

conquer  
breast

## 1<sup>st</sup> GBO Meeting

1st European Course for MDs in training  
Going Beyond in Oncology

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**NOVEMBER, 28<sup>th</sup>**  
**2018**  
**MILANO**

ROSA GRAND HOTEL MILANO  
Piazza Fontana, 3

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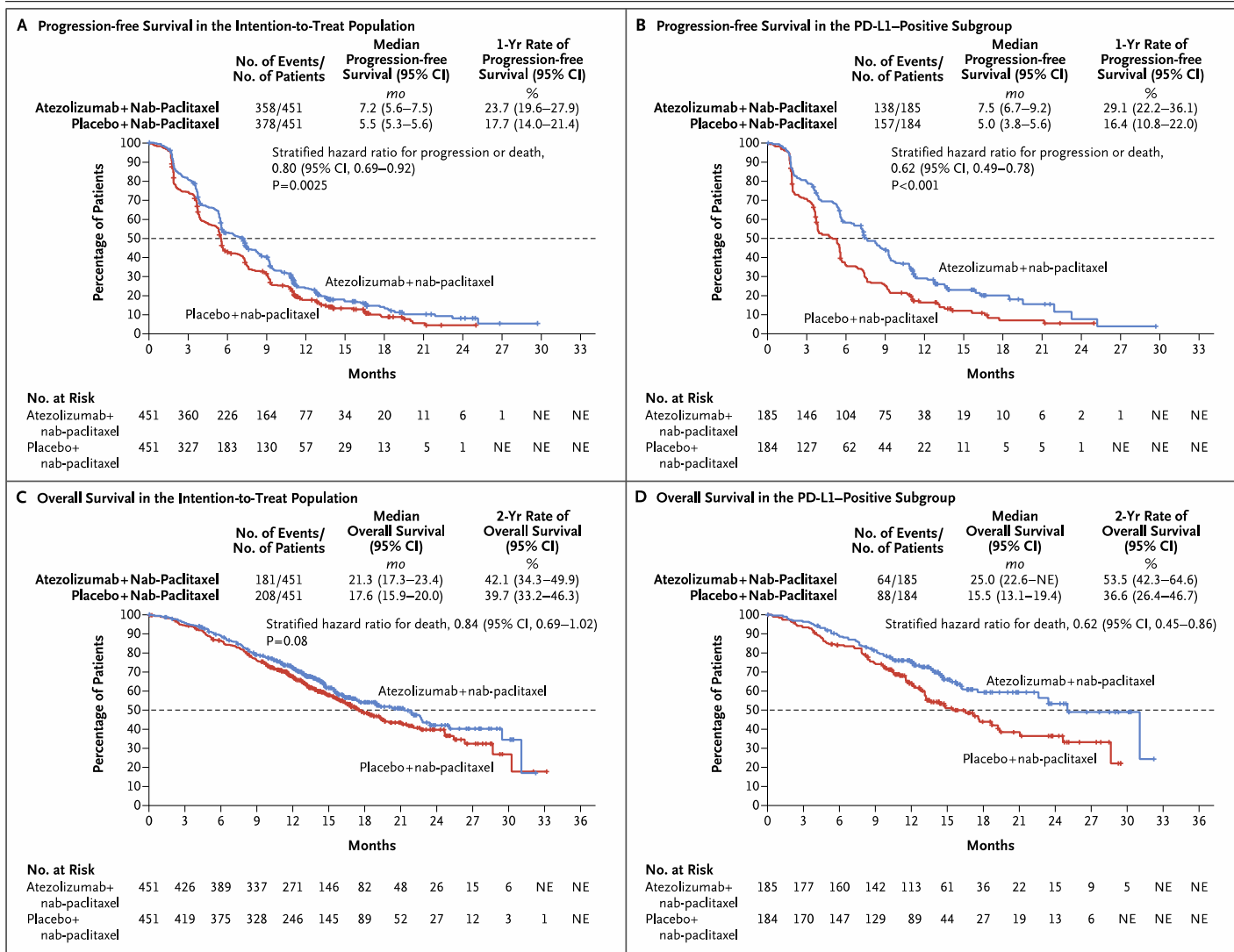
# Tissue biomarkers for immunotherapy

Giancarlo Pruneri, MD

University of Milan

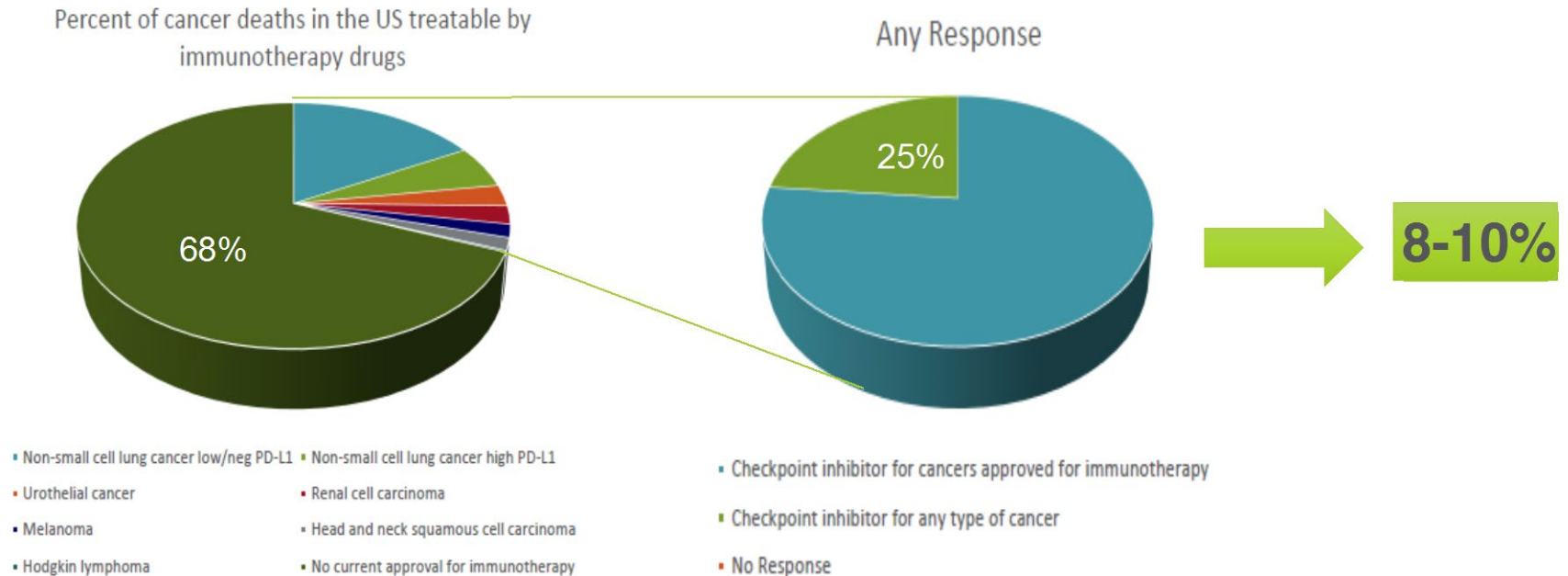
National Cancer Institute INT, Milan

# Breast cancer: immunotherapy goes on stage



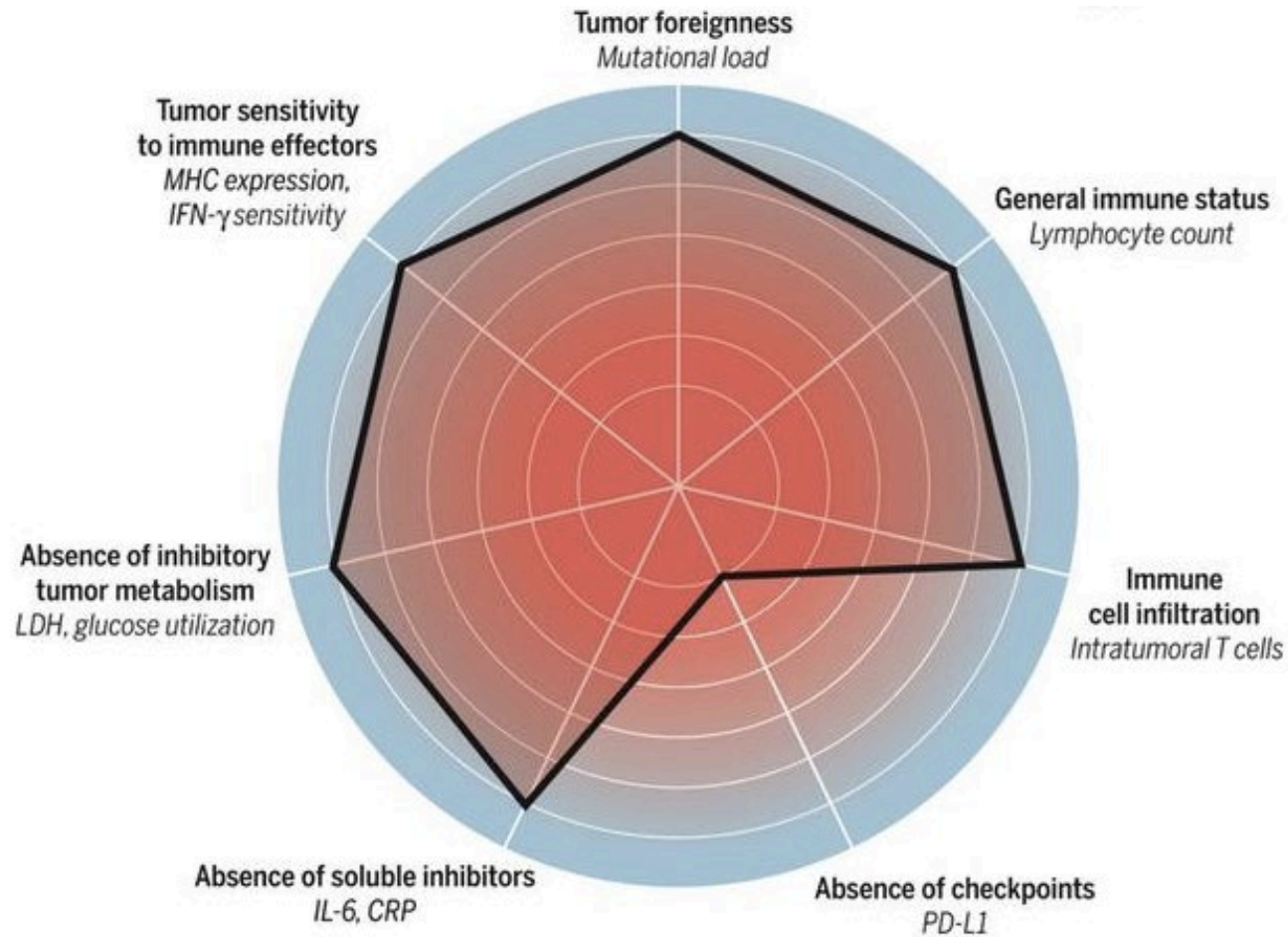
# Immuno-Oncology's Substantial Clinical Impact

## Few Patients Actually Benefit from Currently Approved IO Drugs



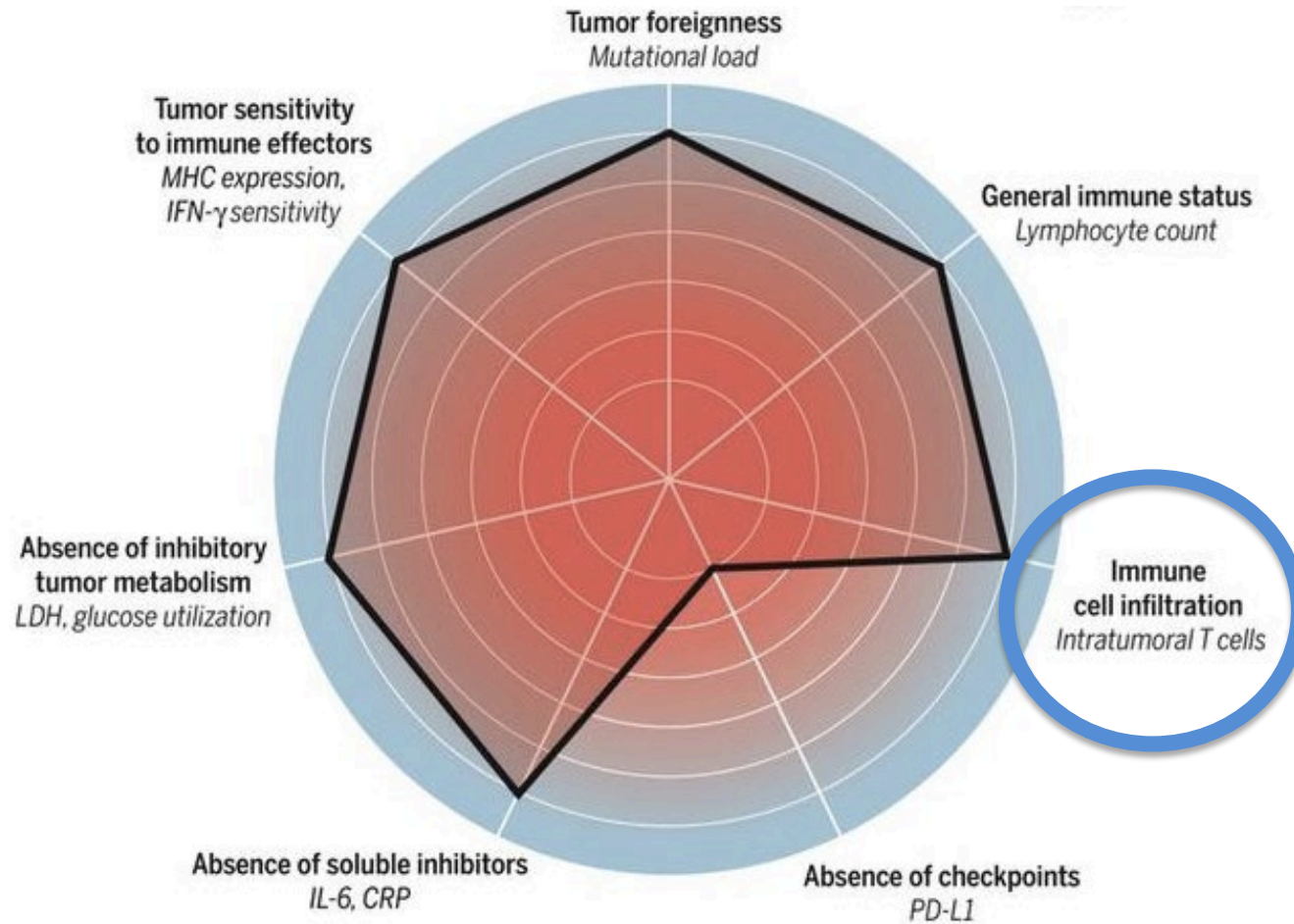
Source: Adapted from Abola and Prasad, JAMA, 2016

# The cancer immunogram



Christian U. Blank et al. Science 2016;352:658-660

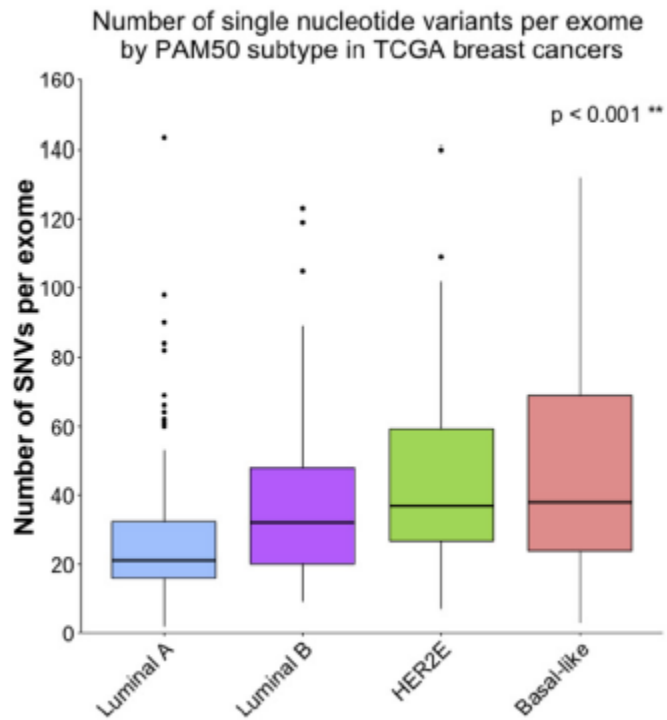
# The cancer immunogram



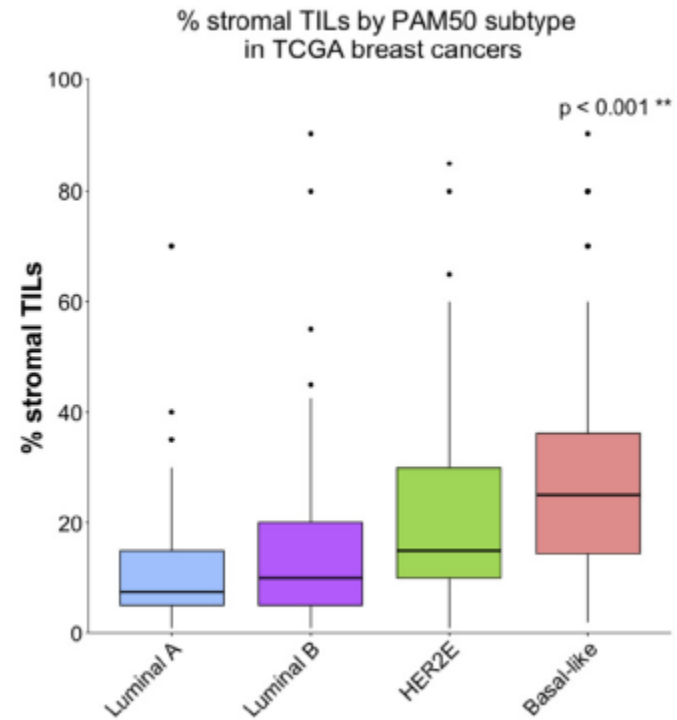
Christian U. Blank et al. Science 2016;352:658-660

# TILs and mutational load in BC subtypes

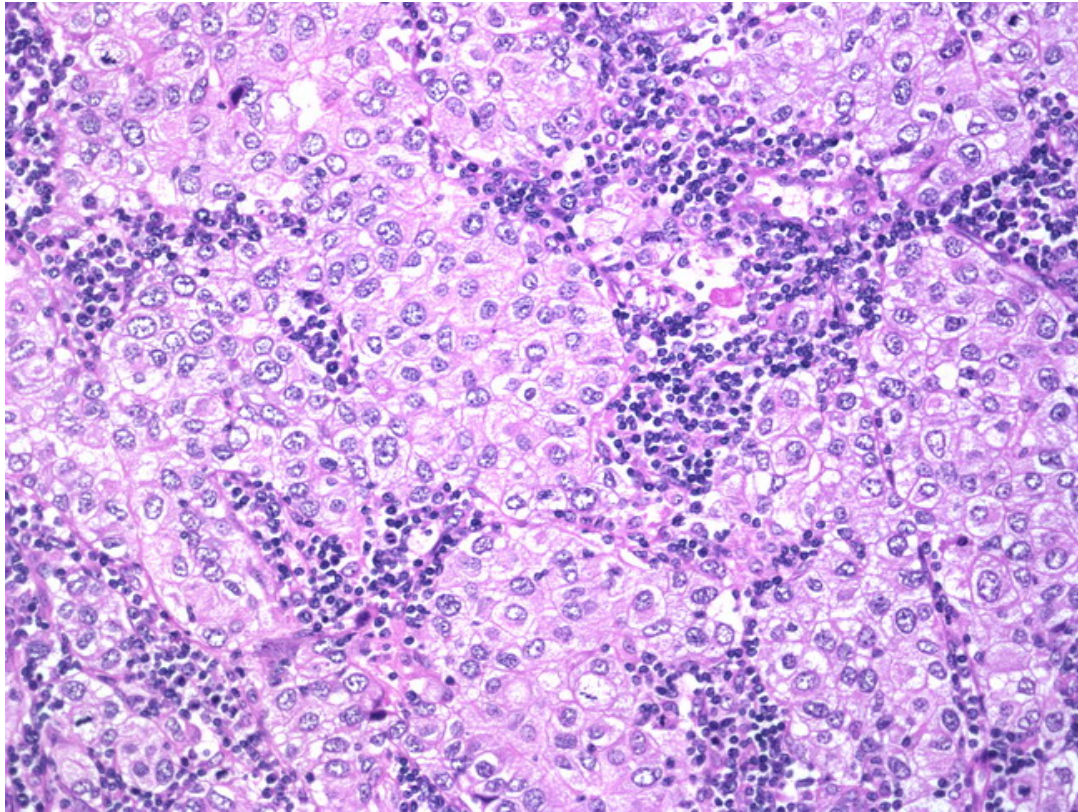
**A**



**B**



# TILs: what if...



## Do TILs have validity?

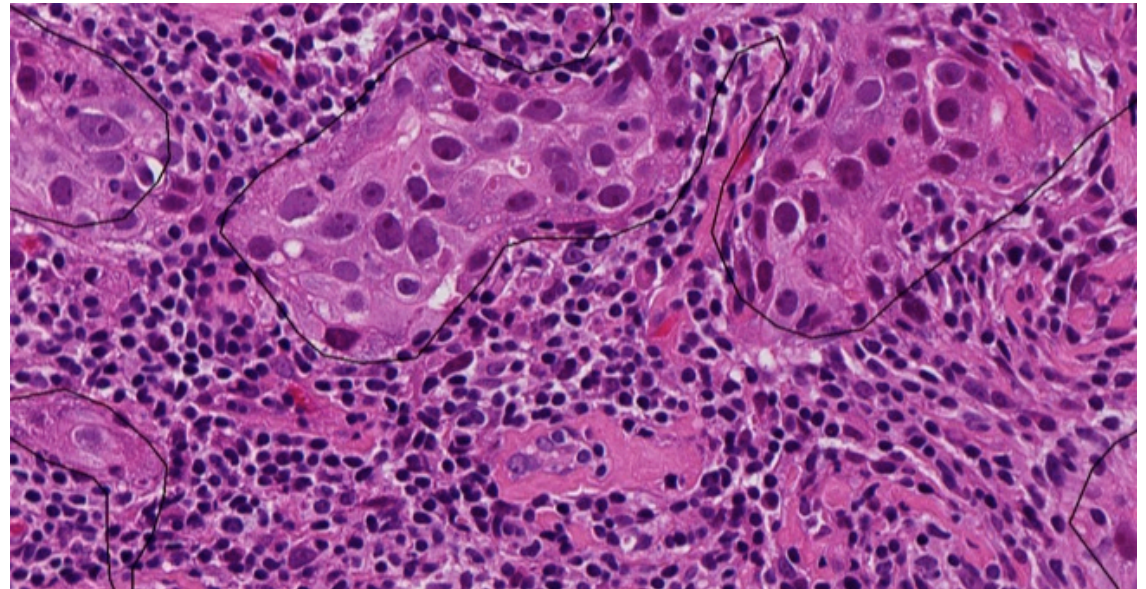
- Analytical validity refers to the accuracy, reliability, and reproducibility of the assay as demonstrated by preanalytic, technical, and scoring or interpretation methods
- Clinical validity refers to the ability of a tumor biomarker test to divide one population into two or more groups that differ either biologically or clinically. CV alone is insufficient to recommend that the test be used to guide treatment decisions



# Pre-defined parameters for TILs evaluation in breast cancer

## **Stromal TILs**

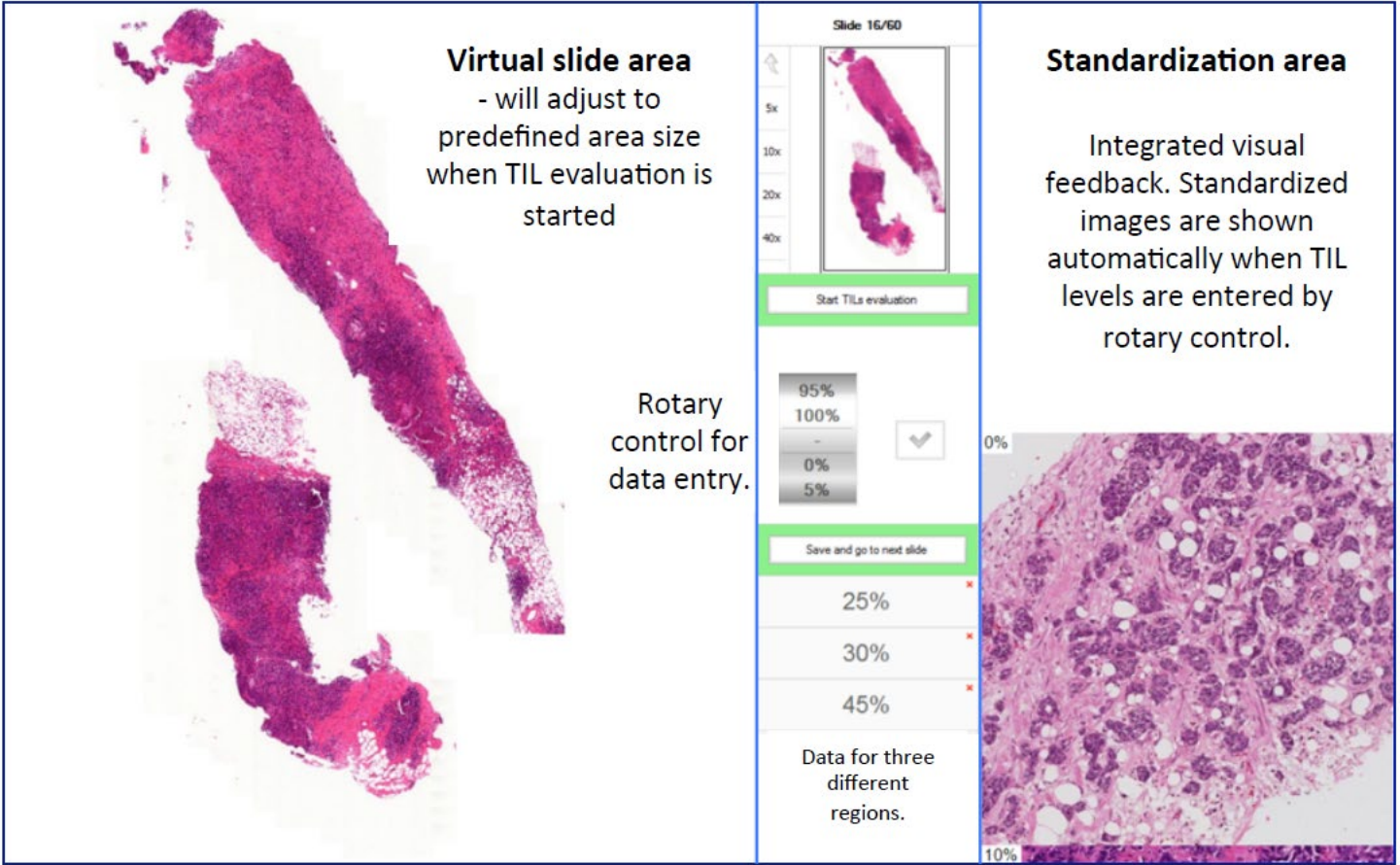
Between the tumor cells but within tumor stroma



**Virtual slide area**  
- will adjust to predefined area size when TIL evaluation is started

Rotary control for data entry.

**Standardization area**  
Integrated visual feedback. Standardized images are shown automatically when TIL levels are entered by rotary control.



Slide 16/60

5x  
10x  
20x  
40x

Start TILs evaluation

95%  
100%  
-  
0%  
5%

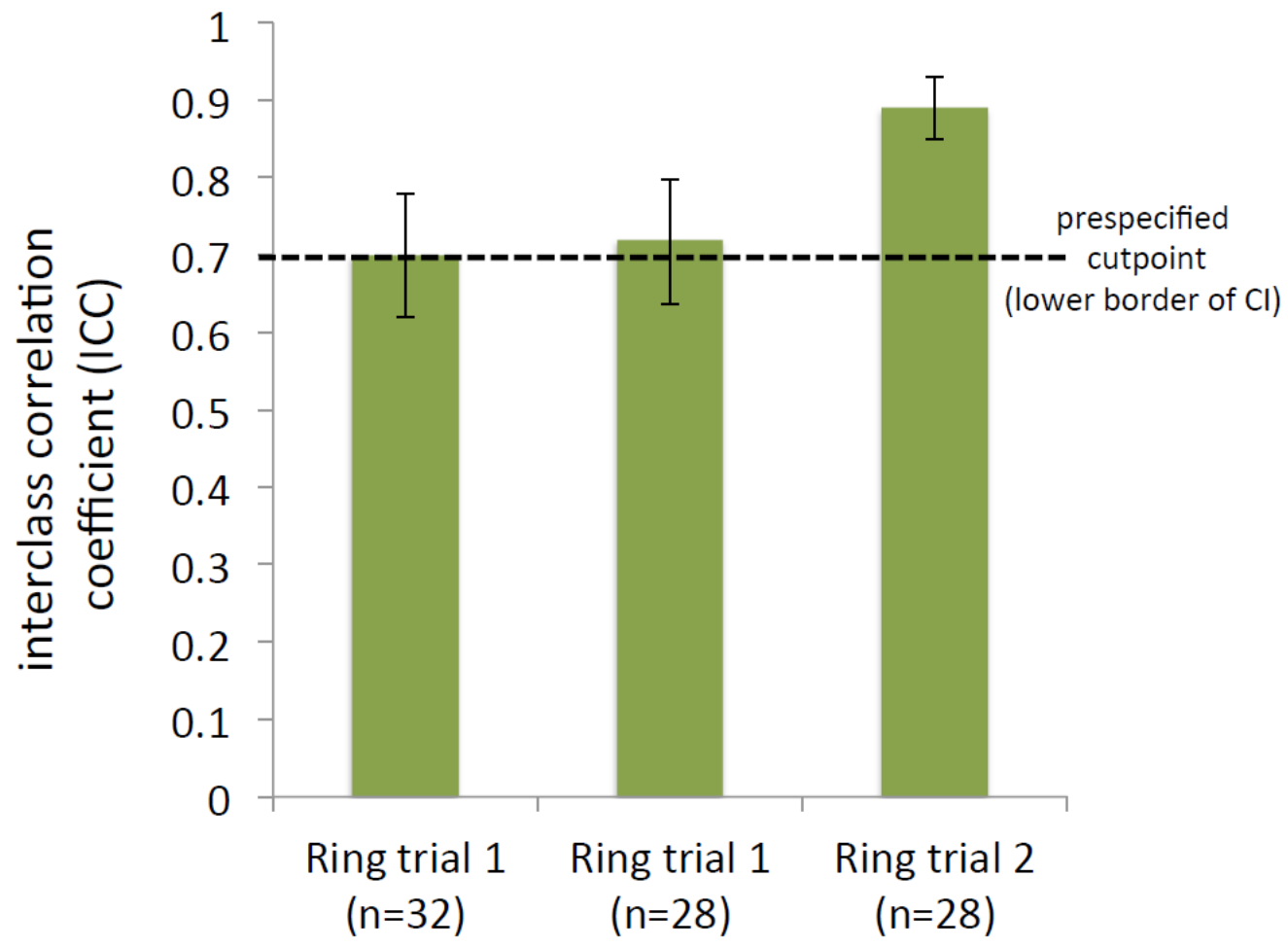
Save and go to next slide

25%  
30%  
45%

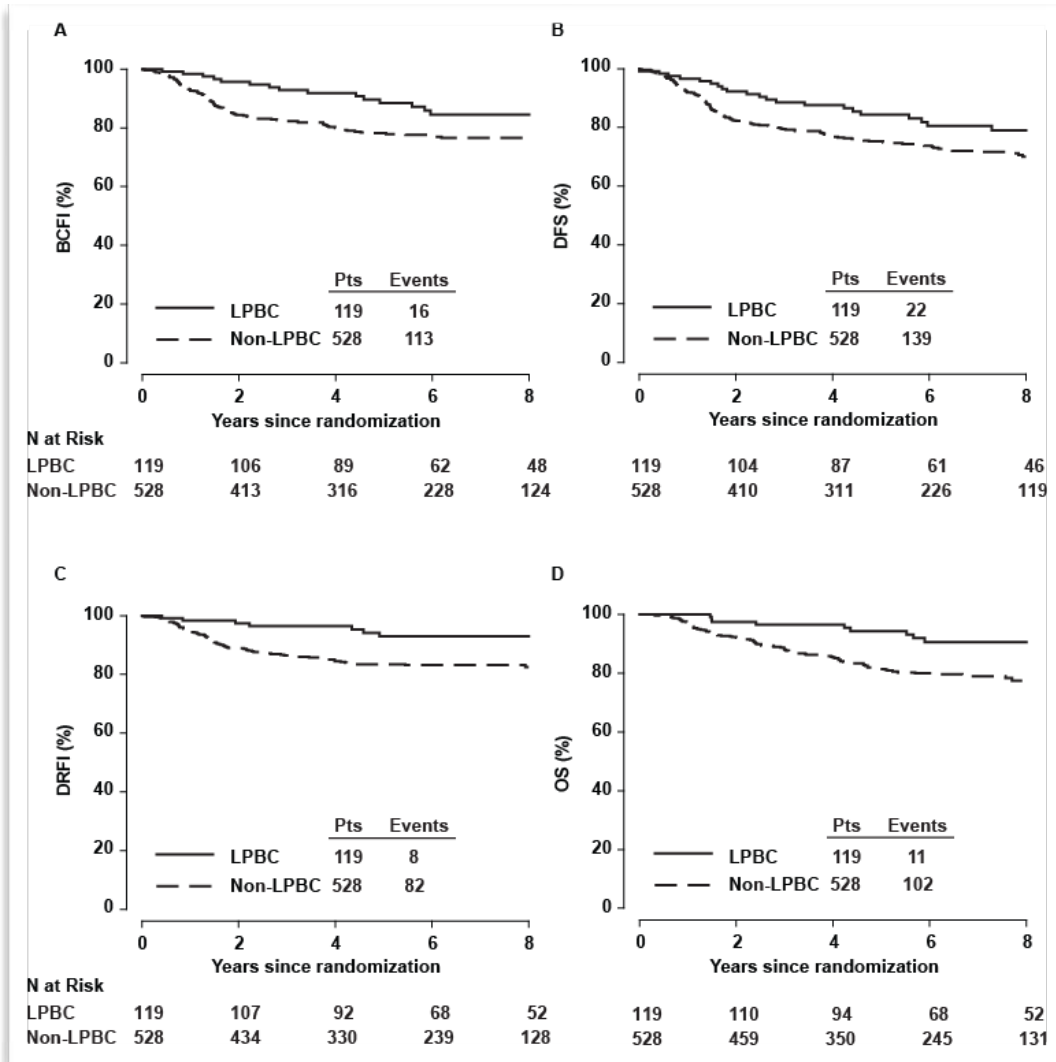
Data for three different regions.

0%

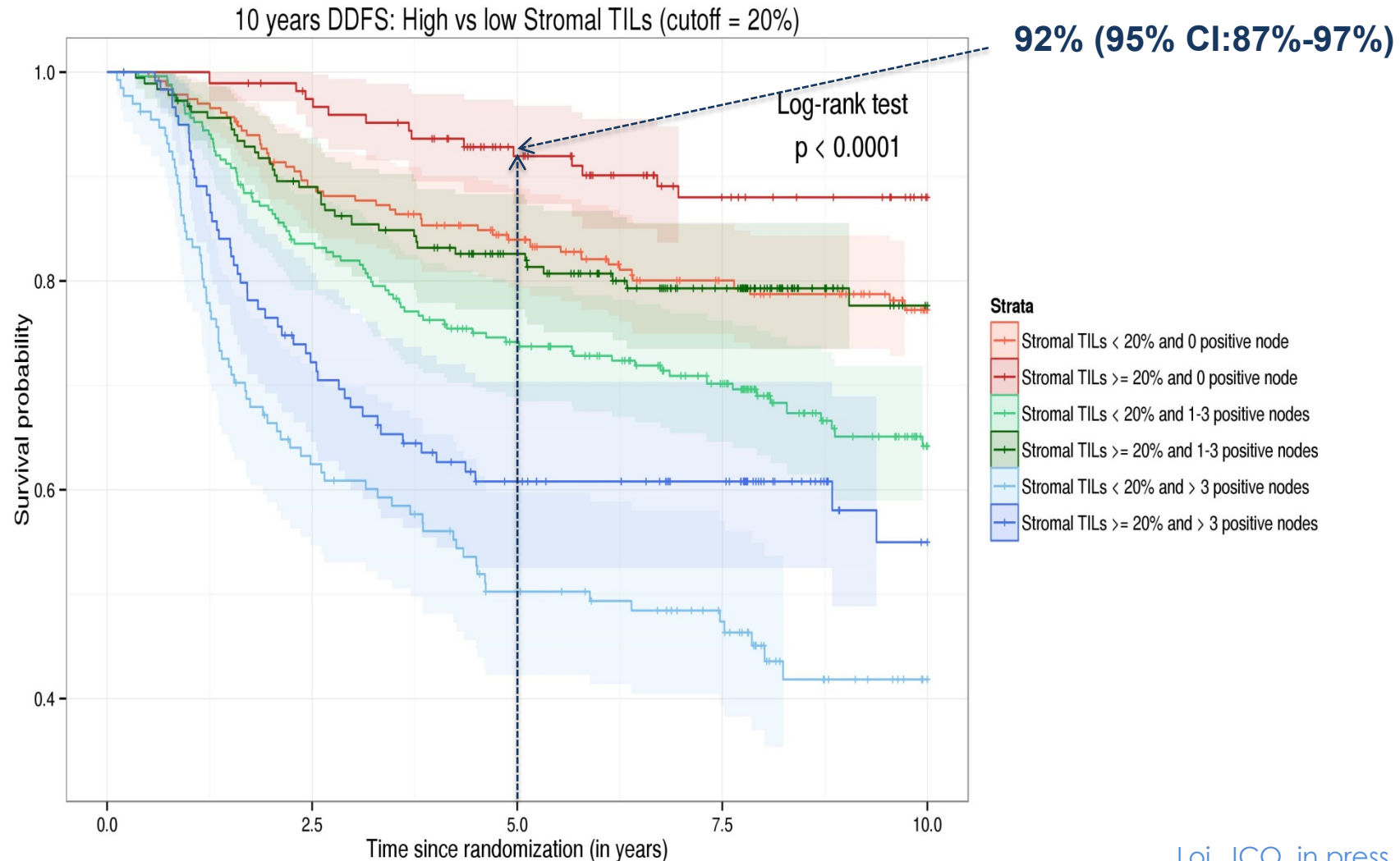
10%



# TILs guidelines in prospective trials: IBCSG 22-00



# Stromal TILs $\geq 20\%$ in node negative TNBC patients have excellent 5 yr D-DFS estimated by Kaplan-Meier



# Clinical utility

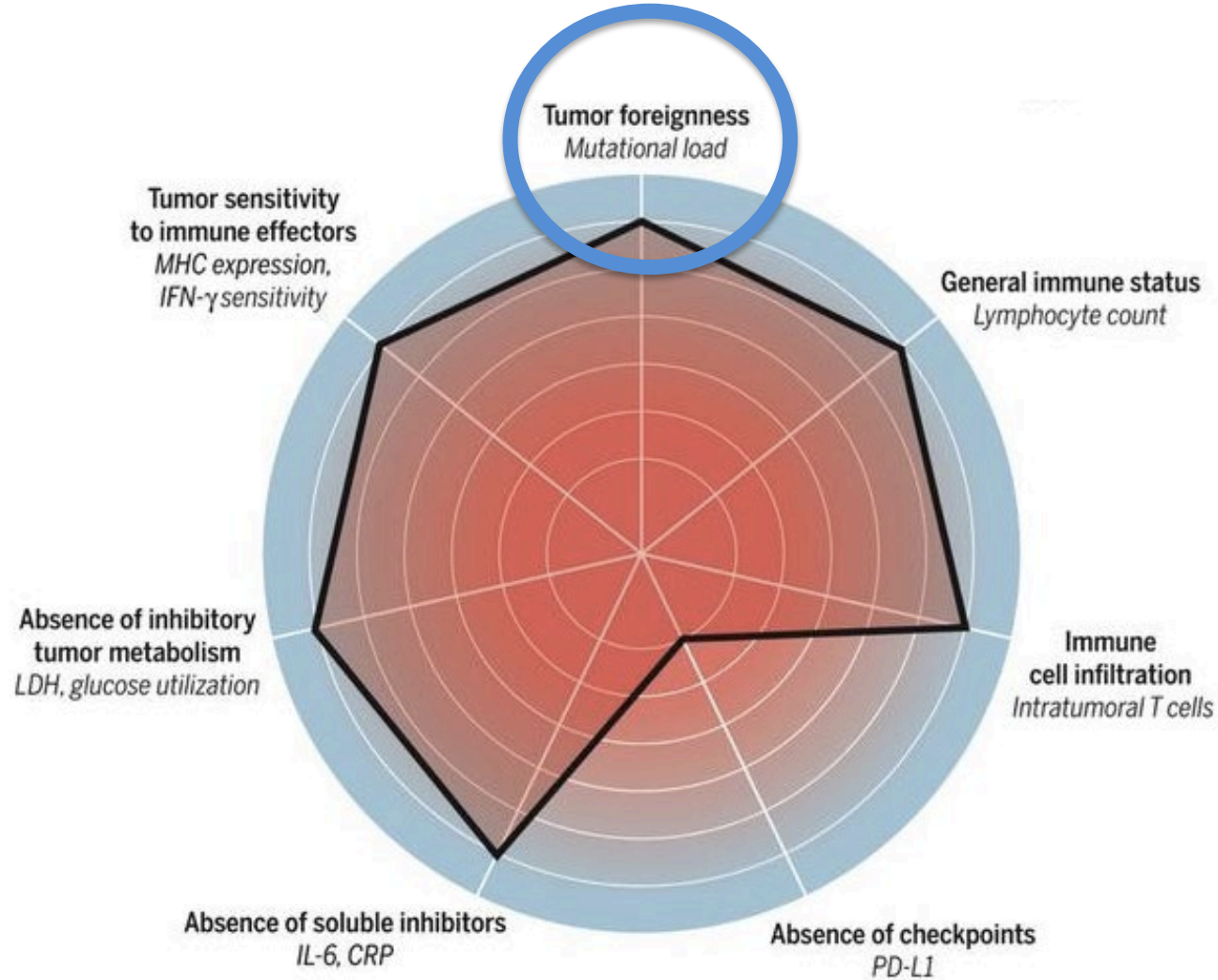
- A biomarker-based test has clinical utility if its use is associated with a favorable balance of benefits to harms compared with treatment of the patient in the absence of the biomarker test results
- Benefits may include improvement in survival end points (EFS, DFS, PFS, OS)
- A biomarker must contribute clinically useful information beyond that already provided by clinical or pathologic indicators in standard use, **unless the new test can provide equivalent information at lower cost**, less invasively, or with less inconvenience or risk. The magnitude of the benefit must be clinically meaningful and outweigh risks, costs, and/or inconvenience associated with use of the test

Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline

*Lyndsay N. Harris, Nofisat Ismaila, Lisa M. McShane, Fabrice Andre, Deborah E. Collyar, Ana M. Gonzalez-Angulo, Elizabeth H. Hammond, Nicole M. Kuderer, Minetta C. Liu, Robert G. Menzel, Cathy van Poznak, Robert C. Bast, and Daniel F. Hayes*

- If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use TILs lymphocytes to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong.
- If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use TILs to guide decisions on adjuvant systemic therapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: strong.

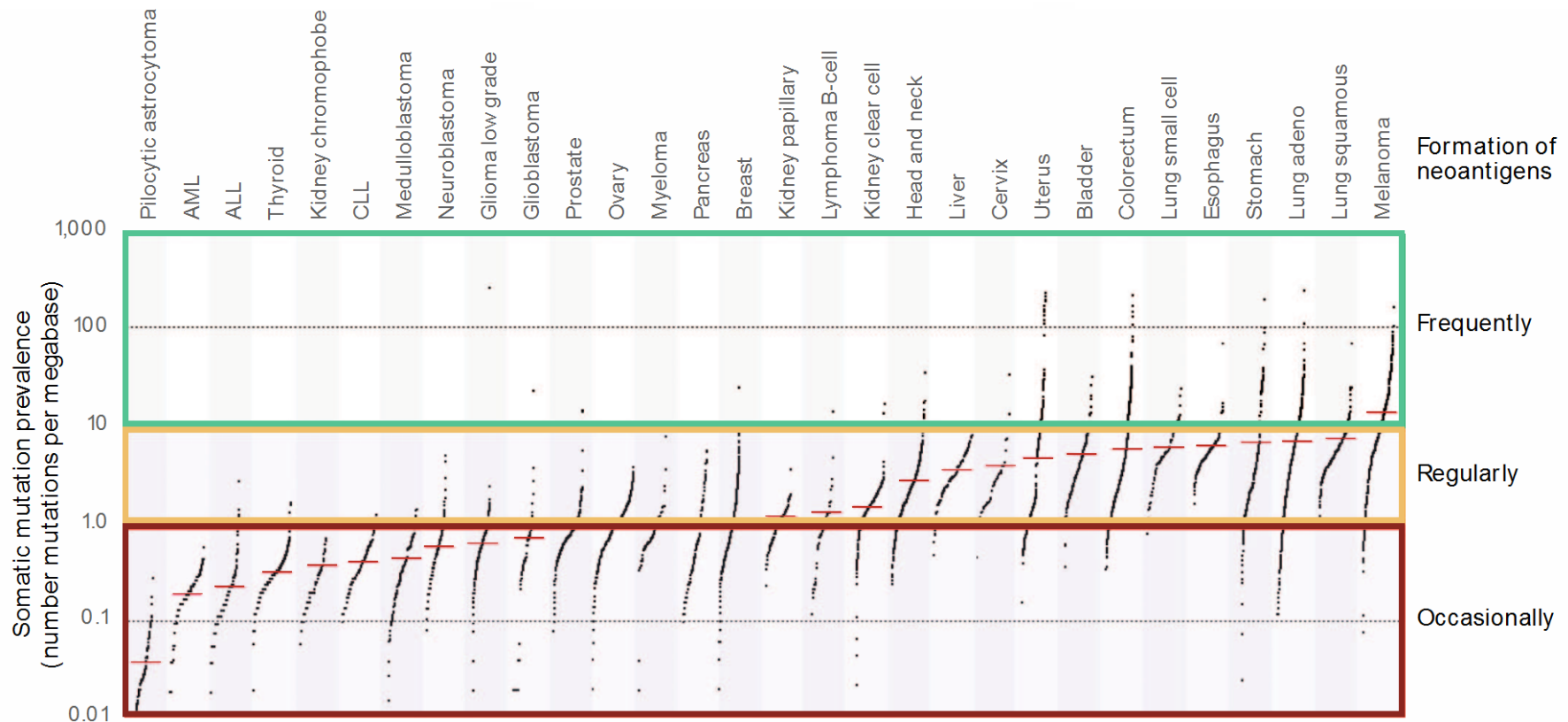
# The cancer immunogram



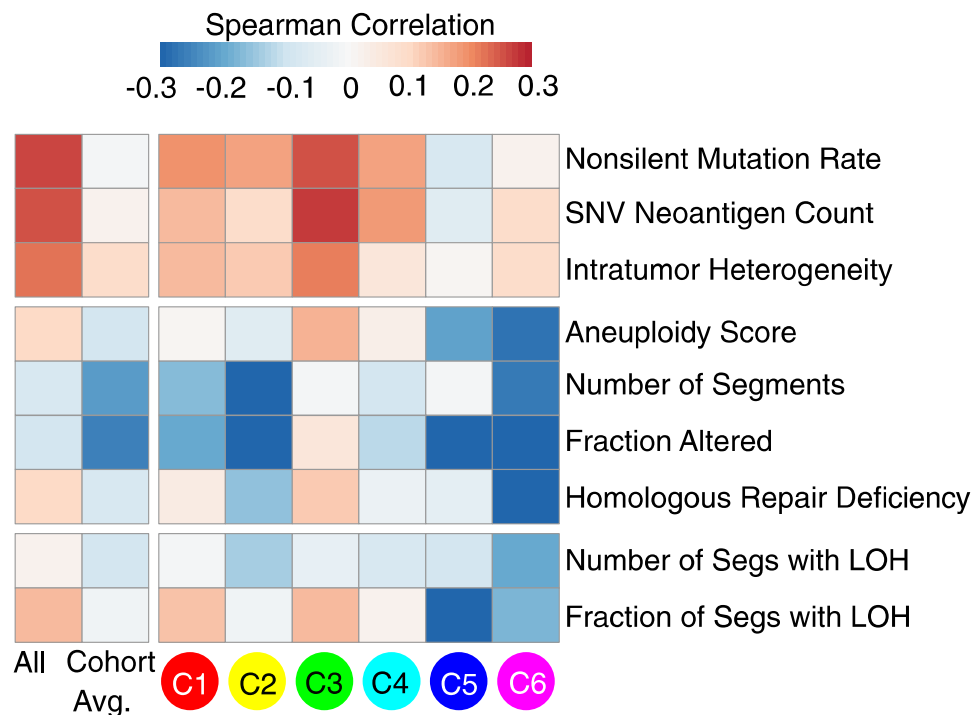
Christian U. Blank et al. Science 2016;352:658-660



# Tumors with $>150$ non-syn mut (10 mut/mb DNA) form neoantigens

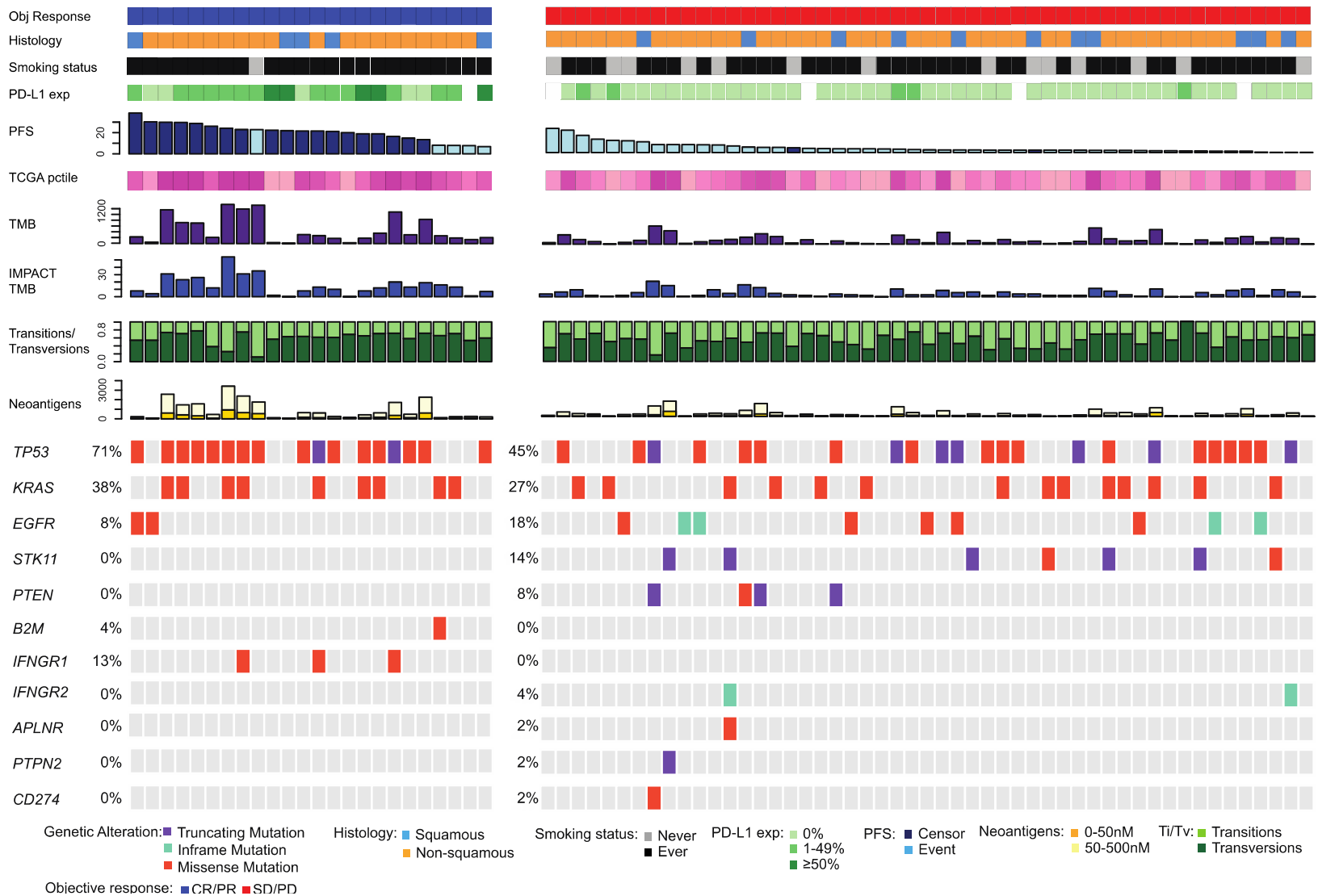


# Immune infiltration correlates with tumor somatic variation

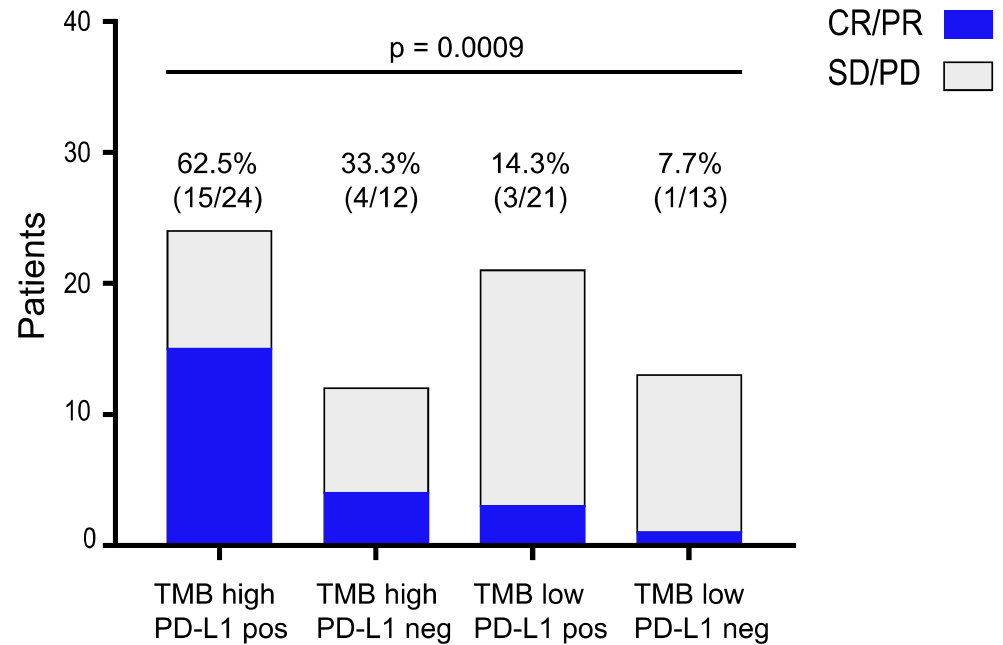
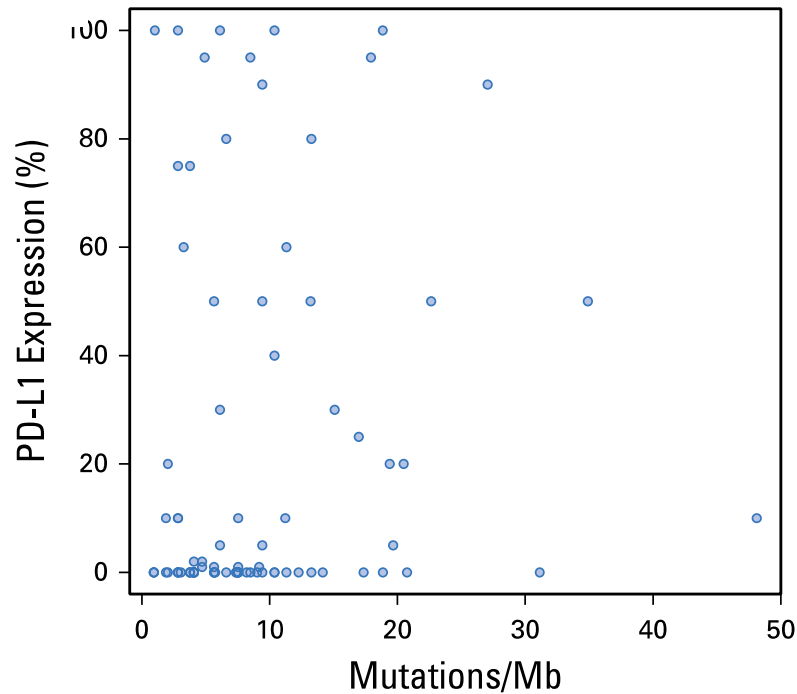


# TMB in clinical trials

## CheckMate-012 Nivo+Ipi NSCLC



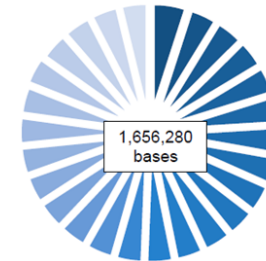
# PDL-1 IHC and TMB: towards an integrate approach?



# TMB in the real world

## INT experience on 35 early NSCLC patients

**The OncoPrint Tumor Mutation Load Assay covers 1.7Mb across 409 cancer-driver genes, relevant across major cancer types**



- signaling cascades
- apoptosis genes
- DNA repair genes
- transcription regulators
- inflammatory response genes
- growth factor genes

- Set up
- TAT
- Data interpretation
- Reproducibility

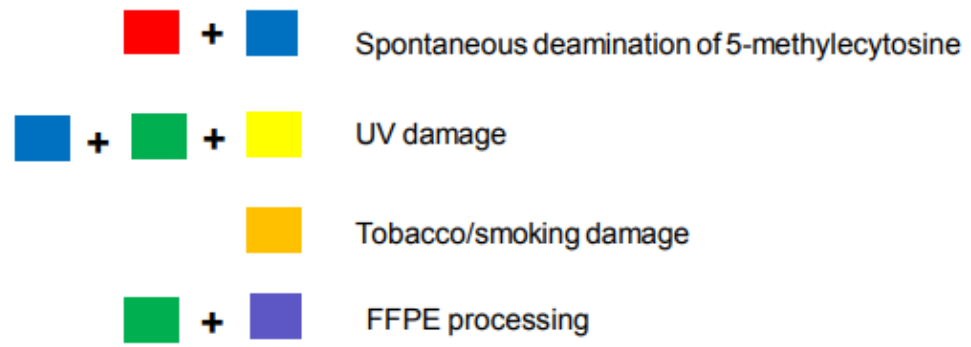
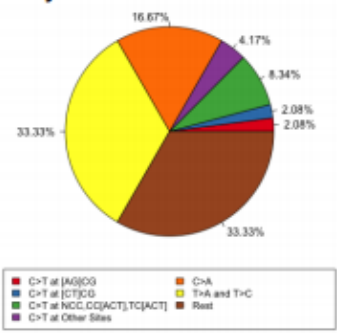
# Signature Pattern of Somatic Mutations - Background

## Carcinogens/Biological Processes have unique Patterns

- High C>T at CpG is consistent with Spontaneous deamination of 5-methylcytosine<sup>1</sup>
- High C>T at CpC, CpC, TpC, T>A, and T>C is consistent with UV damage<sup>2</sup>
- High C>A is consistent with smoking damage<sup>3</sup>
- High C>T (site independent) is consistent with FFPE processing<sup>4</sup>

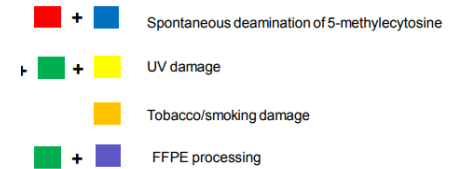
<sup>1</sup> Alexandrov LB et al. *Nature*. 2013;<sup>2</sup> Hayward NK et al. *Nature*. 2017; <sup>3</sup> Alexandrov LB et al. *Cancer Etiology*. 2016; <sup>4</sup> Wong SQ et al. *BMC Medical Genomics*. 2014;

Signature Pattern of Somatic Mutations if each mutation from 96 classes is equally likely

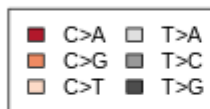
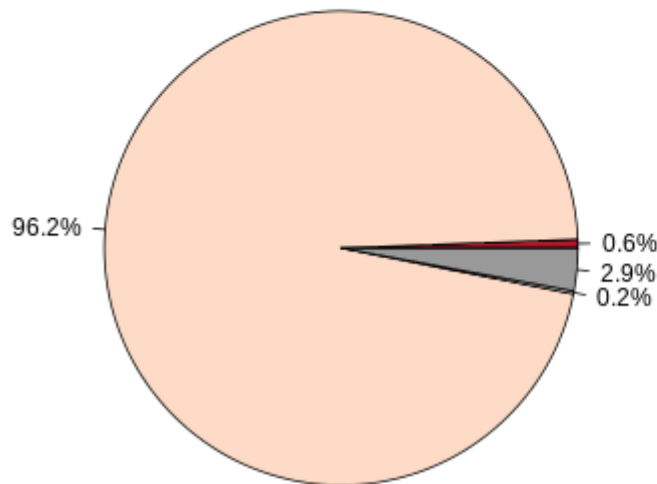


# Real world hurts

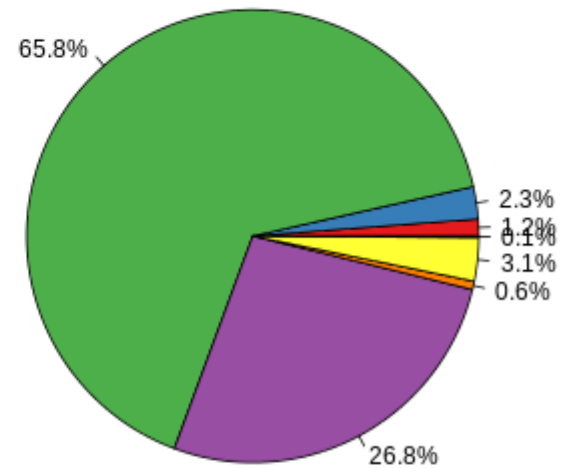
Mutation Load (Mutations/Mb): 1902.52



## Somatic Mutations across Substitution Type



## Signature Pattern of Somatic Mutations



### Additional Information:

- High C>T at CpG is consistent with Spontaneous deamination of 5-methylcytosine<sup>1</sup>
- High C>T at CpC, CpG, TpC, T>A, and T>C is consistent with UV damage<sup>2</sup>
- High C>A is consistent with smoking damage<sup>3</sup>
- High C>T (site independent) is consistent with FFPE processing<sup>4</sup>

<sup>1</sup>Alexandrov LB et al. *Nature*. 2013; <sup>2</sup>Hayward NK et al. *Nature*. 2017; <sup>3</sup>Alexandrov LB et al. *Cancer Etiology*. 2016; <sup>4</sup>Wong SQ et al. *BMC Medical Genomics*. 2014;

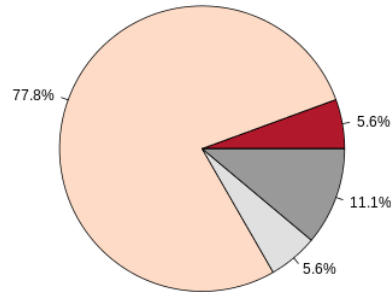
S07-9455 8-4\_v1\_c1476\_2018-06-15-16-51-43-489

Mutation Load (Mutations/Mb): 11.04

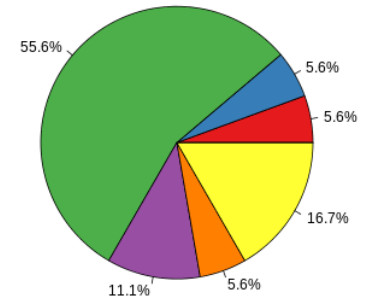
Sample Results Sample QC

Views: Substitution Type and Signature Pattern of Somatic Mutations

Somatic Mutations across Substitution Type



Signature Pattern of Somatic Mutations



Workflow  
10% standard

Red + Blue Spontaneous deamination of 5-methylcytosine

Green + Yellow UV damage

Orange Tobacco/smoking damage

Dark Green + Purple FFPE processing

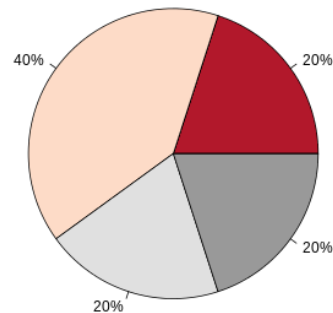
7-9455 8-4\_v1\_c3271\_2018-09-24-18-57-17-958

Mutation Load (Mutations/Mb): 3.07

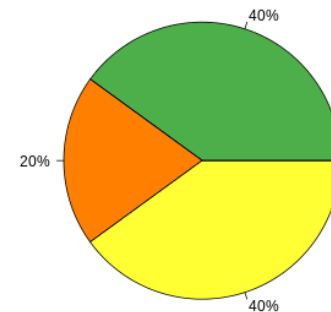
Sample Results Sample QC

Views: Substitution Type and Signature Pattern of Somatic Mutations

Somatic Mutations across Substitution Type



Signature Pattern of Somatic Mutations

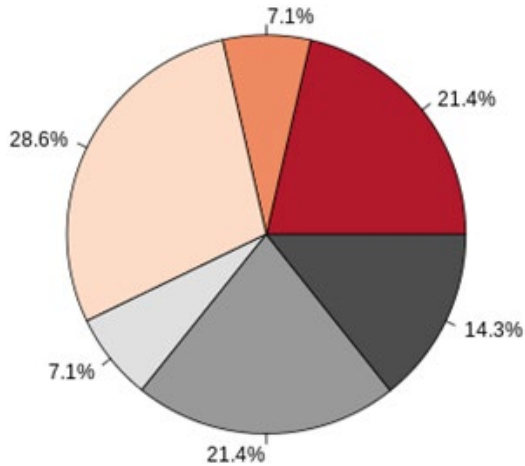


Workflow  
10% modified

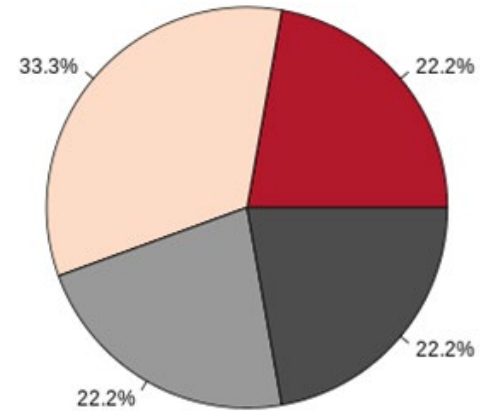


# Formalin fixation

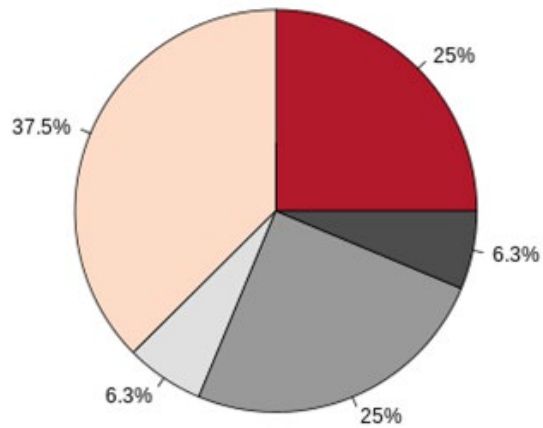
8 hrs MUTATION LOAD (Mutations/Mb): 8.54



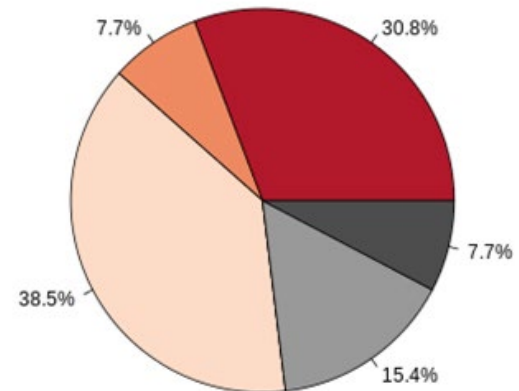
24 hrs MUTATION LOAD (Mutations/Mb): 5.63



72 hrs MUTATION LOAD (Mutations/Mb): 9.78



1 week MUTATION LOAD (Mutations/Mb): 7.94



# Immunotherapy in breast cancer, take-home

- Immunotherapy is entering the clinical arena for BC patients
- TILs will be likely included among predictive/prognostic biomarkers
- Integrating biomarkers is warranted (GEP, TILs, TMB)
- Clinical practice: cancer centers with a NGS lab and large volumes vs, biotech companies
- Tissue workflow critical (IHC for diagnosis HRs, PDL-1, ISH, TMB)
- Multidisciplinary team mandatory (surgeons, oncologists, radiologists, pathologists, molecular biologists)