Development and evaluation of a high-throughput method for rapid detection of surface antigen expression in fixed cells

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Introduction

Cell surface proteins are increasingly being targeted using antibody-drug conjugates (ADCs), enabling the delivery of cytotoxic agents to specific tumor sites. During the preclinical development of ADCs, a rapid detection of the target expression and distribution is essential. Here we report on the development of **oncoFLOW-Profiler**™ our novel 96-well screening platform to rapidly detect target antigen expression across 160 cancer cell lines in just one day.

This proof-of-concept study performed on the HER2 receptor was set up to generate an archived cell library and investigate the impact of PFA fixation and storage conditions over a period of three months.

HER2 data from **oncoFLOW-Profiler** were validated by comparison with ERBB2 RNA expression and *erbb2* gene amplification data from the Cancer DataMiner platform (4HF Biotec). Finally, the flow cytometry data generated served as a predictive biomarker screen of response to clinically approved ADC targeting HER2: Trastuzumab-Emtansine (T-DM1/ Kadcyla).

The aim of this study was to develop a high-throughput flow cytometry method for the rapid detection of surface antigen expression in fixed cells. HER2 was used as a proof-of-concept target for biomarker analysis and to validate our results with drug sensitivity towards the HER2 targeted drug T-DM1 using our 160 Cell line panel ProLiFiler™.

References

1. Barok et. al., (2014) Breast Cancer Research 16(2)
2. Tanner et al. (2004) Molecular Cancer Therapeutics 3(12)
3. D´Amico et al. (2020) Cells 9(5)
4. Boyer et al. (2021) Breast Cancer Research and Treatment 188(1)
5. Li et al. (2021) Antibody Therapeutics 4(3)

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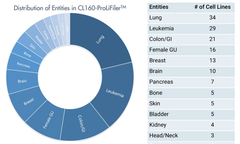
Material and Methods

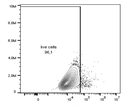
Cell line origin



Entities	# of Cell Lines
Lung	34
Leukemia	29
Colon/GI	21
Female GU	16
Breast	13
Brain	10
Pancreas	7
Bone	5
Skin	5
Bladder	5
Kidney	4
Head/Neck	3
Prostate	3
Soft Tissue	3
Liver	2

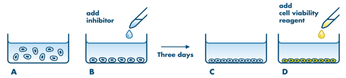
Cell treatment

Cells were stained with a fixable viability dye (Zombie Violet Fixable Viability Kit (Biozol)), which enables the exclusion of dead cells in later assays.



2D Cell Proliferation Assay for T-DM1 correlation analysis

Cells were seeded in 384-well plates and incubated with compounds for 3 days. Promega's CellTiter-Glo dye was used to determine the number of viable cells by detecting a luminescent signal that is proportional to the amount of ATP present in culture.

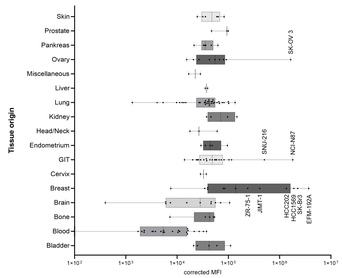


First oncoFLOW-Profiler™ on HER2

We conducted an initial screen on a full panel of 160 fixed cell lines, to detect HER2 expression, and obtained data within a day. The box plot diagram illustrates HER2 median fluorescence intensity (MFI) data sorted by the 17 tumor origins.

Breast cancer cell lines exhibits the highest median MFI. All cell lines surpassing the median threshold originate from breast, ovary, and GIT tumors and are explicitly named.

→ Our assay affirmed the prevailing scientific expectations.

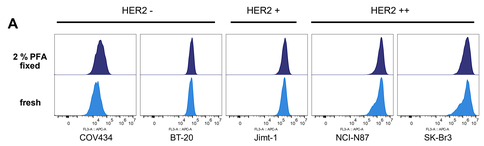


Influence of PFA fixation and storage on the structural integrity of HER2

2 % PFA fixation | The impact of 2% PFA fixation on cell structural integrity and its consequent effect on fluorescence signal in flow cytometry experiments were investigated in comparison to fresh cells (panel A). It was found that the detectability of HER2 is not influenced by 2% PFA fixation.

The slight left shift of histograms of the HER2++ cell lines NCI-N87 and SK-Br3 in fresh cells (panel A), could be attributed to the internalization of Trastuzumab, used as the primary antibody in the staining process.

→ HER2 detectability of is not influenced by 2 % PFA fixation.

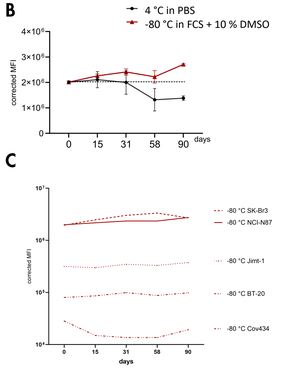


Storage | The structural integrity of the HER2 receptor was assessed under two different storage conditions: storing the cells at 4°C in PBS and storing them at -80°C in FCS + 10% DMSO for a duration of 90 days, with measurements taken at four time points using flow cytometry experiments.

In panel B, data for the NCI-N87 cell line is displayed, showing that storing cells at -80°C in freezing medium preserves receptor signal better compared to storage at 4°C.

Panel C highlights that even after PFA fixation and 90 days of storage at -80°C in freezing medium, the ability to distinguish between high and low expressors of HER2 remains intact.

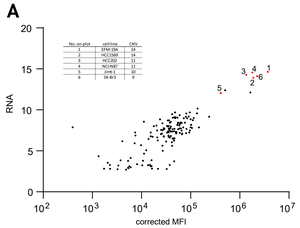
→ The detectability of HER2 is not influenced by storage in freezing medium at -80 °C.



oncoFLOW-Profiler data validation

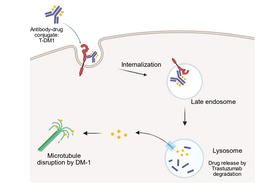
Correlation of HER MFI with ERBB2 mRNA data | A spearman correlation analysis of oncoFLOW-Profiler data with ERBB2 RNA expression (4HF Biotec) revealed a strong positive correlation. Previously unexplored cell lines align very well with their mRNA expression level. Cell lines with *erbb2* gene amplification (red dots and table within the graph) are among those with the highest HER2 MFI levels (A).

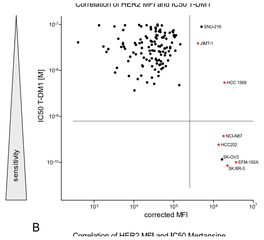
→ Correlation of HER2 oncoFLOW-Profiler™ data and ERBB2 mRNA strengthens the functionality of the novel assay.



Correlation of HER MFI with IC50 T-DM1 data | Additionally, the HER oncoFLOW-Profiler data was validated with drug sensitivity towards the HER2 targeted drug T-DM1. IC_{50} values were measured within our 160 Cell line panel ProLiFiler. The mode of action of T-DM1 is dependent on the HER2 receptor expression on the cell`s surface. Upon internalization of the ADC, the antibody Trastuzumab gets proteolytically digested within the lysosome, so that the payload Mertansine gets released into the cytoplasm, where it can disrupt microtubules (B)[1]. The analysis revealed five cell lines, exhibiting high HER2 expression levels on their cell surface along with high sensitivity towards T-DM1 (lower right quadrant). In contrast, three cell lines were identified as being T-DM1 resistant, characterized by high HER2 MFI values but low T-DM1 sensitivity (C). These findings are mostly consistent with data from literature[2,3,4,5].

→ The result of the proliferation assay indicates the reliability of the novel oncoFLOW-Profiler.





Conclusion

Our novel oncoFLOW-Profiler enables biomarker detection over a panel of 160 fixed cell lines. Initially, the method establishment was successfully performed on HER2, this receptor is stable under 2 % PFA fixation and storage for 3 months at -80 °C in freezing medium.

The first staining of HER2, over the whole panel was validated through correlation with an ERBB2 mRNA data set (4HF Biotec), revealing a strong positive correlation. In addition, for most cell lines the sensitivity against HER2 targeted drugs, aligns with the measured HER2 MFI level. However, we could identify three cell lines that exhibit resistance against T-DM1. Our findings are consistent with the data in literature, thereby oncoFLOW-Profiler has proven to be a reliable tool for biomarker analysis.

It can not be guaranteed that other markers might have been influenced by the fixation and storage protocol. Therefore, we suggest to first conduct a pilot experiment, comprising stains performed on fresh and stored cells to compare their detectability.