Genomic and Immune characterization of metastatic breast cancer (mBC): An ancillary study of the SAFIR01 & MOSCATO trials

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Background

- **Extensive efforts have been done in order to profile primary breast cancers**
- **Mutational landscape may evolve and some arguments suggest that metastatic "lethal" breast cancer dramatically differ from primary**
- **The molecular landscape of metastatic breast cancer is unknown**

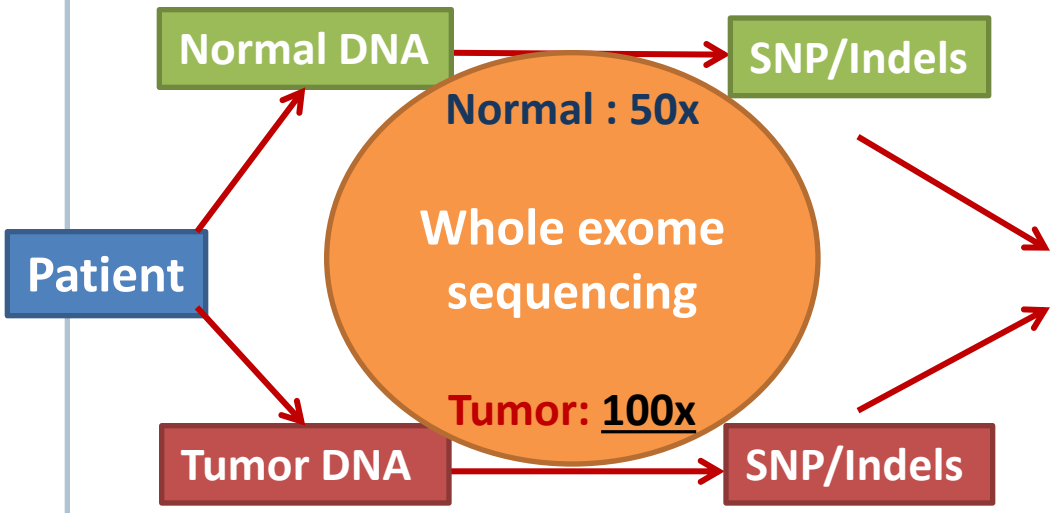
The aim of this study is to analyze the genomic and immunologic profiles of « lethal » metastatic breast cancers in order to identify new targets and unmet medical needs

Outline

- Whole exome sequencing
  - Genomic landscape
  - Mutational signatures
- Immunological markers
  - MHC I
  - TIL
  - PDL1/PD1

Whole Exome Sequencing

HiSeq technology (Integragen)



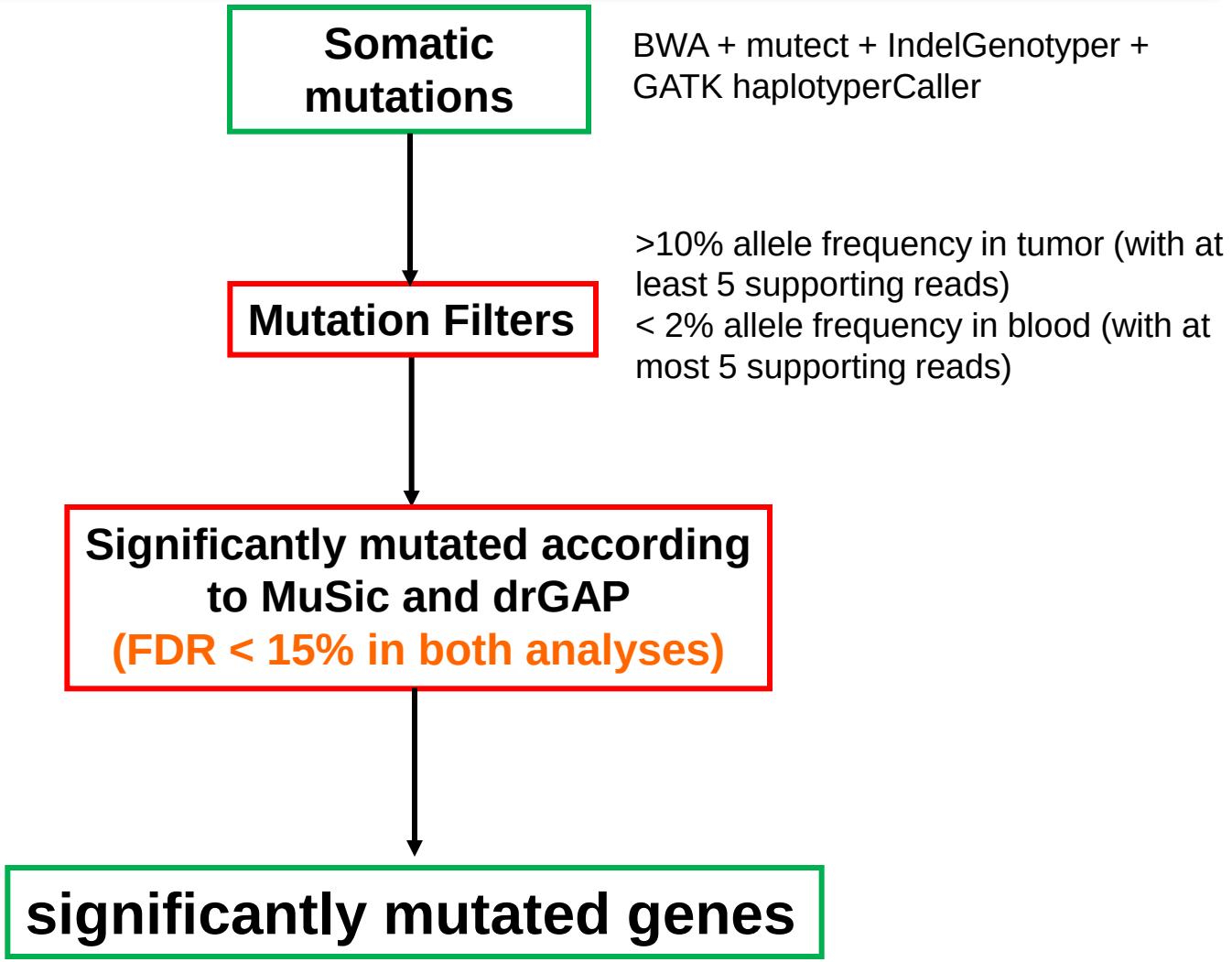
- Compare genotype
- Determine variant status

CASAVA1.8.2 (Illumina Software)

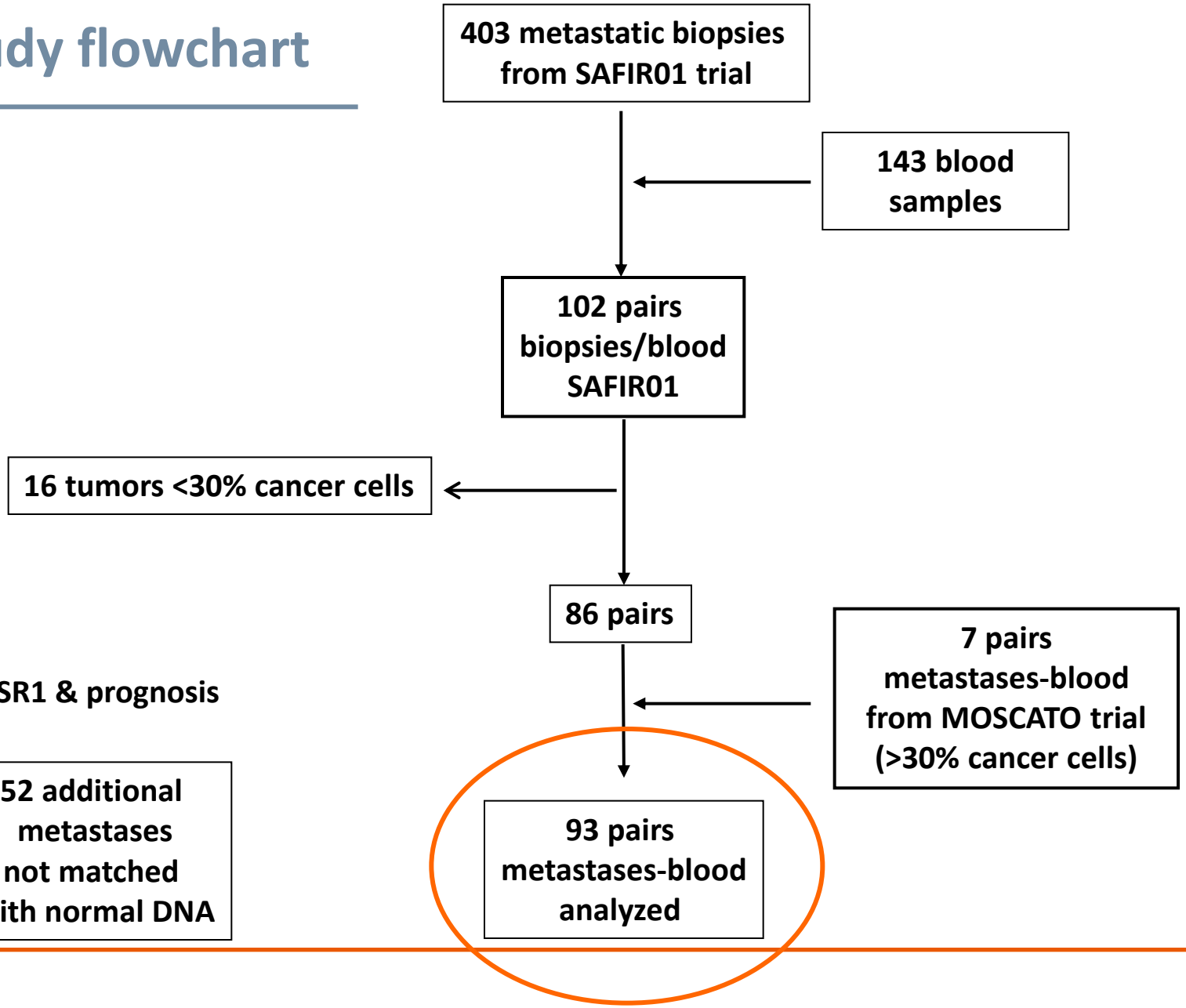
	Mean coverage +/- SD	% of the exome covered >25X
Tumor samples	102x	89%
Normal samples	53x	79%

Quality control with Sanger	
PIK3CA	14/18
AKT	3/4

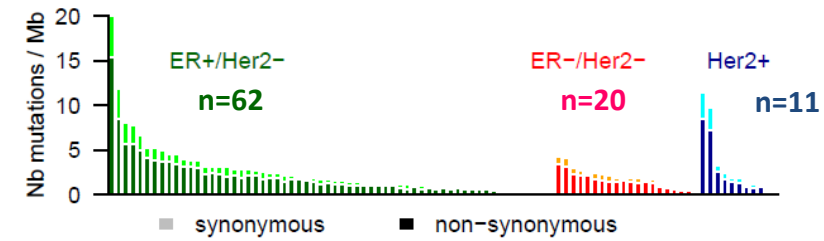
Identification of significantly mutated genes

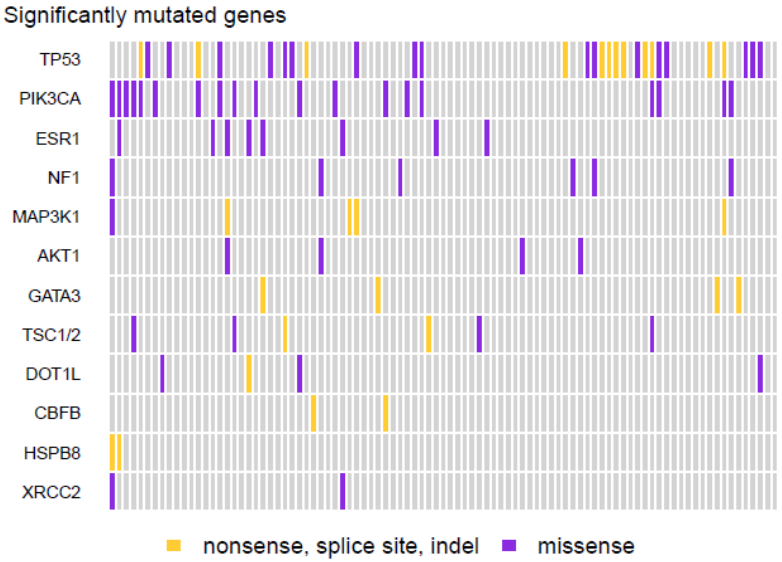


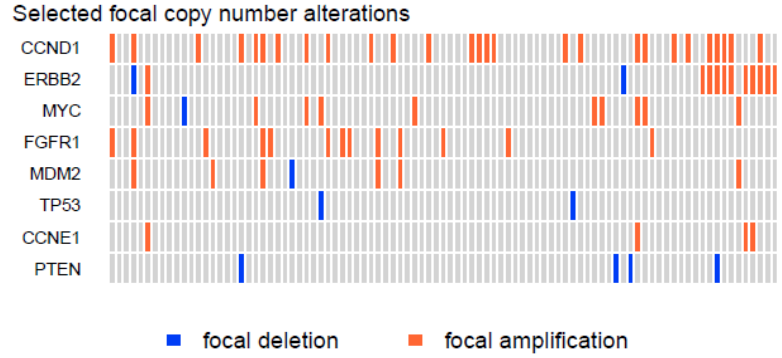
Study flowchart



Mutational landscape and significantly mutated genes





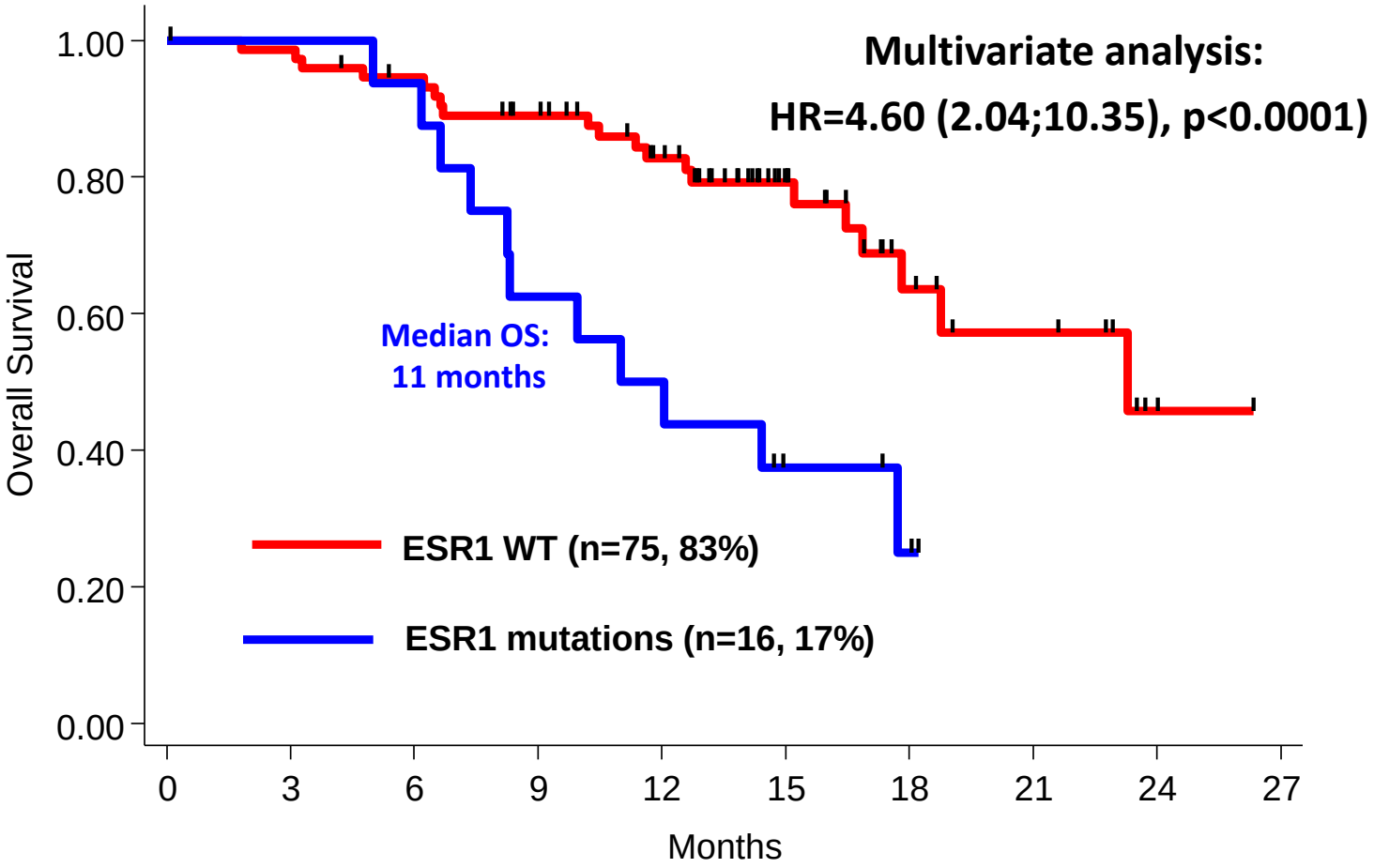


FDR<15%
Recurrent alterations

⇨ **Mutations found in <1% of early breast cancer (TCGA)**

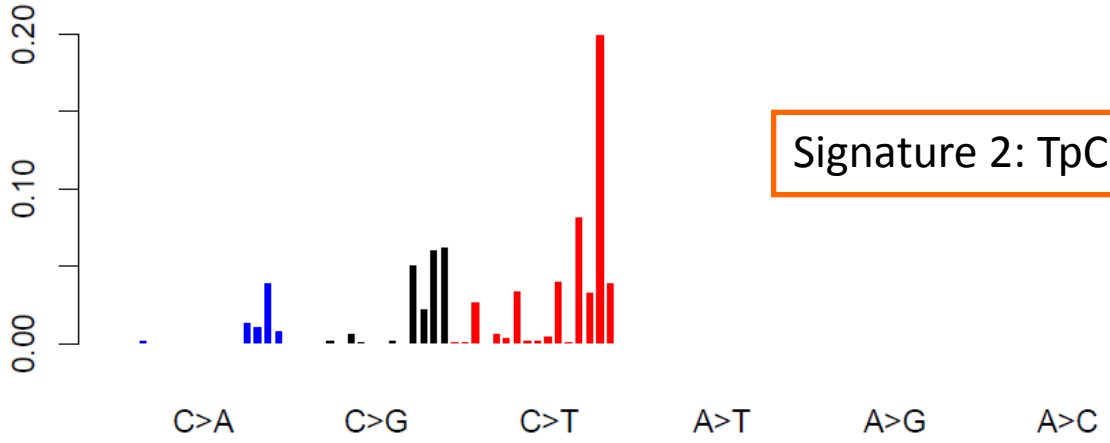
- **ESR1, TSC1/2 and DOT1L are found mutated in at least 5% of mBC but <1% early breast cancers (TCGA)**
- **Using a 15% FDR as cut-off, we could not identify other recurrent « metastasis-specific » drivers**

ESR1 mutations & patient outcome

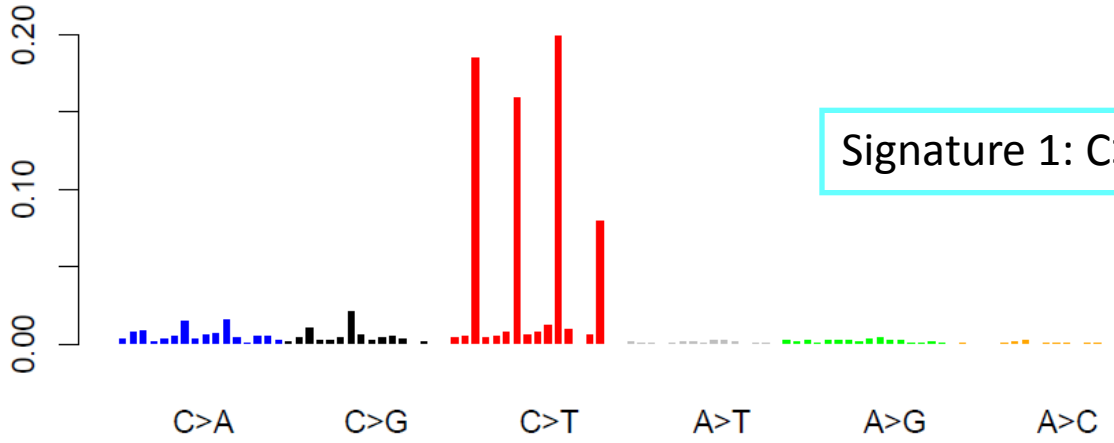


ESR1 mutations are associated with poor outcome

Analysis of mutation signatures (EMu algorithm) revealed two mutational processes

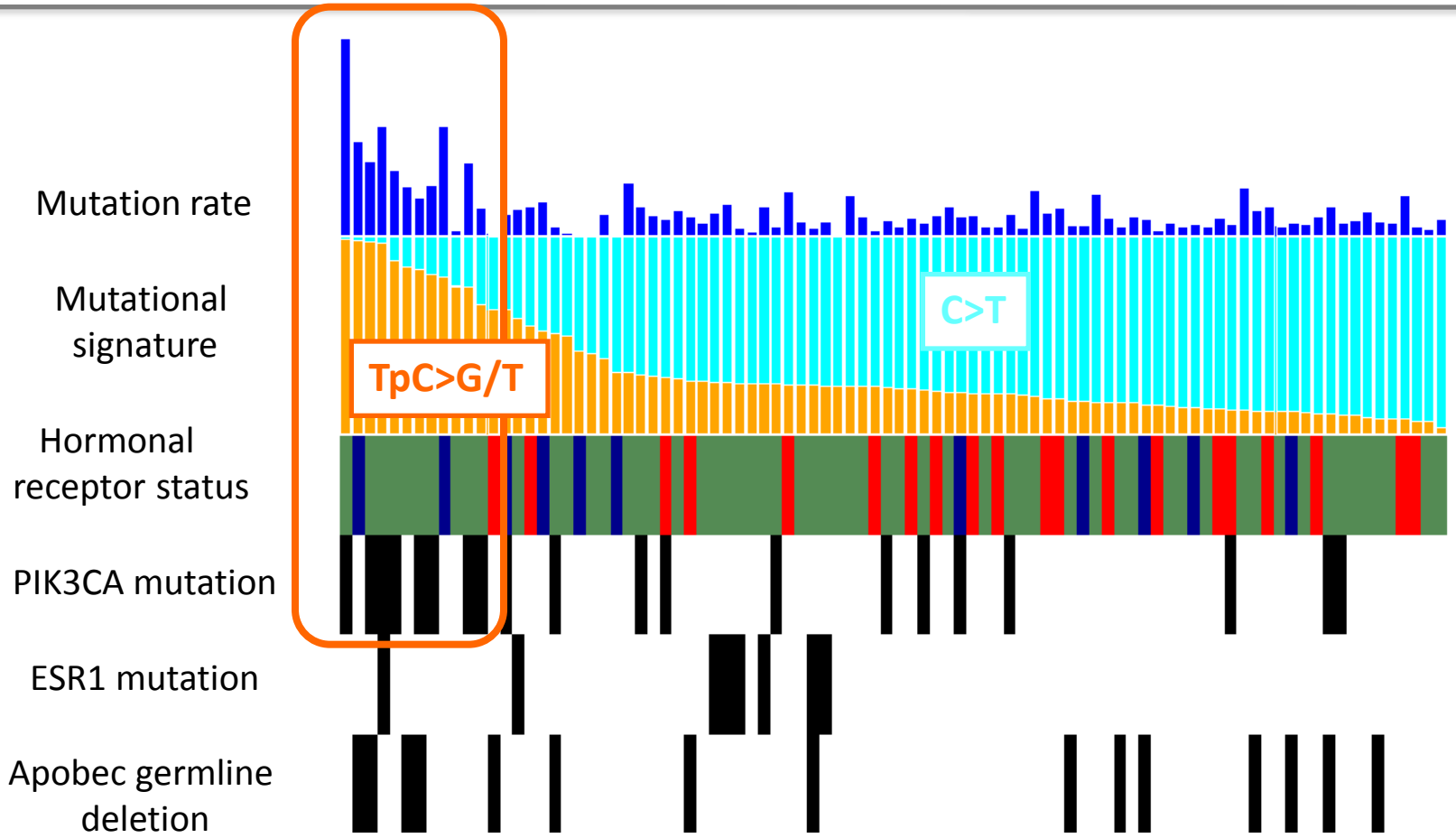


Signature 2: TpC>G/T



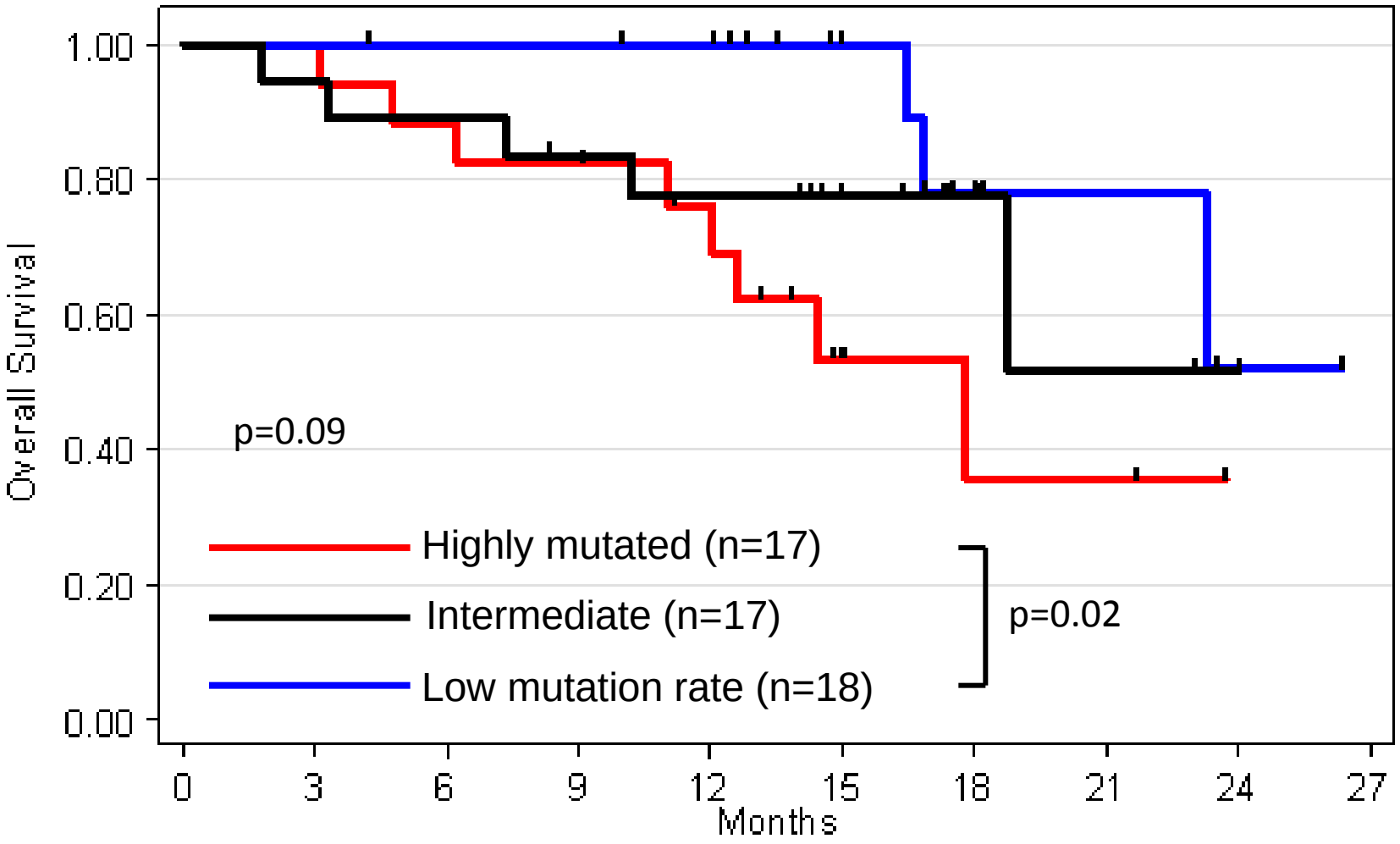
Signature 1: C>T

Correlation betwen mutation rate, mutational signature and genomic alterations



Cluster of patients present with ER+, PIK3CA mutations, high mutation rate, TpC>G/T mutations,

Mutation number & patient outcome



Outline

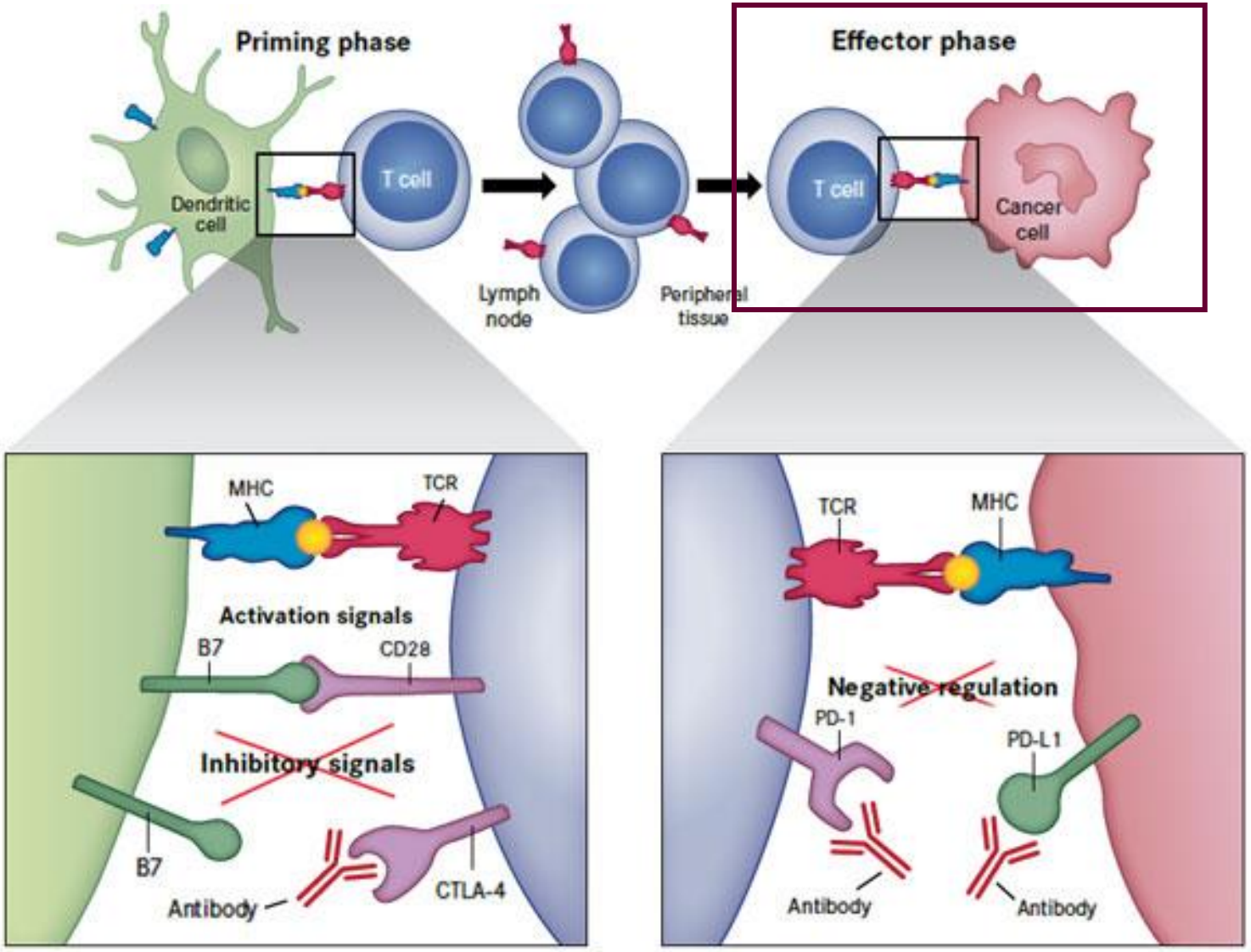
- Whole exome sequencing
  - Genomic landscape
  - Mutational signatures
- Immunological markers
  - MHC I
  - TIL
  - PDL1/PD1

Methods: Immune characterization

- 333 samples were stained for immune analyses
- intratumoral and stromal TILs was assessed according to criteria previously described and published by Denkert et al[1]
- PD-L1 and PD-1 were performed by Medimmune using internal protocols
  - PD-L1: anti-human PD-L1 monoclonal antibody (CAMP-1). Samples were considered PD-L1 (+) if 5% or more of the tumor cells showed staining at the surface membrane
  - PD-1: mouse anti-human PD-1 (clone Nat105). Average number of cells were then categorized in a 0-3 scoring system.
- MHC class I: mouse monoclonal antibody (clone EMR8-5).
  - Semi-quantitative evaluation of percentage of stained cells (moderate to strong intensity). Internal positive control (lymphocytes and endothelial cells)

1. Denkert C, et al *J Clin Oncol 2010;28:105-113.*

Question: which patients could be eligible to modulators of immune checkpoints and expansion of adaptive immune response



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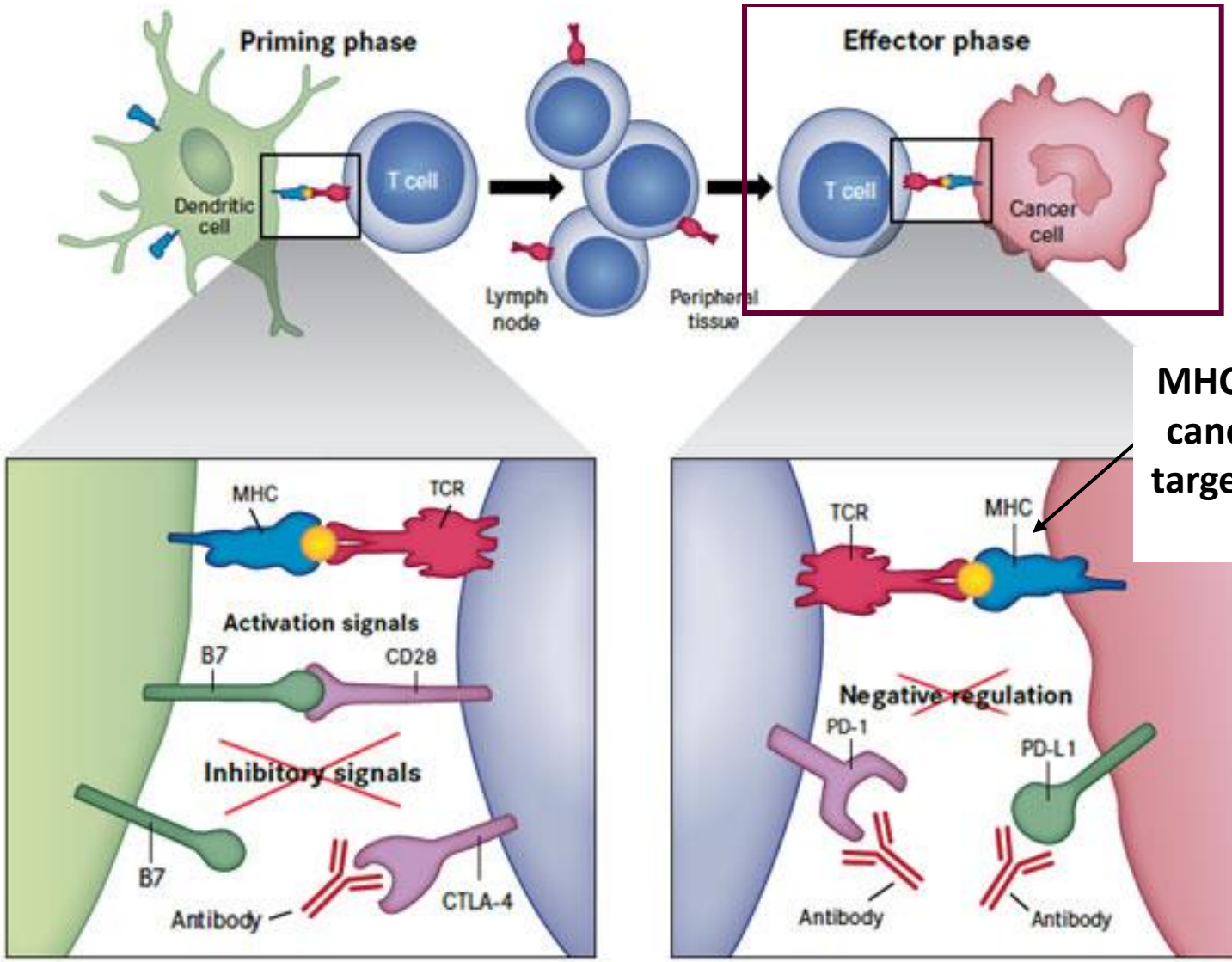
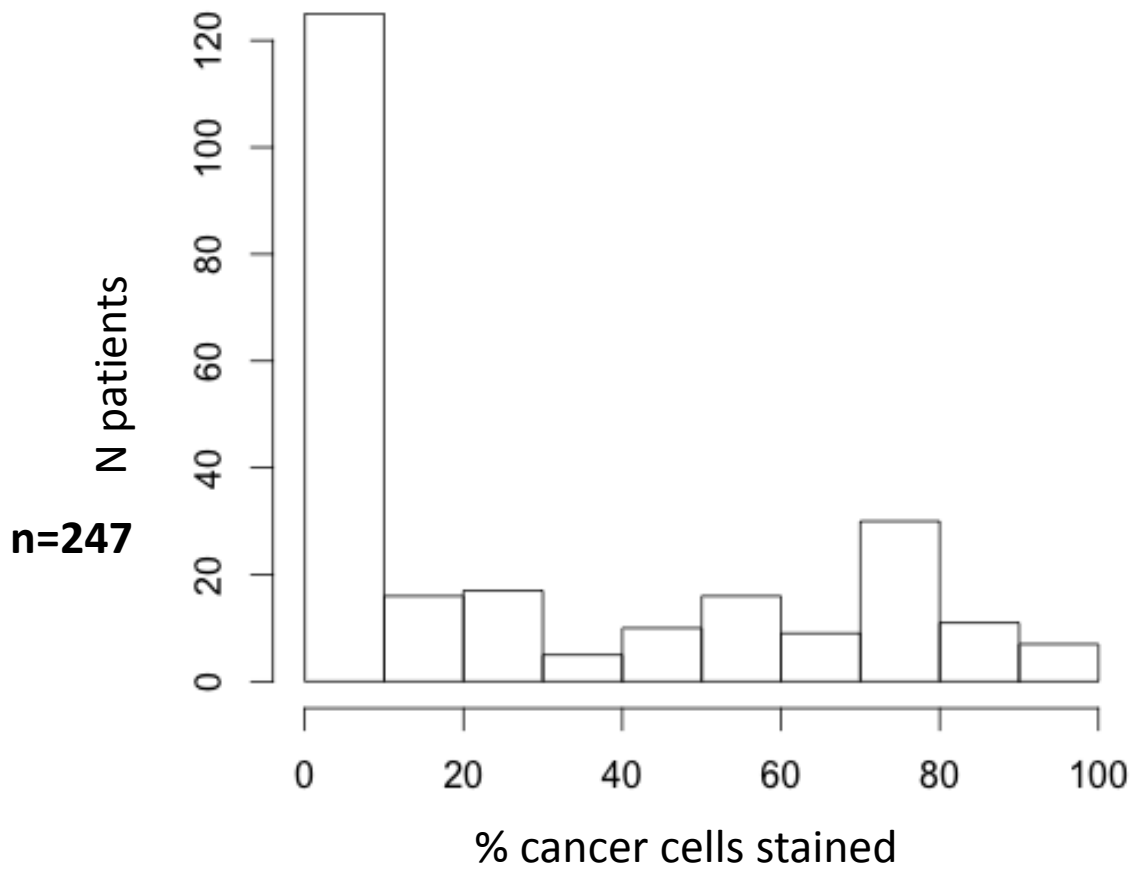


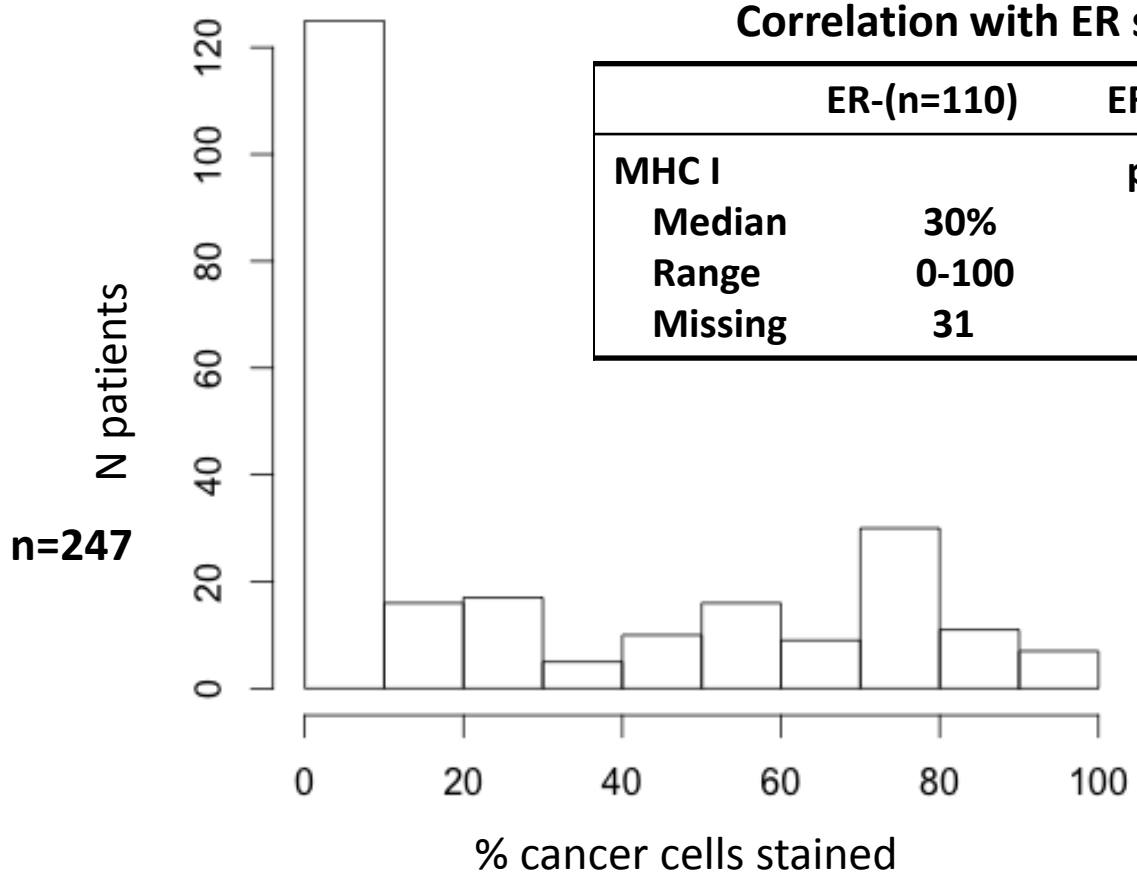
MHC I: can the cancer cell be targeted by the CTL ?

MHC class I expression by metastatic breast cancers



A majority of metastatic breast cancers have lost MHC I expression

MHC class I expression by metastatic breast cancers

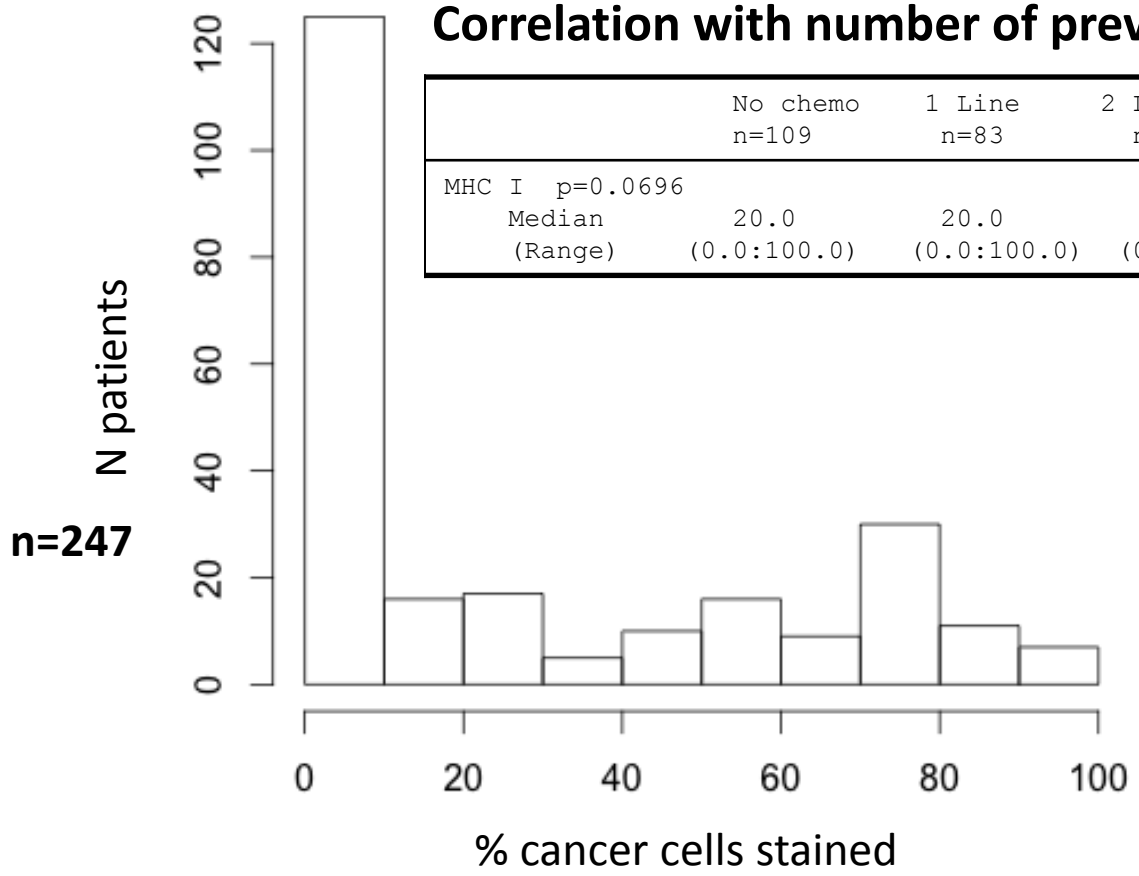


Correlation with ER status

	ER-(n=110)	ER+ (n=178)
MHC I		**p=0.0324**
Median	**30%**	**10%**
Range	**0-100**	**0-100**
Missing	**31**	**47**

MHC I expression is higher in ER-negative breast cancer

MHC class I expression by metastatic breast cancers

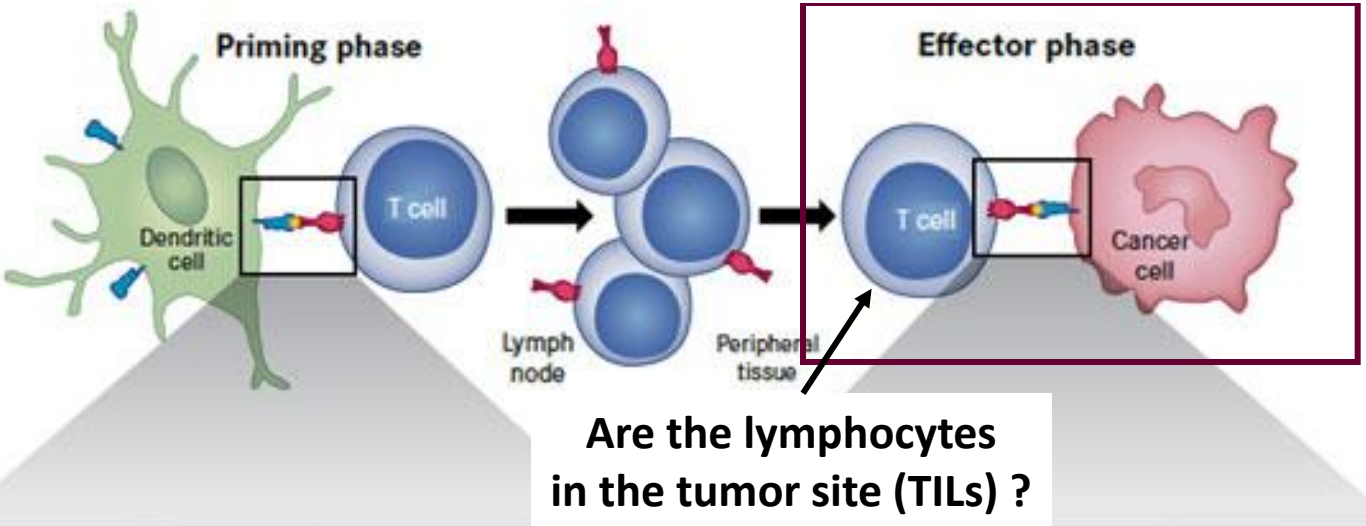


Correlation with number of previous chemotherapy

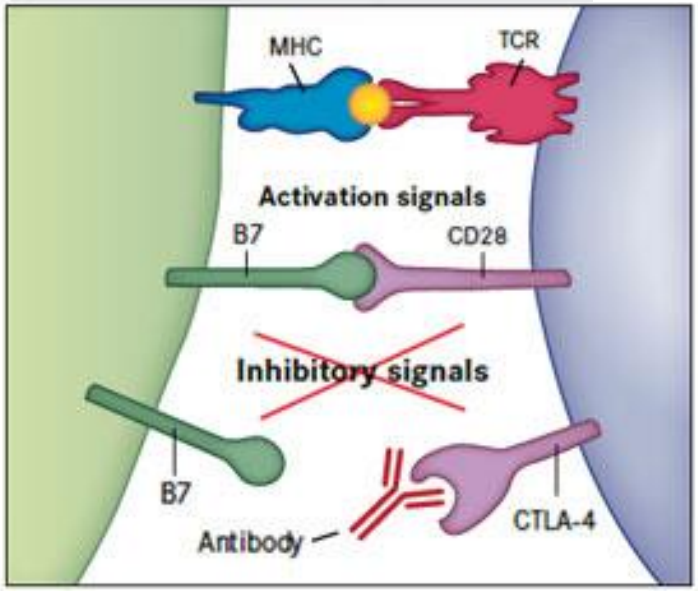
	No chemo n=109	1 Line n=83	2 Lines n=52	>2 Lines n=46
MHC I p=0.0696				
Median	20.0	20.0	15.0	5.0
(Range)	(0.0:100.0)	(0.0:100.0)	(0.0:100.0)	(0.0:100.0)

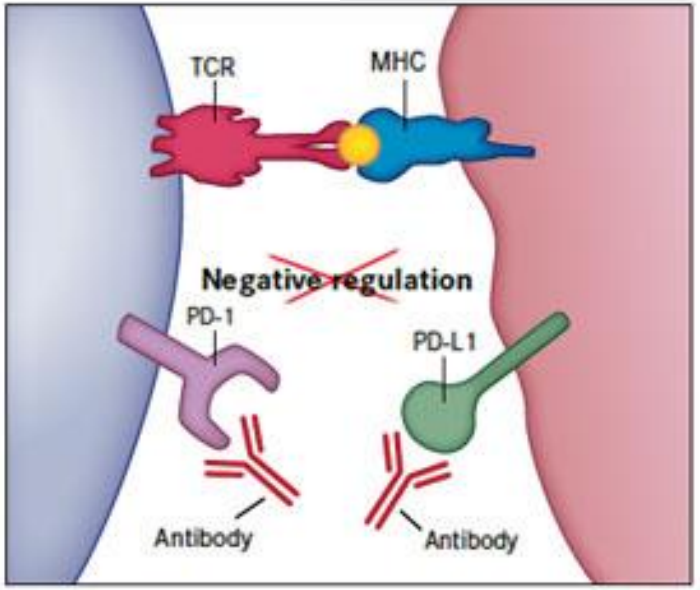
MHC I expression is lower in heavily pretreated patients

Question: which patients could be eligible to modulators of immune checkpoints and expansion of adaptive immune response

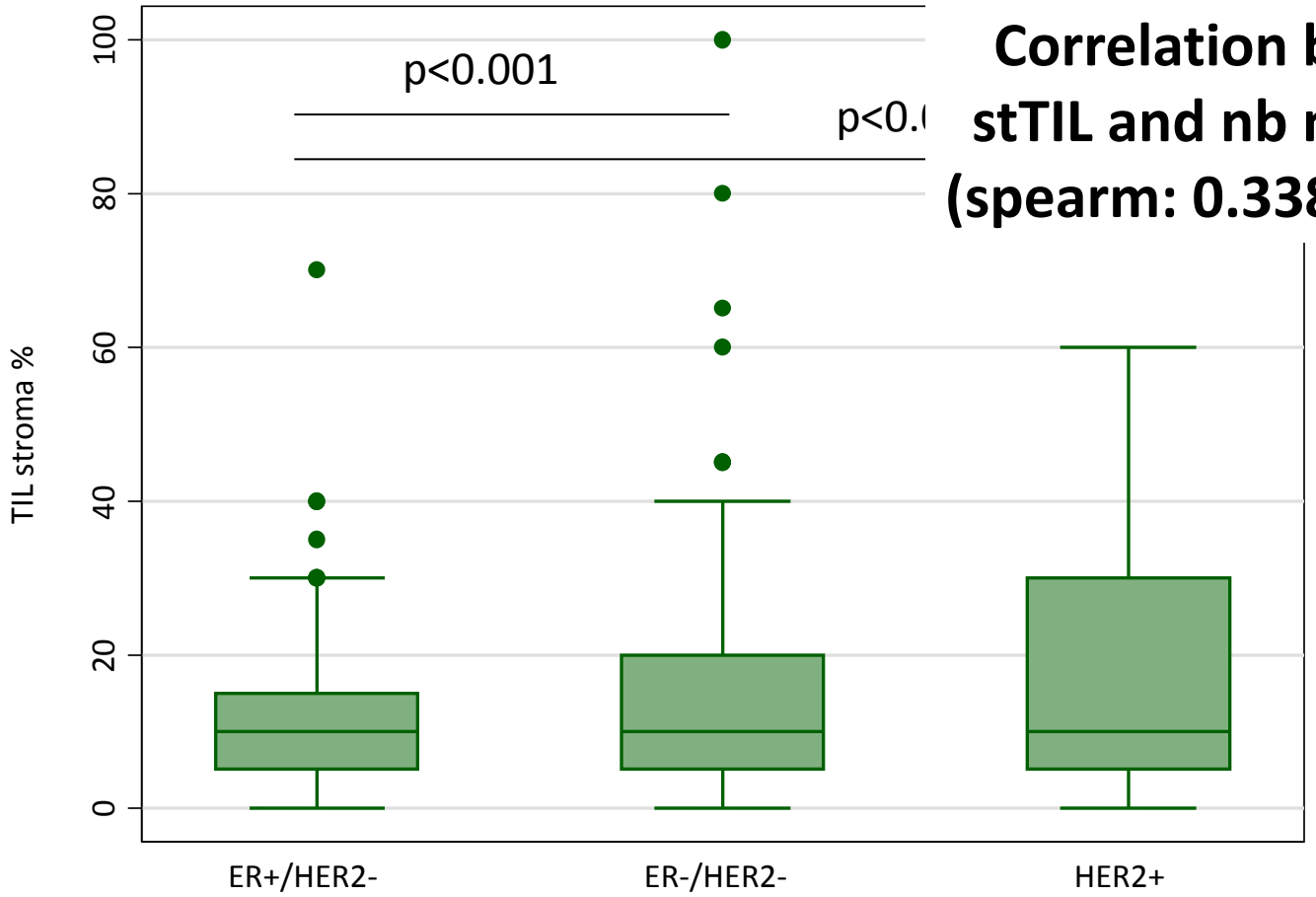


Are the lymphocytes in the tumor site (TILs) ?





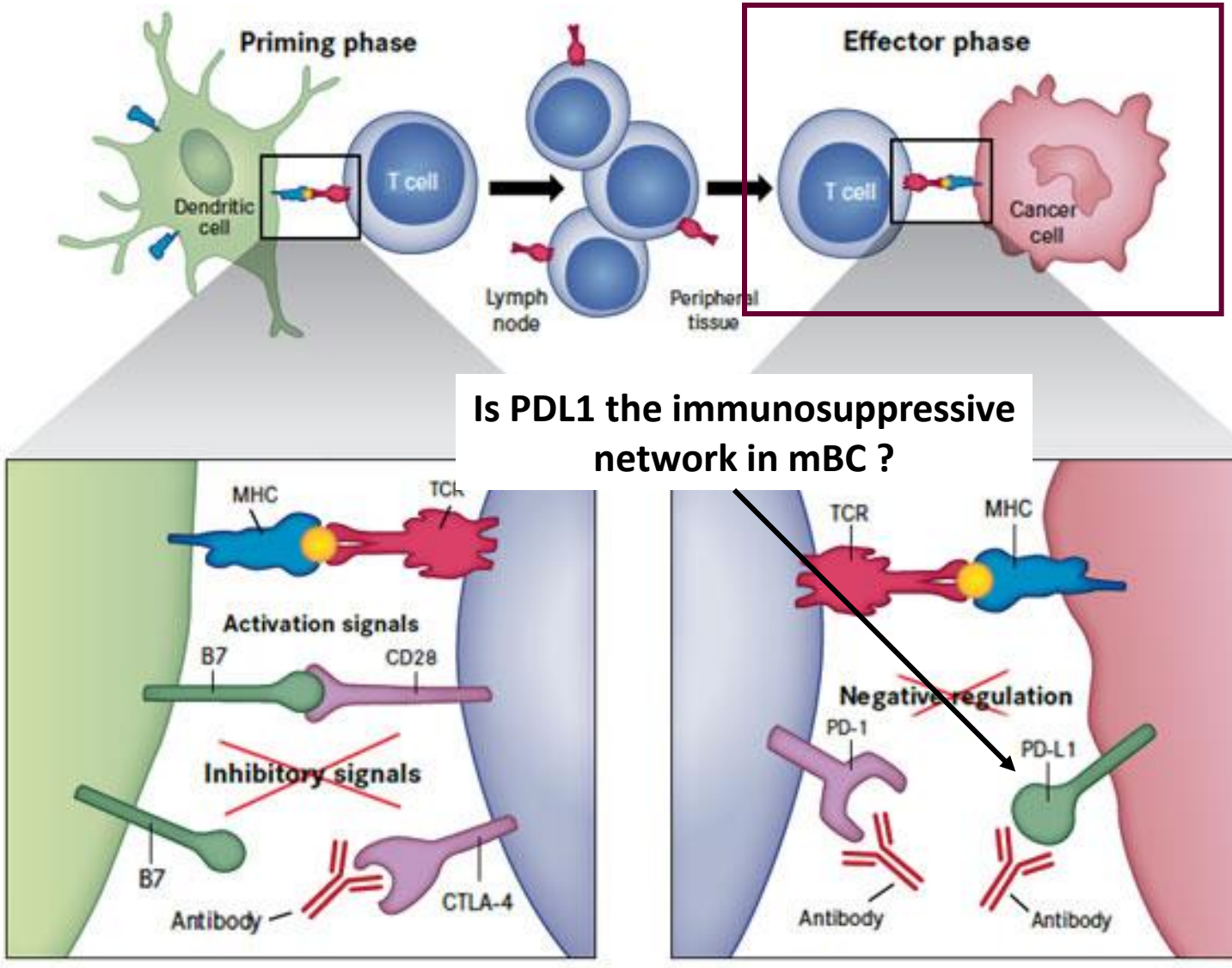
Stromal TIL and metastatic breast cancers



Correlation between stTIL and nb mutations (spearm: 0.338, p=0.008)

Median	10%	10%	10%
Q3	15%	20 %	30%

Questions: which patients could be eligible to modulators of immune checkpoints and adaptive immune response



PD1/PDL1 expression in metastatic breast cancers

	ER+/Her2- (n=145)	TNBC (n=66)	Her2-overexpressed (n=37)
PDL1 cancer cells (>5%)	2 (1%)	2 (3%)	3 (8%)
PDL1 immune cells (>0 cell)	104 (71%)	46 (69%)	25 (69%)
PD1 immune cells (>0 cell)	30 (20%)	20 (31%)	13 (36%)

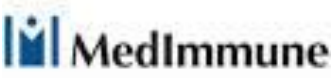
Conclusion

- **ESR1, TSC1/2 and DOT1L mutations are enriched in metastatic samples**
- **In this preliminary analysis (93 samples), we could not identify additional « metastasis-specific » drivers**
- **ESR1 mutation is associated with poor outcome**
- **A subset of PIK3CA mutated mBC clusters in a group defined by high mutation rate and TpC>G/T mutational signature**
- **Ideal population to develop immunotherapeutics could TNBC / Her2+++ treated with <2 lines chemotherapy (TIL+ / MHC I+)**

Questions generated by the study

- Is it possible to identify new recurrent « metastases-specific » drivers in metastatic samples ? Need for more samples before excluding they exist (aim >200 Q1 2015)
- Does ESR1 mutated BC define a genomic segment with very poor outcome that would deserve drug approval based on phase II ?
- Should PIK3CA mutated mBC be stratified according to the mutational process ?
- Should trials on immunotherapeutic stratify the patients based on MHC I ?
- Should trials on immunotherapeutics include interferon in the strategy ?
- Which immune targets in mBC ? (CD73, NK0

Acknowledgements



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