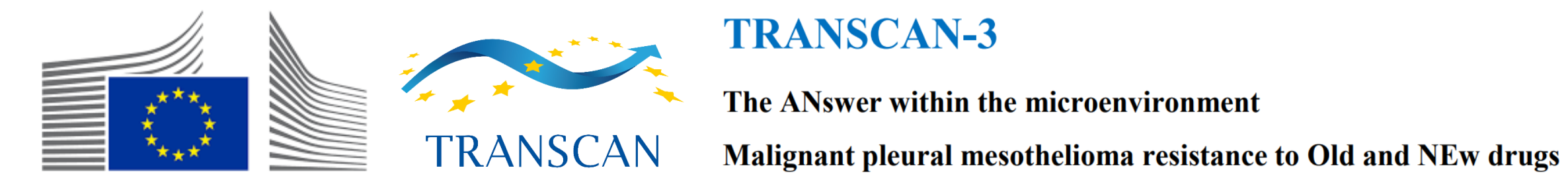


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INTRODUCTION

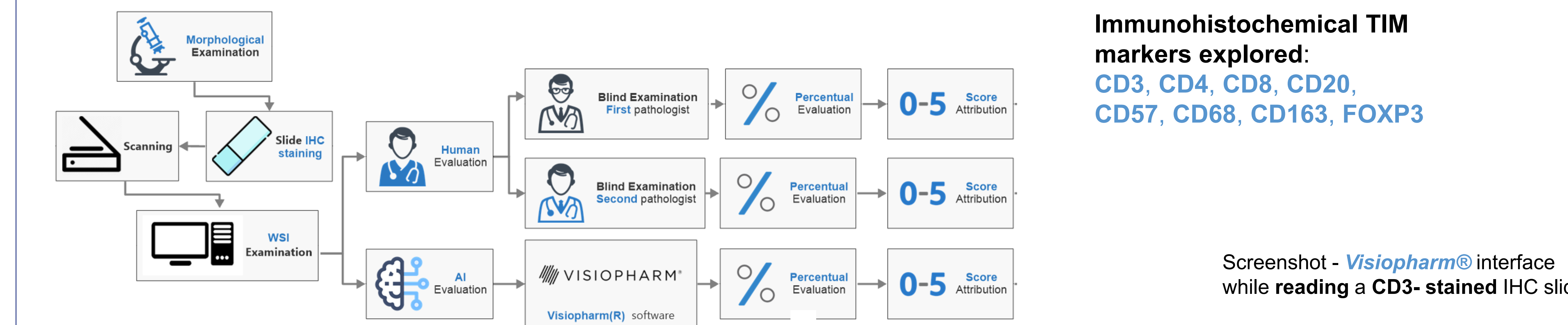
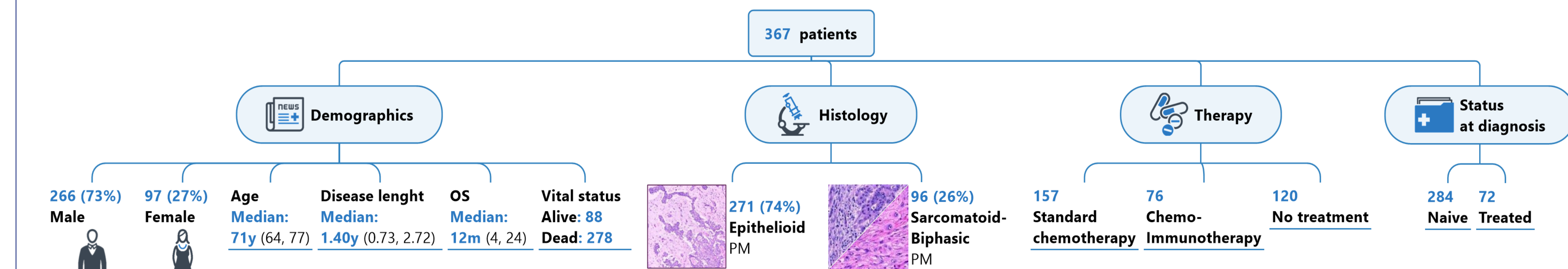
- Pleural mesothelioma (PM) is an aggressive neoplasia with a **severe** prognosis.
- Although **rare**, a **high peak** incidence is expected in **2020-2025**.
- **Asbestos** exposure is a **key risk factor**: the **protracted immune response**, makes PM a **candidate** for immunotherapeutic approaches.
- However, to date the **overall response rates** to treatment with immune checkpoint inhibitors (ICI) are **only 10-20%**.

Hypothesis: tumour microenvironment (TME), particularly tumour immune microenvironment (TIM), plays a **crucial role** in the **development/progression** of PM. **No universally accepted method** exists for a **standardized evaluation** of the **TIM**.

Aim: **Evaluate** the **concordance** between **human** and **AI**- whole slide image (WSI) TIM quantification. Which **TIM marker** matter for **response**, and which for **survival**?

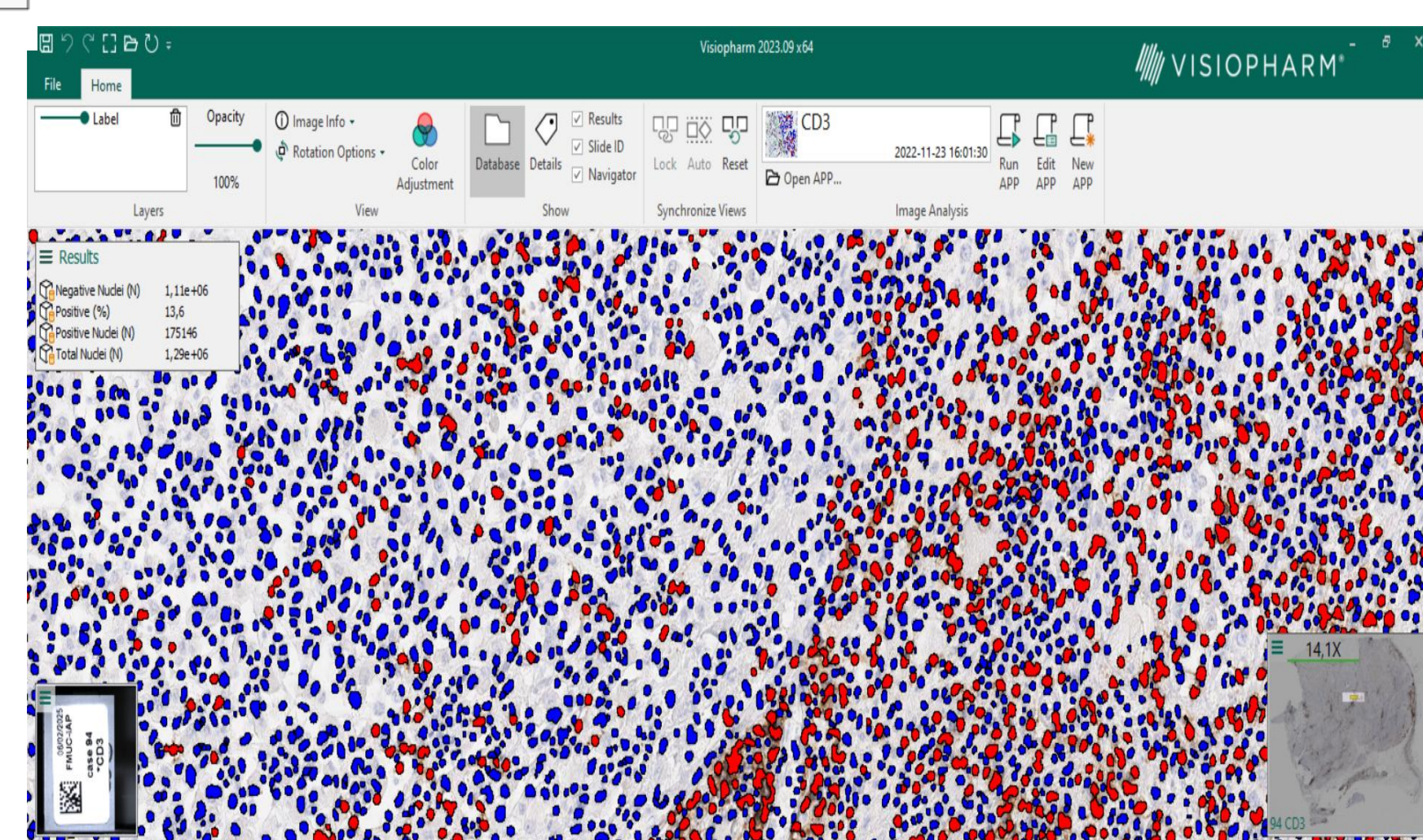
METHODS

COMPLETE
Clinical-pathological database of the population



Immunohistochemical TIM markers explored:
CD3, CD4, CD8, CD20, CD57, CD68, CD163, FOXP3

Screenshot - Visiopharm® interface while reading a CD3- stained IHC slide



Statistical methodolgy

- **AI scoring**: Visiopharm, percentages and ordinal scores
- **Response**: 217 evaluable; adjusted ordinal model n=212
- **Survival**: Cox models; splines and cutoff bootstrap (1,000 iterations)
- **Nested models**: response n=199; OS n=275; LRT, C-index and AIC
- **Exploratory response model**: 200 bootstrap iterations

DISCUSSION AND CONCLUSION

Take Home Messages

CD20 >3.6% (AI; %) identified patients with **significantly longer OS** (17.4 vs 11.7 months; HR 0.64): candidate **prognostic marker**.

CD68-high/CD163-low MFs: independently associated with treatment response. TIM improved response prediction beyond treatment type, whereas treatment type remained the dominant determinant of overall survival.

Critical Points and Future Directions

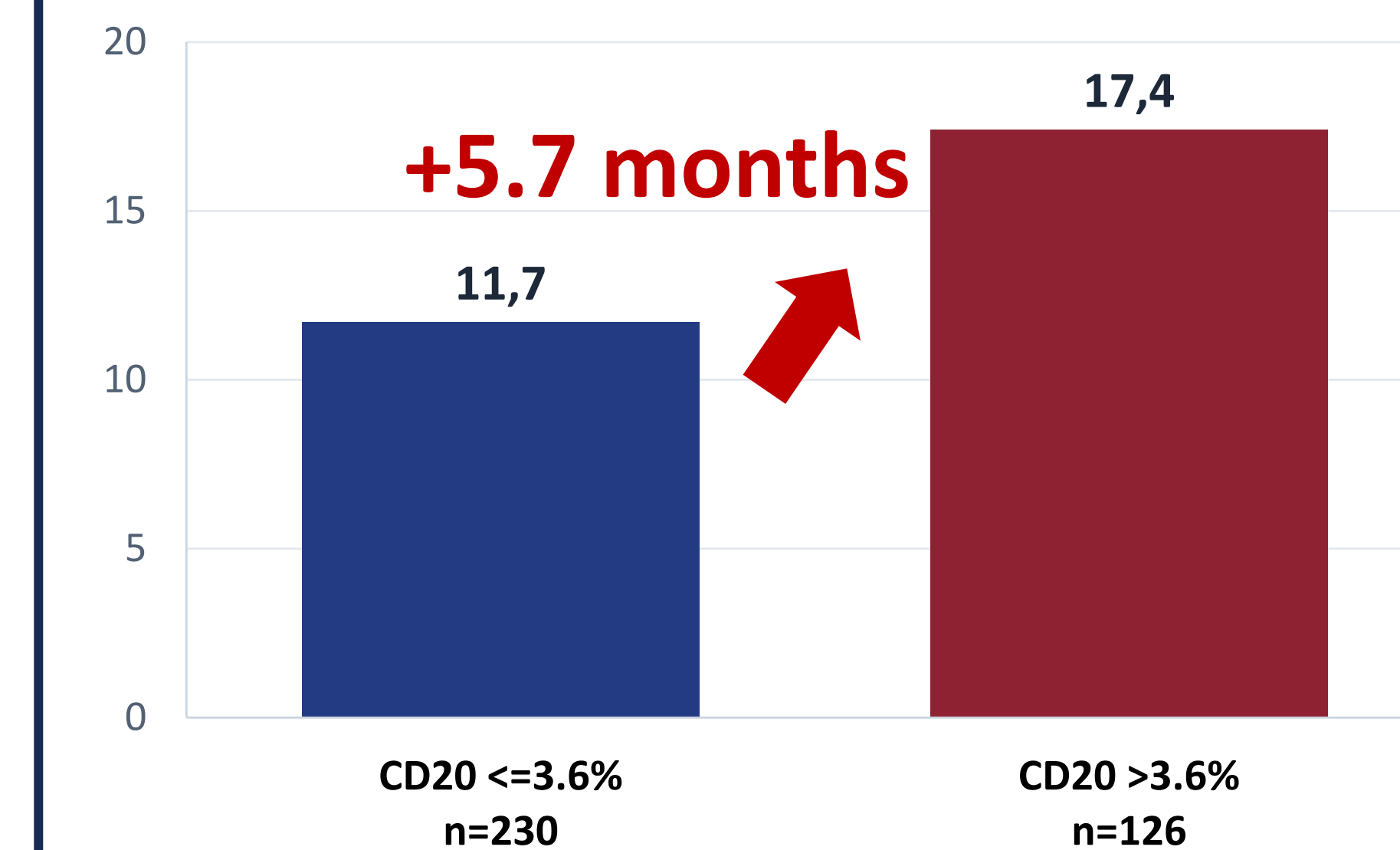
These **pareliminary data** and require considering:
(1) **non-random treatment** and **surgery** allocation;
(2) **modest discrimination** of the response model (bootstrap-corrected AUC 0.637);
(3) the **underpowered** chemo-immunotherapy subgroup.

Surgery modified the observed associations: **CD68** predicted **survival and response in non-operated patients**, while **no TIM** marker predicted survival **after surgery**. Overall, **AI-based TIM** profiling of diagnostic biopsies identifies complementary biomarkers for response and survival, suggesting **prospective external validation before clinical use**.

RESULTS

CD20 and survival threshold

Median overall survival (months)



Higher CD20 AI scores were associated with longer OS
HR 0.79; p=0.019
A non-linear effect identified an optimal cutoff at **CD20 >3.6%**
Median OS: 17.4 vs 11.7 months
HR 0.64 (95% CI 0.49–0.83); p<0.001

CD20 >3.6% represents a candidate prognostic (+5.7m in OS) biomarker requiring prospective validation.

Macrophage (MFs) polarization predicts response

MODEL	MARKER	OR	p	INTERPRETATION
Ordinal response, n=212	CD20	1.70	0.026	favorable
Ordinal response, n=212	CD163	0.64	0.008	unfavorable
Binary response, n=199	CD68	1.58	0.024	favorable
Binary response, n=199	CD163	0.61	0.006	unfavorable

Continuous AI percentages confirmed the signal: CD68 OR 1.03 per % (p=0.011); CD163 OR 0.97 per % (p=0.002).

MF profile characterized by **higher CD68** and **lower CD163** expression may be associated with a **greater likelihood of treatment response**. However, the modest AUC indicates limited discriminatory performance, and these exploratory associations require validation in independent prospective cohorts.

Higher CD68 + lower CD163 = M1 > M2 pattern associated with response

TIM and response – Treatment and survival

OUTCOME	TIM ADDED BEYOND TREATMENT TYPE	TREATMENT TYPE ADDED BEYOND TIM	INTERPRETATION
Treatment response n=199	SIGNIFICANT IMPROVEMENT LRT p=0.004	No significant improvement LRT p=0.377	TIM provides additional information for predicting response
Overall survival n=275	No significant improvement LRT p=0.409	SIGNIFICANT IMPROVEMENT LRT p<0.001	Treatment type provides most additional prognostic information for OS.

LRT = likelihood-ratio test comparing nested models. Treatment allocation was non-random; interpretation is associative, not causal.

TIM primarily informs **treatment response**, whereas treatment type remains the dominant source of prognostic information for overall survival.