

LBCA Advocate Chat Series 2023

**Introduction to Cancer Basic Science and Immunology:
A Conversation with Dr. Ramlah Nehring and Dr. Sasha Stanton**



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Introduction to Cancer Basic Science and Immunology



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Disclaimer

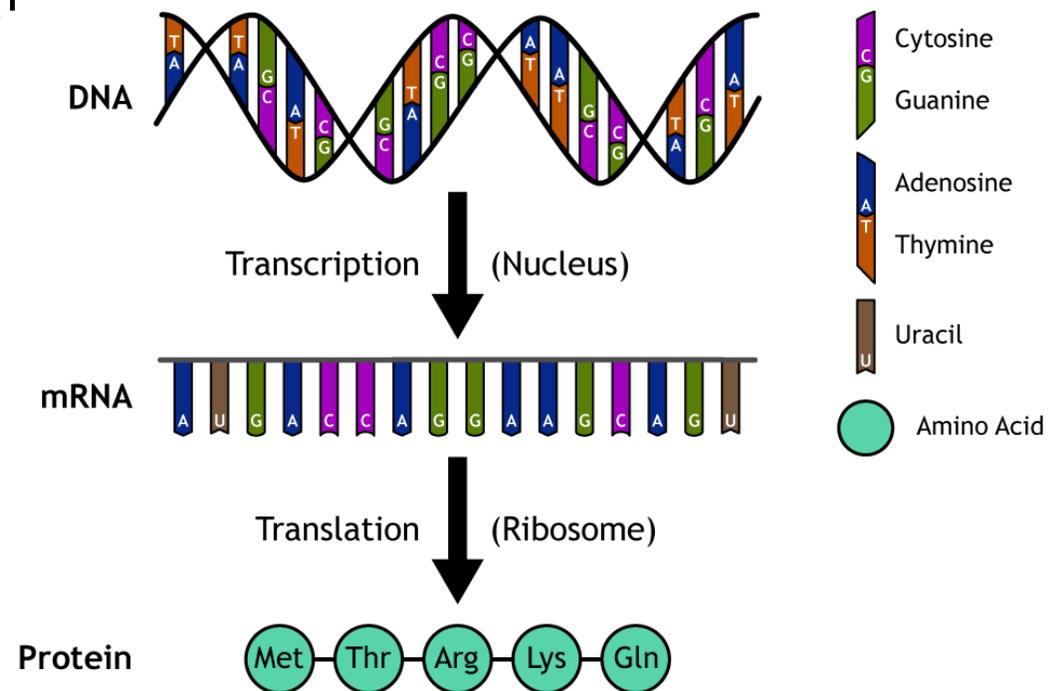
- Ramlah Nehring is an employee of Roche Diagnostics.
- The views and opinions expressed in the following PowerPoint slides are those of the individual presenter and should not be attributed to her employer or seen as an endorsement of any products.
- The information provided here is for general purposes only. It is not medical advice and is not a substitute for consulting with your healthcare team. Only your healthcare team can make a diagnosis or treatment recommendation. Always discuss individual symptoms and any questions you may have with your healthcare team.

Outline Cancer Basic Science

1. Overview of DNA, RNA, Proteins and epigenetics
2. DNA basis of cancer, mutations, and resistance
3. Tumor suppressors and oncogenes
4. Known genomic / molecular hallmarks of IDC and ILC
5. Diagnostic testing and applications
 1. Hereditary genetic testing
 2. Prognostic
 3. Therapy selection
 4. MRD / monitoring and circulating DNA (challenges with breast cancer)

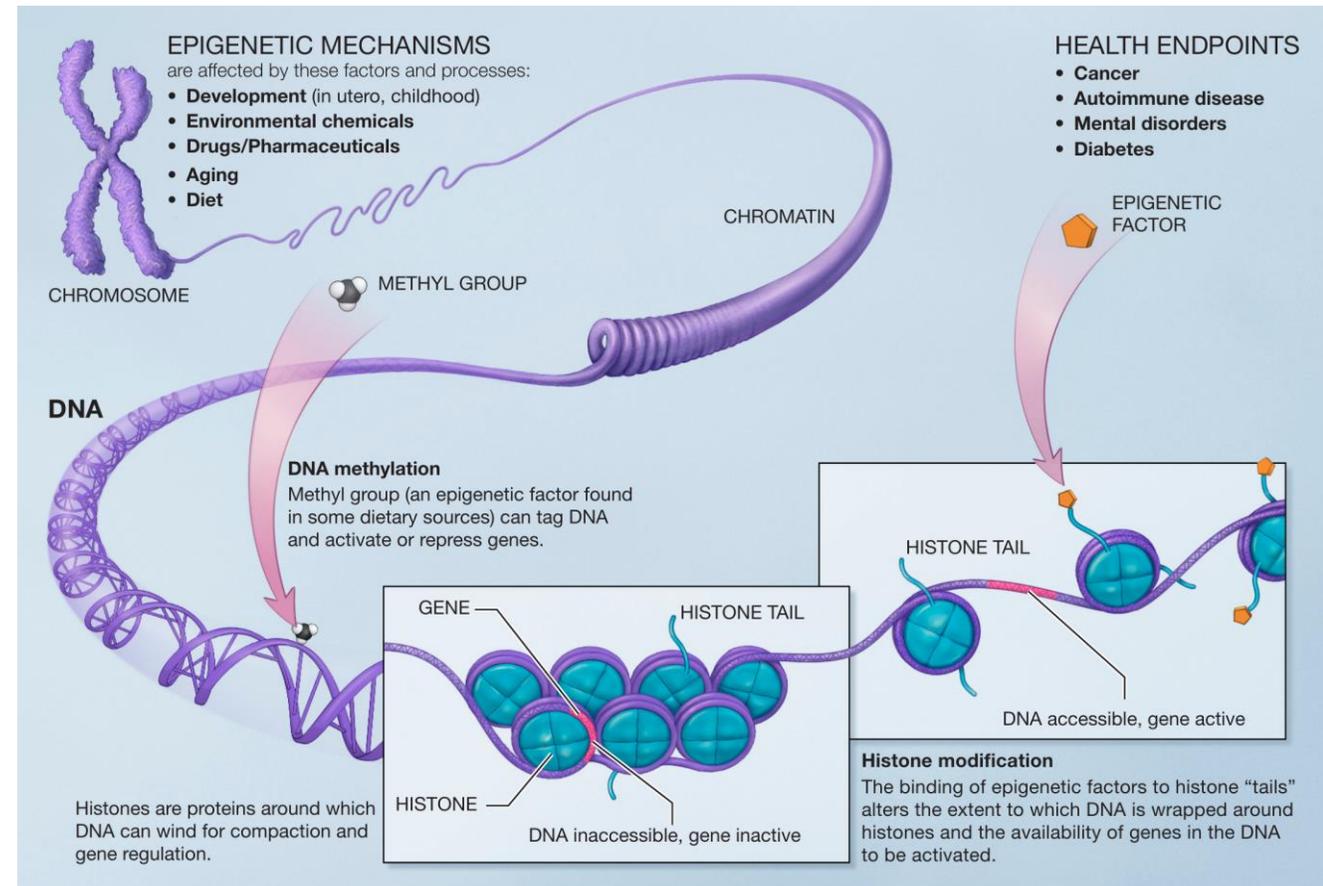
DNA -> RNA -> Proteins and all the “omics”

- **The central dogma of genetics:** DNA is transcribed into RNA, which is translated into protein.
- DNA is composed of the nucleotides adenosine, thymine, guanine and cytosine (A,T,G,C). RNA is composed of the nucleotides cytosine, guanine, adenosine, and uracil. Protein is composed of amino acids.
- Genomics = study of genome
- Transcriptomics = study of transcriptome (RNA)
- Proteomics = study of proteome (Proteins)



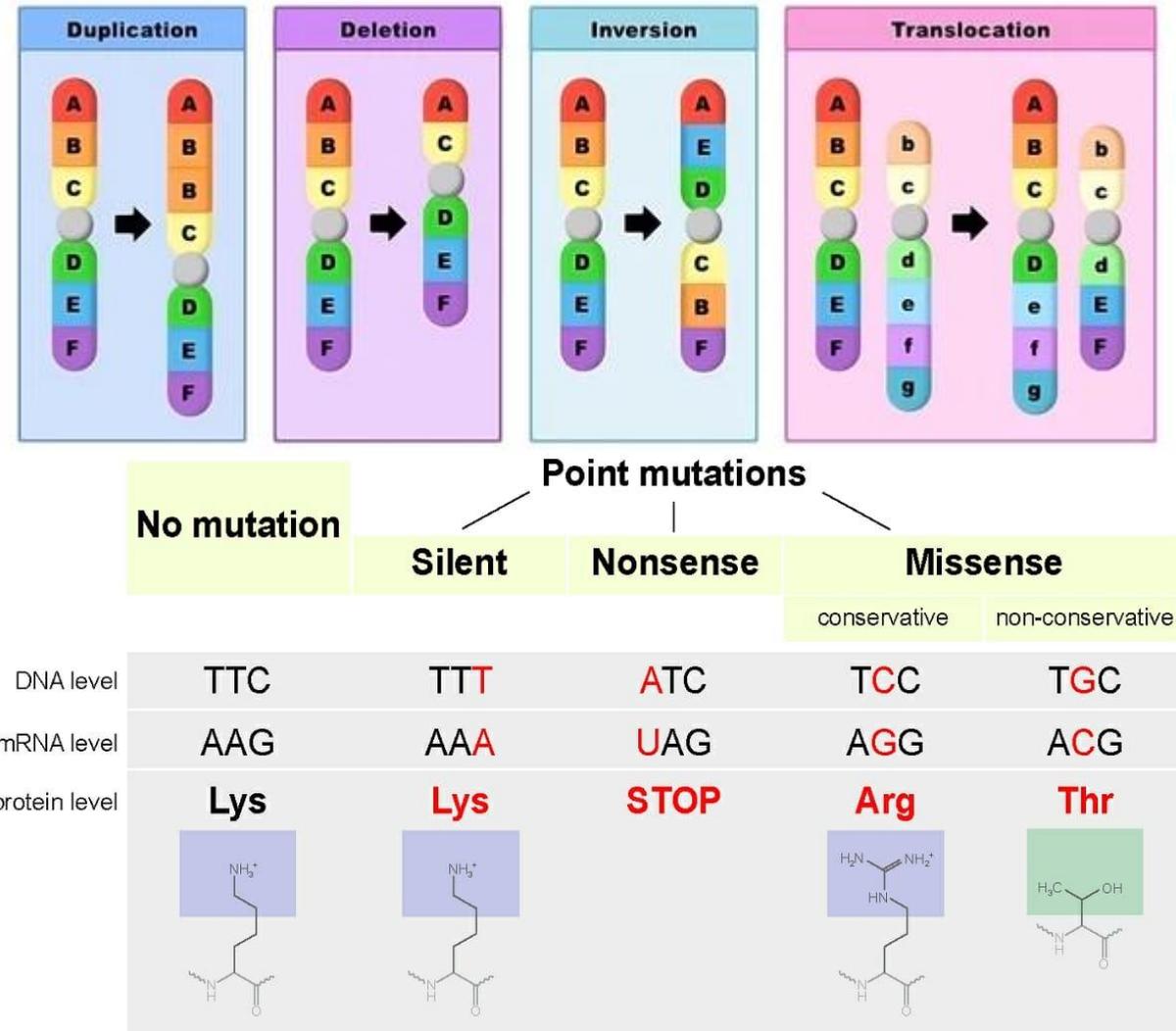
Histones, DNA packaging and Epigenetics

- Epigenetics is **the study of how your behaviors and environment can cause changes that affect the way your genes work**. Unlike genetic changes, epigenetic changes are reversible and do not change your DNA sequence, but they can change how your body reads a DNA sequence.
- Epigenetic processes, including DNA methylation, histone modification and various RNA-mediated processes, are thought to influence gene expression at the level of transcription.



Mutations

- Gene variants (also known as mutations) can have varying effects on health, depending on where they occur and whether they alter the function of essential proteins.
- In addition to gene mutations there can also be copy number changes, chromosomal changes or epigenetic changes that impact proteins.
- Example: Covid delta vs omicron

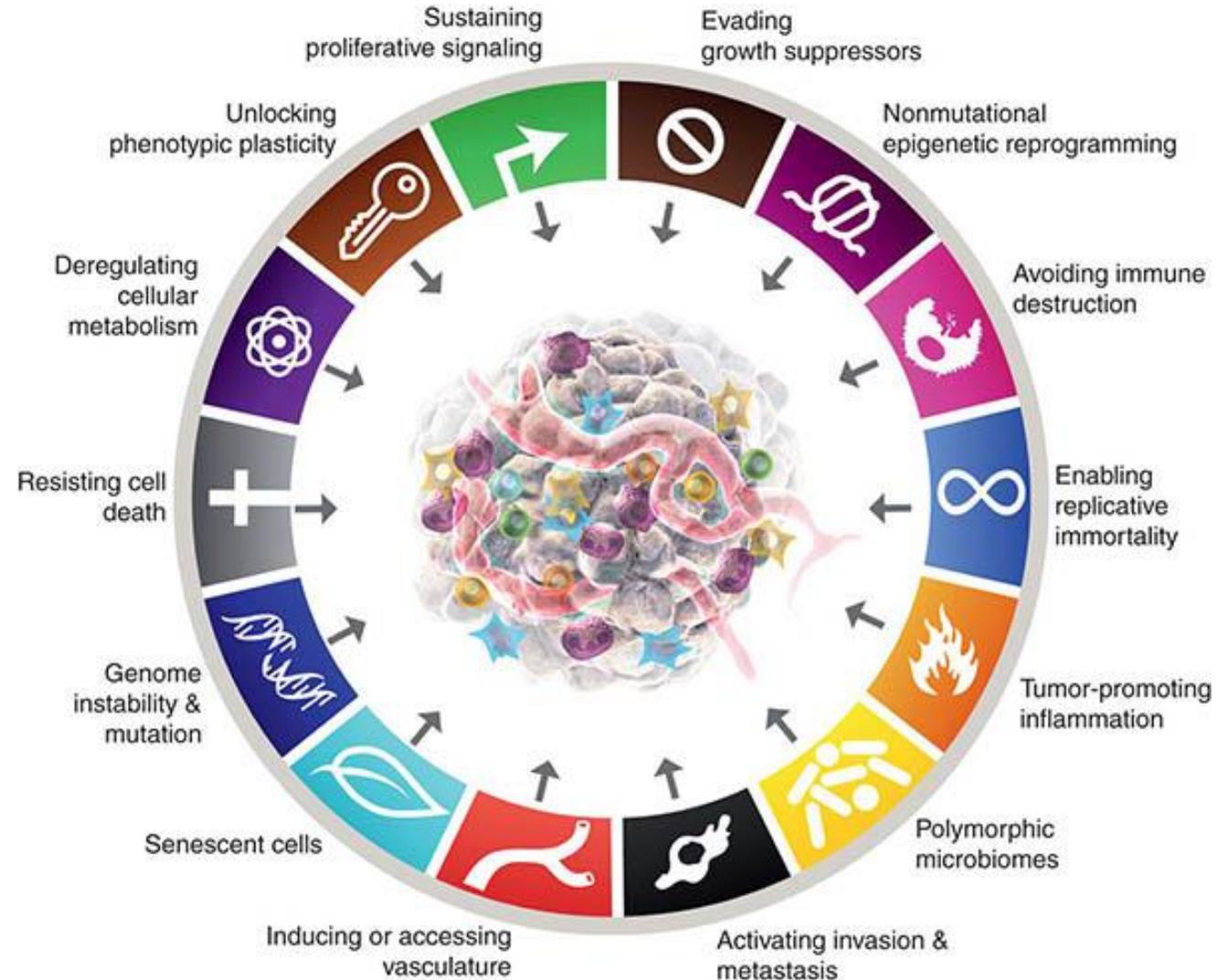


Neoplasm / Tumor / Carcinoma

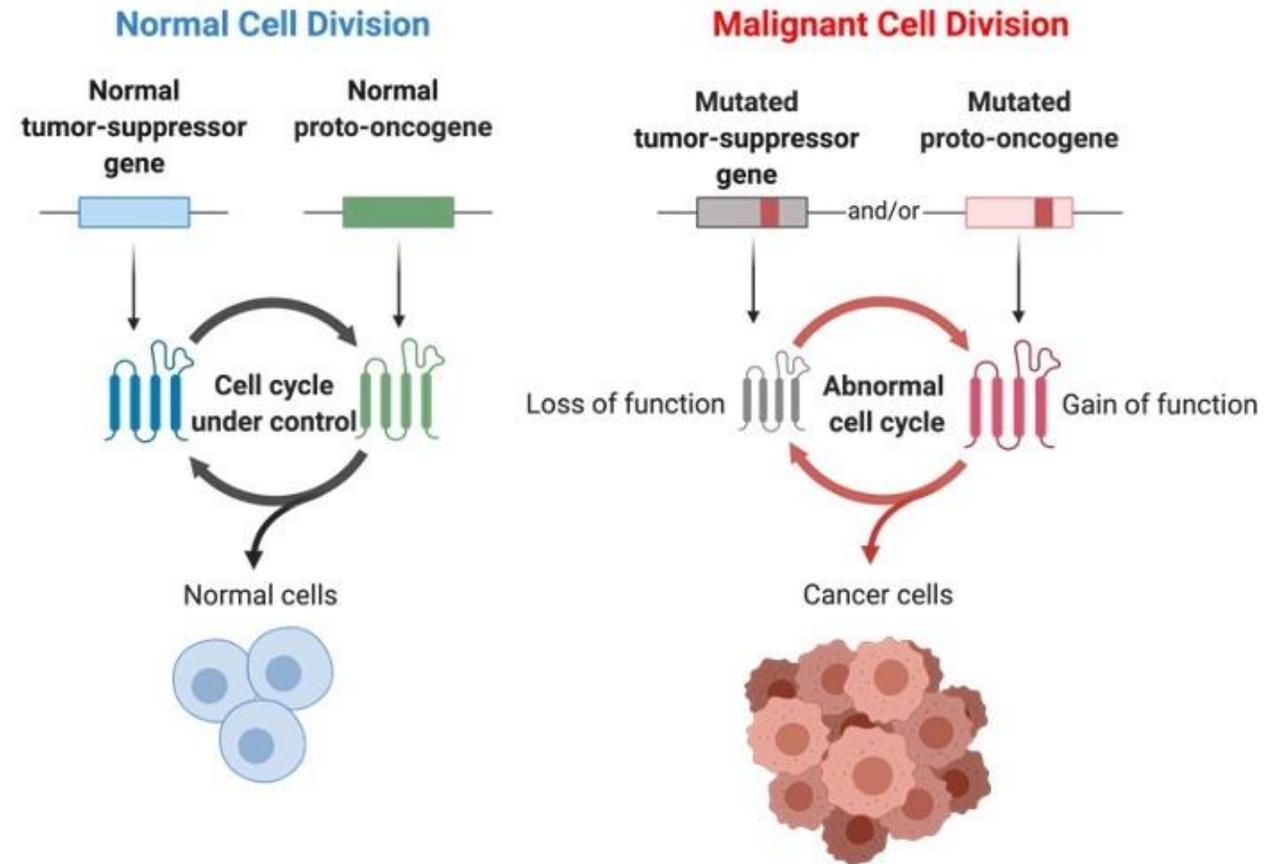
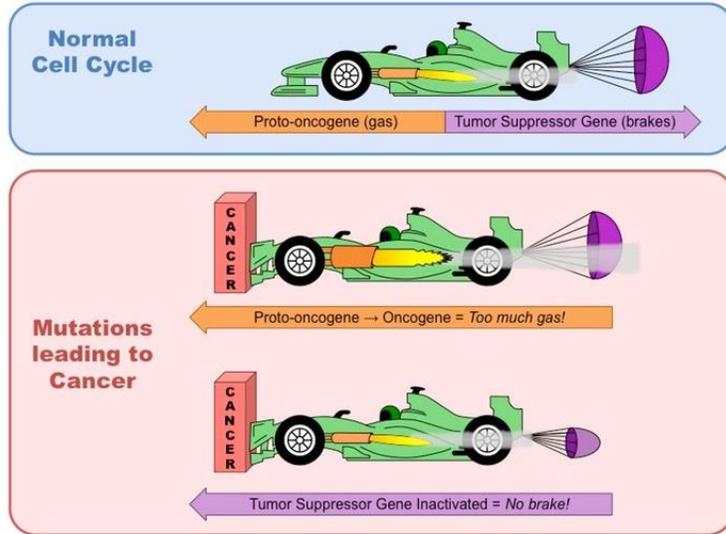
- A neoplasm is an abnormal mass of tissue that forms when cells grow and divide more than they should or do not die when they should. Neoplasms may be benign (not cancer) or malignant (cancer).
- Carcinoma is cancer that forms in epithelial tissue. Epithelial tissue lines most of your organs, the internal passageways in your body (like your esophagus), and your skin. Most cancers affecting your skin, breasts, kidney, liver, lungs, pancreas, prostate gland, head and neck are carcinomas.
- Cancer emerges when regulatory systems that keep cell division in check are broken and the cells divide in an uncontrolled manner (cells in metastases look like each other, external agents can give rise to cancer, gain or loss of chromosomes (aneuploidy) can give rise to cancer, cancer can be heritable from one generation to another)
- Cancer arises from the accumulation of genetic aberrations (mutations, chromosomal changes) in somatic cells, over 500 genes are now known to be involved in cancer development.

Hallmarks of Cancer

- Include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.
- Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions.



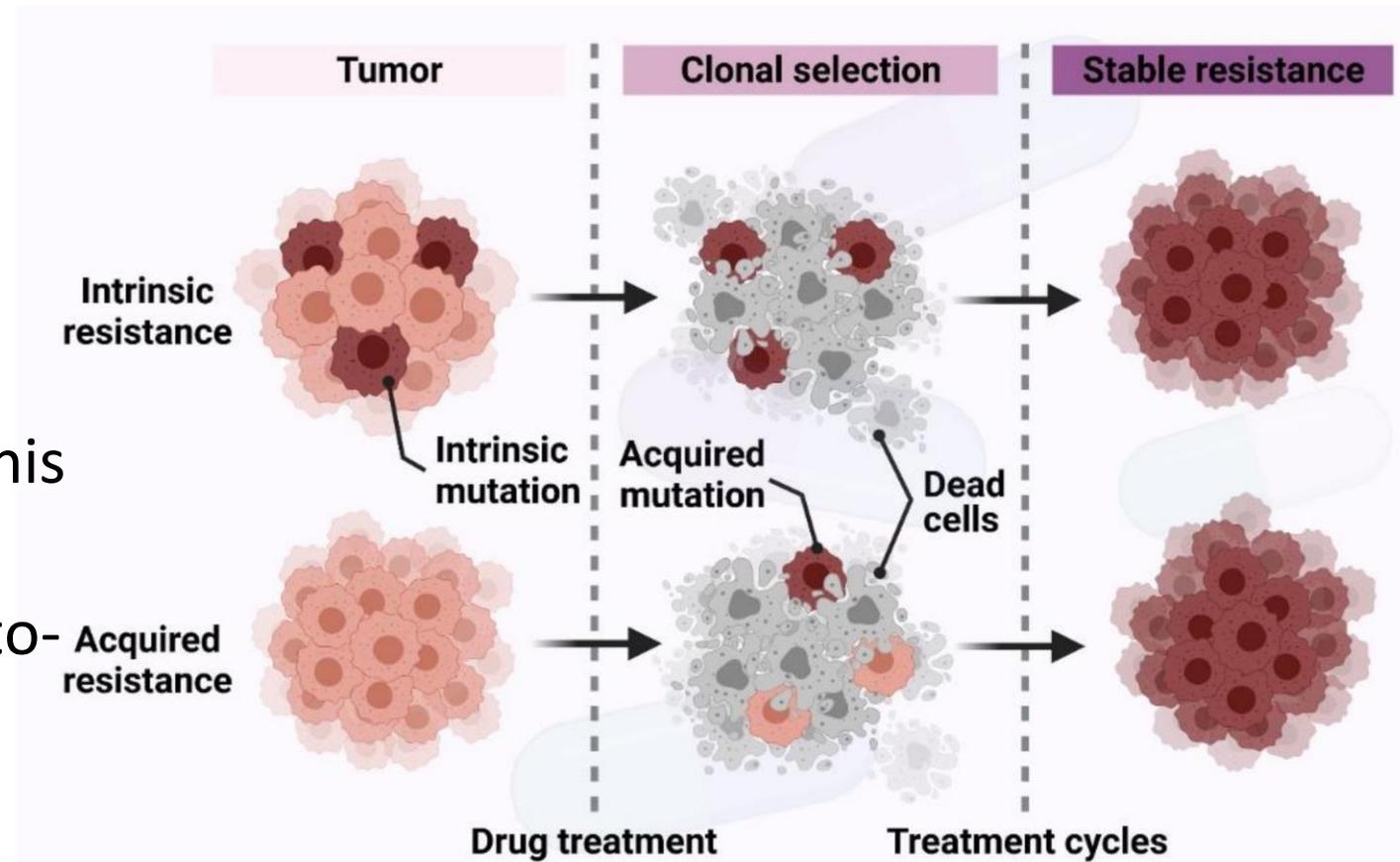
Tumor suppressors, oncogenes and DNA repair



Type of Gene	This Mutation Causes	Examples
Oncogene	Uncontrolled growth: step on the gas	Her2-neu, Ras, Myc, Src, Htert
Tumor Suppressor	Uncontrolled growth: remove the brake	P53, Rb, APC
DNA Repair	No longer able to correct cell division mistakes	BRCA1 and BRCA2

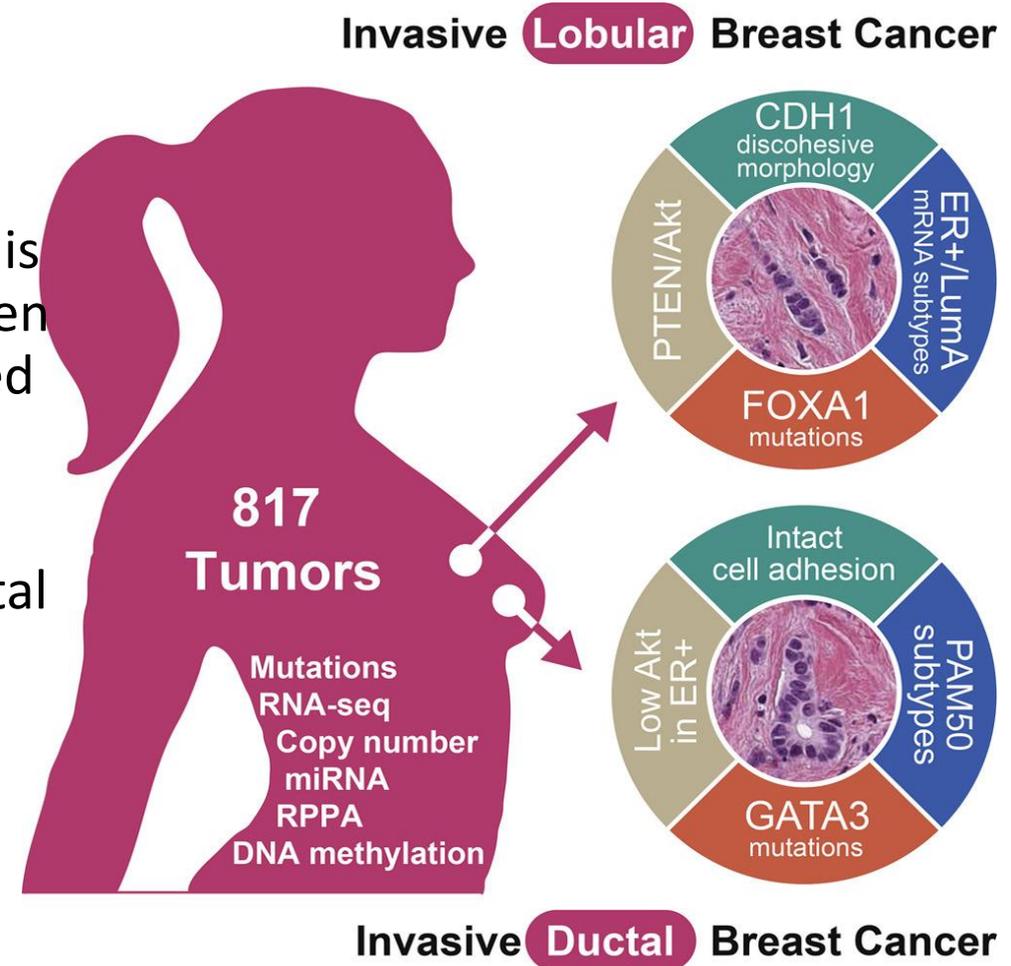
Mutation based treatment resistance

- Intrinsic resistance exists before the drug/therapy has been administered to the patient.
- Acquired drug resistance occurs after chemotherapy treatment. The reasons for this type of resistance are mutations of drug targets, activation of the second proto-oncogene, changes in the tumor microenvironment, epigenetic alterations and others.



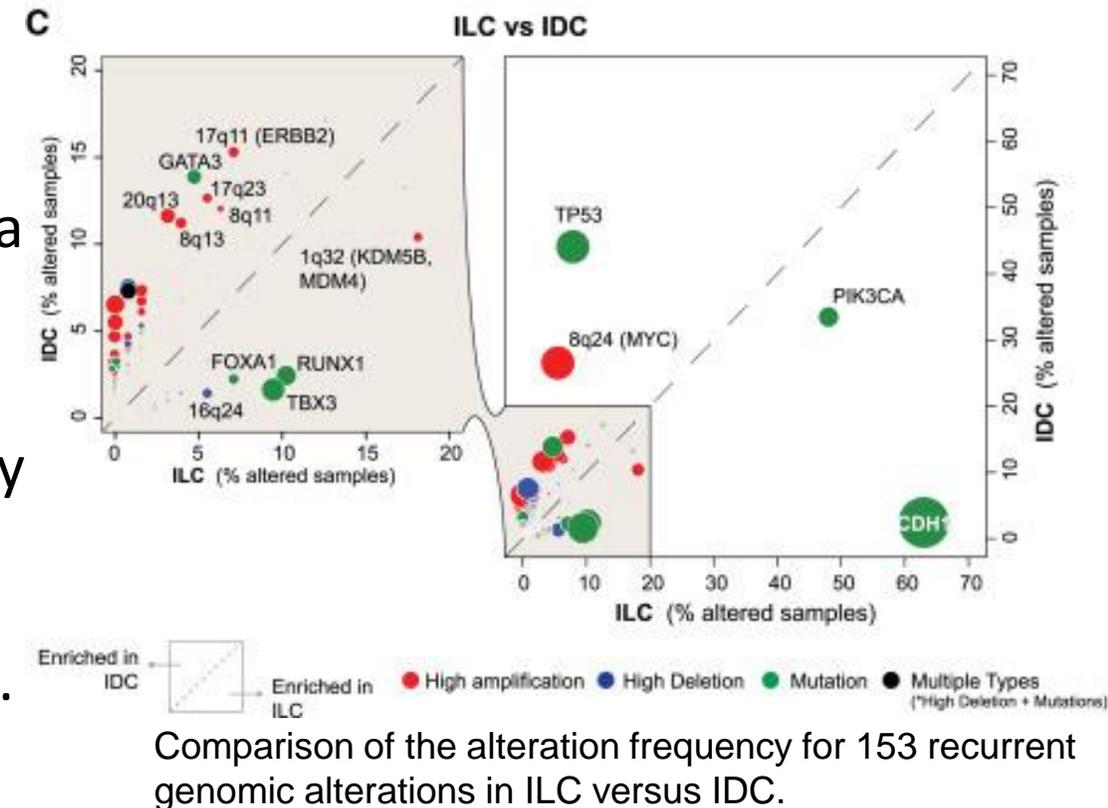
Known Molecular Hallmarks of IDC and ILC

- ILCs demonstrate small discohesive neoplastic cells invading the stroma in a single-file pattern. Discohesion is due to dysregulation of cell-cell adhesion, primarily driven by lack of E-cadherin (CDH1) protein expression observed in ~90% of ILCs. Classical lobular tumors are luminal in molecular subtype, being ER / PR +, and HER2 -.
- Lobular tumors are more likely to be grade I/II than ductal tumors.
- Numerous studies have characterized the molecular fingerprints unique to lobular tumors. These include changes to FOXA1, PTEN/Akt, PIK3CA in addition to alterations in oncogenes such as *ERBB2* and *TP53*.



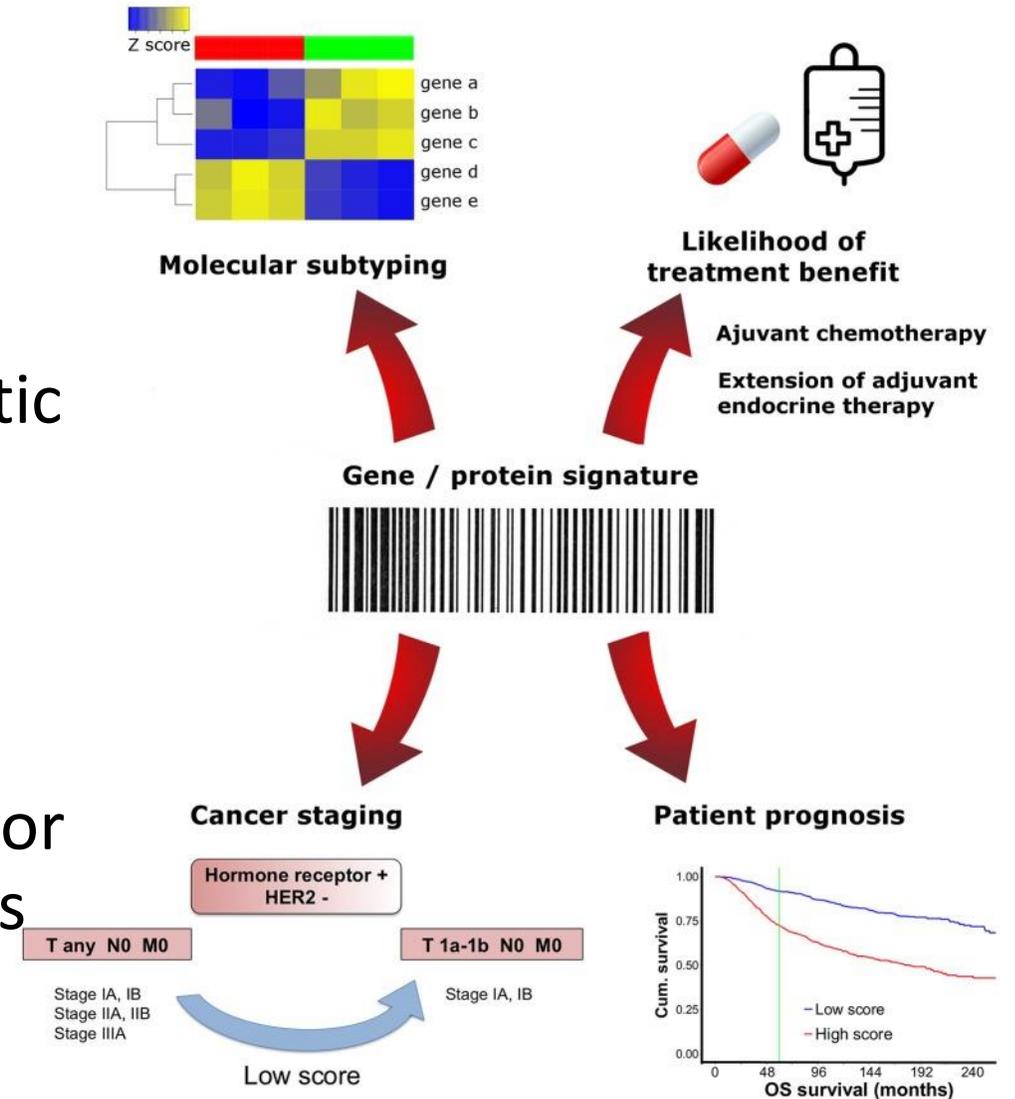
Unique Molecular Characteristics of ILC

- 66% (107/162) of ILCs harbor mutation in *CDH1* compared with only 3% (22/741) of IDCs.
- *FOXA1*, an ER transcription modulator, plays a key role in cancer progression and development of endocrine resistance
- ILC is characterized by loss of E-cadherin expression and function (*CDH1* gene), and key early somatic alterations involving gain of chromosome 1q, loss of 16q, and mutations in *PIK3CA*, *AKT1*, or *PTEN*. Amplifications of 8p12 (*FGRF1* locus) and 11q13 (*CCND1* locus). *PIK3CA*, *PTEN*, and *AKT1* are collectively mutated in over half of all ILC.

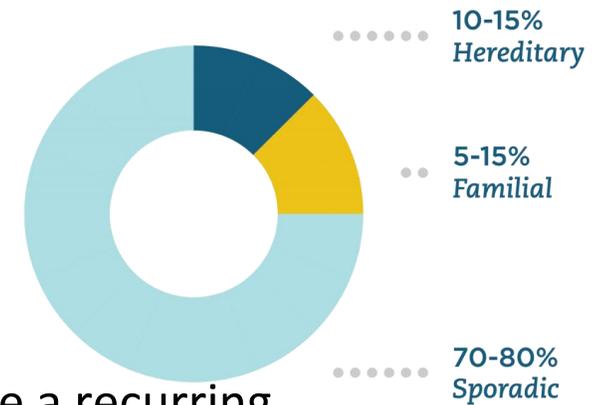


Diagnostic testing for oncology

- Clinical applications for oncology genetic tests include: prognostic information, therapy decision, molecular subtyping, and patient staging.
- Assays / tests may include analysis of DNA / RNA / Proteins, from either tumor biopsies or blood along with algorithms



Hereditary Genetic Testing



- Hereditary cancer is caused by an inherited genetic mutation. It is typical to see a recurring pattern of cancer across two to three generations—like multiple individuals diagnosed with the same type of cancer(s) and individuals diagnosed with cancer much younger than average.
- Genetic counseling can help patients make informed decisions about genetic testing for BRCA1, BRCA2, and other inherited mutations.
- Cascade testing for BRCA1, BRCA2, and other genetic changes that cause hereditary breast and ovarian cancer can find family members who are more likely to get breast, ovarian, and other cancers.
- Four high-penetrance genes are tested in clinical practice when genetic susceptibility to breast cancer is suspected, BRCA1, BRCA2, TP53 and CDH1. Germline mutations in BRCA1 and TP53 are predominantly associated with invasive ductal carcinoma, **while BRCA2 mutations are associated with both ductal and lobular cancers. CDH1 mutations are associated with invasive lobular carcinoma, but never with ductal carcinoma.** The risk of invasive lobular carcinoma is high in female mutation carriers, as about 50% are expected to develop the disease.

Prognostic Tests

- Genetic prognostic tests are biomarkers commercially available in the form of medical devices/tests. The prognosis of breast cancer, the assessment of patients where chemotherapy will be beneficial, as well as the identification of the molecular subtype can be provided by such molecular tests. These tests have been well validated in IDC to select patients who would benefit from use of adjuvant chemotherapy.
- Research for these tests in ILC has been mostly retrospective, due to the extended time between diagnosis and relapse or recurrence.
- A majority of genomic prognostic tests are able to identify a high risk group for ILC, but additional prospective trials are needed.
 - *“...The distribution of risk score (RS) in patients with ILC is different than in those with IDC. Far fewer patients with ILC had a high RS than those with IDC. Although there is a tendency by oncologists to underplay the significance of RS in ILC, the authors demonstrated that in this very large sample of patients (1037), RS was a prognostic factor.” (Weiser et al, Cancer, 09 Feb 2022 <https://doi.org/10.1002/cncr.34127>)*

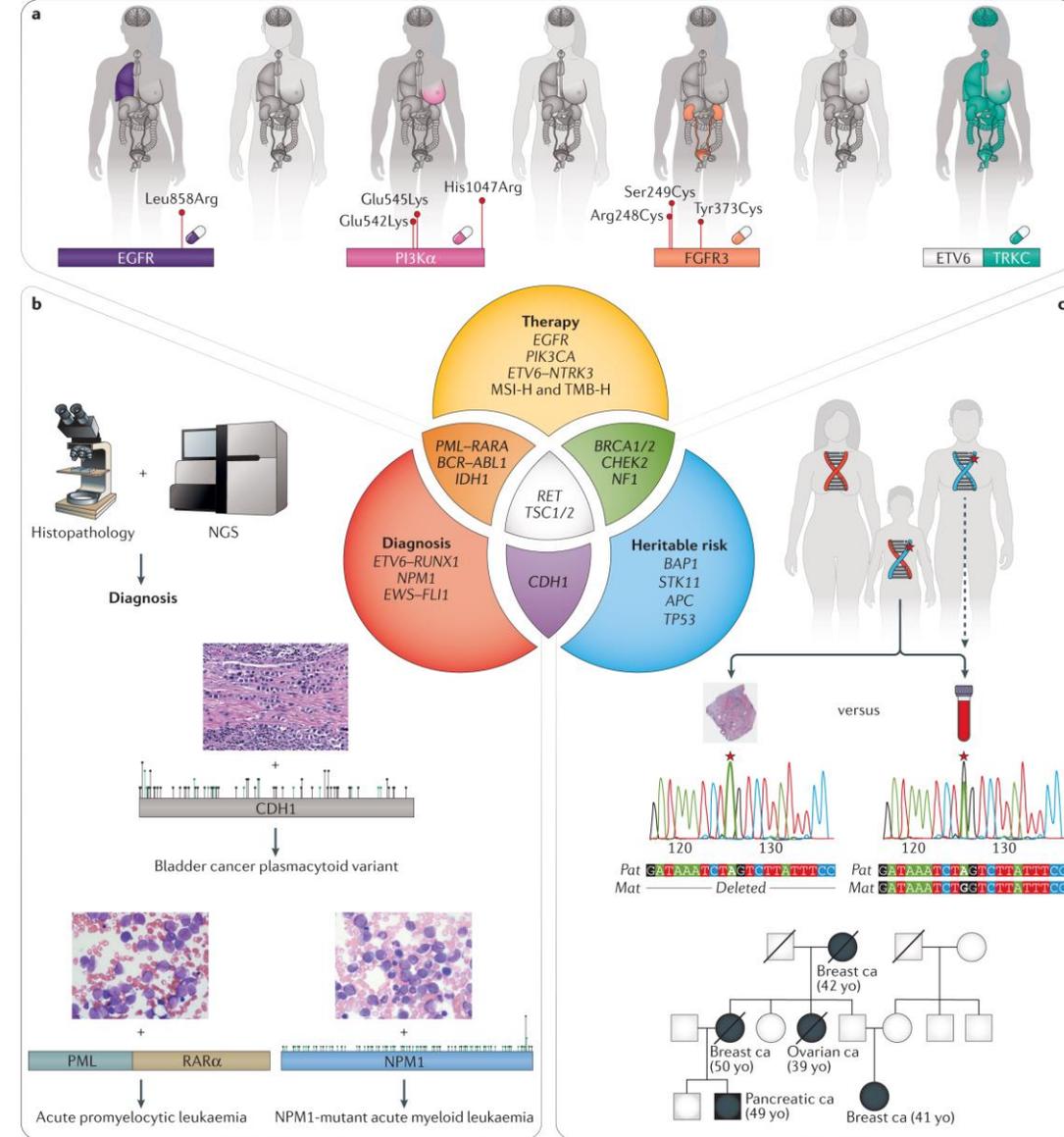
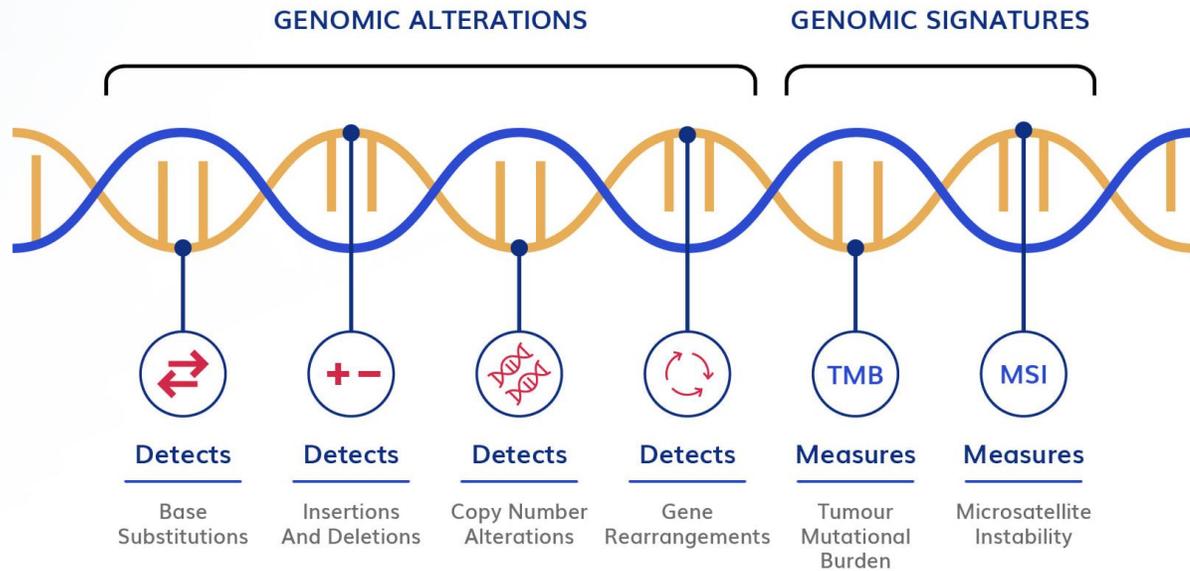
Tumor Profiling

- Tumor profiling tests are available as medical devices or laboratory developed tests, from a number of commercial providers, they can use either tumor tissue or liquid biopsy (circulating tumor DNA collected via a blood sample) to show somatic mutations
- Recommendation to employ genomic sequencing in advanced cancer patients by a certified lab to identify genomic biomarkers **to guide the use of or exclusion from treatments for their disease** (ASCO Provisional Clinical Opinion, Feb 2022)
- Comprehensive Genomic Profiling tests can identify mutations by DNA sequencing that could be missed by routine clinical testing immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH), such as activating mutations in *ERBB2*
- Therapy selection results show both approved therapies (along with CDx claims) and clinical trials appropriate for the unique molecular fingerprint of a tumor

Tumor Profiling

Comprehensive Genomic Profiling

is a next-generation sequencing (NGS) approach that uses a single assay to assess relevant cancer biomarkers.

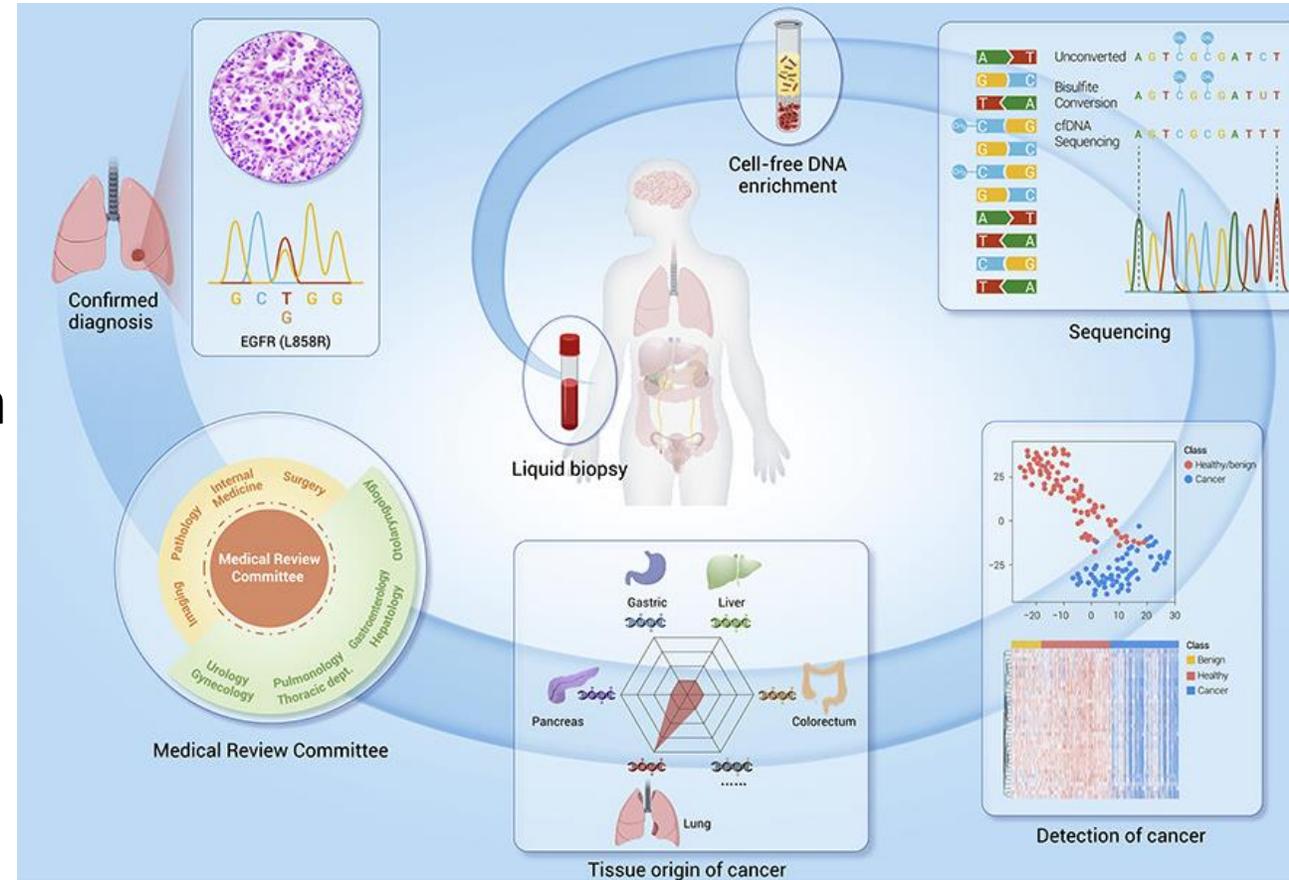


Minimal Residual Disease and Monitoring

- The overall survival for ILC is favorable compared with IDC within 5 years of diagnosis, but is worse after 5 years. One reason is disseminated cancer cells (DCC) which are dormant for prolonged periods and can result in delayed relapse at distant metastatic sites
- Minimal residual disease (MRD) after potentially curative surgery for BC is thought to contribute to disease relapse and to be the target of adjuvant treatment. MRD is defined as micrometastatic cells undetectable by conventional imaging and laboratory tests (imaging)
- Surrogates of MRD are tumor cells detected in the bone marrow (disseminated tumor cells (DTCs)) and peripheral blood (circulating tumor cells (CTCs)), along with DNA changes that can be tracked in the blood (personalized tests)
- Testing for MRD is still exploratory and requires clinical validation, although commercial companies are starting to bring tests to the market

Non-invasive Early Cancer Detection

- The “holy grail” of cancer would be to detect many cancer’s early from a single blood draw and determine the tissue of origin
- Early cancer detection screening exists for colorectal cancer via stool based testing
- New scientific methods are being tested to develop blood based tests, early and need clinical validation



Dr. Sasha Stanton

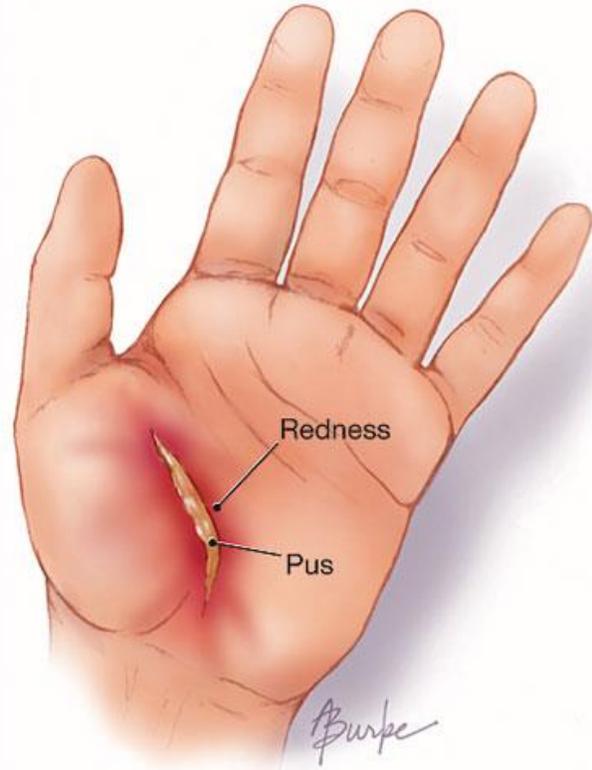


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The immune system at work

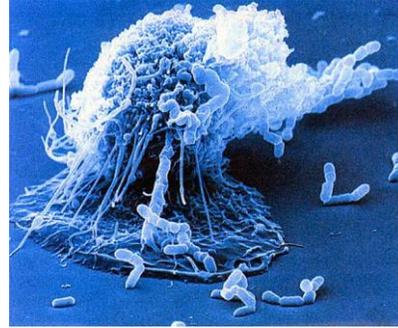
- Innate



- Adaptive



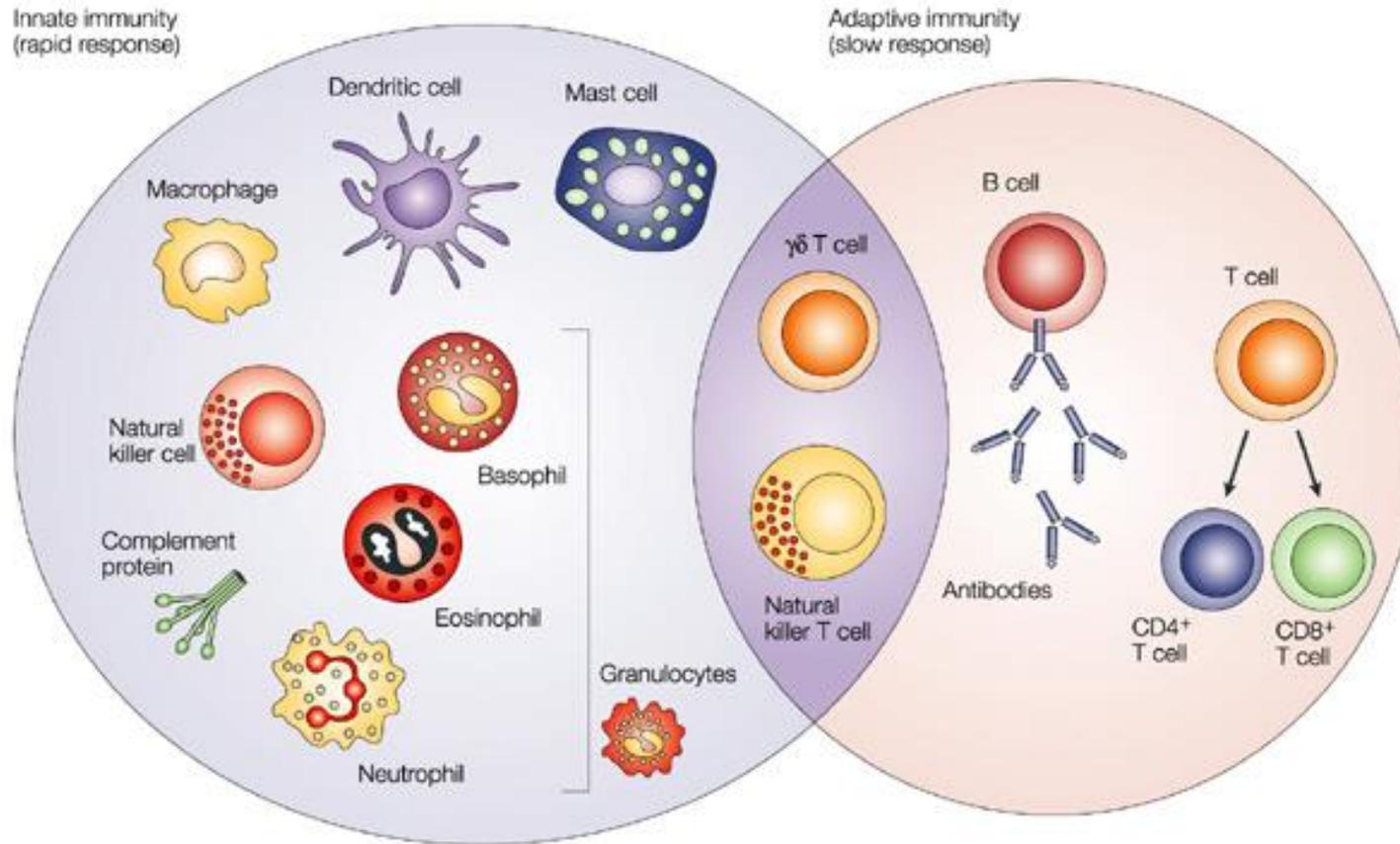
The immune system



CHARACTERISTICS	INNATE	ADAPTIVE
Specificity	Non-specific	Specific
Antigens	Not needed	Required
Memory	None	Generated
Time course	Immediate	Slowly developing
Duration	Transient	Lifelong

Courtesy of M.L. Disis

The key players of the immune system

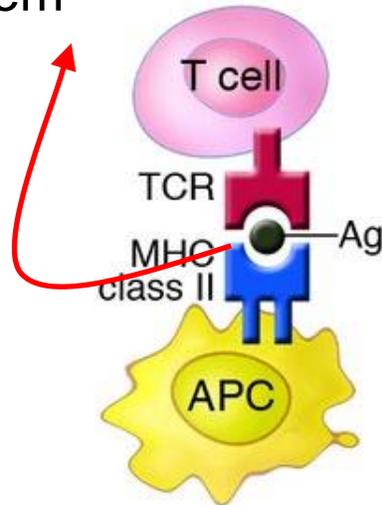


Nature Reviews | Cancer

How the immune system communicates

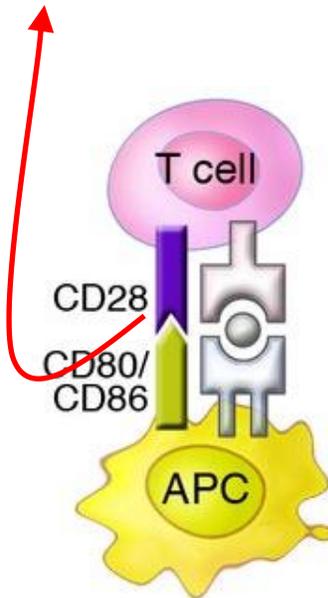
- **Antigens**

From the pathogen, piece of protein in a context that triggers the adaptive immune system



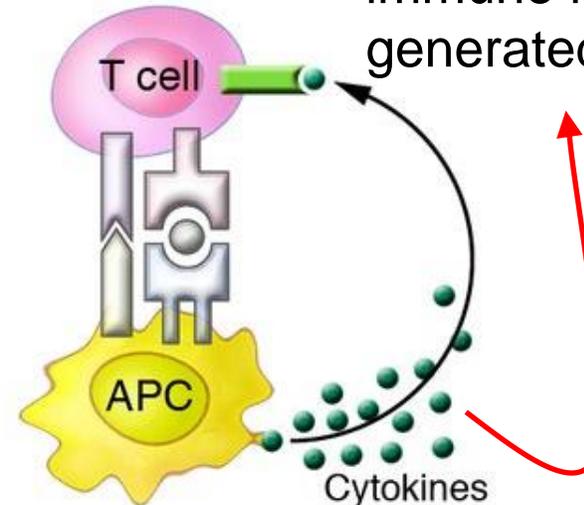
- **Receptors**

how cells communicate to each other/ their environment



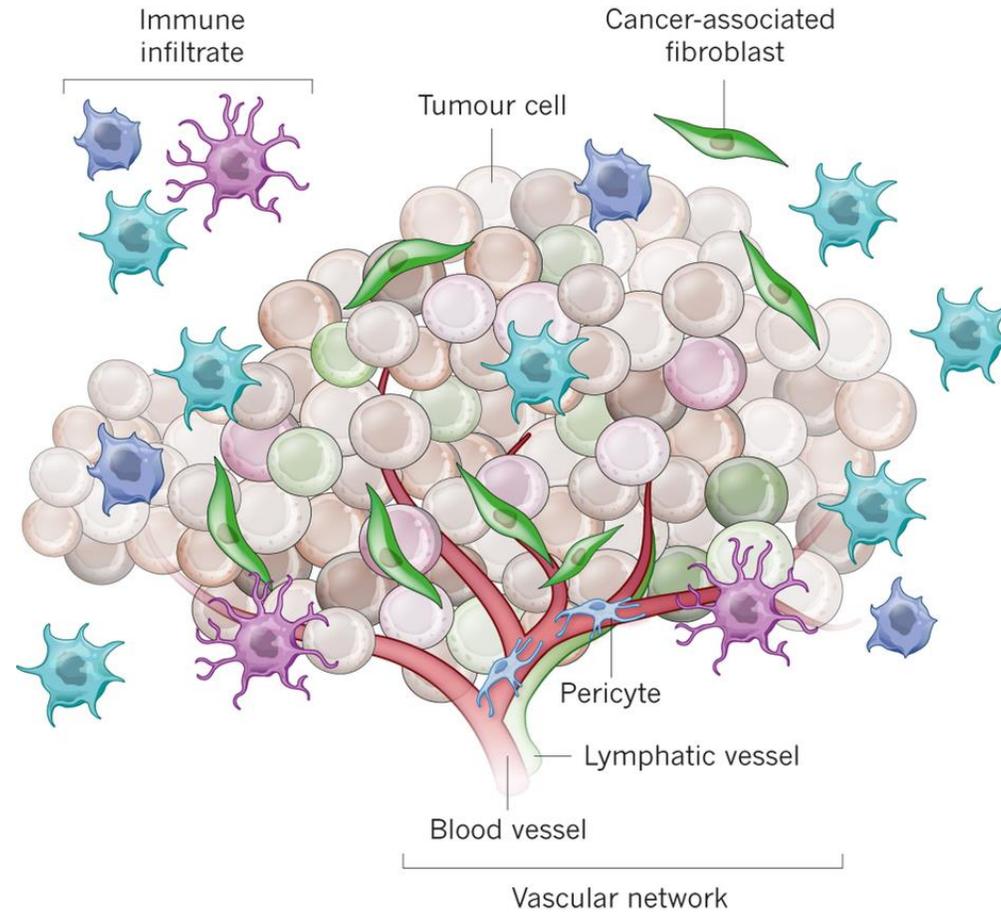
- **Cytokines**

how immune cells communicate
Small proteins that direct the type of immune response generated



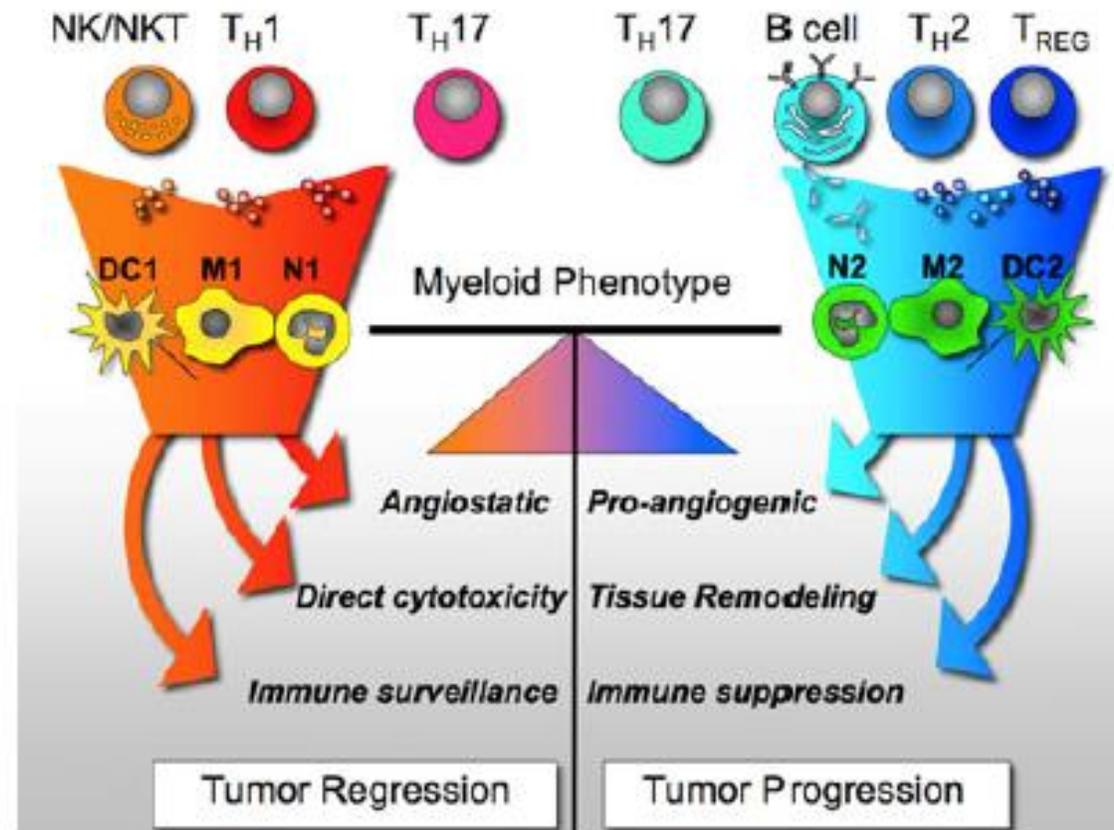
Modified from Gutcher et al *J Clin Invest.* 2007

Tumor immune environment



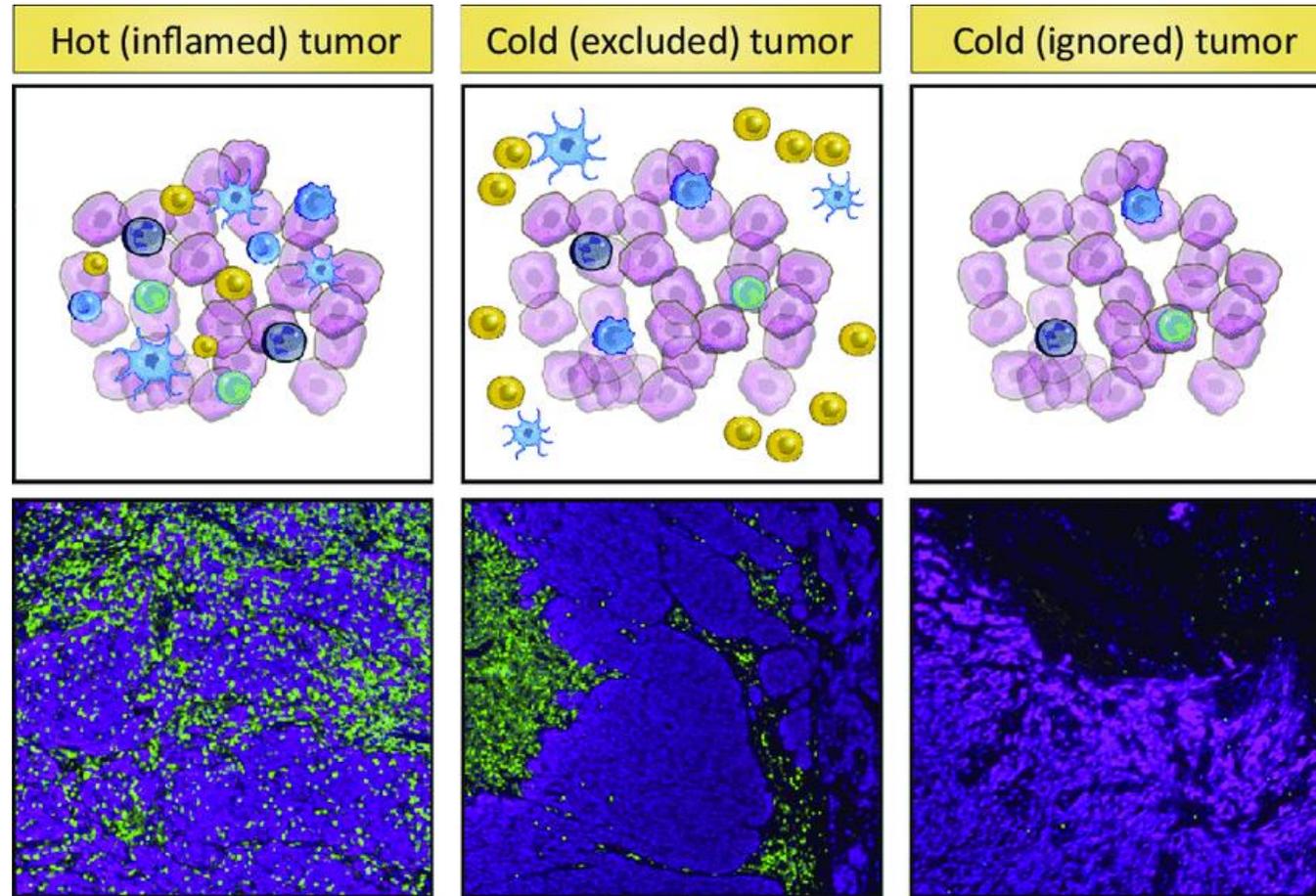
Juntilla et al Nature (2013) 501: 346

Th1 verses Th2 environment



DeNardo Cancer Metastasis Rev (2010) 29:309

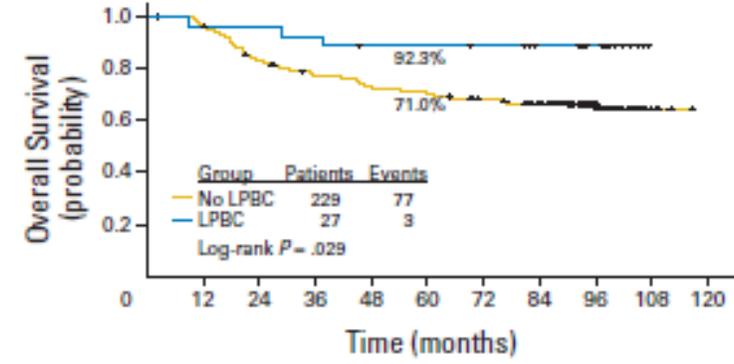
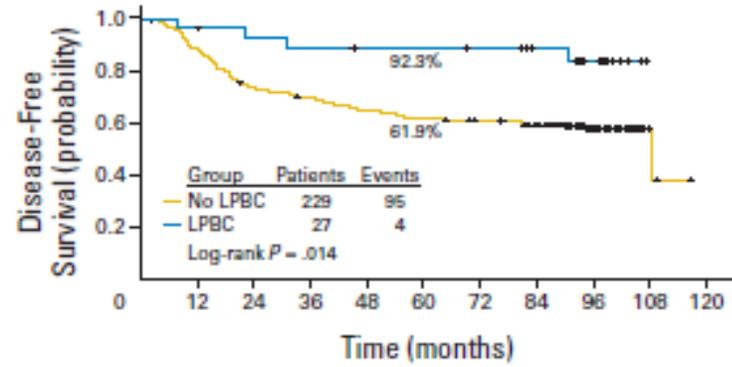
Types of tumor immune environments



Van der Woude Trends in Cancer 2017

TILS (Tumor infiltrating lymphocytes) in breast cancer

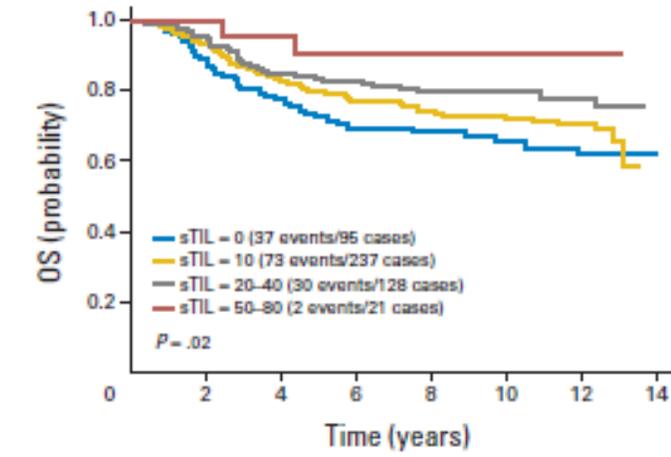
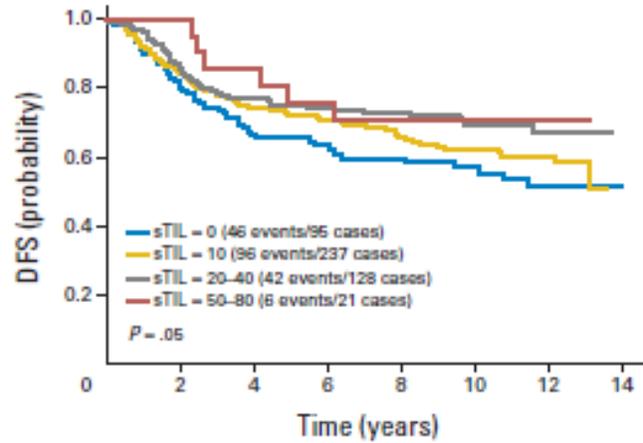
>50% TILS



No. at risk	0	12	24	36	48	60	72	84	96	108	120
No LPBC	229	202	167	156	146	138	134	116	41	3	0
LPBC	27	26	24	23	22	22	21	18	11	0	0

No. at risk	0	12	24	36	48	60	72	84	96	108	120
No LPBC	229	219	188	173	163	157	148	131	50	4	0
LPBC	27	26	25	24	22	22	21	18	12	0	0

Increasing TILS



Loi JCO 2013; Adams JCO 2014



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Immune infiltrate (and impact on prognosis) varies depending on subtype

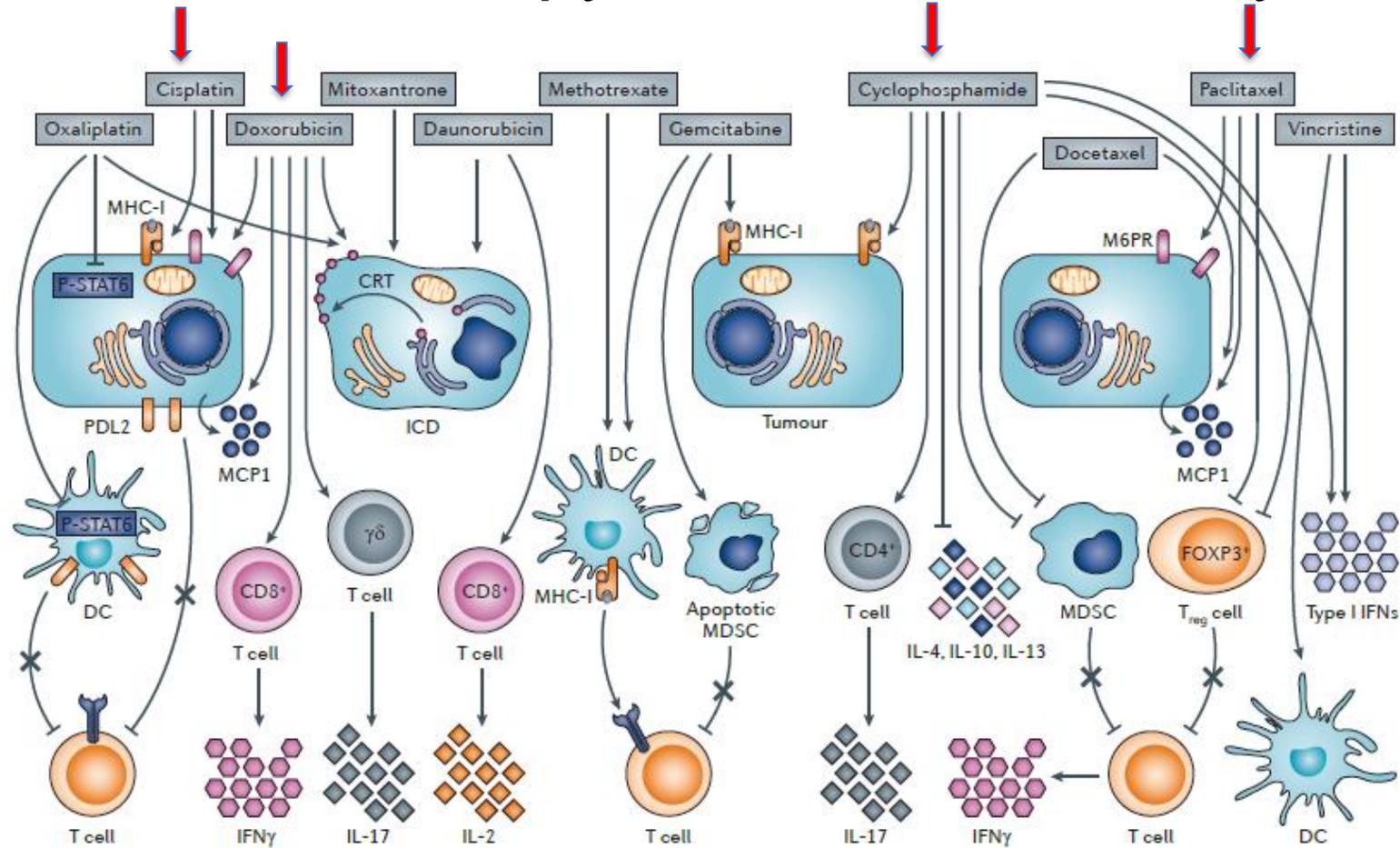
Table 1. Effect on Outcome of LPBC, CD8+, or FOXP3 Tumor Infiltrate by subtype

Immune Infiltrate	Breast Cancer Subtype					
	TN		HR+		HER2+	
	DFS or RFS	OS or DSS	DFS or RFS	OS or DSS	DFS or RFS	OS or DSS
LPBC	+++	+++			++	+
Elevated CD8+	+	+++		+	+ (TBET)	
Elevated FOXP3			-	-		

Abbreviations: DFS, disease free survival; RFS, relapse free survival; OS, overall survival; DSS, disease specific survival; LPD, lymphoplasmacytoid infiltrate; TN, triple negative; HR, hormone receptor. +++ Increased (>2 sources); ++ (increased 2 sources) + Increased (one source); - Decreased (one source).

Stanton *JITC* 2016

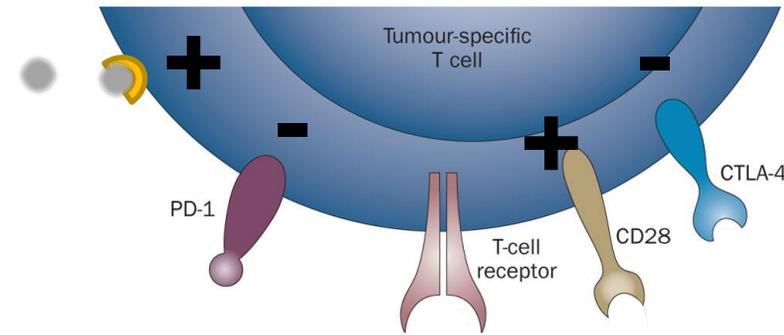
Role of chemotherapy and the immune system



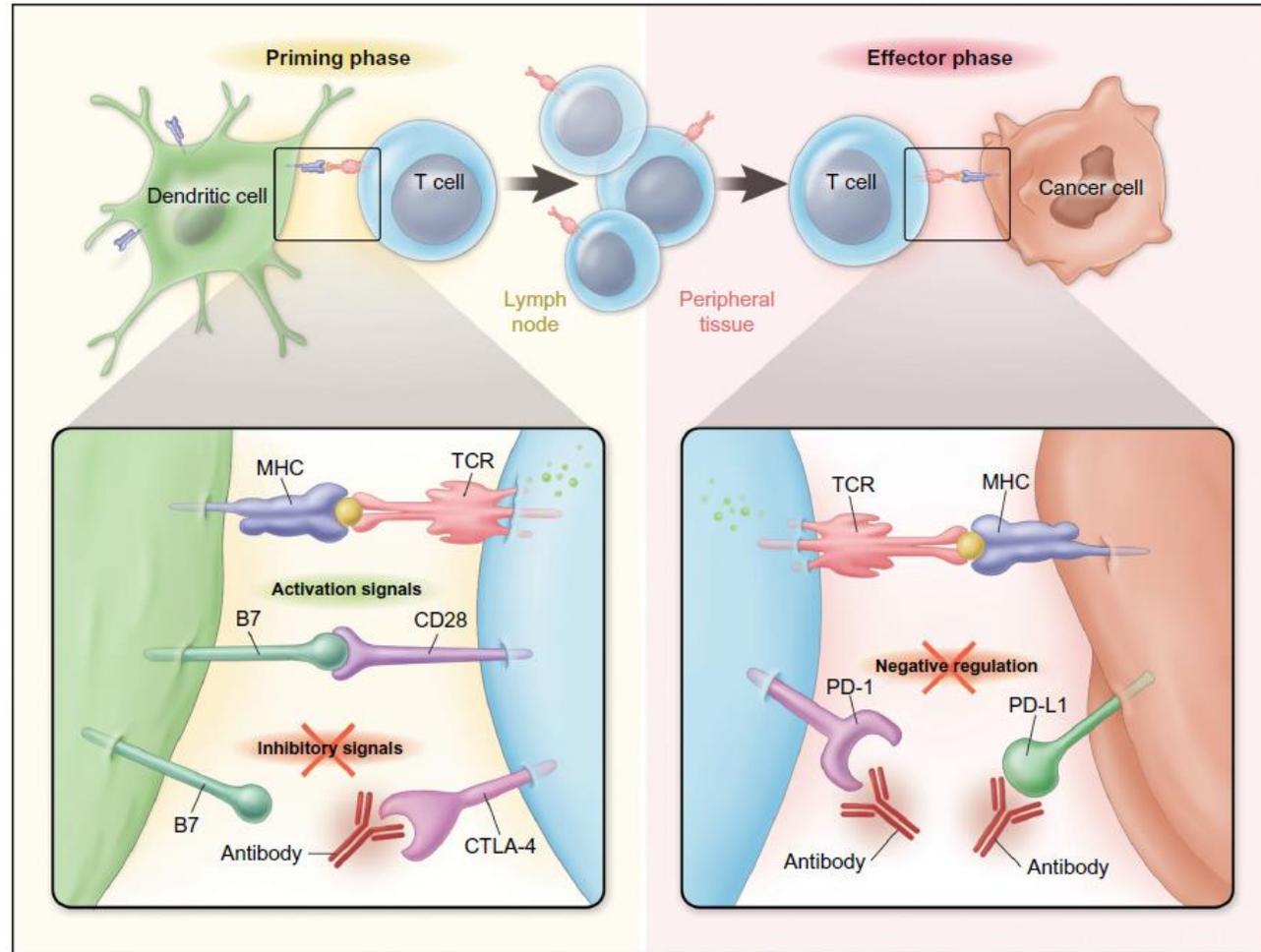
Galluzzi Nature Reviews Drug Discovery (2012)

Immunotherapy in cancer

- Non-specific stimulation of immune system
 - checkpoint blockade
 - antibodies
 - cytokines
- Specific response by own immune system
 - cancer vaccines
- Passive transfer of activated cells
 - adoptive immunotherapy

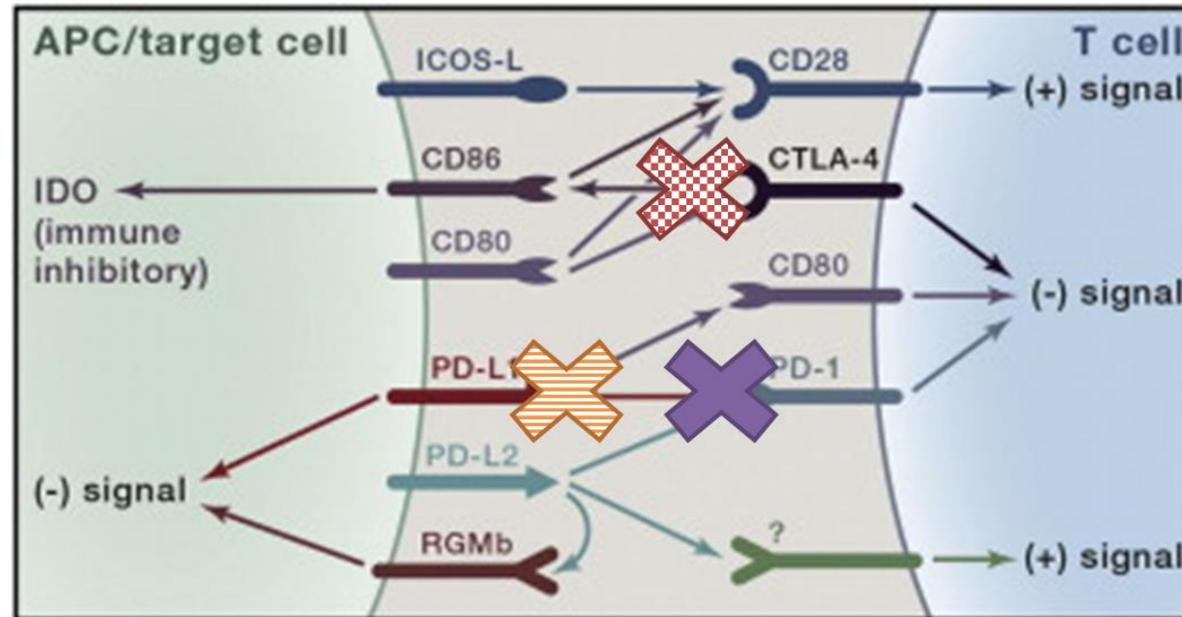


Immune checkpoint inhibitors and where they function



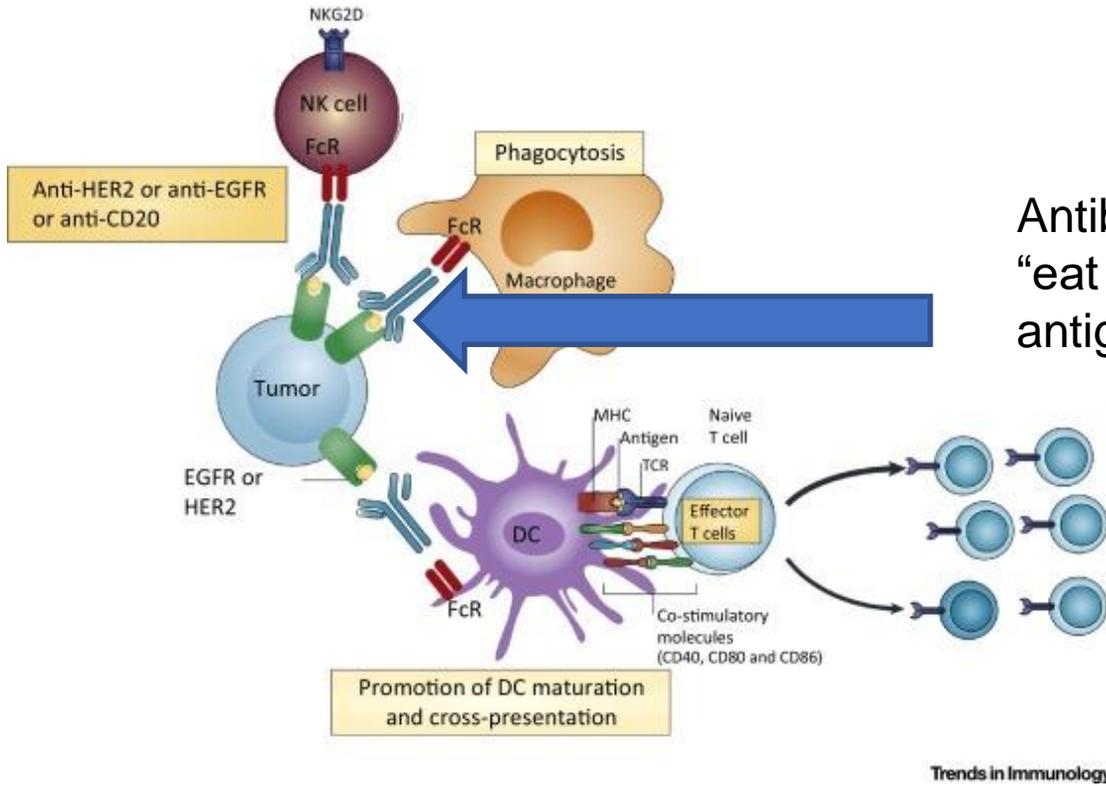
Tykodi Oncotargets and Therapy 2014

Immune Checkpoint inhibitors



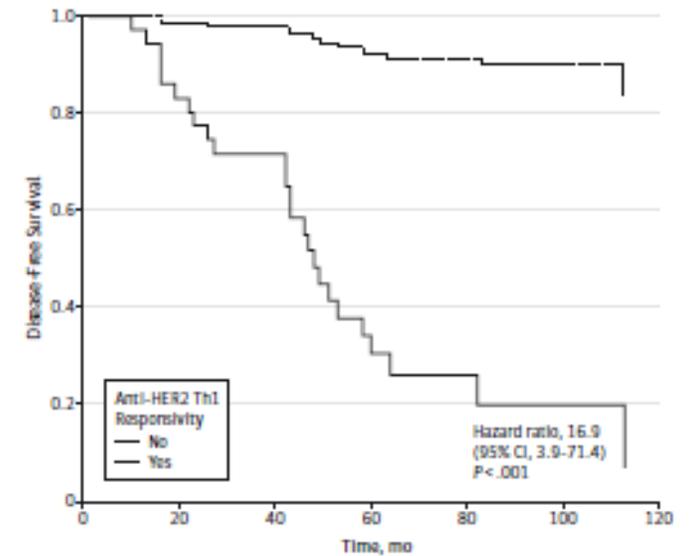
- ✘ Anti-CTLA-4: ipilimumab (Yervoy), tremelimumab
- ✘ Anti-PD-1: nivolumab (Opdivo), pembrolizumab (Keytruda)
- ✘ Anti-PD-L1: atezolizumab (Tecentriq), avelumab (Bavencio), durvalumab (Imfinzi)

Antibodies in breast cancer therapy



Antibodies help increase the “eat me” signal, providing antigens for T cells

Xu Trends in Immunology 2016; Datta JAMA Oncology 2016



Patients that develop HER2-specific T-cells have better disease-free survival

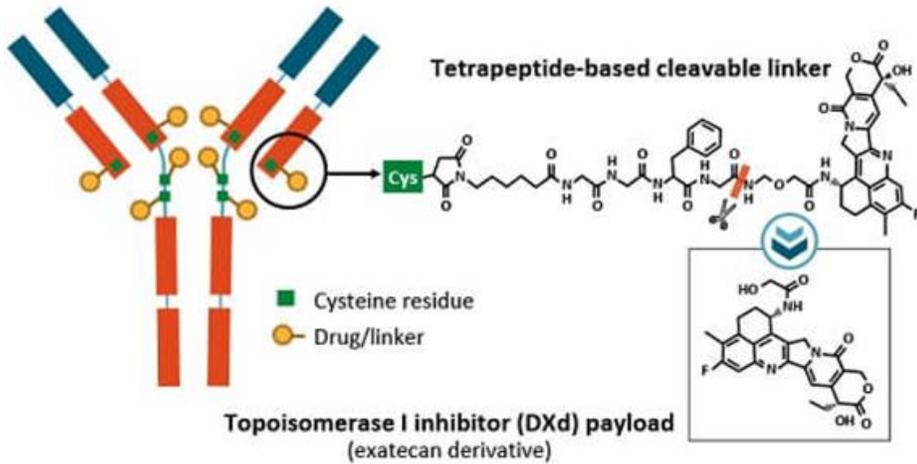


ADC (antibody-drug conjugate)

Enhertu

HER2-Targeted ADC: Trastuzumab Deruxtecan (DS-8201)

Humanized anti-HER2 IgG1 mAb
with same AA sequence as
trastuzumab



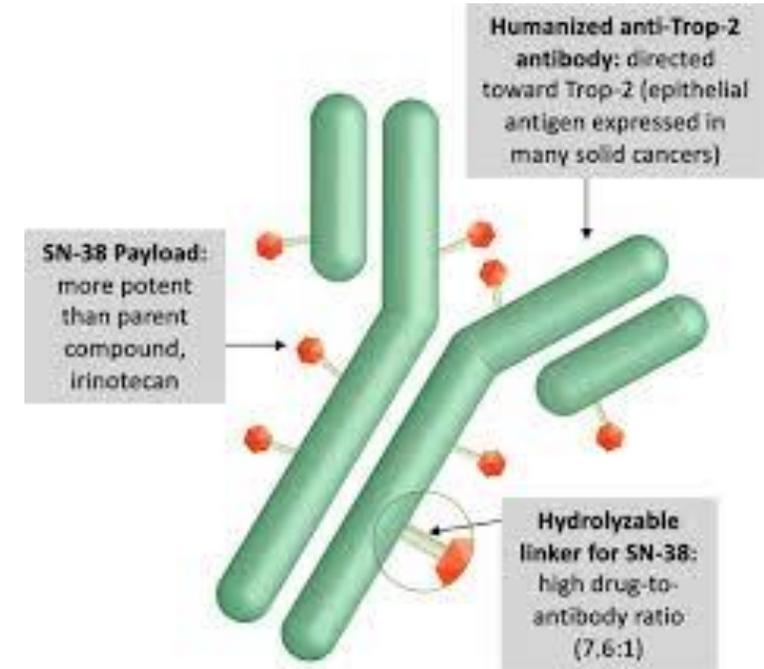
- High drug:antibody ratio: ~ 8
- Stable linker-payload
- Tumor-selectable cleavable linker
- High potency, membrane-permeable payload with short systemic half-life
- Bystander killing effect

Nakada. Chem Pharm Bull (Tokyo). 2019;67:173. Trail. Pharmacol Ther. 2018;181:126. Ogitani. Cancer Sci. 2016;107:1039.

Slide credit: clinicaloptions.com

Modi NEJM 2022

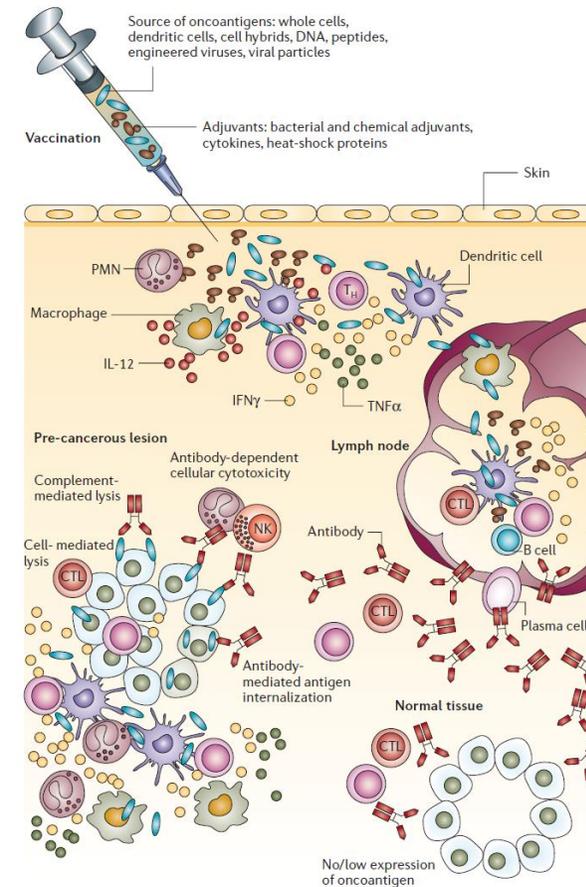
Trodelvy



Bardia NEJM 2021

Components of a cancer vaccine

1. type of vaccine
2. type of adjuvant
3. type of immune response
4. response specifically against tumor



Lollini Nature Reviews Cancer 2006

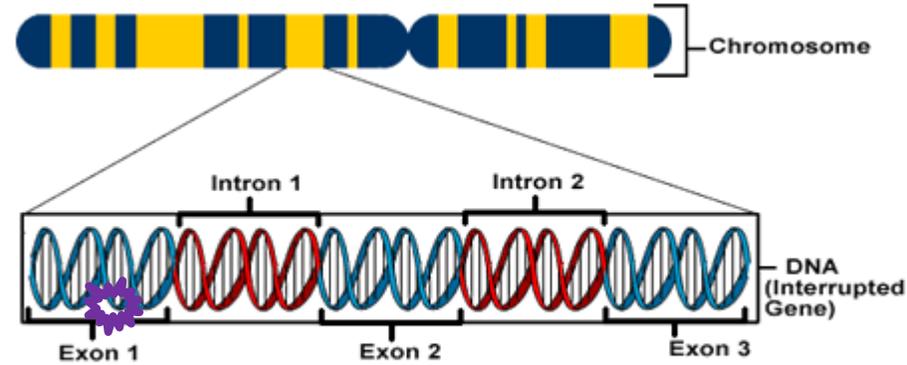
Neoantigen Vaccines

A small DNA
change...

that makes
a protein...

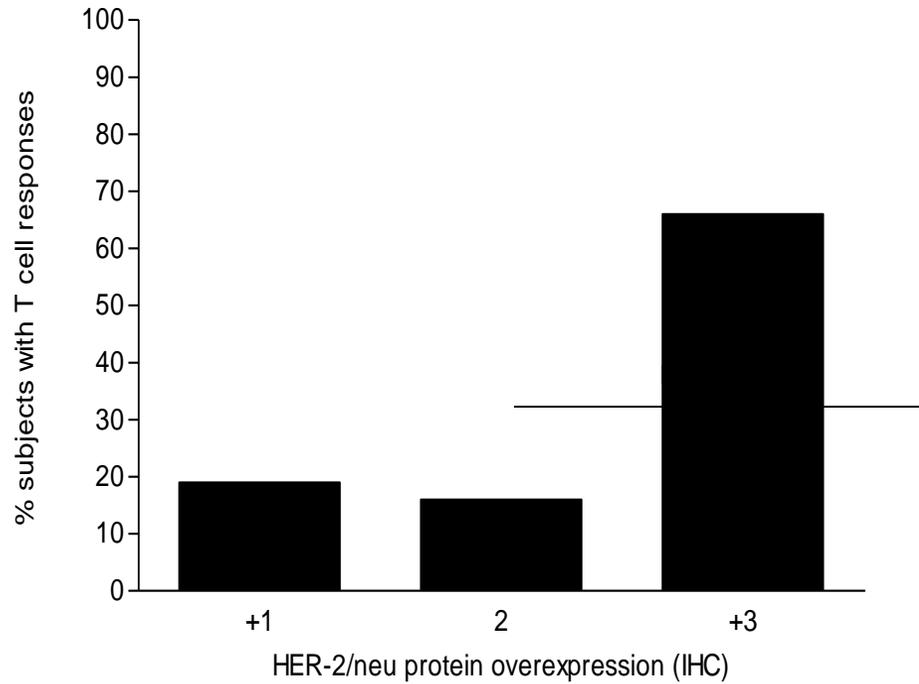
different enough for
the immune system to
find it...

and want to destroy it.

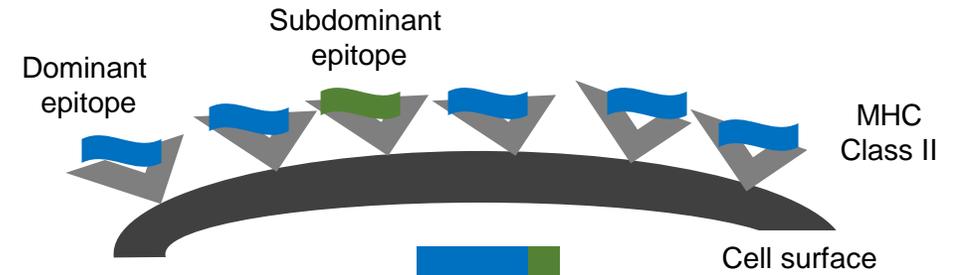


Vaccines against overexpressed tumor proteins

TOLERANCE

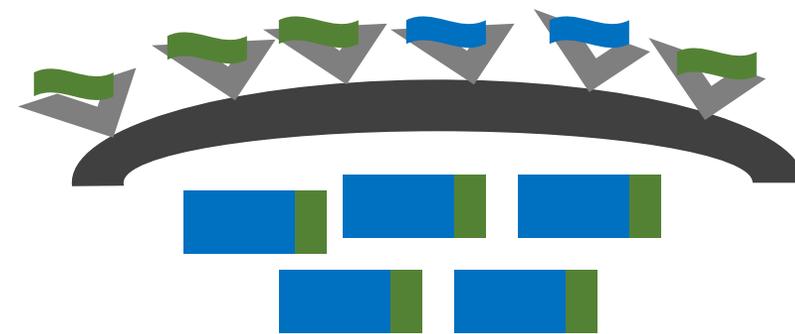


Overexpression: independent predictor of immunity



BASAL expression

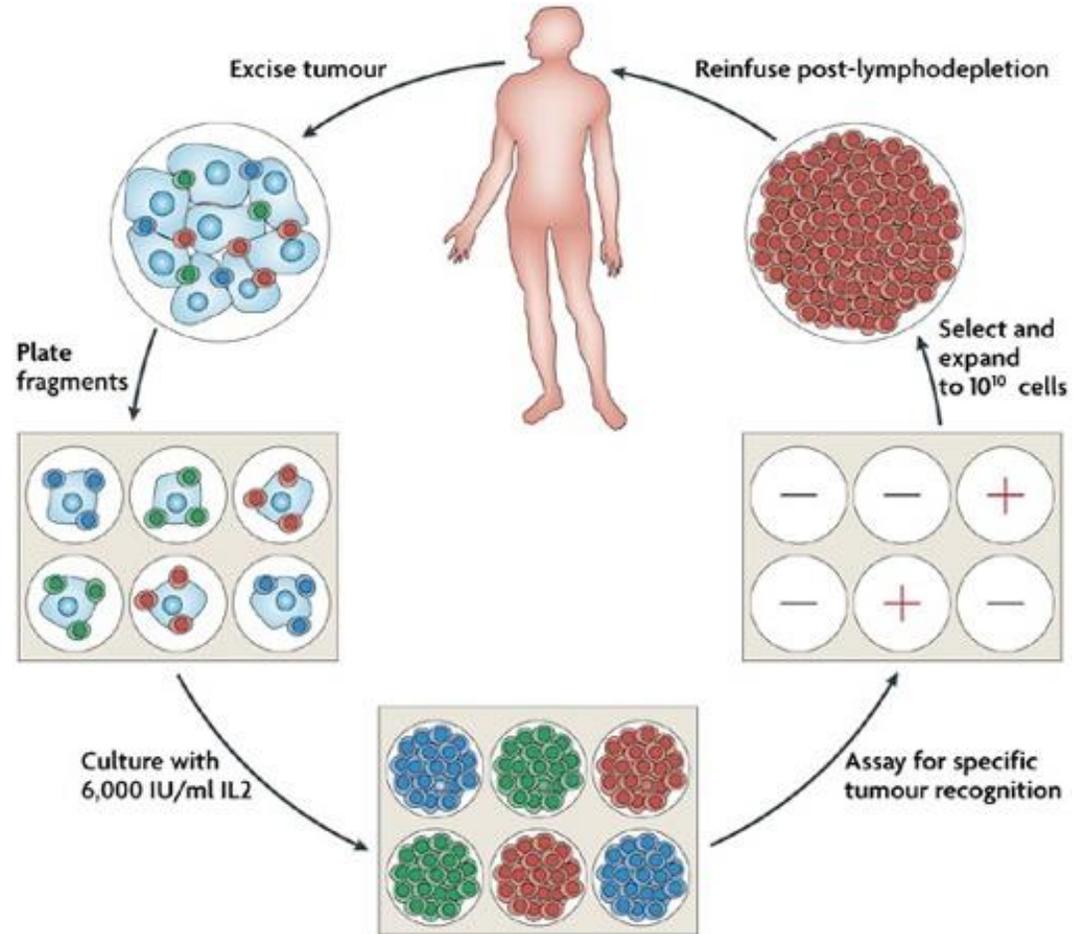
IMMUNE RESPONSE



OVER expression

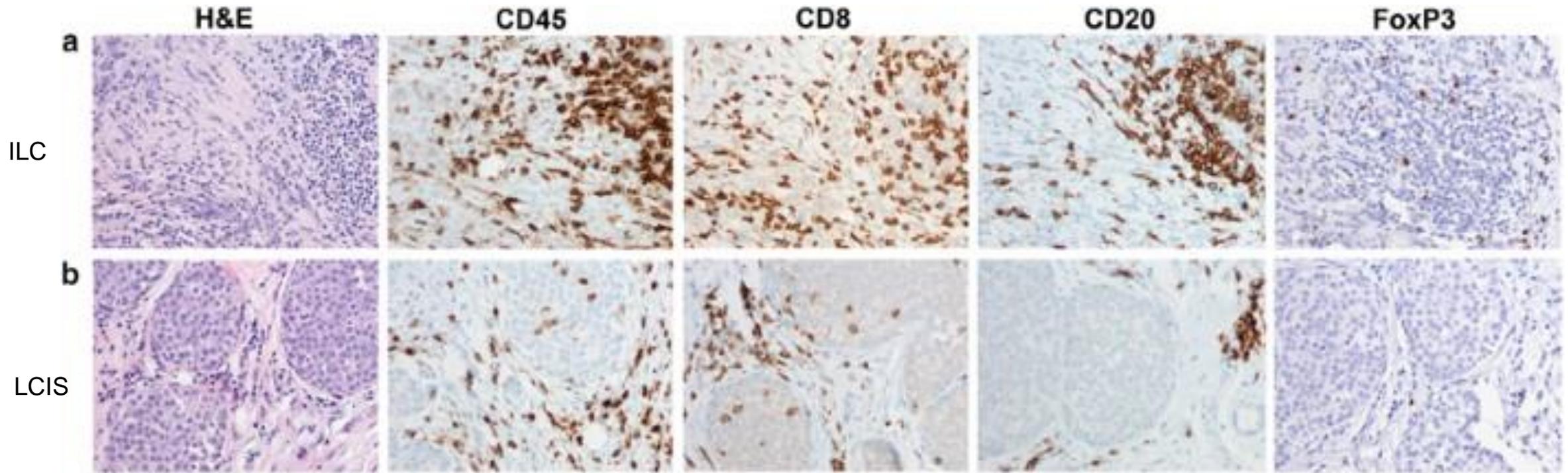
Goodell et al, Mol Ca Ther, 2008; Disis 2014

Adoptive immunotherapy



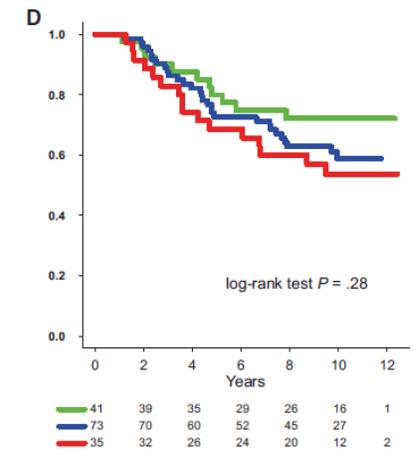
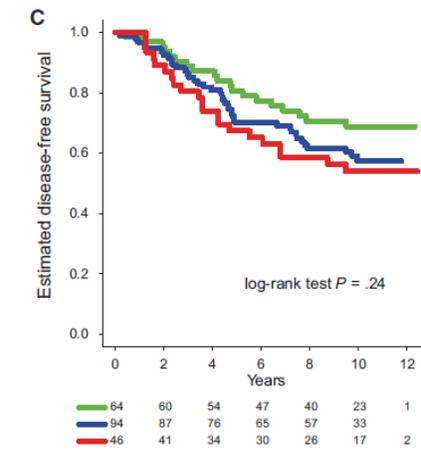
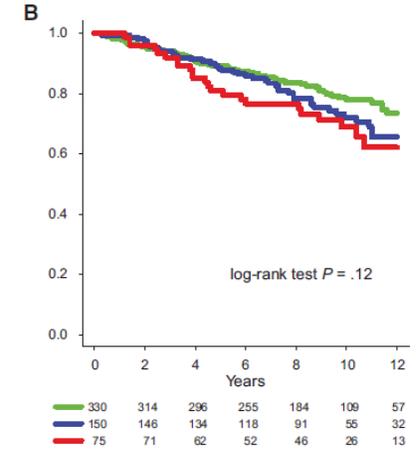
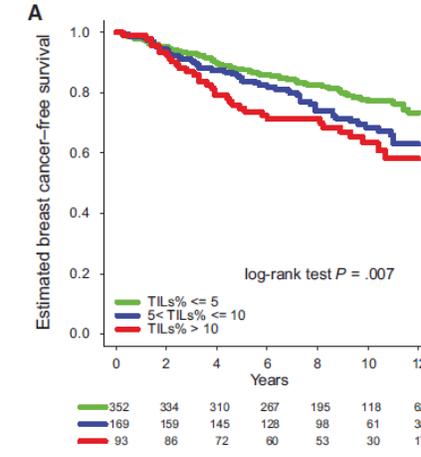
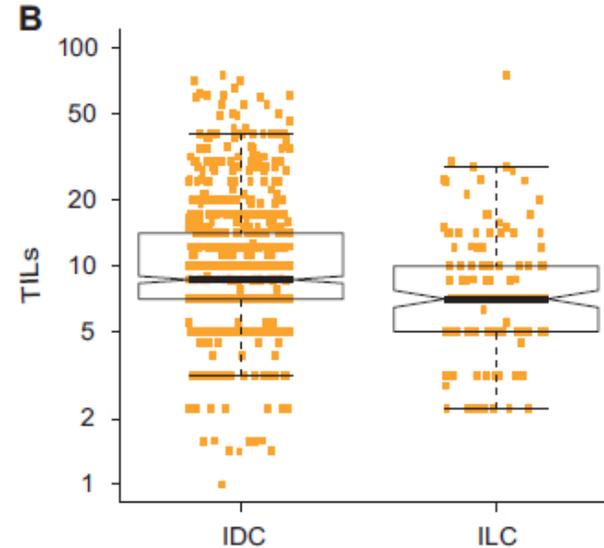
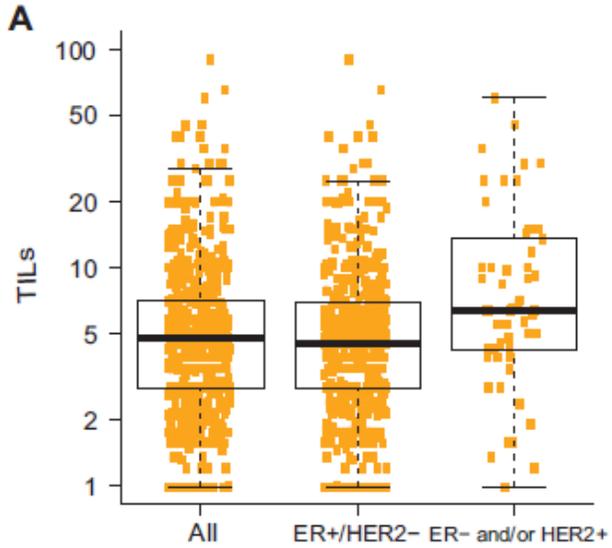
Rosenberg et al, *Nat Rev Cancer* 2008

Now what about lobular and the immune system



Thompson Modern Pathology 2017

TILs in Lobular



Desmedt JNCI 2018



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Questions?