

# THERAPY and TMB

A STUDY ON THEIR **RELATIONSHIP**  
AND HOW TMB AFFECTS **EFFECTIVENESS**.

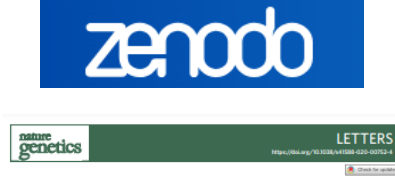


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*Bio-Statistics project*  
*BCG Course*  
*Università degli Studi di Milano*  
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# Data origin

All the data is freely available at: DOI  
10.5281/zenodo.4074183



The association between tumor mutational burden and prognosis is dependent on treatment context

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In multiple cancer types, high tumor mutational burden (TMB) is associated with longer survival after treatment with immune checkpoint inhibitors (ICIs). The association of TMB with survival outside of the immunotherapy context is poorly understood. We analyzed 10,233 patients (80% non-ICI-treated, 20% ICI-treated) with 17 cancer types before/without ICI treatment or after ICI treatment. In non-ICI-treated patients, higher TMB (higher percentile within cancer type) was not associated with better prognosis; in fact, in many cancer types, higher TMB was associated with poorer survival, in contrast to ICI-treated patients in whom higher TMB was associated with longer survival.

In multiple cancer types, high TMB is associated with higher rates of treatment response and longer survival in patients who receive treatment with ICI<sup>1-4</sup>. This association has been attributed to higher numbers of potentially immunogenic neoantigens that may facilitate antitumor immune responses. However, an open question is whether this association might also reflect a general prognostic benefit to high TMB in cancer, irrespective of treatment with immunotherapy. To the best of our knowledge, no studies have examined the association between TMB and survival in cohorts including both non-ICI-treated and ICI-treated patients by using contemporary clinical and genetic data.

Interpreting the recent ICI<sup>1-4</sup> Food and Drug Administration approval of pembrolizumab for solid tumors of any histology with TMB ≥ 10 mutations per megabase requires that we understand whether high TMB might also be associated with longer survival in patients receiving other therapies besides ICI. This understanding would be important to better understand the role of TMB in cancer.

Survival differentiation of T cells, leading to improved immunological surveillance<sup>5</sup>. Results of previous studies have suggested that high TMB may be associated with poorer outcomes in some cancer types, but these studies have not controlled for ICI therapy<sup>6-8</sup>. We hypothesized that, in some cancer types, high TMB would have opposite associations with survival, depending on immunotherapy context. As a caveat to simply comparing the effect of TMB in patients receiving or not receiving ICI treatment is that immunotherapy has now become integrated into standard of care for many types of cancer, in some cases as first-line therapy. Thus, a selected cohort of patients never treated with ICI would be unrepresentative of patients with cancer types where ICI is now standard of care. To analyze the effect of high TMB with and without ICI, we undertook an analysis of overall survival among a large contemporary cohort of cancer patients, rather than a subcohort of those never treated with ICI. We used a Cox proportional hazards regression model that included ICI treatment as a time-dependent covariate together with high versus low TMB and their interaction (ICI × TMB) ( $p < 0.0001$ ) as covariates in this model allowed us to isolate the effect of high TMB on survival in cancer patients who had received ICI therapy versus after ICI therapy. Because TMB distributions differ across cancer types<sup>9-11</sup>, high TMB was defined as the top 20th percentile within cancer types, as described previously<sup>12</sup>. Because survival time differs among various cancer types, overall survival in the model was stratified by cancer type. Overall survival was calculated from time of diagnosis to the earliest between death or death after the first ICI dose (for the analysis after ICI) to death of any cause (patients alive at time of analysis who were never treated with ICI) or death of any cause.

## The study

It was performed in **retrospect** on already available data.

## Patients

We have a cohort of 8,693 patients

## Consent

Patients provided informed consent permitting return of results from sequencing analyses for research.

Memorial Sloan Kettering institutional review board (IRB) **approval** of study

Patients with **solid tumors** diagnosed during 2015–2018 who had MSK-IMPACT testing.  
(n = 14,577)

History of > 1 cancer  
(n = 3,425)

Cancer types with < 100 cases  
(n = 797)

Unknown primary origin  
(n = 122)

Study cohort:  
**n = 10,233**  
19 variables

Least 7 frequent cancer types  
(n = 1540)

Our cohort:  
**n = 8,693**  
7 variables

# Variables

<b>Age</b>	Continuous	0 - 90
<b>Sex</b>	Categorical	Male and Female
<b>Treatment</b>	Categorical	ICI* and Non-ICI
<b>Cancer type</b>	Categorical	NSCLC, Colorectal, Ovarian...
<b>Stage</b>	Categorical	I - II - III - IV
<b>MSI</b>	Categorical	Stable, Unstable, Unknown
<b>TMB</b>	Continuous	0 - 424.8

*\*ICI: Immune Checkpoint Inhibitors*

# A summary of our workflow

## FIND OUT HOW TMB INFLUENCES SURVIVAL, DEPENDING ON THERAPY

### 1. Observe our variables

- Visually compare our variables to select which ones to work on.

### 2. Perform log rank tests on TMB

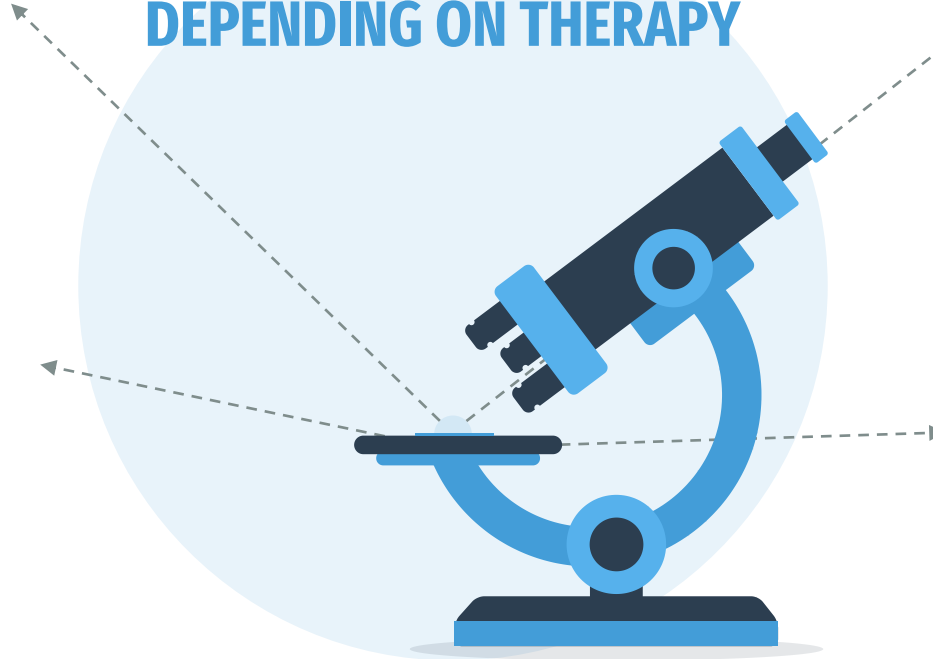
- Statistically show that TMB influences therapy.

### 3. Fit a cox model

- Fit a cox model to study the impact of the different covariates.

### 4. Investigate relations

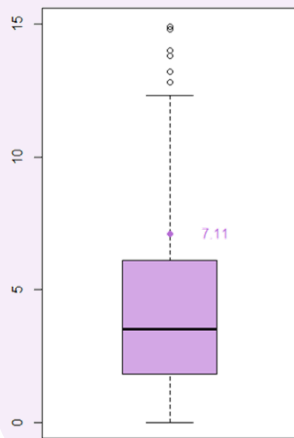
- Identify the relationship between two variables: TMB and MSI.



# TMB (Tumor Mutational Burden)

## TMB

Boxplot TMB



Min: 0

Max: 424.8

Outliers: anything over 14.8

**TMB** is defined as the **number of somatic mutations per megabase** of interrogated genomic sequence.

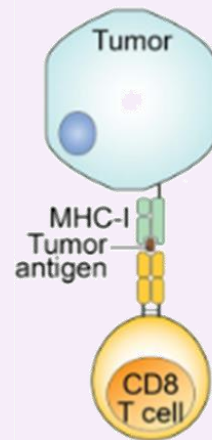
It varies across malignancies. [...] TMB could predict the efficacy of immune checkpoint inhibitors (ICIs) because it is believed to induce the generation of **immunogenic neoepptides** displayed on **major histocompatibility complexes** (MHC) on the tumor cell surface that influence patient response to ICIs.

Tumor Mutational Burden (TMB) as a Predictive Biomarker in Solid Tumors (2020). Dan Sha, et al.

There's no such thing as an "absolute" high or low TMB: it changes for every type of tumor. So, we're going to consider TMB populations by dividing them in **quantiles** within the type of cancer.

High 20%

Bottom 80%

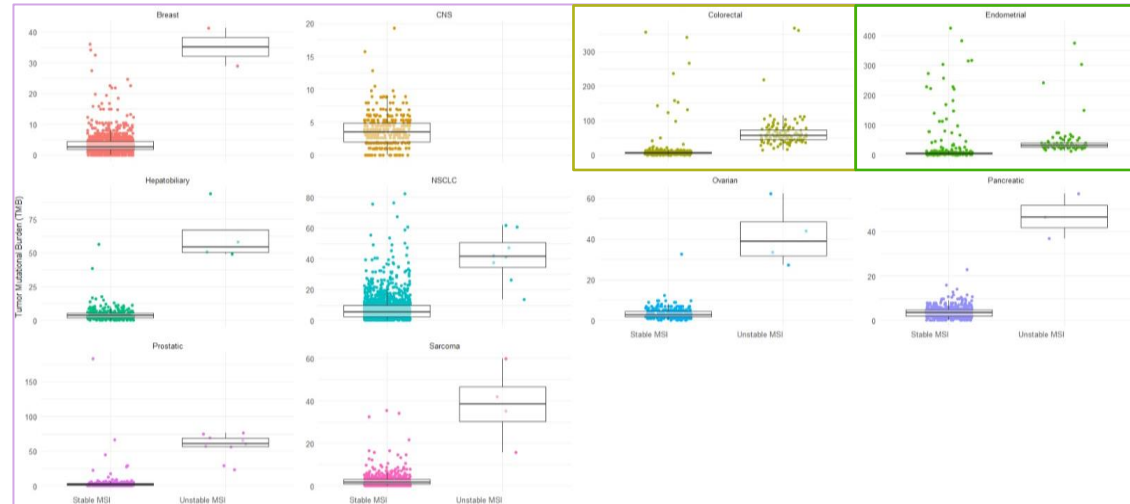
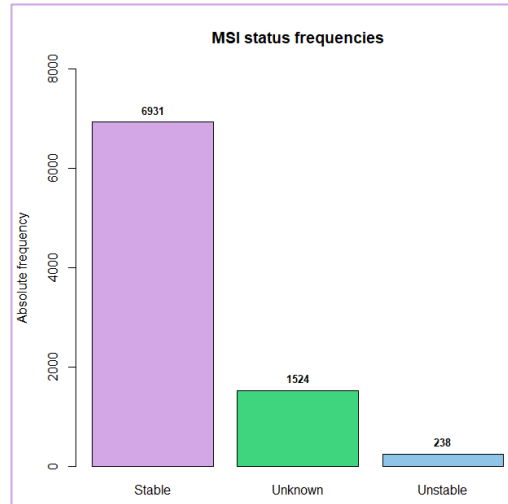


# MSI (Micro-Satellite Instability)

## MSI

**Microsatellite instability (MSI)** results from impaired DNA mismatch repair and causes an accumulation of mutations in microsatellites (MS). MSI occurs among various tumor types [...]. Most cases of MSI are sporadic [...]. The highest prevalence of MSI is in colorectal cancer, followed by endometrial. [...] MSI is one of the **best predictive biomarkers of immune checkpoint inhibitors' (ICIs) efficacy**.

Microsatellite Instability: A Review of Molecular Epidemiology and Implications for Immune Checkpoint Inhibitor Therapy (2023). Alexandra Kavun, et al.

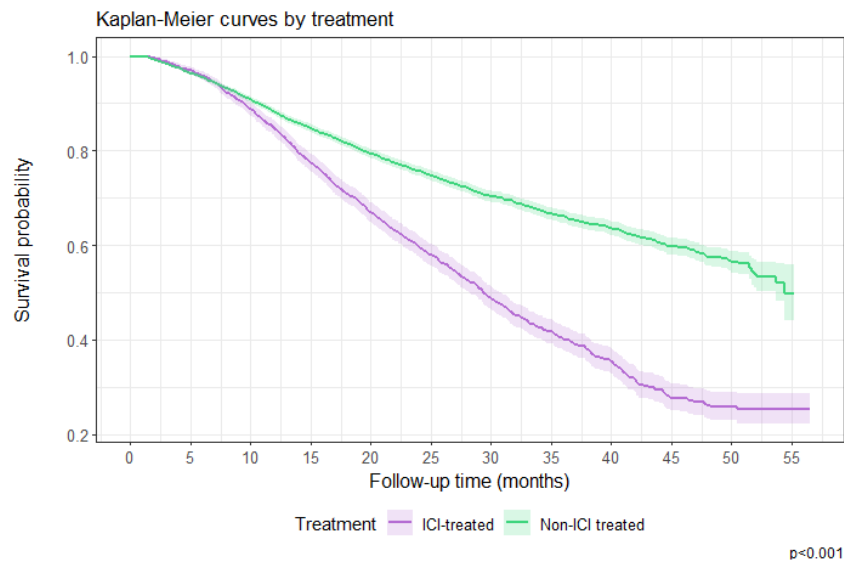


## 2. Log rank tests

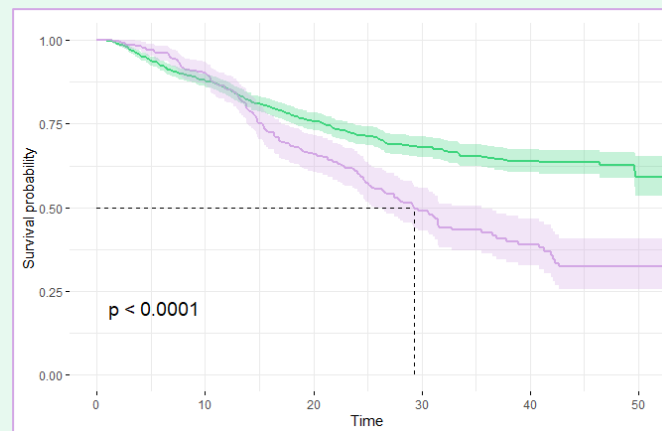
# High TMB vs Low TMB



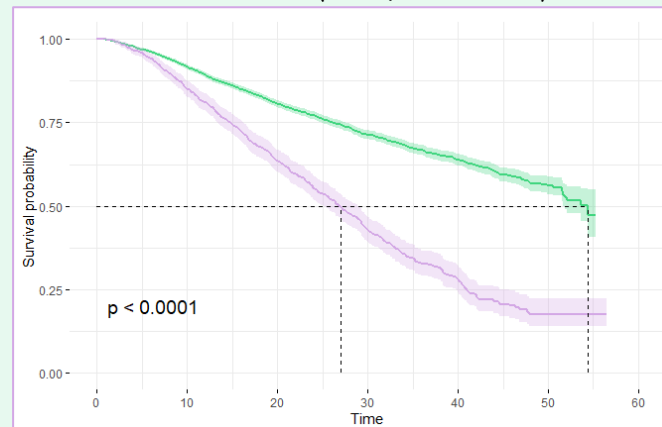
● Non-ICI treated **80.2%**  
● ICI treated **19.8%**



## HIGH TMB (HR ICI/Non-ICI = 1.74)

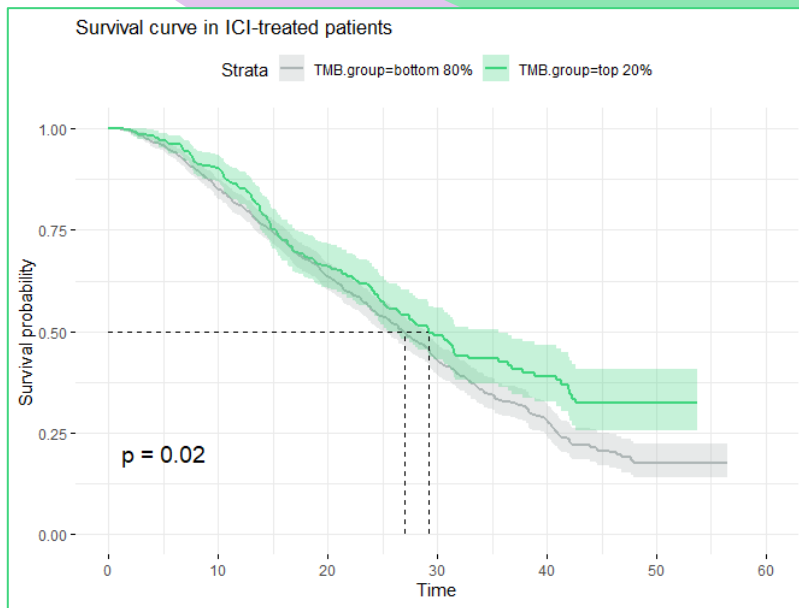


## LOW TMB (HR ICI/Non-ICI = 2.47)

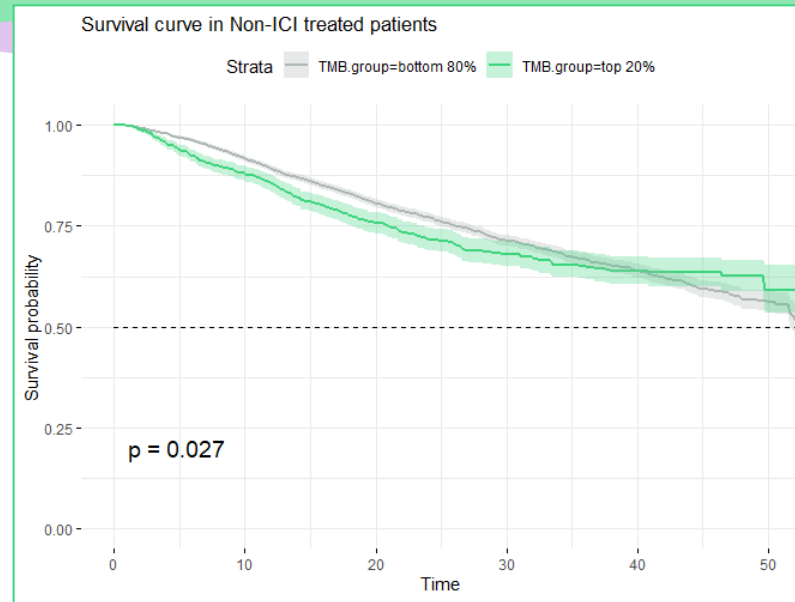


\*ICI: Immune Checkpoint Inhibitors

# ICI-treated vs Non-ICI treated



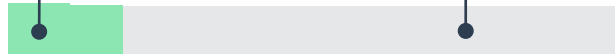
(HR High/Low = 0.70)



(HR High/Low = 1.14)

High 20%

Bottom 80%





# How does TMB affect the hazard ratio (ICI)?

## Model for ICI patients

$$HR = e^{-0.337x_{Top20\%TMB} + 0.010x_{age} + 0.432x_{stageIV}}$$

where  $x_{Top20\%TMB} = \begin{cases} 1, & \text{if TMB is high (top 20\%)} \\ 0, & \text{if TMB is low (bottom 80\%)} \end{cases}$

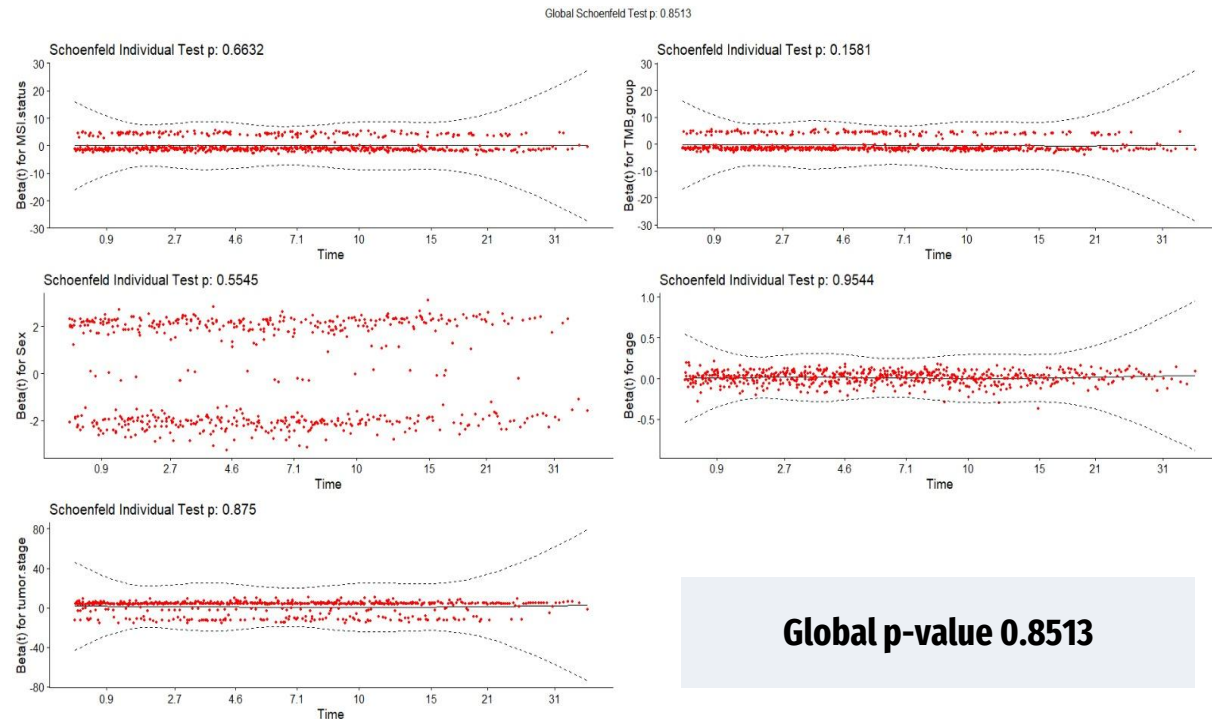
where  $x_{stageIV} = \begin{cases} 1, & \text{if the tumor is at stage IV} \\ 0, & \text{if the tumor is at any other stage} \end{cases}$

(Stratified by cancer type)

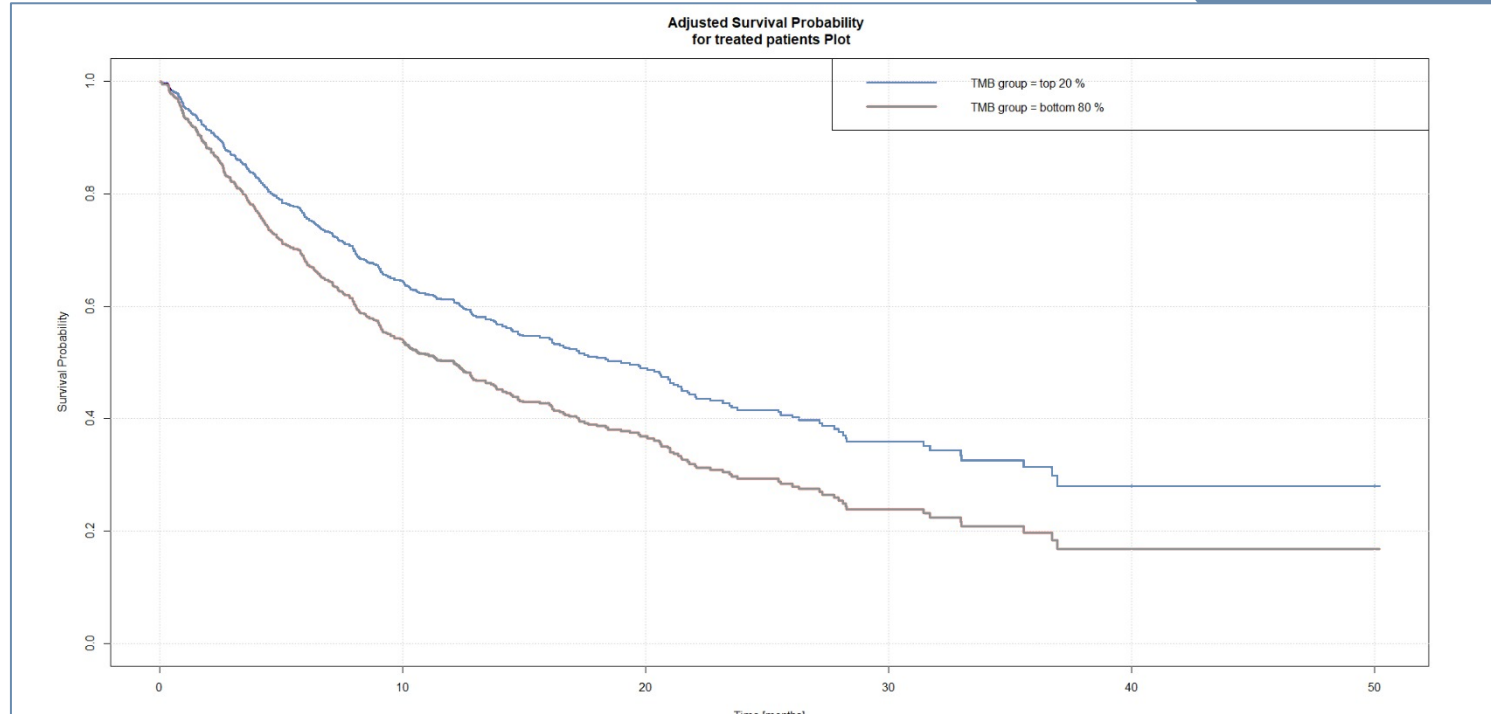
Covariates:

- **TMB (high) [HR = 0.71]**
- **Age [HR = 1.01]**
- **Tumor stage (IV) [HR = 1.54]**
- MSI status
- Sex

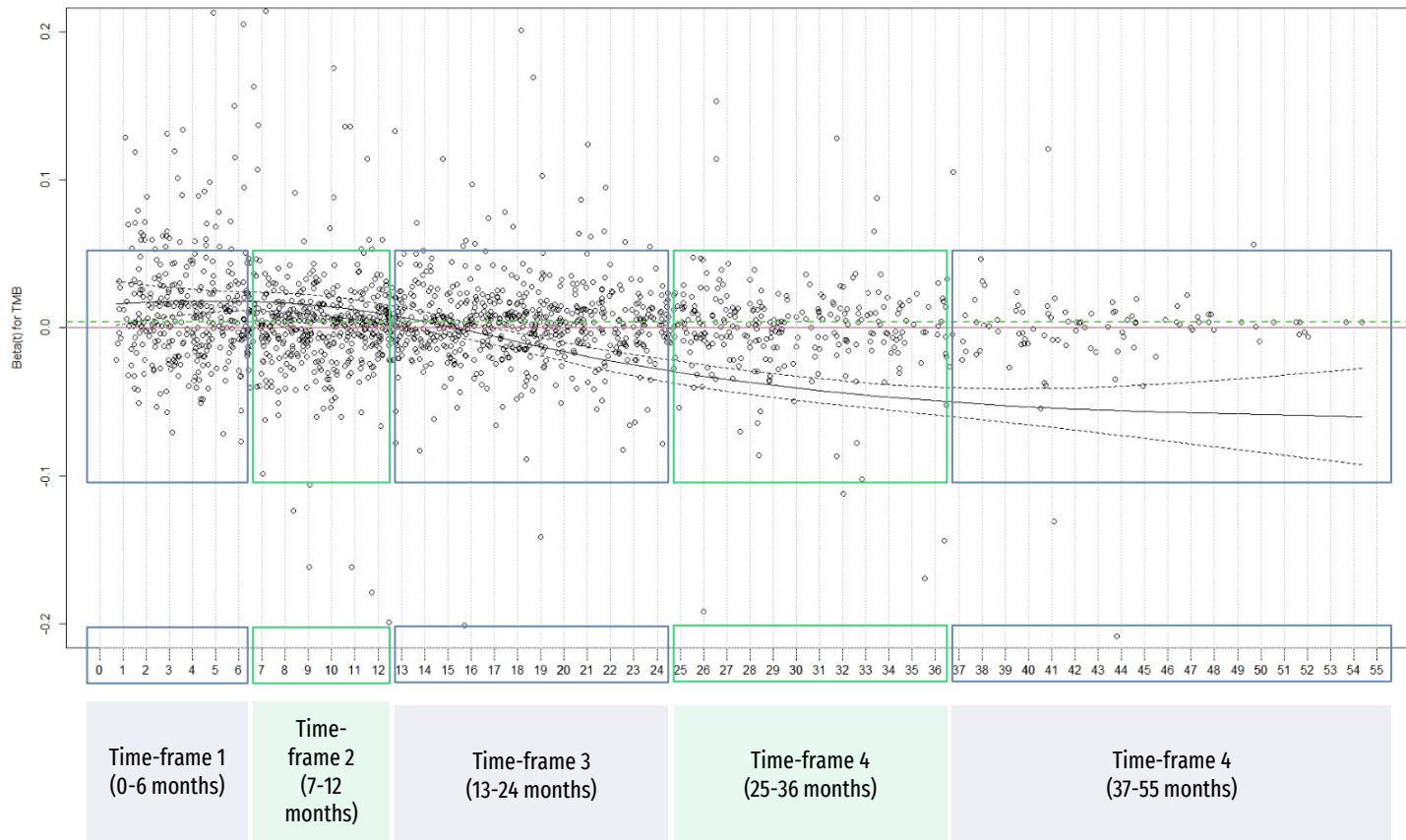
## Schoenfeld Test



# Adjusted survival probability for ICI patients: the influence of TMB



# Important! In non-ICI treated patients, TMB coefficient is time-dependent



# How does TMB affect the hazard ratio (Non-ICI)?

## Model for Non-ICI patients

$$HR = e^{0.143x_{male} + 0.016x_{age} + 0.011x_{1st\ TMB\ tgroup}}$$

$$where\ x_{male} = \begin{cases} 1, & \text{if the patient is male} \\ 0, & \text{if the patient is female} \end{cases}$$

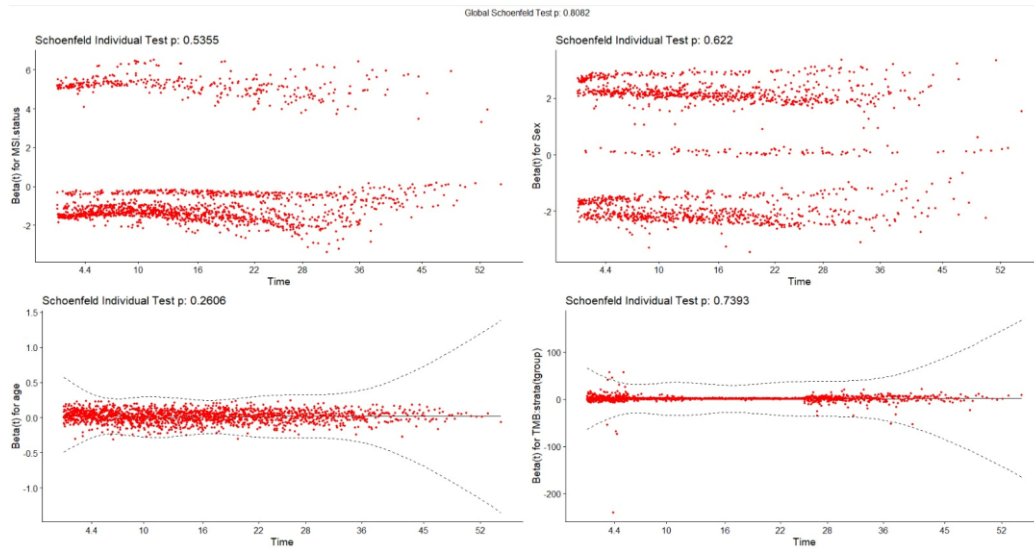
$$where\ x_{1st\ TMB\ tgroup} = \begin{cases} 1, & \text{if we're considering the first 6 months} \\ 0, & \text{if not} \end{cases}$$

(Stratified by cancer type)

Covariates:

- **Sex (Male) [1.15]**
- **Age [1.02]**
- **TMB (high, in the first time frame) [1.01]**
- MSI status
- Other time-frames for TMB

## Schoenfeld Test



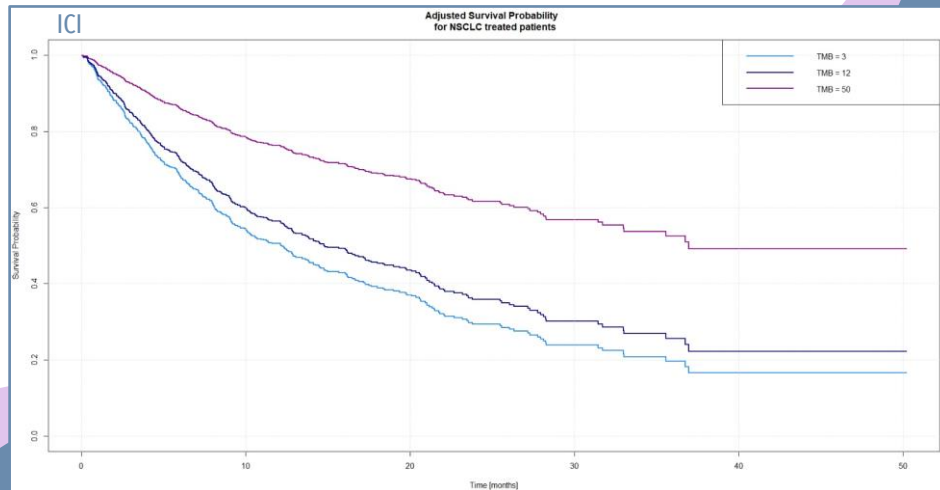
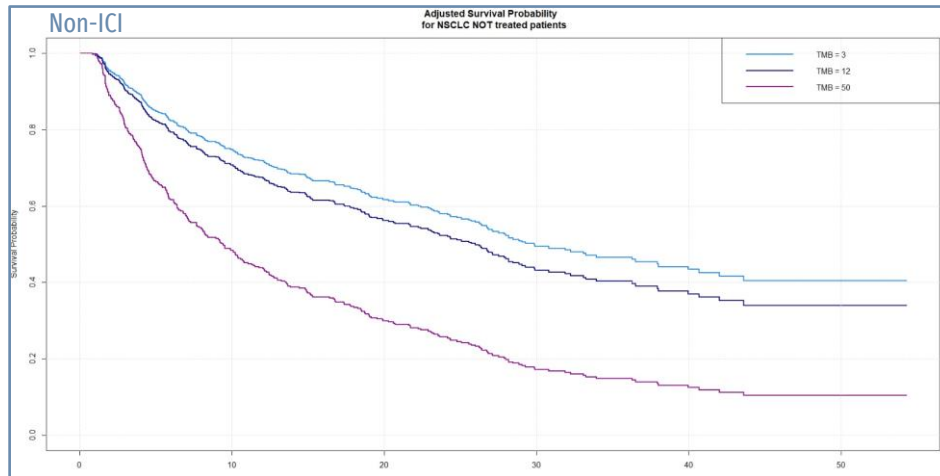
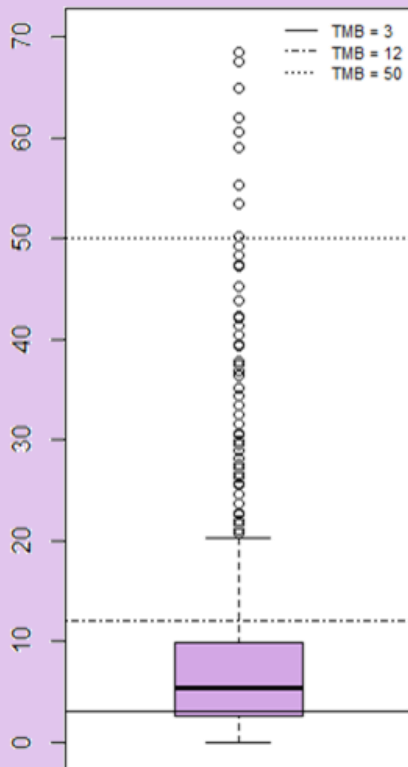
**Global p-value 0.8082**

# Cancer-specific analysis of TMB impact on survival

Cancer type	#ICI	Significance	#Non-ICI	Significance
<i>Endometrial</i>	69	Not significant	357	Not significant
<i>Colorectal</i>	74	Not significant	1277	Not significant
<i>Ovarian</i>	91	Not significant	282	Not significant
<i>Pancreatic</i>	39	Not significant	809	<b>High TMB increases HR</b>
<i>Hepatobiliary</i>	58	Not significant	349	Not significant
<i>Prostatic</i>	10	Not sufficient data	551	Not significant
<i>CNS</i>	24	Not significant	407	Not significant
<i>Breast</i>	33	Not significant	1516	Not significant
<i>NSCLC</i>	715	<b>High TMB decreases HR</b>	1347	<b>High TMB increases HR</b>
<i>Sarcoma</i>	85	Not significant	638	<b>High TMB increases HR</b>

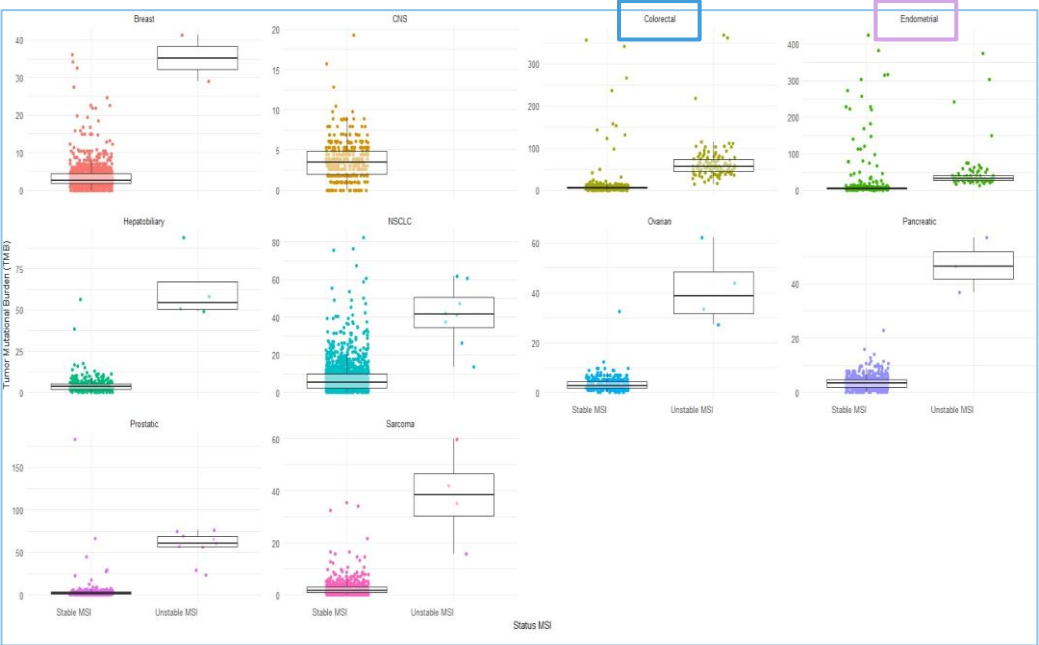
# The example: NSCLC

Boxplot TMB in NSCLC



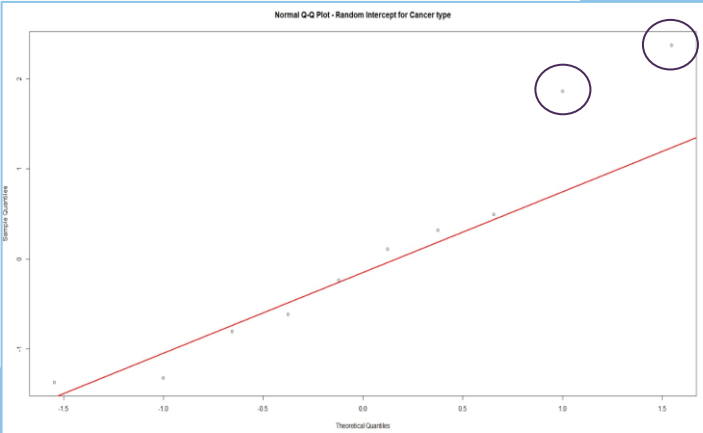
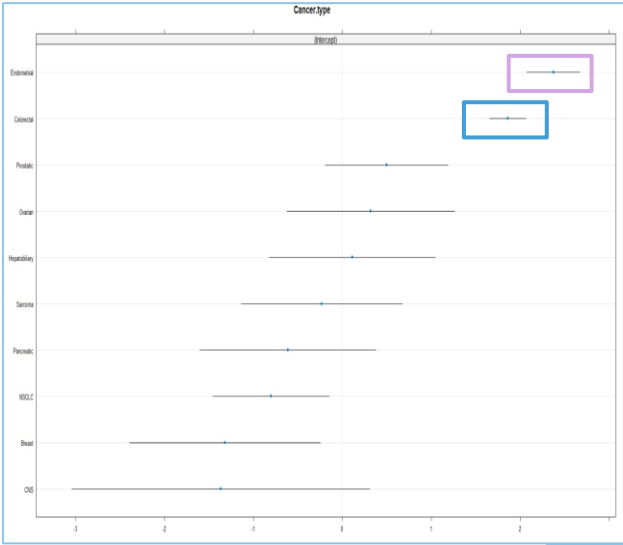
# Can we use MSI instead of TMB to predict results?

We tried to fit a mixed effect logistic model to predict the odds of having an MSI status of “Unstable” knowing the amount of TMB. Random intercept VPC\* = 0.35



\*VPC = Variant Partition Coefficient

Odds-ratio (OR) for an increase of 10 units of TMB = 1.5



# Logistic model for presence of «Unstable» MSI (Example: Colorectal Cancer)

We deemed better to focus only on a simple logistic model fit for each cancer type.

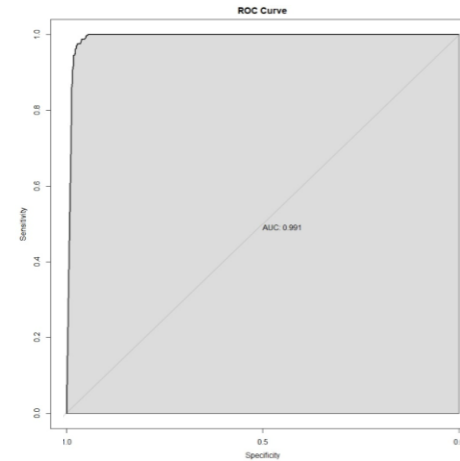
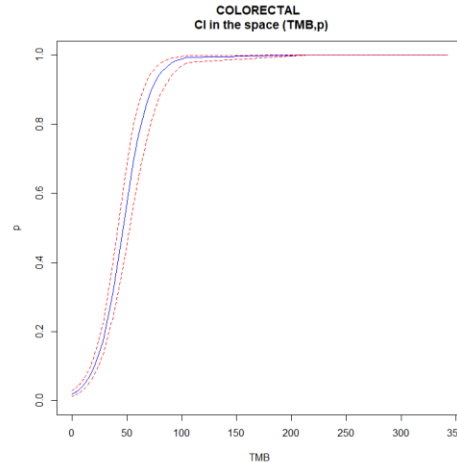
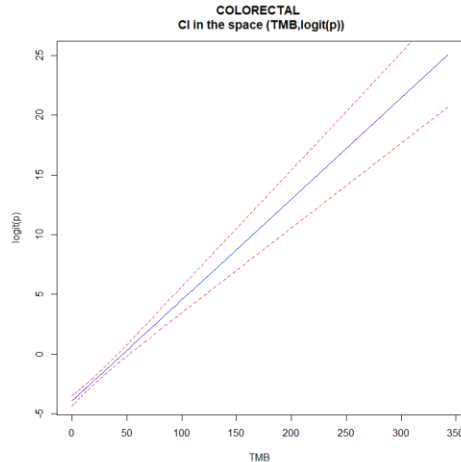
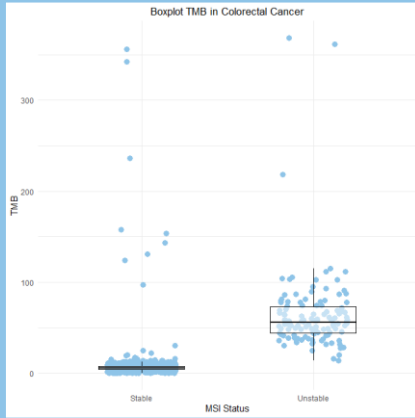
Probability of MSI status = Unstable

$$p = \frac{1}{1 + e^{-(-3.817 + 0.076 \cdot x_{TMB})}}$$

Interpretation of the coefficients (OR)

$$\text{Odds Ratio for a 10 units of TMB increment} = \frac{p(\text{MSI} = \text{Unstable})}{p(\text{MSI} = \text{Stable})} = 2.14$$

$$\text{with } CI_{95\%} = [1.92, 2.38]$$



Specificity: 95.9%  
Sensitivity: 89.6%





# Conclusions

1

As for 2021 (the date of the study), ICI isn't associated with a better prognosis. **TMB** levels and other variables, though, can influence therapy: a **high** level of **TMB** influences **positively ICI therapy**, while **Chemotherapy** is influenced **negatively**.

## ICI

$$HR = e^{-0.337x_{Top20\%TMB} + 0.010x_{age} + 0.432x_{stageIV}}$$

$$\text{where } x_{Top20\%TMB} = \begin{cases} 1, & \text{if TMB is high (top 20\%)} \\ 0, & \text{if TMB is low (bottom 80\%)} \end{cases}$$

$$\text{where } x_{stageIV} = \begin{cases} 1, & \text{if the tumor is at stage IV} \\ 0, & \text{if the tumor is at any other stage} \end{cases}$$

## NON-ICI

$$HR = e^{0.143x_{male} + 0.016x_{age} + 0.011x_{1st\ TMB\ tgroup}}$$

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2

Our dataset isn't prepared for a cancer-specific analysis: only **Non Small Cell Lung Cancer** (NSCLC), being the most frequent cancer type, follows the conclusions reached. We don't have enough data to confirm our hypothesis on the other types, but neither to reject it.



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3

Additionally, instead of using high or low levels of TMB (in case datasets don't report it), the presence or not of **unstable MSI** can be used. It is proved that high TMB is positively associated with unstable MSI, here's reported a logistic model for **Colorectal cancer**.

$$p = \frac{1}{1 + e^{-(3.817 + 0.076 \cdot x_{TMB})}}$$

$$\text{Odds Ratio for a 10 units of TMB increment} = \frac{p(\text{MSI} = \text{Unstable})}{p(\text{MSI} = \text{Stable})} = 2.14$$

**THANK YOU**  
for your attention

