020)
4. **Kirurgisk behandling, link til DMCG** (Indeholdet sidst opdateret 16. september 2019)
5. **Postoperativ strålebehandling** 310 KB (Indholdet sidst opdateret 21. juni 2019)
6. **Medicinsk behandling** 412 KB (Indholdet sidst opdateret 21. februar 2019)
7. **Neoadjuverende kemoterapi ved brystkræft mhp. down-sizing og down-staging** 864 KB (nyt kapitel, 4. Oktober 2016)
8. Kapitlet er udgået (29. februar 2016)
9. **Opfølgning** 111 KB (Indholdet sidst opdateret 08. maj 2015)
10. **Duktalt carcinoma in situ (DCIS)** 55 KB (Indholdet sidst opdateret 13. december 2012)
11. **Lobular carcinoma in situ (LCIS)** 50 KB (Indholdet sidst opdateret 01. januar 2006)
12. Kapitlet er udgået (December 2019)
13. **Rekommandationer vedr. brystrekonstruktion** 101 KB (Indholdet sidst opdateret 01. januar 2006)
14. **Primær lokal og regional fremskreden cancer mammae, link til DMCG** (Indholdet sidst opdateret 15. september 2020)
15. **Behandling af brystkræft hos kvinder på 75 år eller ældre og/eller kvinder uanset alder med høj komorbiditet** 56 KB (Nyt kapitel 12. oktober 2009)
16. **Udredning og behandling af brystkræft i forbindelse med graviditet** 53 KB (Indholdet sidst opdateret 01. januar 2011)
17. **Lokoregionært recidiv** 85 KB (Indholdet sidst opdateret 27. april 2011)
18. **Behandling af cancer mammae med fjernmetastaser** 561 KB (Indholdet sidst opdateret 21. februar 2019)
19. **Arvelig cancer mammae - ovarii** 424 KB (Indholdet sidst opdateret 23. september 2016)
20. **Anbefaling om bevægelse/træning til kvinder opereret for brystkræft indenfor de første 8 uger postoperativt** (Indholdet sidst opdateret 02. oktober 2014)
21. **Forløbsbeskrivelse for fysioterapi under brystkirurgiske patientforløb** 82KB (Nyt kapitel; 5. december 2011)
22. **Vejledning til udarbejdelse af retningslinjer** 38 KB (Indholdet sidst opdateret 01. januar 2006)

KLINISKE RETNINGSLINJER | KRÆFT

Patologiprocedurer og molekylærpatologiske analyser ved brystkræft

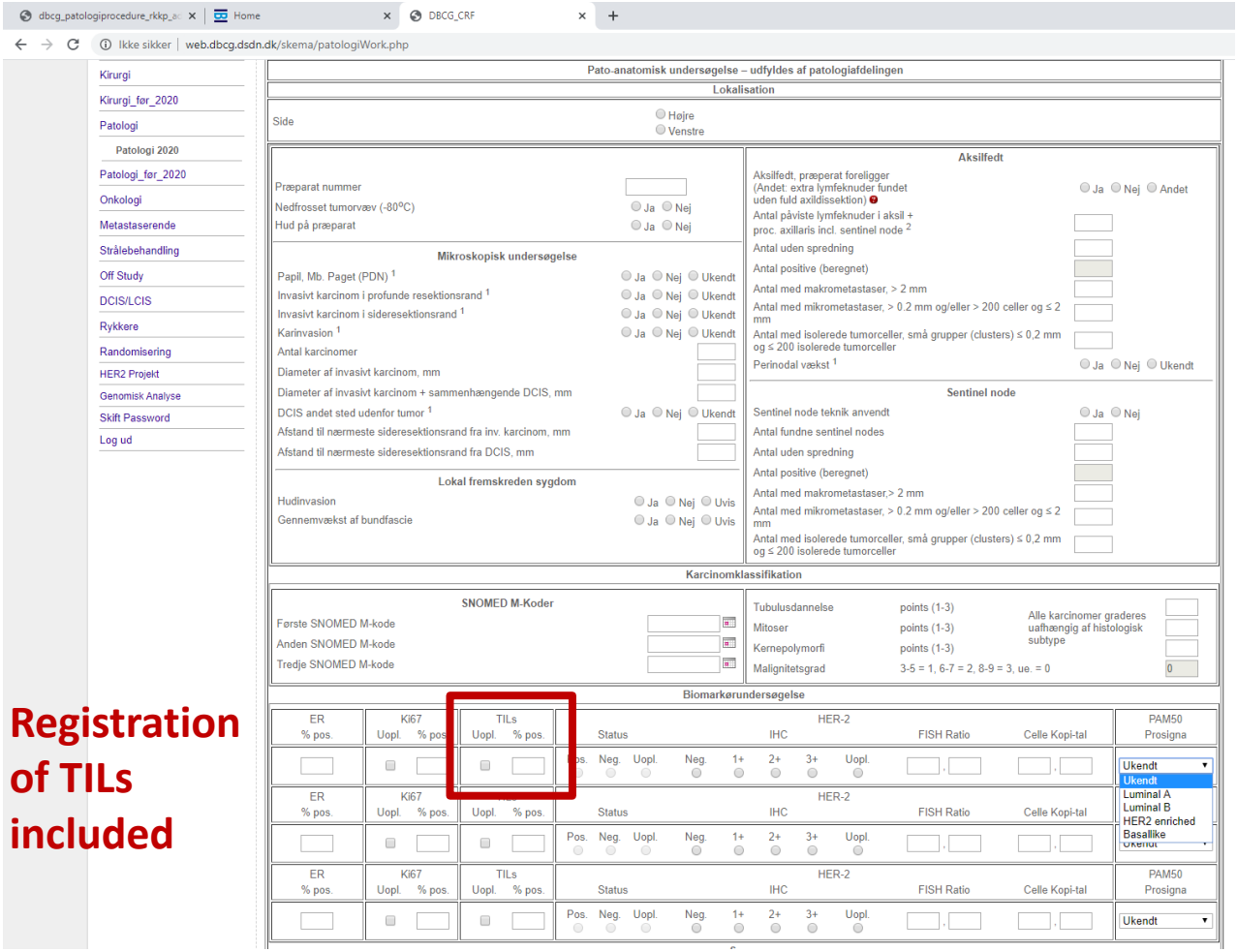
Version 1.0

GODKENDT
Faglig godkendelse
26. februar 2020 (DBCG)
Administrativ godkendelse
30. april 2020 (Sekretariatet for Kliniske Retningslinjer på Kræftområdet)

REVISION
Planlagt: 1. marts 2023

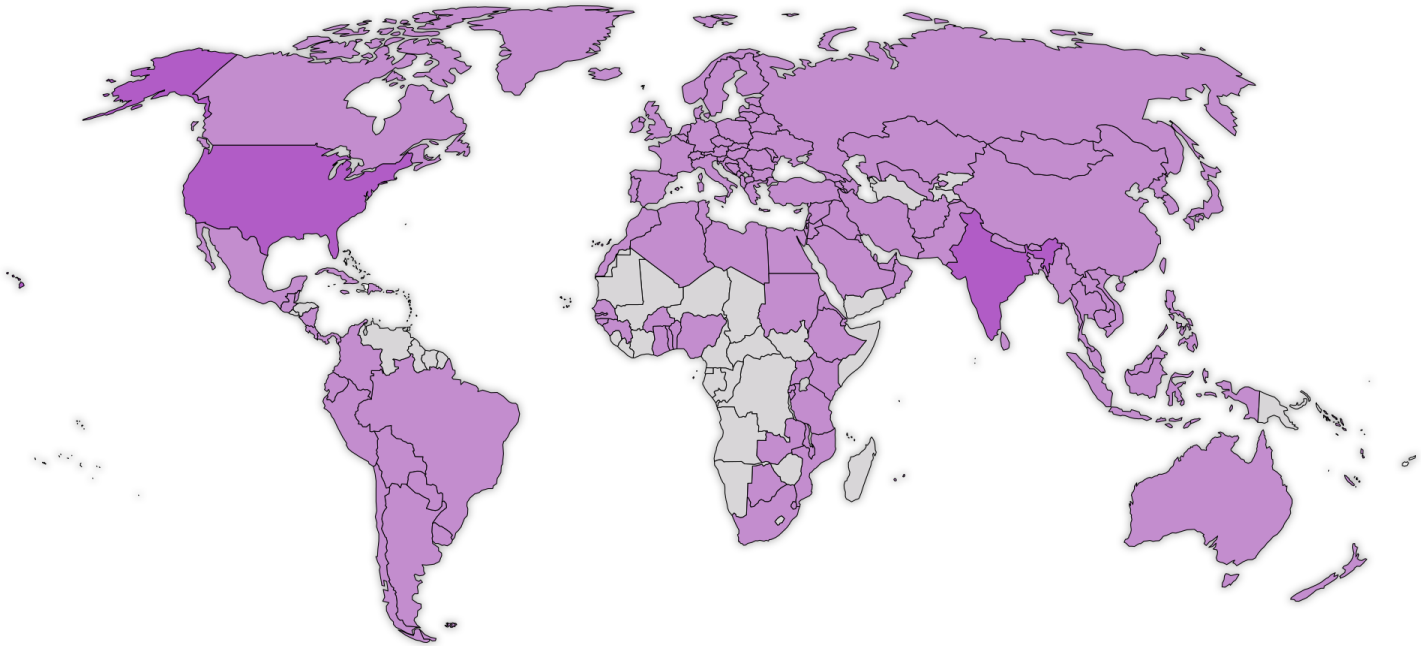
INDEKSERING
Anbefaling, patologi, molekylærpatologi, brystkræft

- All clinico-pathological data are registrered in the DBCG database (web-registration).
- Pathology web-schemes for each procedure, here exemplified by registration of pathology data after lumpectomy.
- Separate web-schemes in the neo-adjuvant setting including reporting of RCB class.
- The biomarker section is identical for all schemes (for up to three tumours in multifocal disease).
- The pathology report and web-scheme also include information of prognostic gene signature.
- **Annual publication regarding quality af the reported data – as part of the The Danish Clinical Quality Program – National Clinical Registries (RKKP) which constitutes the infrastructure of the Danish clinical quality registries and the Danish Multidisciplinary Cancer Groups (DMCG).**



Registration of TILs included

Pathologists from all these countries are involved in the TIL-WG



KU LEUVEN

Bristol-Myers Squibb

EQA to optimise TIL and PDL1 estimation in Breast Cancer for prognosis and therapy response prediction

TILS BREAST CANCER
INTERNATIONAL IMMUNO-ONCOLOGY WORKING GROUP

Dr. Roberto Salgado, co-chair of the International Immuno-Oncology Working Group (TILs-WG), co-chaired with Sherene Loi, Carsten Denkert, Stefan Michiels. Co-leads of this project: Hugo Horlings, Netherlands Cancer Institute; Siziopikou Kalliopi, Northwestern University, US; John Bartlett, Canada/UK (Biobank), Giuseppe Floris, UZ Leuven.

Prof. Dr. Els Dequeker, KU Leuven

Inne Nauwelaers, KU Leuven

eqa KU LEUVEN BIOMEDICAL QUALITY ASSURANCE

Start early 2021

To Industry and Regulatory

Who defines clinical practice?

How current assay approval policies are leading to unintended imprecision medicine

*Roberto Salgado, Andrew M Bellizzi, David Rimm, John M S Bartlett, Torsten Nielsen, Moch Holger, Anne-Vibeke Laenkholm, Cecily Quinn, Gábor Cserni, Isabela W Cunha, Isabel Alvarado-Cabrero, Ian Cree
roberto@salgado.be

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Panel: Solutions to improve the current assay approval pathway

- Industry should be mandated to do concordance studies with other similar assays or standardised controls before a drug is approved
- Industry should support, in concert with all stakeholders, relabelling or revising approved companion diagnostics if evidence exists that the labelling might lead to uncertainty in the identification of patients for treatments
- Industry should support, in concert with all stakeholders, relabelling or revising of the companion diagnostics if equivalent clinical validity has been shown with other biomarkers or standards, providing access to clinical trial tissues to validate other assays
- Industry, when considering the incorporation of assays in their trials, should communicate and share assay information when using an assay that identifies the same molecule (eg, epitope, antigen, DNA, RNA) as in other competitive trials—eg, method information related to the binding sites of the antibodies used in the companion diagnostic assay should be made public, even if this information is commercially sensitive
- Pathways for regulatory acceptance of other assays that are equivalent, but less expensive and easier to implement in daily practice, should be developed by governments and regulatory agencies, ideally before a drug is labelled together with a companion diagnostic
- Early engagement by all stakeholders in external quality control schemes to allow rapid development of guidelines and quality standards is essential, preferably before an assay is approved by the regulatory agencies
- Clinical practice guidelines developed by professional organisations like the American Society of Clinical Oncology and the European Society for Medical Oncology should endorse not just a companion diagnostic assay used in the trial, but any rigorously and technically validated equivalent laboratory assays that can define essentially the same population as the companion diagnostic
- Regulators should require data confirmation of the analytical validity of the companion diagnostic in the distributed setting in which it would be applied, at a level of rigor similar to that required to show efficacy of the drug in question

In conclusion

Importance of **collaboration**

Taking a new biomarker into daily practice should be in agreement between **pathologists, oncologists, patients, regulatory** and **industry.**

FROM THE TIL-MOVIE
COMING SOON!

Thank you to all of you
Thank you to many others also

Matthew Goetz, Matthew Dacy, Cynthia Chauchan, Bobbi-Ann Jebens, Jodie Carter, Nakhleh Raouf, Roberto LF, Aubrey Thompson, Edith Perez, Sherene Loi, Peter Savas, David Moore, Paula, Melinda, Crispin Hiley, Maise Al-Bakir, Charles Swanton, Stephen Luen, Jeannette, Sylvia Adams, Sandra Demaria, Sunil Badve, Giuseppe Floris, Christine Desmedt, Iris, Leonie, Jan, Marleen, Hugo, Stefan, Fabrice, Beppe V., TIL-WG, Carsten Denkert, Sybille, Fraser, Elia, Jorge RF, Mark R, David R., Federico R., Lajos P., David S., Zuzana, Torsten, John B., Rim K., Tracy L., Ken Emancipator, Jon J., Luciana M., Ian Cree, Giancarlo, Maria Vittoria, etc…

THANK YOU

Roberto@salgado.be

All wellcome to join the TIL-WG
Just mail me

BCRF BREAST CANCER RESEARCH FOUNDATION

TILS BREAST CANCER
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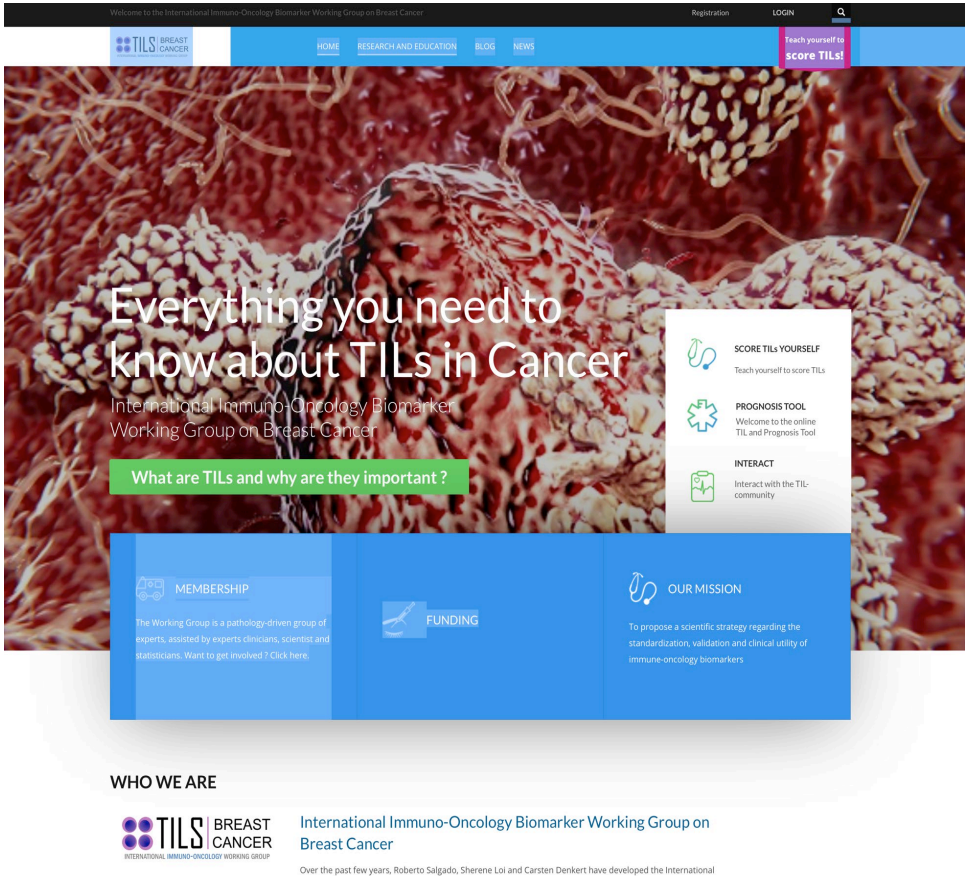

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How current assay approval policies are leading to unintended imprecision medicine

Lancet Oncol 2020
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Pathologists are responsible for selecting the assays for the optimal identification of patients for targeted therapy. The current paradigm of regulatory assay approval is that when a clinical trial involving a drug and a biomarker, using a specific assay to identify patients that might respond to the drug, meets its endpoint, the assay is approved concomitantly as a companion diagnostic. Private health insurance bodies or public health systems then decide on reimbursement of the assay when they decide on the reimbursement of the drug. Use of US Food and Drug Administration (FDA)-approved assays is obligatory in some countries, like the USA and Japan, to gain access to the drug. In the EU, the use of an FDA-approved assay is not mandatory to gain access to the drug, as long as the laboratory-developed test or assay that is used is validated.

Thresholds for defining a positive biomarker in a clinical trial, and what constitutes a positive biomarker, are not standardised. Moreover, companion assays are co-developed with a drug, as determined by the pharmaceutical company in collaboration with the company contracted to produce the assay, without regard to the other assays being developed for the same biomarker. For example, PD-L1 assay kits are approved by the FDA in 15 different cancer types but the PD-L1 staining patterns, scoring methods, and positivity thresholds are different in almost all of these cancer types. Moreover, the various assays and scoring systems are not equivalent, despite being matched to the same specific drug. There are at least five non-equivalent assays for PD-L1, each with its own scoring system and tumour site indications.

Absence of assay standardisation is an emerging issue for triple-negative breast cancer. In 2019, considering the results of the IMpassion130 trial, the FDA approved the Ventana PD-L1 (SP142) assay (Ventana Medical Systems, Tucson, AZ, USA) and cut-point (1% of tumour-infiltrating immune cells) to assess PD-L1 in patients with triple-negative breast cancer treated with atezolizumab.[1] However, following the Keynote 355 breast cancer trial,[2] the results of which were publicised in 2020, investigating pembrolizumab in the same patient population, the FDA is likely to approve the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, Carpinteria, CA, USA) and its combined positivity score-scoring system to assess PD-L1. Using more than one assay for the same biomarker is problematic because the assays have different positive prevalence rates. In the IMpassion130 trial, 46% of patients with triple-negative breast cancer were deemed to be positive using the Ventana PD-L1 (SP142) assay; when using other assays (eg, the PD-L1 IHC 22C3 pharmDx assay) in the same patients, the PD-L1 positive prevalence increased to nearly 80%.[3] The cause of these inconsistencies is multifactorial and includes reproducibility issues and variable antibody and assay sensitivity, even when different assays use the same antibody.[4-6] One issue is the balance of risk, costs, and benefit. If treatment recommendations differ depending on the assay that is used, it is difficult for health-care providers to reliably analyse the cost-effectiveness for reimbursement of that particular treatment. Costs are arguably even more important in low-income and middle-income countries. Some private insurance companies or governments insist on the use of FDA-approved assays, which are more expensive than laboratory-developed tests. A concerning situation is if patients underwent unnecessary toxicity and extra costs due to potentially false-positive tests. However, lower sensitivity of an assay, with potentially false-negative results, could lead to fewer patients receiving therapy and benefit. Some oncologists might prefer their pathologist to use an FDA-approved assay, despite being unaware of the analytical validity of the assay and the fact that many laboratory-developed tests can perform as well as FDA-approved companion diagnostics.[7] Others might prefer an assay with a higher positive prevalence to identify more patients that can be treated.

Different assays, different platforms, different positivity thresholds, and a divergent international approach to reimbursement of these assays suggest that patients are not well served by the current system. Industry, regulatory agencies, governments, clinicians, and patients also need to be aware that a positive phase 3 trial does not guarantee consistency, reproducibility, and practicality of the biomarker-specific assay used in

Panel: Solutions to improve the current assay approval pathway

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For more on the **International Immuno-Oncology Biomarker Working Group** see www.tilsinbreastcancer.org

the trial. Furthermore, the use of a suboptimal assay might lead to inconsistent trial outcomes when used in different trials investigating the same drug in the same patient population leaving, for example, the best method to select patients for immunotherapy still uncertain.

We propose solutions to industry (pharmaceuticals and diagnostics), academia, patients, governments, and regulatory agencies (panel) who currently hold the keys to resolving the issues outlined here. The current assay approval pathway should be updated to reflect current realities, including mandating a detailed assessment of the analytical validity of an assay before it is considered as a companion diagnostic.

Although PD-L1 is the latest diagnostic challenge, it is neither the first nor will it be the last such challenge to face the community unless focused efforts in a partnership between all stakeholders are directed towards standardisation of assay development for both current and future applications.

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**Roberto Salgado, Andrew M Bellizzi, David Rimm, John M S Bartlett, Torsten Nielsen, Moch Holger, Anne-Vibeke Laenkholm, Cecily Quinn, Gábor Cserni, Isabela W Cunha, Isabel Alvarado-Cabrero, Ian Cree*
roberto@salgado.be

Division of Research, Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia (RS); Department of Pathology, GZA-ZNA Hospitals, Wilrijk, Antwerp, Belgium (RS); Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA, USA (AMB); Department of Pathology, Yale School of Medicine, New Haven, CT, USA (DR); Diagnostic Development, Ontario Institute for Cancer Research, Toronto, ON, Canada (JMSB); Department of Pathology and Laboratory Medicine, Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, BC, Canada (TN); Department of Pathology and Molecular Pathology, University Hospital Zurich, University of Zurich, Zurich, Switzerland (MH); Department of Surgical Pathology, Zealand University Hospital, Roskilde, Denmark (A-VL); Department of Pathology, St Vincent's University Hospital, Dublin, Ireland (CQ); Department of Pathology, Bács-Kiskun County Teaching Hospital, Kecskemét, Hungary (GC); Department of Pathology, University of Szeged, Szeged, Hungary (GC); Institute of Anatomical Pathology, Rede D'Or São Luiz Hospitals Network, São Paulo, Brazil (IWC); D'Or Institute for Research and Education, Rio de Janeiro and São Paulo, Brazil (IWC); Department of Pathology, Hospital de Oncología, CMN, SXXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico (IA-C); and International Agency for Research on Cancer, World Health Organization, Lyon, France (IC)

1 Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab- paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018; **379:** 2108–21.
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3 Rugo HS, Loi S, Adams S, et al. Performance of PD-L1 immunohistochemistry (IHC) assays in unresectable locally advanced or metastatic triple-negative breast cancer (MTNBC): post-hoc analysis of Impassion130. *Ann Oncol* 2019; **30** (suppl 5)**:** v851–59 (abstr).

4 Reisenbichler ES, Han G, Bellizzi A, et al. Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. *Mod Pathol* 2020; **33:** 1746–52.

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7 Kim AS, Bartley AN, Bridge JA, et al. Comparison of laboratory-developed tests and FDA- approved assays for BRAF, EGFR, and KRAS testing. *JAMA Oncol* 2018; **4:** 838–41.

The Planning Committee would like to thank you for attending and participating in the Workshop. Please contact the Committee any questions.

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