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
2023/2024

Data origin

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

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

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

**ICI : Immune Checkpoint Inhibitors*

A summary of our workflow

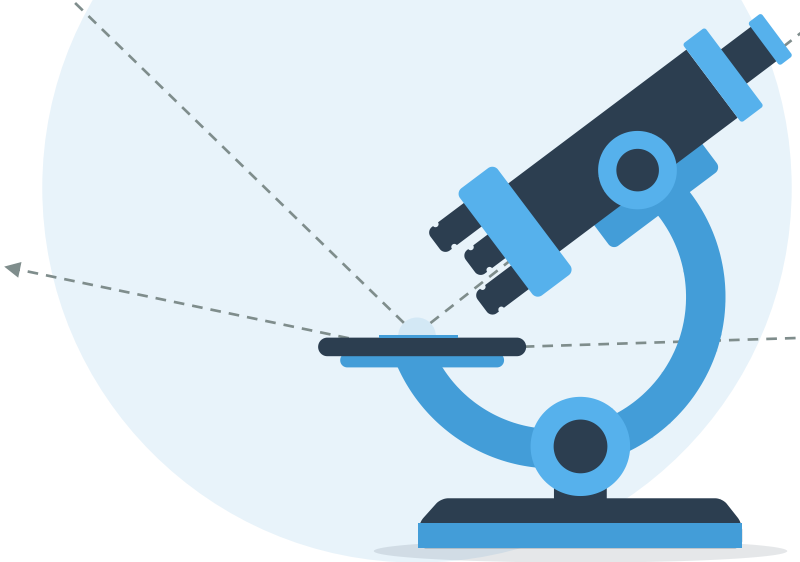
FIND OUT HOW TMB INFLUENCES SURVIVAL, DEPENDING ON THERAPY

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

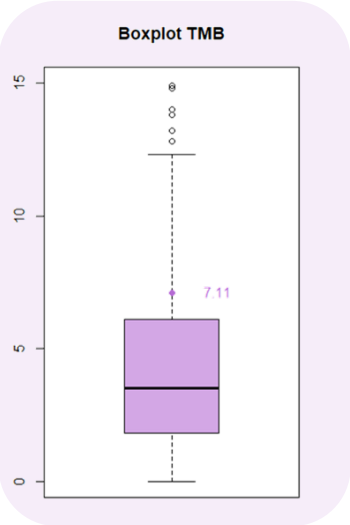
- Fit a cox model to study the impact of the different covariates.

4. Investigate relations

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

TMB (Tumor Mutational Burden)

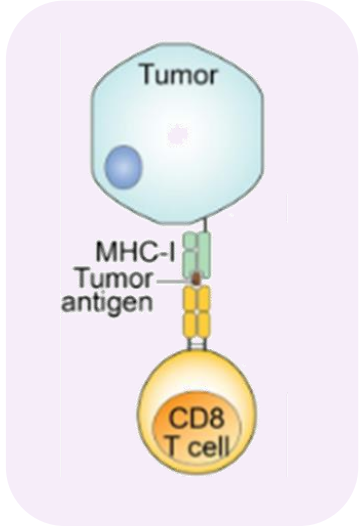
TMB



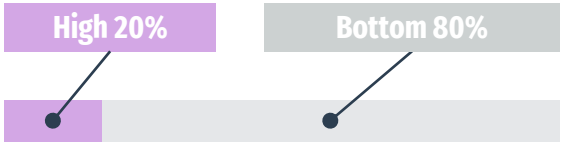
Min: 0
Max: 424.8
Outliers: anything over 14.8

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 neopeptides** 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.

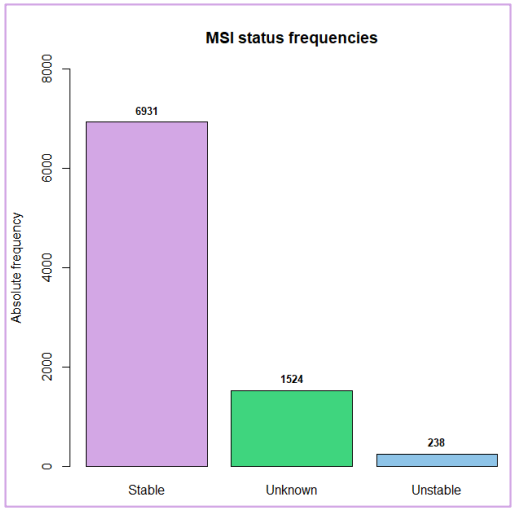


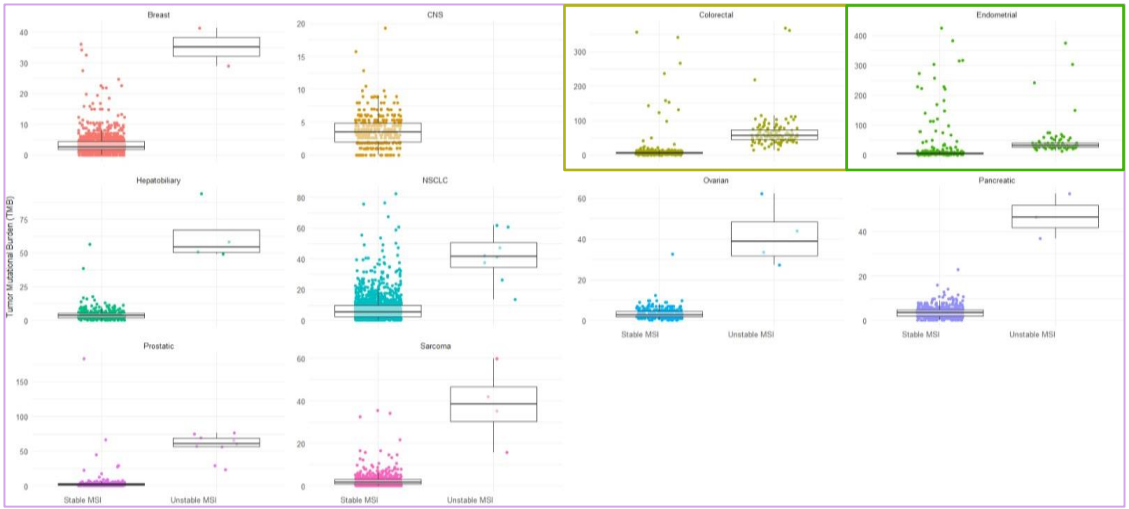
MSI (Micro-Satellite Instability)

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.*



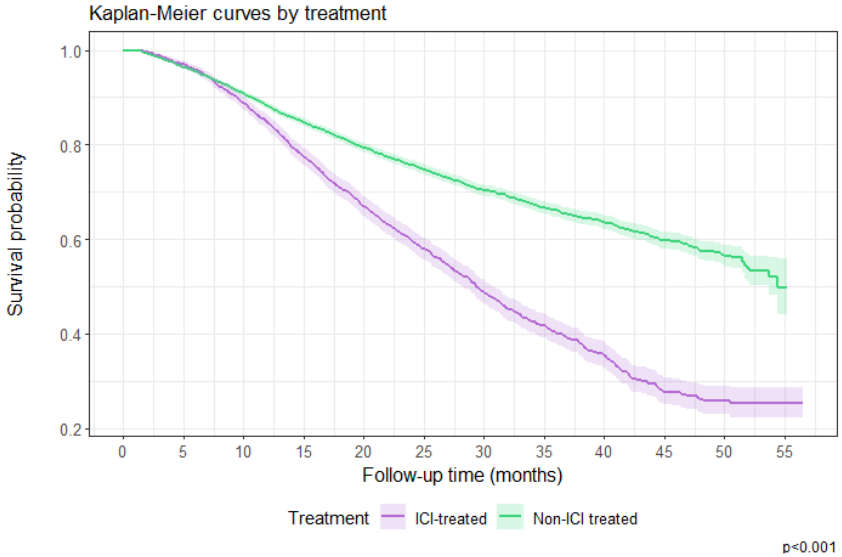


2. Log rank tests

High TMB vs Low TMB

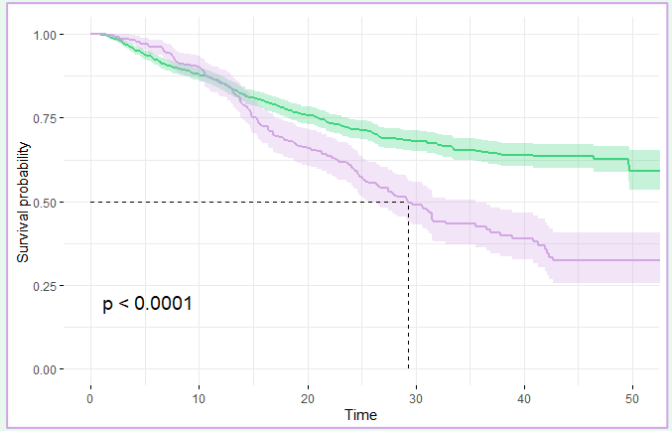
Non-ICI trated 80.2%

ICI treated 19.8%

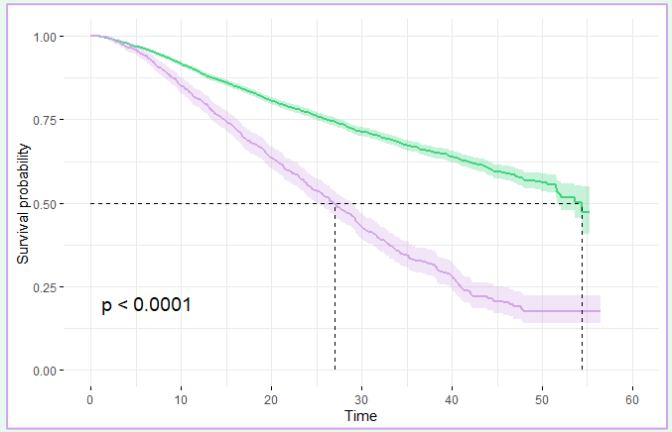


*ICI : Immune Checkpoint Inhibitors

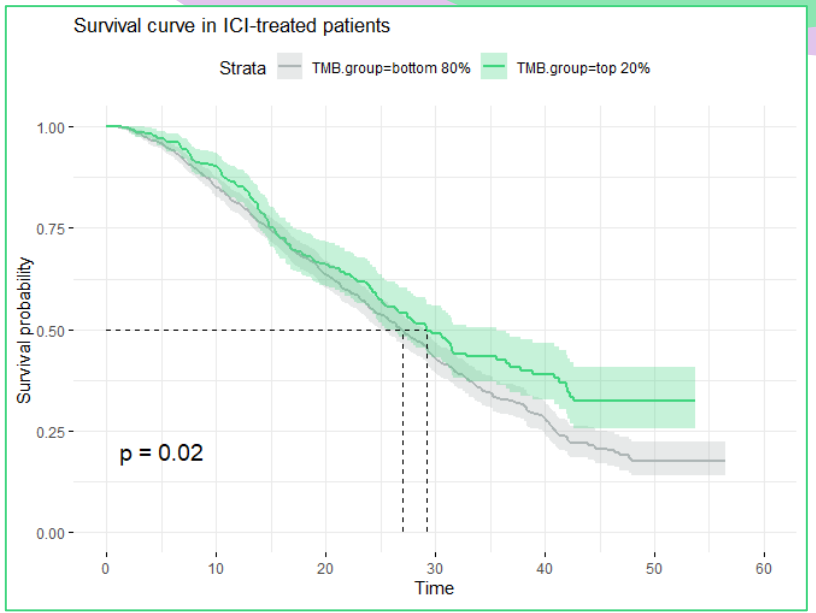
HIGH TMB (HR ICI/Non-ICI = 1.74)

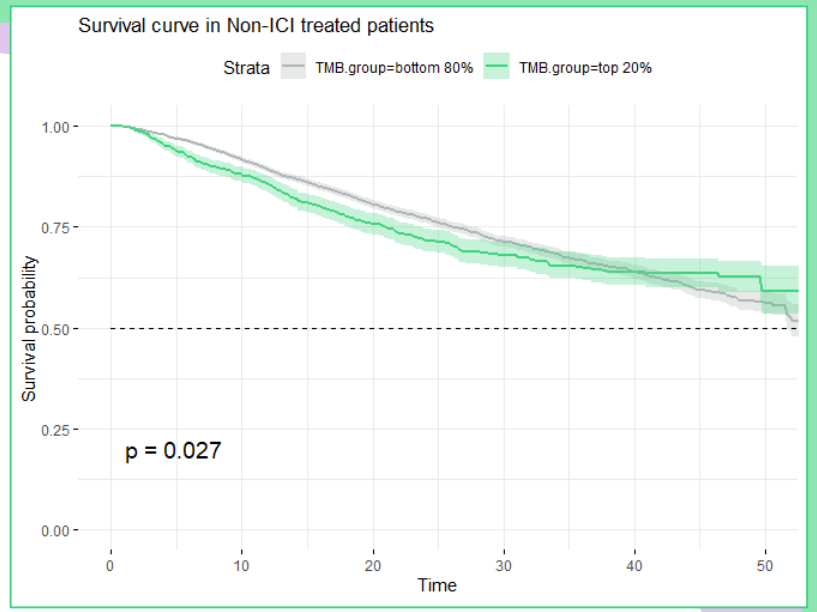


LOW TMB (HR ICI/Non-ICI = 2.47)



ICI-treated vs Non-ICI treated





(HR High/Low = 0.70)

(HR High/Low = 1.14)

How does TMB affect the hazard ratio (ICI)?

Model for ICI patients

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

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

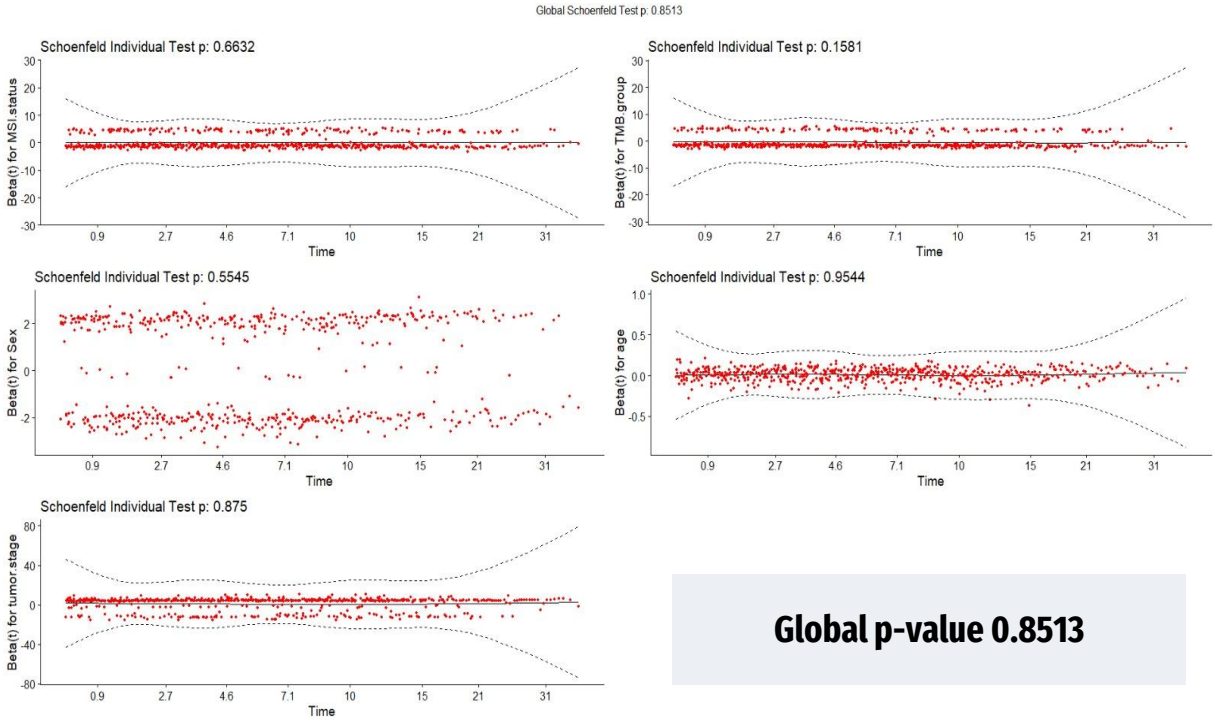
$$where\ x_{stageIV} = \begin{cases} 1, & if\ the\ tumor\ is\ at\ stage\ IV \\ 0, & if\ the\ tumor\ is\ at\ any\ other\ stage \end{cases}$$

(Stratified by cancer type)

Covariates:

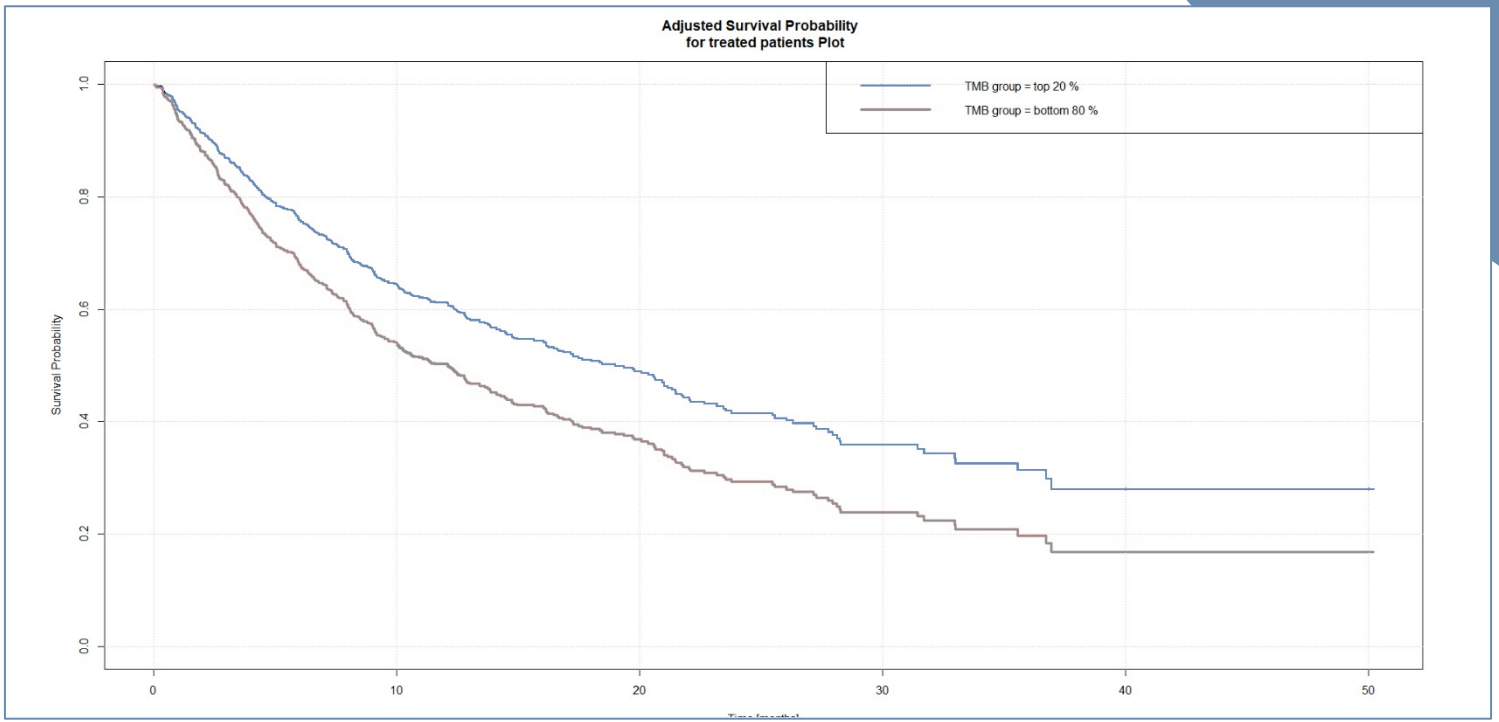
- **TMB (high) [HR = 0.71]**
- **Age [HR = 1.01]**
- **Tumor stage (IV) [HR = 1.54]**
- MSI status
- Sex

Schoenfield Test

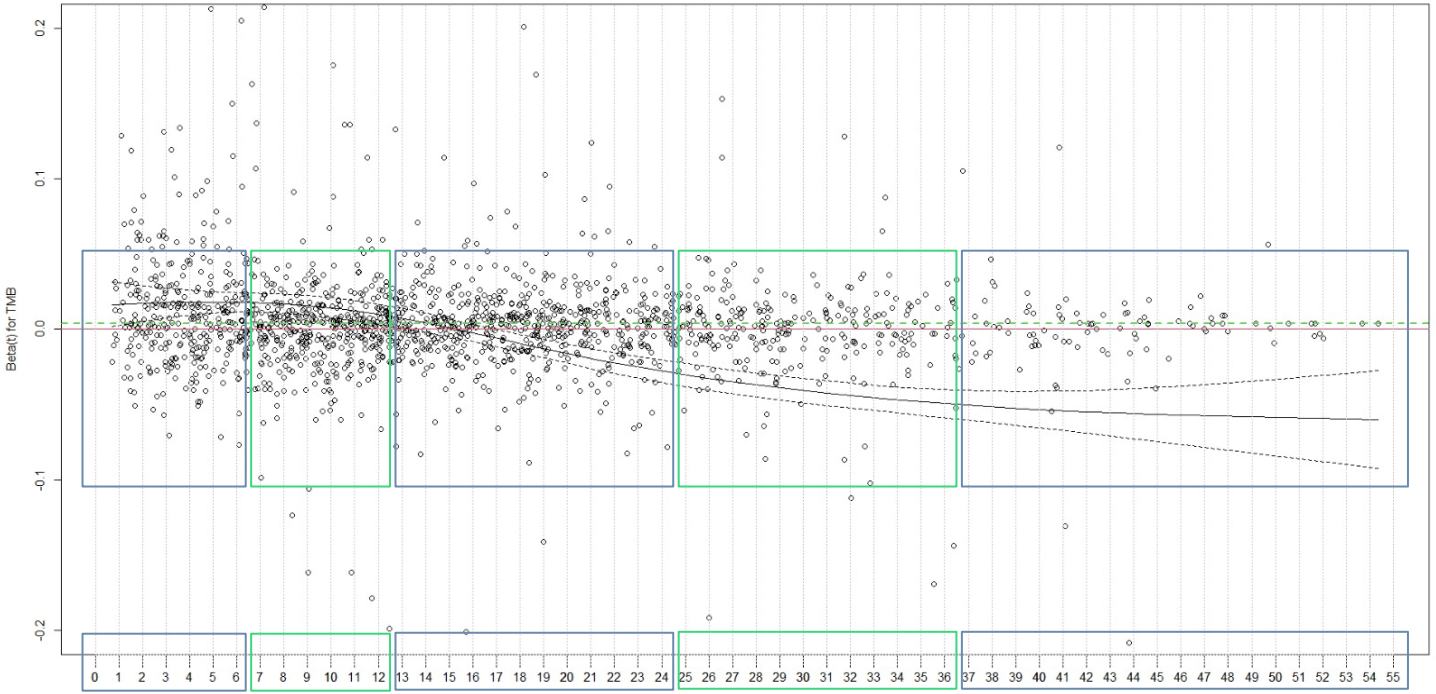


Global p-value 0.8513

Adjusted survival probability for ICI patients: the influence of TMB



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



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

Model for Non-ICI patients

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

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

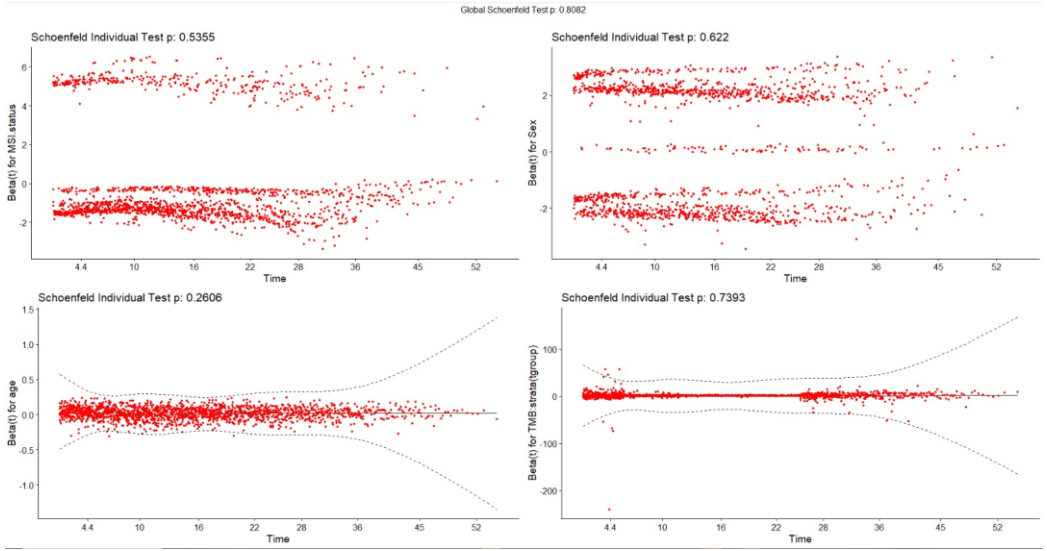
$$where\ x_{1st\ TMB\ tgroup} = \begin{cases} 1, & if\ we're\ considering\ the\ first\ 6\ months \\ 0, & if\ not \end{cases}$$

(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

Schoenfield Test

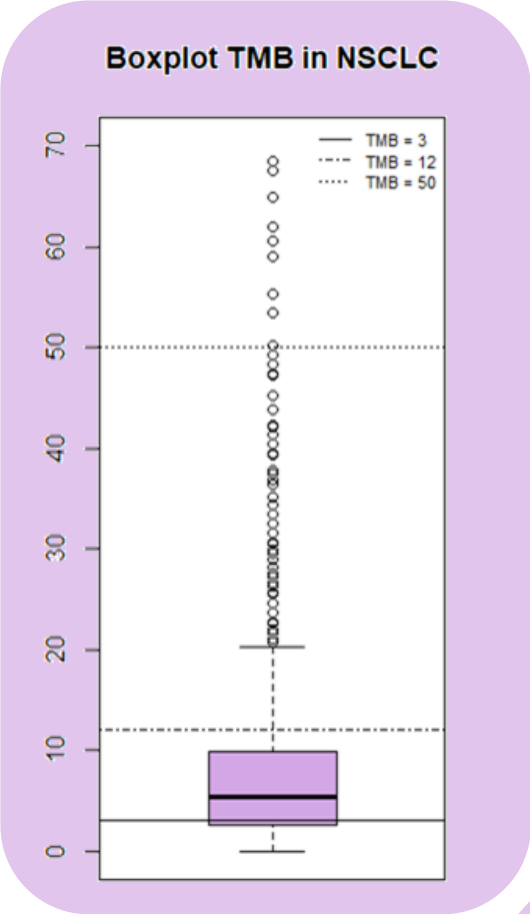


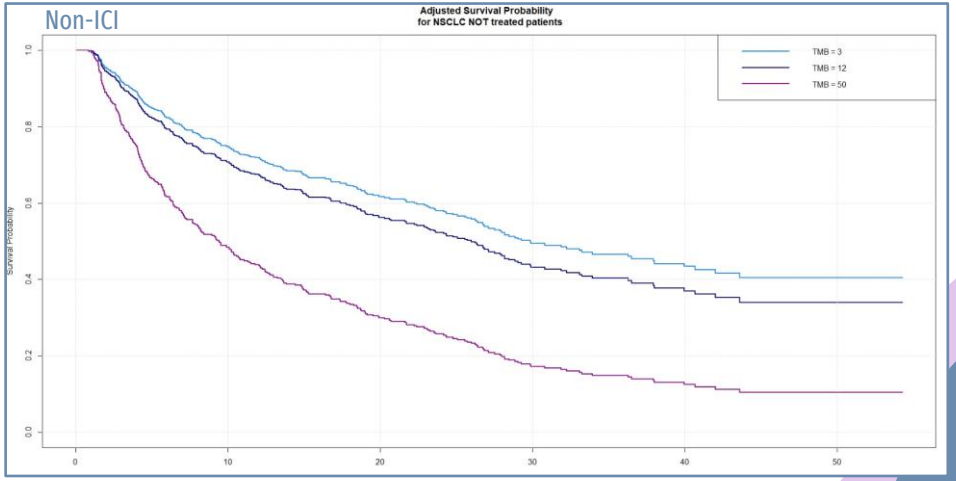
Global p-value 0.8082

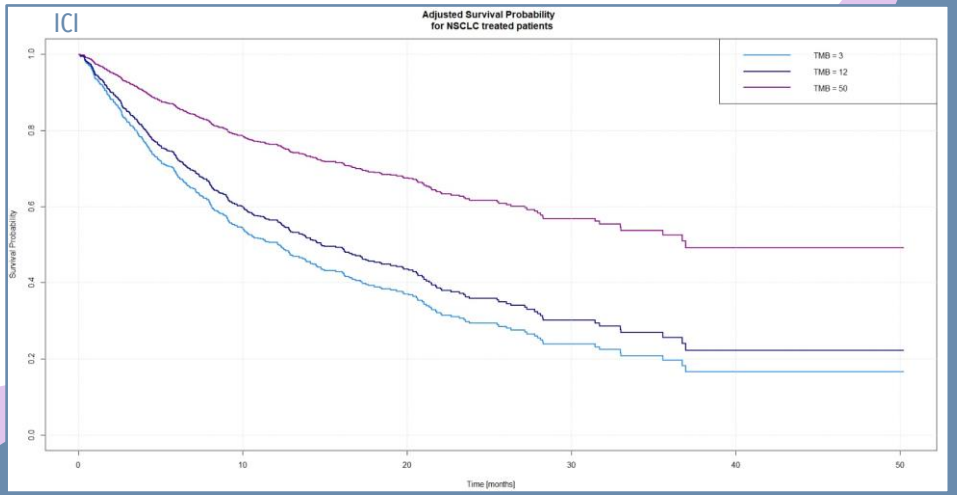
Cancer-specific analysis of TMB impact on survival

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

The example: NSCLC

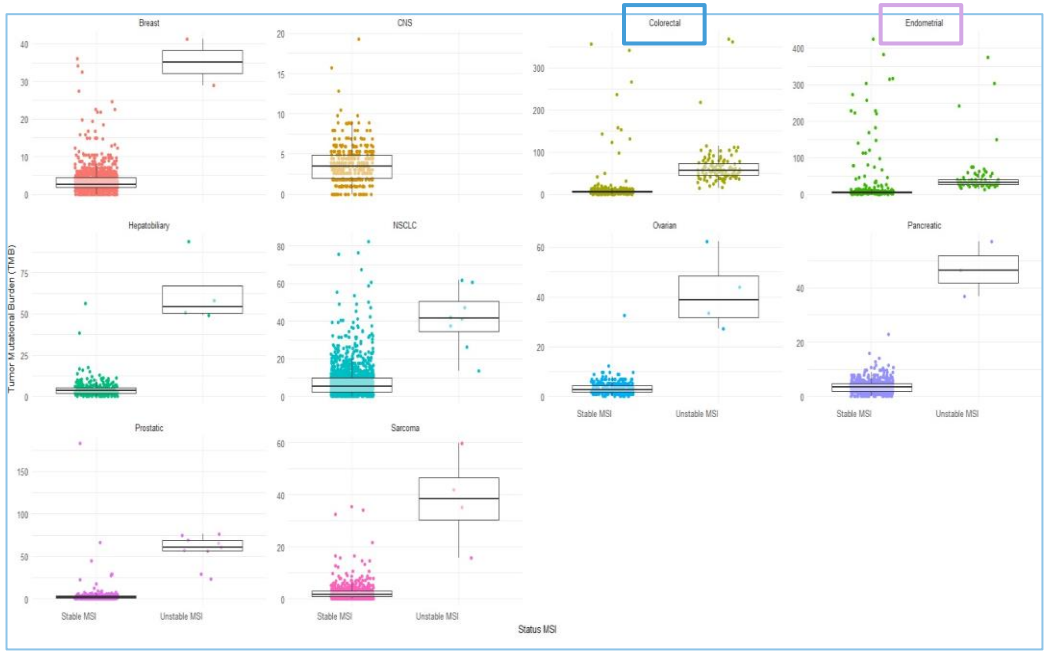




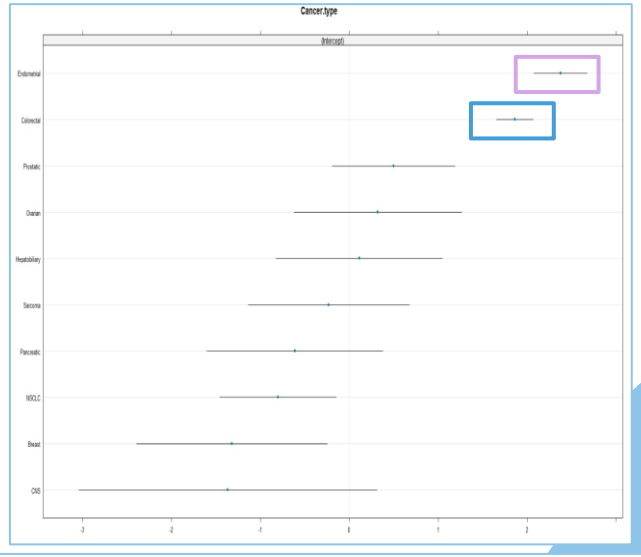


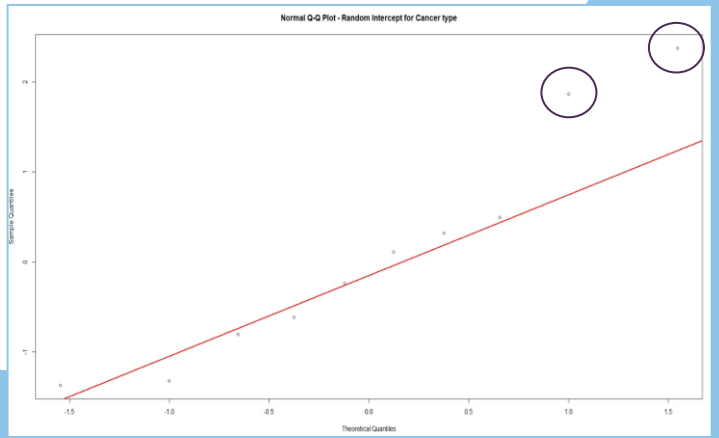
Can we use MSI instead of TMB to predict results?

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

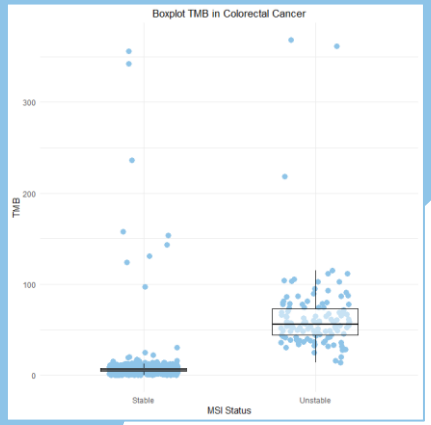


*VPC = Variant Partition Coefficient





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.

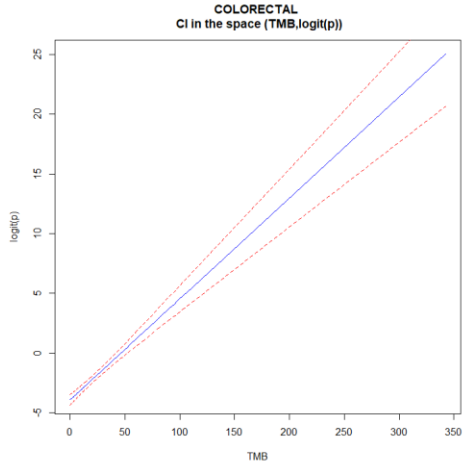
Probability of MSI status = Unstable

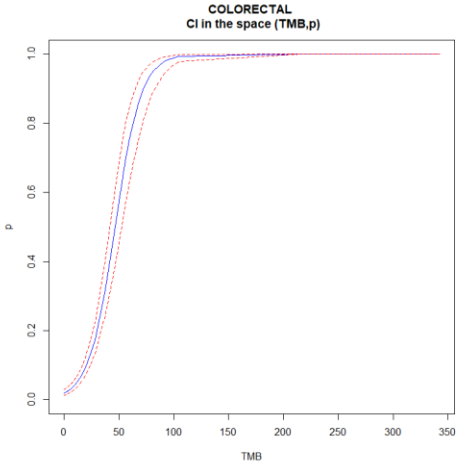
$$p = \frac{1}{1+e^{-(-3.817+0.076 \cdot x_{TMB})}}$$

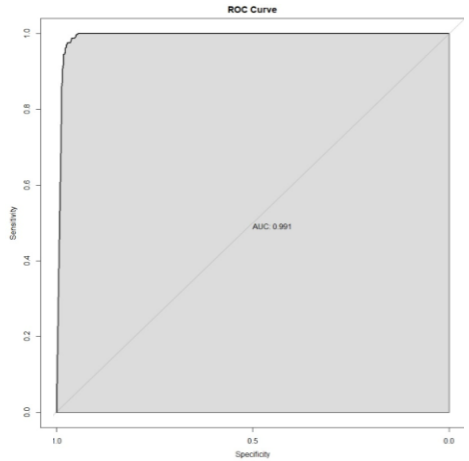
Interpretation of the coefficients (OR)

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

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







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

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

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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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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**.

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.

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**.

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

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

THANK YOU

for your attention