

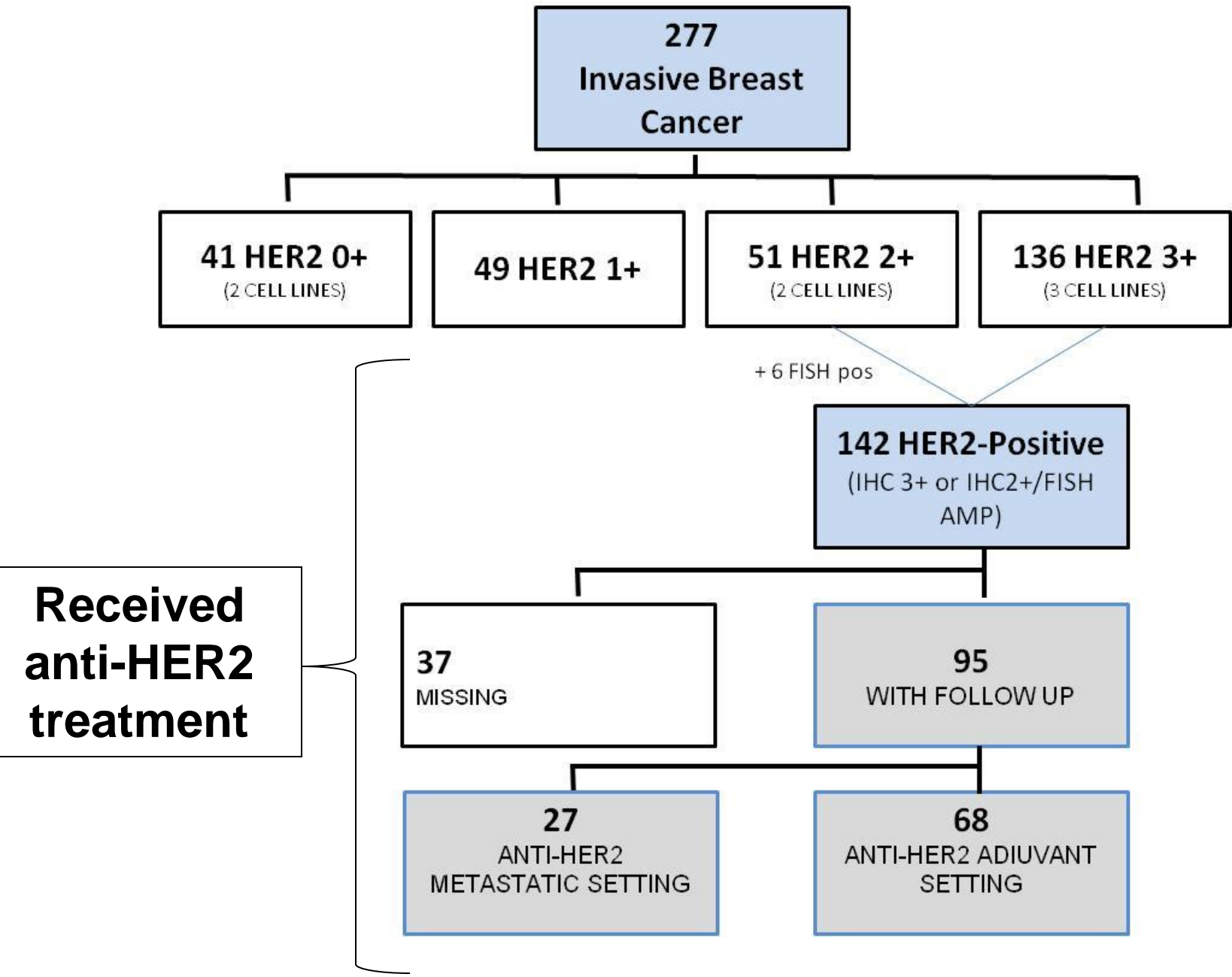
# Quantitative measurement of HER2 levels by multiplexed mass spectrometry from FFPE tissue predicts survival in patients treated with anti-HER2 based therapy

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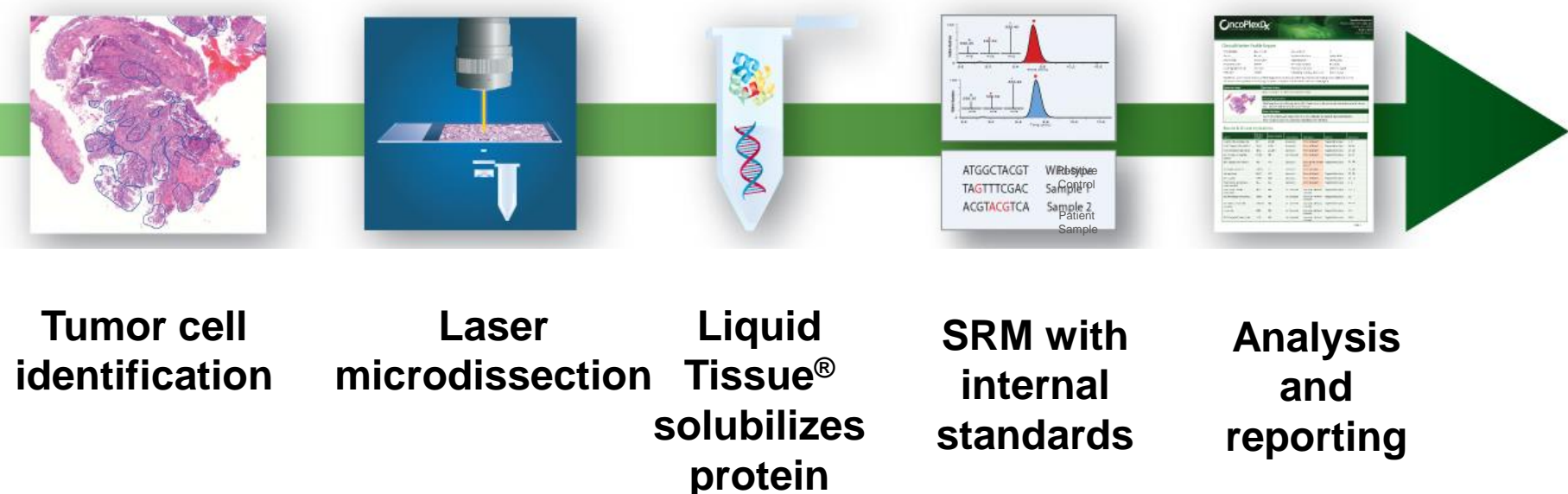
## Background

Approximately 20% of breast cancer patients overexpress HER2 and are treated with anti-HER2 therapies. However, there is a great deal of disparity of HER2 levels in the patients that are classified as HER2 positive (IHC3+). Techniques like FISH or IHC do not allow for HER2 quantification and a significant proportion of patients are wrongly classified as HER2 positive. Liquid Tissue-Selected reaction monitoring (LT-SRM) is a multiplexed mass spectrometric technique that can objectively quantify levels of HER2 and other targets (e.g. EGFR, HER3 etc.) simultaneously from formalin fixed paraffin embedded (FFPE) sections. Accurate quantification of the expression levels of HER family proteins and knowledge of its impact on response to anti-HER2 therapies may be beneficial for clinicians to better personalize the treatment to the patient.

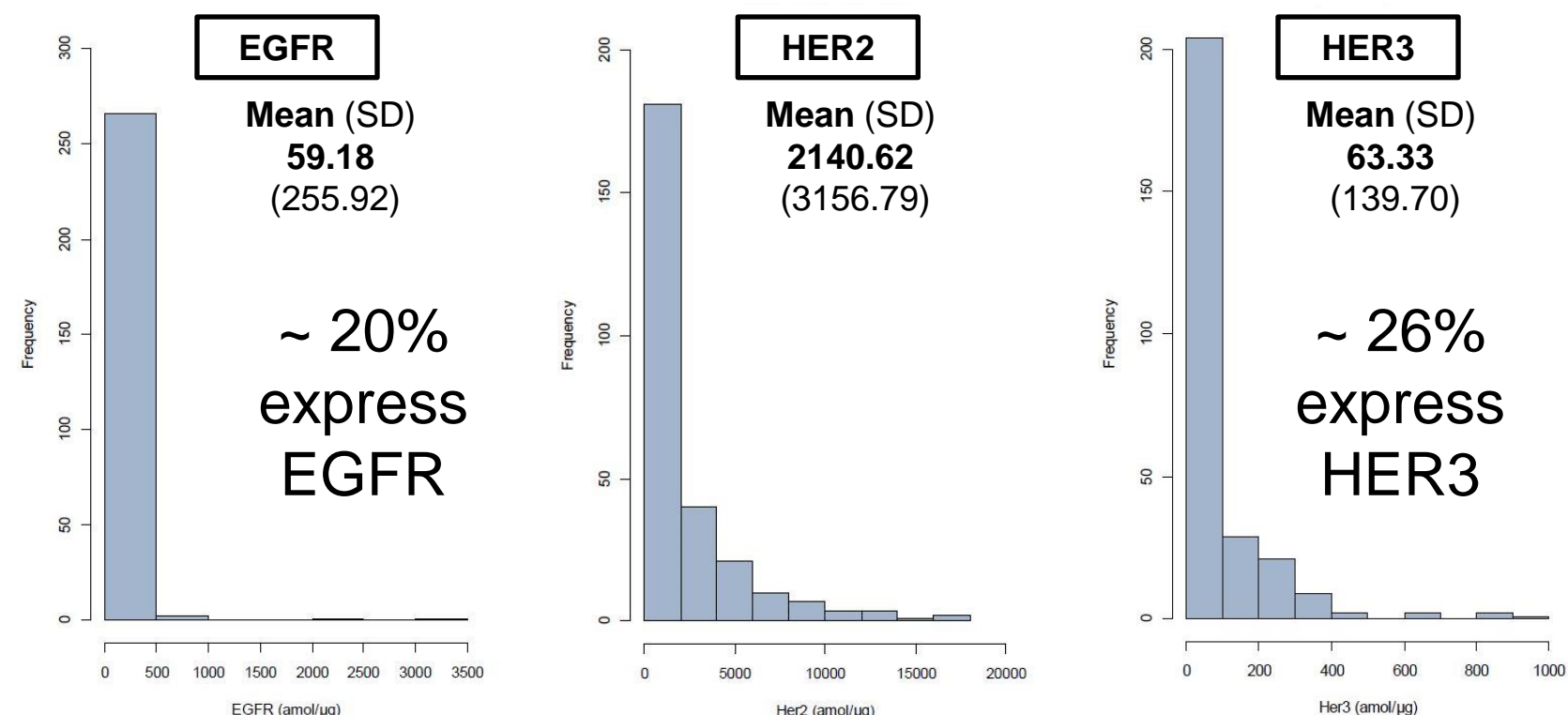
## Study population



## Quantitative Multiplex Mass Spec Assay



## HER family proteins abundance in BC (n=277)

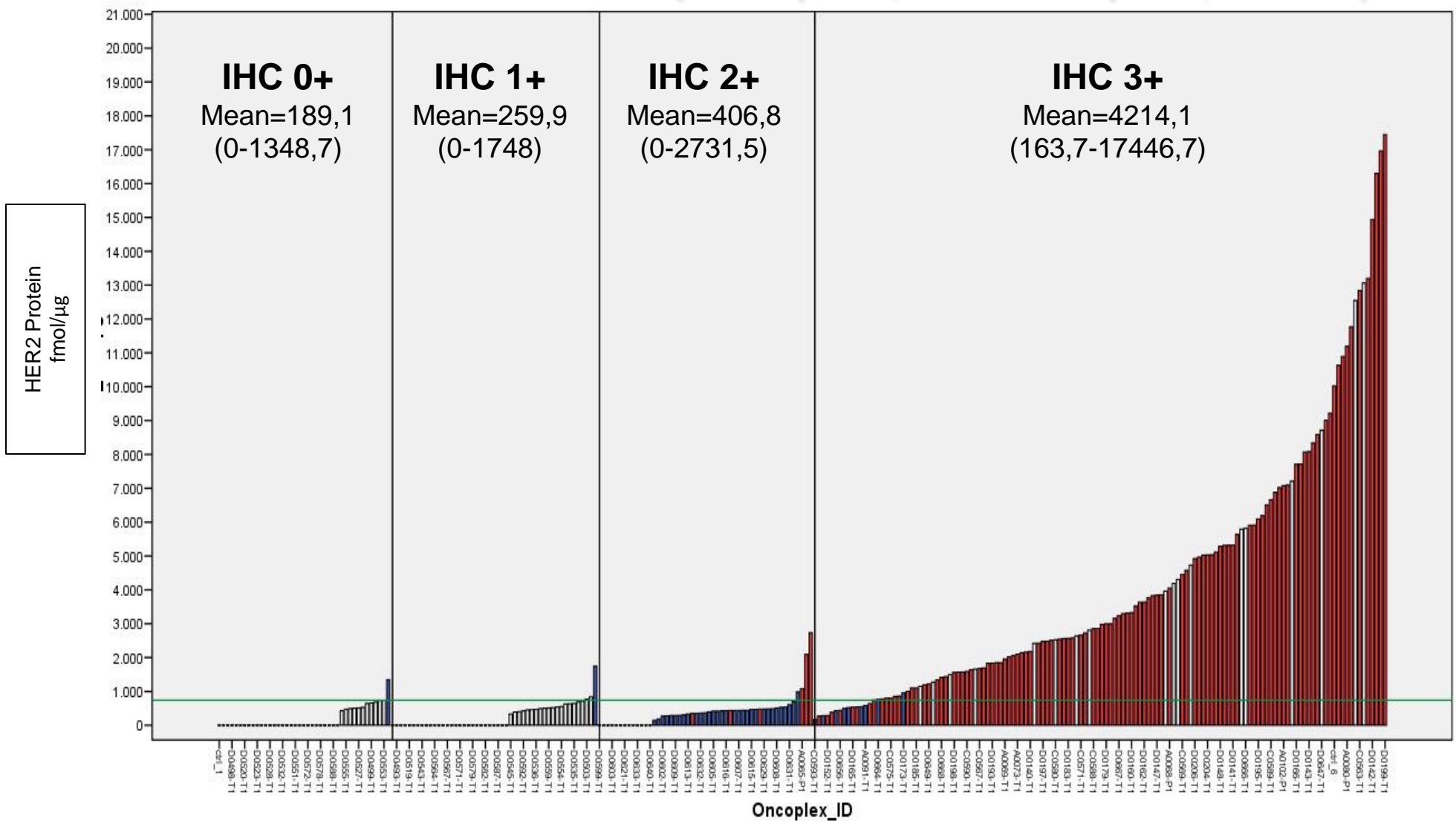


### HER2-pos receiving anti-HER2 treatment

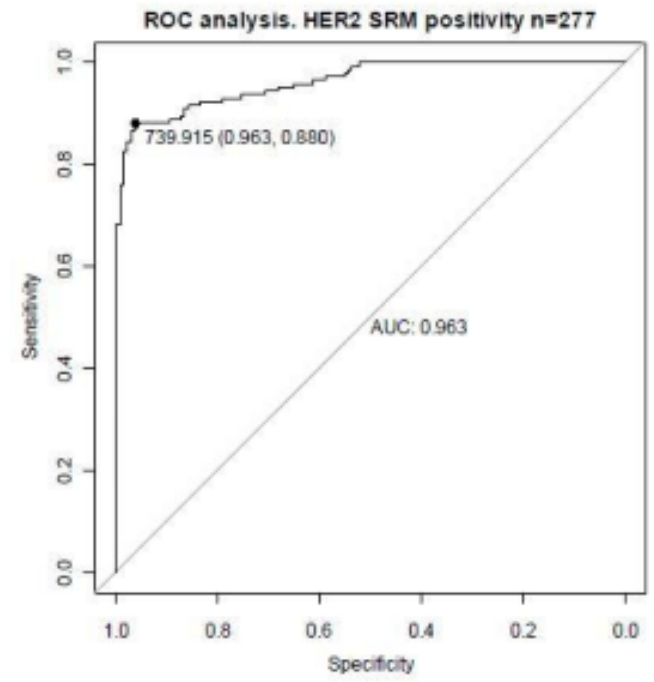
|      | Treatment setting | Mean    | SD      |
|------|-------------------|---------|---------|
| EGFR | Adjuvant (n=68)   | 97.95   | 290.92  |
|      | Metastatic (n=27) | 56.18   | 103.52  |
| HER2 | Adjuvant (n=68)   | 3934.08 | 3712.50 |
|      | Metastatic (n=27) | 4157.35 | 3248.83 |
| HER3 | Adjuvant (n=68)   | 70.20   | 123.79  |
|      | Metastatic (n=27) | 61.95   | 93.52   |

## Quantitative HER2 level distribution by IHC groups

(red=amplified; blue=non-amplified; blank=na)



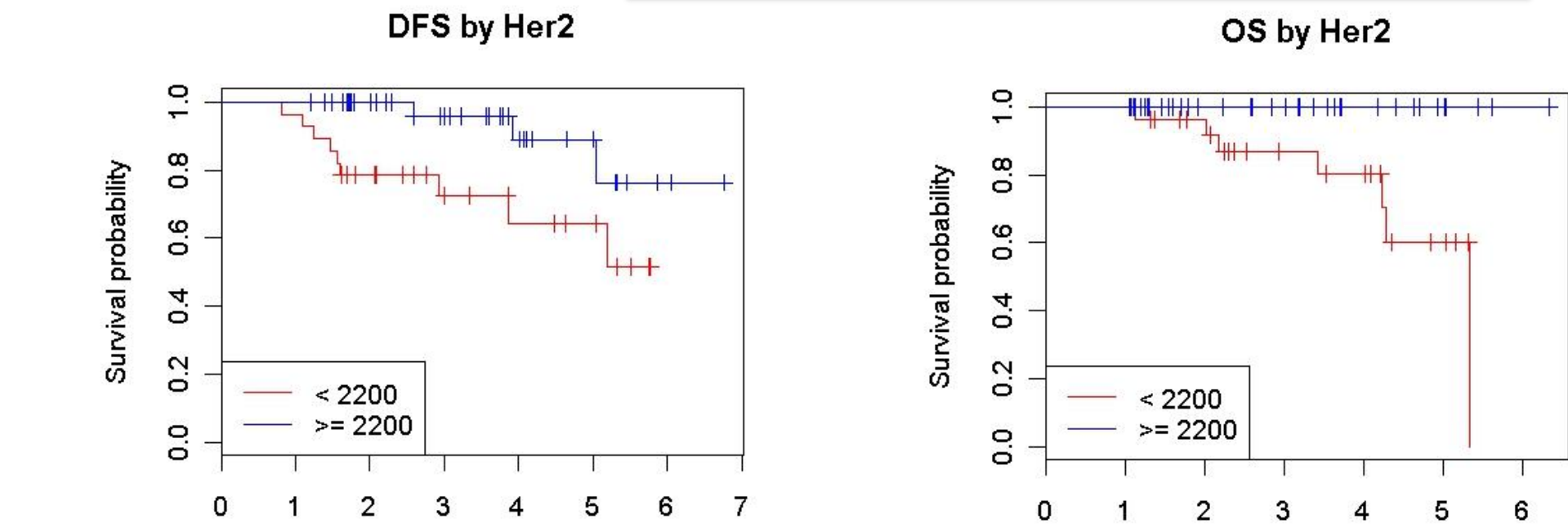
HER2\_FISH\_DX\_CL



SRM threshold (740 amol/μg) that correlates with HER2 status by IHC/FISH was determined using ROC analysis

## Survival analyses

### ANTI-HER2 ADJUVANT SETTING

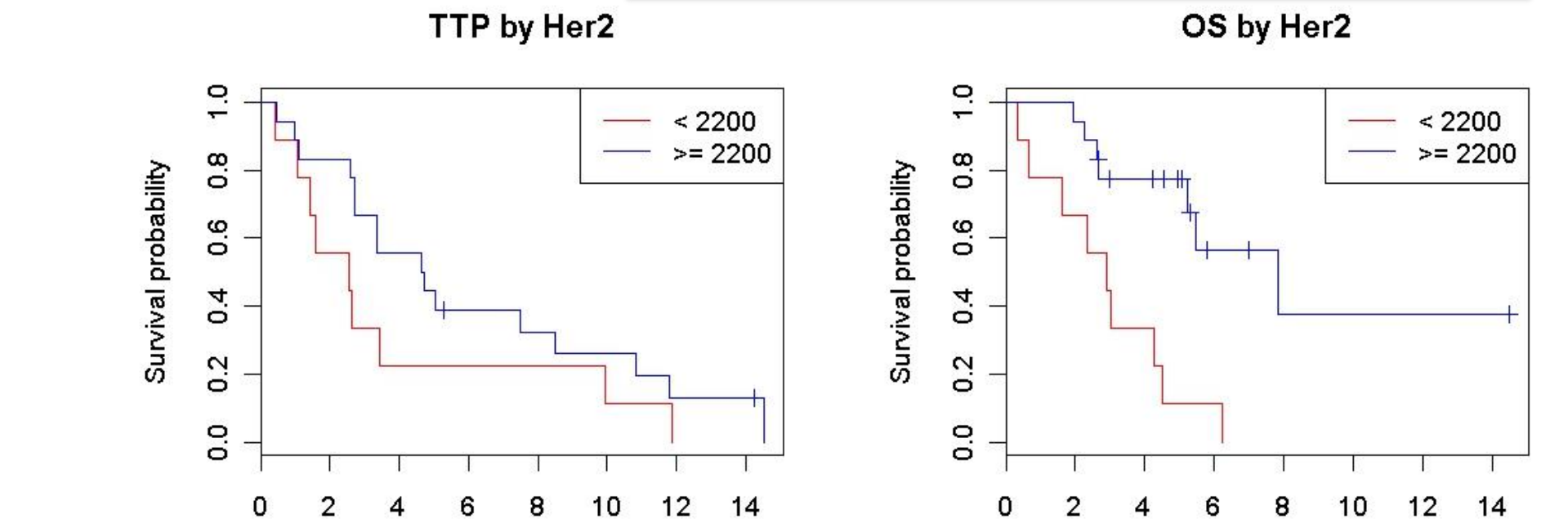


| No. at Risk | Time (years)         | No. at Risk | Time (years)       |
|-------------|----------------------|-------------|--------------------|
| < 2200      | 28 27 19 12 8 6 0 0  | < 2200      | 28 28 21 13 11 4 0 |
| >= 2200     | 40 40 29 22 13 7 2 0 | >= 2200     | 40 40 25 21 11 5 1 |

| DISEASE FREE SURVIVAL (DFS) |             |         |
|-----------------------------|-------------|---------|
| HR                          | CI 95% (HR) | p value |
| 0.22                        | 0.06-0.81   | 0.013   |
| OVERALL SURVIVAL (OS)       |             |         |
| HR                          | CI 95% (HR) | p value |
| na*                         | na          | 0.001   |

\*Hazard ratio for OS can't be determined because all patients with >2200 amol/μg of HER2 are alive after 6 years of anti-HER2 therapy

### ANTI-HER2 METASTATIC SETTING



| No. at Risk | Time (years)       | No. at Risk | Time (years)       |
|-------------|--------------------|-------------|--------------------|
| < 2200      | 9 5 2 2 2 1 0 0    | < 2200      | 9 6 3 1 0 0 0 0    |
| >= 2200     | 18 15 10 6 5 4 2 2 | >= 2200     | 18 17 12 4 1 1 1 1 |

| TIME TO PROGRESSION (TTP) |             |         |
|---------------------------|-------------|---------|
| HR                        | CI 95% (HR) | p value |
| 0.56                      | 0.24-1.29   | 0.16    |
| OVERALL SURVIVAL (OS)     |             |         |
| HR                        | CI 95% (HR) | p value |
| 0.20                      | 0.07-0.57   | <0.001  |

## Conclusions

- Quantification of HER2 protein by SRM (>740 amol/μg) accurately predicts HER2 expression status compared to standard IHC (3+)/ISH (≥2.0) in 277 FFPE samples.
- High HER2 protein levels (>2200 amol/μg) predict DFS (HR= 0.22; p=0.013) and OS (HR =<0.10; p=0.001) benefit with HER2 targeted therapy in the adjuvant setting.
- Patients who highly express HER2 protein (>2200 amol/μg) also have significant OS benefit (HR=0.20 ; p<0.001) from HER2 targeted therapy in the metastatic setting.
- The OncoPlexDx test can identify HER2 moderate expressers (740 to 2200 amol/μg) who may not receive complete therapeutic benefit from anti-HER2 therapy.
- These moderate expressers may benefit from co-treatment with targeted therapies based on coexpressed targets.
- Upfront multiplex SRM testing will reveal what these options are and support physicians in making informed treatment decisions.

### Agreement between SRM and HER2 status by IHC/FISH

|             | HER2 Status | n   | SRM Agreement, n (%) |
|-------------|-------------|-----|----------------------|
| Diagnostic  | Negative    | 135 | 130 (96%)            |
|             | Positive    | 142 | 125 (88%)            |
| Central Lab | Negative    | 144 | 137 (95%)            |
|             | Positive    | 133 | 123 (92%)            |

### Correlation between HER2 SRM and FISH ratio

|              |            | HER2 FISH |      |       | pvalue |
|--------------|------------|-----------|------|-------|--------|
|              |            | NO AMPL   | AMPL | total |        |
| HER2 amol/μg | neg (<740) | 53        | 10   | 63    | <0.001 |
|              | pos (>740) | 5         | 105  | 110   |        |
|              | total      | 58        | 115  | 173   |        |

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