Comparison of single-cell ERBB2 mRNA expression levels with HER2 status by immunohistochemistry reveals heterogeneity of the HER2-low status |

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14P

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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) HER2expression 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 :

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

PATIENTS AND METHODS

- We retrospectively analyzed 22 untreated BC samples with single cell RNAsequencing 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*

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



Figure 3. (A,B) Percentage of tumor cells expressing ERBB2 and ERBB2 median expression level according to the ER IHC status (ERneg=negative, ERpos=positive). (C) Distribution of the ERBB2 expression level per cell for each HER2 IHC and FISH status, colored by ER IHC status (red=negative, blue=positive). (A,B) are colored by patient ID according to the scheme in Figure 1.

CONCLUSIONS

HER2 IHC scores and single cell data correlate overall with correlation

Tumor classified as HER2-undectable still present tumor cells with comparable *ERBB2* expression level as HER2-low tumors.

Single cell data might provide more granularity into the tumor-specific

Future research is needed to investigate whether single-cell ERBB2 expression could serve as a predictive biomarker for HER2-ADC.

REFERENCES 1. Modi, et. al. N Engl J Med (2022); 2. Moutafi, et. al. Laboratory Investigation ACKNOWLEDGEMENTS