

INTRODUCTION

- HER2-targeting antibody-drug conjugates (HER2-ADC) were approved to treat HER2-low breast cancer (BC).
- HER2-low is currently defined by the immunohistochemical (IHC) HER2-expression ASCO/CAP scores of 1+ or 2+ without *HER2/ERBB2* amplification¹.
- While the HER2 IHC assay was optimized to detect protein overexpression, concerns exist regarding the use of this assay to reliably detect HER2-low BC².

OBJECTIVES

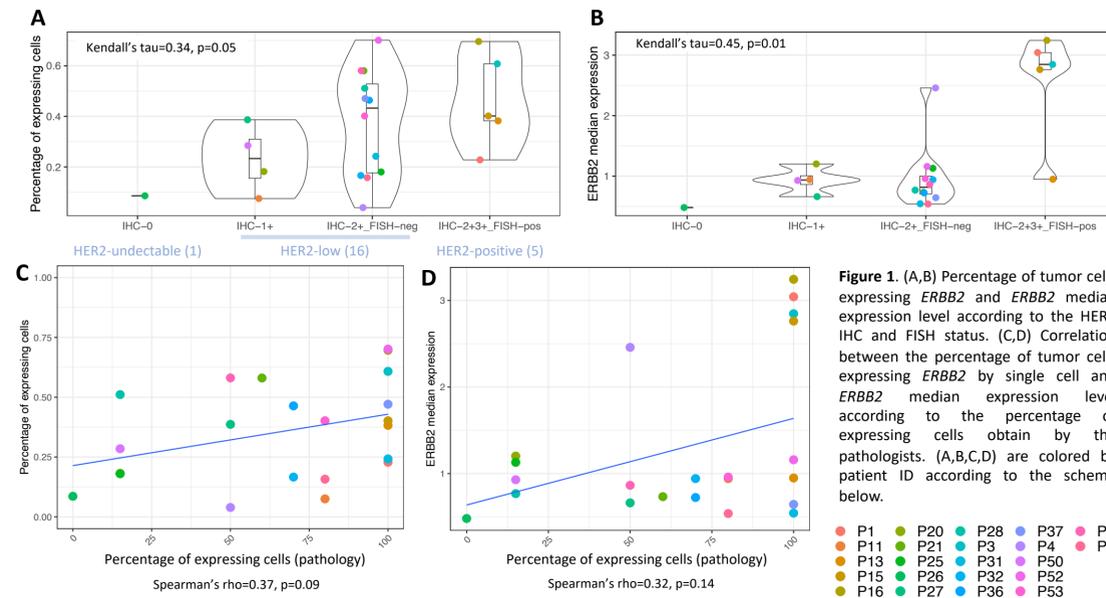
We aimed at :

- investigating the correlation between the IHC classification and the tumor cell expression levels of *ERBB2* mRNA at the single cell level.
- characterizing the inpatient heterogeneity of the *ERBB2* expression level
- characterizing the effect of ER status on *ERBB2* expression level

PATIENTS AND METHODS

- We retrospectively analyzed 22 untreated BC samples with single cell RNA-sequencing and centralized HER2 IHC data.
- IHC staining for HER2 (Agilent, GA0485, RTU) was performed and scored according to ASCO/CAP 2018 guidelines.
- Single cell data were retrieved from the original publication³ (BioKey, NCT03197389) and only the cancer cells (n= 31016) were retained.
- ERBB2* expression was considered among cells where *ERBB2* could be detected (non-zero normalized expression).
- Two metrics were systematically assessed:
 - The percentage of cells expressing *ERBB2*
 - The median of *ERBB2* expression among cells expressing *ERBB2*

A correlation is observed between HER2 IHC scores and single cell data...



...but inpatient heterogeneity of *ERBB2* mRNA expression is high with some *HER2*-undetectable tumor cells having expression level comparable at *HER2*-low tumor cells

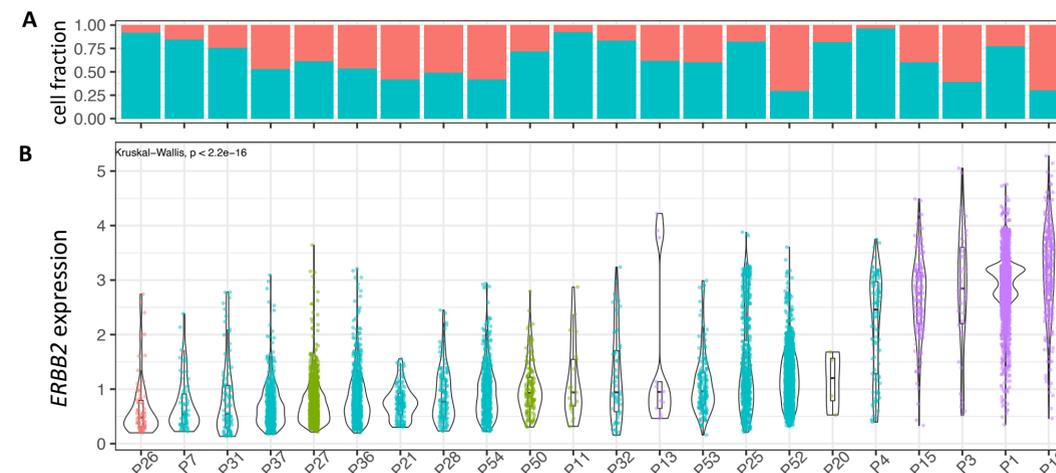
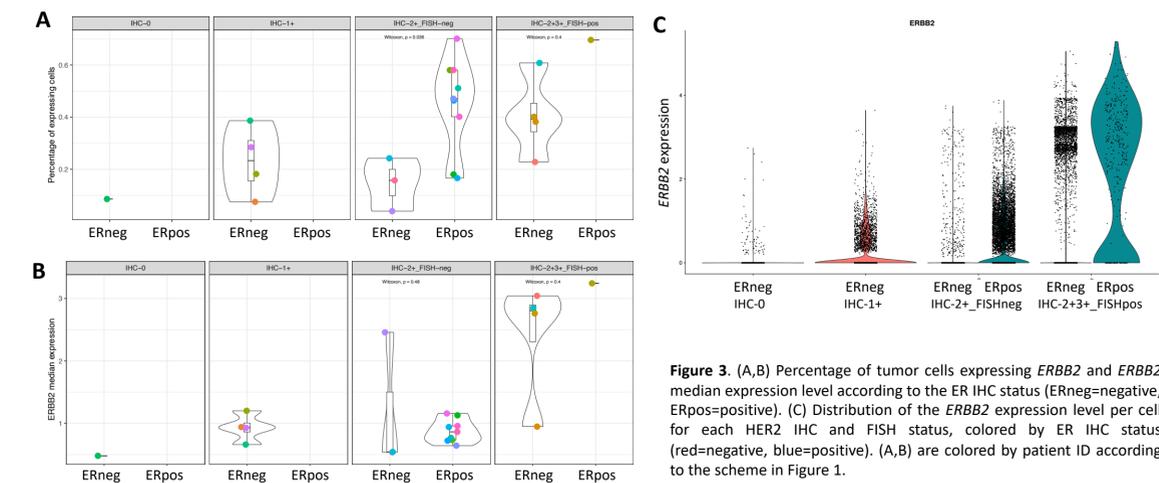


Figure 2. (A) Percentage of tumor cells expressing *ERBB2* (red) or not (blue) per patient. (B) Distribution of the *ERBB2* expression level in cells expressing *ERBB2* per patient, colored by HER2 IHC and FISH status with IHC-0 (red), IHC-1+ (green), IHC-2+_FISH-neg (blue) and IHC-2+_FISH-pos (purple). Patients are ranked according to their median *ERBB2* expression levels.

RESULTS

Positive ER status is associated with higher percentage of *ERBB2* expressing cells but stable *ERBB2* expression level



CONCLUSIONS

- HER2 IHC scores and single cell data correlate overall with correlation coefficients > 0.30.
- Tumor classified as HER2-undetectable still present tumor cells with comparable *ERBB2* expression level as HER2-low tumors.
- Single cell data might provide more granularity into the tumor-specific expression levels of HER2.
- Future research is needed to investigate whether single-cell *ERBB2* expression could serve as a predictive biomarker for HER2-ADC.

REFERENCES

- Modi, et. al. N Engl J Med (2022);
- Moutafi, et. al. Laboratory Investigation 2022;
- Bassez, et. al. Nature Medicine (2021)

ACKNOWLEDGEMENTS

