# Identifying HER2 Expression in Osteosarcoma as Immunotherapy Target

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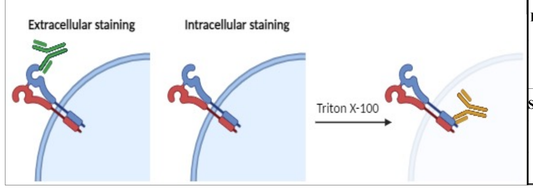
## Introduction

- Osteosarcoma (OS) is the most common pediatric bone sarcoma and accounts for 2% of all childhood cancers (Prater & Mckeon 2023). OS therapy has remained mostly unchanged for decades, highlighting the need for new therapeutic options. HER2 and GD2 have recently emerged as promising targets for therapy.
- Clinical trials have shown some success in antibody-drug conjugates and T-cell therapies targeting HER2 and GD2 in other cancers.
- Recent translational studies have demonstrated that T cells engineered with bispecific antibodies (BsAbs) against HER2 and GD2 can reduce tumor growth in OS.
- Objective: To identify HER2 expression levels among different OS cell lines and patient samples to determine if HER2 can be a promising target in BsAb-mediated immunotherapy in OS therapy.

## Methods & Material

For Immunofluorescence (IF), we used antibodies targeting the intracellular (IC) and extracellular (EC) domains of HER2. We permeabilized the cell membrane with Triton X-100 for IC staining and then used IC HER2-targeting antibodies to detect HER2 expression levels. EC HER2-targeting antibodies detected HER2 surface-level expression while the cell membrane remained intact for EC staining. We compared HER2 expression levels in OS cell lines to our control groups (MCF7 and HEC1B). Western Blot (WB) was used to analyze HER2's presence. GAPDH was analyzed to ensure equal protein concentration in each well. We then analyzed the difference in HER2 and GAPDH for each well to determine HER2's concentration.




| | Reagent | Identity | Usage |
|---|---|---|---|
| **Primary Antibody** | HER2/ErbB2 (29D8) Rabbit mAb #2165 | Cell Signaling | IF IC 1:100 & WB HER2 1:1000 |
| | Purified anti-human CD340 (erbB2/HER-2) | Sony Biotechnology | IF EC 1: 100 |
| | GAPDH (D16H11) XP® Rabbit mAb #5174 | Cell Signaling | WB GAPDH 1:1000 |
| **Secondary Antibody** | Cy™3 AffiniPure™ Donkey Anti-Rabbit IgG (H+L) | Jackson Immunoresearch | IF IC 1:1000 |
| | Cy™3 AffiniPure™ Donkey Anti-Mouse IgG (H+L) | Jackson Immunoresearch | IF EC 1:1000 |
| | Anti-rabbit IgG, HRP-linked Antibody #7074 | Cell Signaling | WB HER2 & GAPDH 1:5000 |

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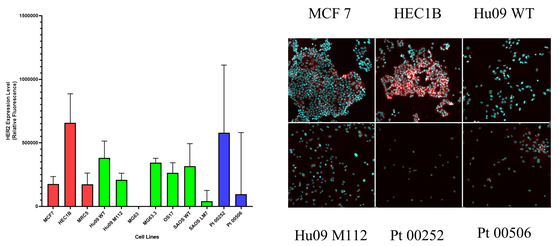
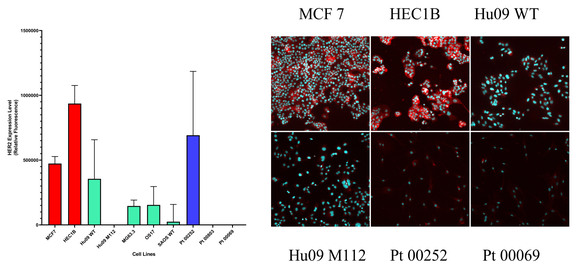
## Results

### Immunofluorescence (IF) Assay




HER2 Intracellular (IC) Antibody

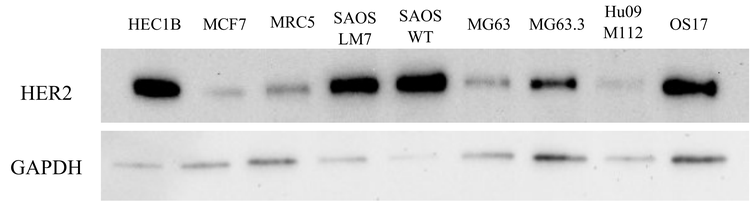
HER2 expression levels vary among OS cell lines, including wild-type and metastatic variants. The IF analysis targeting HER2 IC showed some OS cell lines having higher HER2 expression than MCF7, and one of the OS patient sample-derived cell lines had a higher HER2 expression than MCF7, comparable to HEC1B. Cell lines with HER2 overexpression from IF IC were chosen for IF EC to determine the level of HER2 expression.




HER2 Extracellular (EC) Antibody

The IF analysis targeting HER2 EC showed that almost all OS cell lines had lower HER2 expression than MCF7; however, one of the OS patient sample-derived cell lines had higher HER2 expression than MCF7, which was comparable to HEC1B.

### Western Blotting (WB)




Our WB analysis has suggested the presence of HER2 in OS cell lines, showing varying levels of HER2 concentration, providing a solid foundation for our research findings.

## References

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2. Park, J. A., Cheung, NK. V. 2000. GD2 or HER2 targeting T cell engaging bispecific antibodies to treat osteosarcoma. *J Hematol Oncol.* 13:172.
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## Future Directions

We aim to replicate our experiments and utilize Fluorescence-activated cell sorting (FACS) to validate our findings from IF and WB analyses. Our goal is to extend these studies to additional patient sample-derived cells. Once we establish HER2 expression levels, we will employ HER2 and GD2-targeted BsAbs with T-cell cultures to evaluate the cytotoxic effects on OS cells and investigate the relationship between HER2 expression and cytotoxicity. Additionally, we plan to apply BsAbs in *in vivo* xenograft models. If these approaches prove successful, we will proceed towards clinical trials.

## Discussion

There's an inconsistent correlation between HER2 surface-level expression and total HER2 expression. Some OS cell lines expressed high total HER2 expression but low HER2 expression for surface staining, which may indicate membrane trafficking's important role in surface expression. We can target the membrane trafficking system to enhance HER2 level of expression on the cell surface, which will enhance HER2-targeted immunotherapy and its benefits.