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Background

The incidence of brain metastases (BM) from primary breast cancer (BC) increased over the past decades. Up to 30% of patients with metastatic BC develop BM over time. The patients’ prognosis is still poor and could not be significantly improved over the past decades. Despite the increasing incidence of BM in BC, the biology and development of BM are still poorly understood. Consequently, gaining insight into how BM develop is essential for identifying effective treatments or preventive strategies. The Brain Metastases in Breast Cancer Registry (BMBC) has collected clinical data of 4300 BC patients with BM and tumor tissue samples (n= 400).

The aims of this analysis from the BMBC registry were:

- To identify absolute assignment of BC intrinsic molecular subtype (AIMS) in primary BC and matched BM.
- To assess the HER2 IHC including switches from primary BC to BM in matched pairs that could be relevant for prognosis and treatment decisions
- To identify BM-specific genes or gene clusters that will help us to develop predictive biomarkers for BM development in BC.

Patients and Methods

The study population includes female BC patients from the BMBC registry which is collecting patient’s data from patients with BM and BC as well as biomaterial.

For this study, gene expression data (HTG EdgeSeq Oncology Biomarker Panel) for 2549 genes was generated from **BM and the corresponding primary BC tissue samples (n= 76)**, followed by differential gene expression analysis. Molecular subtypes were classified according to **ER and PR status as well as HER2 IHC (ultralow, 1+, 2+, 3+)** and additionally AIMS subtypes was assessed using HTG data. Bioinformatics analysis and visualization were carried out in R. Differential expression analysis was performed using limma. Volcano plots were generated using the ggplot2 package.

- In total 76 corresponding BM and primary BC tissue samples were included in this analysis. Median age at BM diagnosis was 55 years. 50% of the patients had singular BM, 27.6% 2-3 BM and 22.4% >=4 BM-(see table 1).
- Among significantly upregulated genes (logFC > 0.58) in BMs in comparison to BC, heat shock proteins (HSPA1B), mitochondria-related genes (COX5 and MRPL13) as well as cell cycle-associated factors (CDC20, CCNF and PTTG1) were detected. Significantly downregulated genes included the following: frizzled-protein related genes (SFRP2), collagen-related genes (MMP2/MMP11, COL1A1/2) and extracellular matrix proteoglycan (VCAN), (see figure 3).

Table 1: patients' characteristics in categorical parameters

Parameter at diagnosis	Category	Overall n=76 (%)*
Age at first BC diagnosis, years	Median (Range)	50.5 (26.0-76.0)
Age at first BM diagnosis, years	Median (Range)	55.0 (31.0-77.0)
ECOG	0-1	40 (74.1)
	2-4	14 (25.9)
Number of BM	1	38 (50.0)
	2-3	21 (27.6)
	≥4	17 (22.4)
ECM at first BM**	no	20 (26.3)
	yes	56 (73.7)
Number of BM	1	38 (50.0)
	2-3	21 (27.6)
	≥4	17 (22.4)

*valid percent

**Diagnosis of ECM not later than 60 days after diagnosis of BM

Figure 1a and 1b: Distribution of AIMS subtypes in primary BC and BM

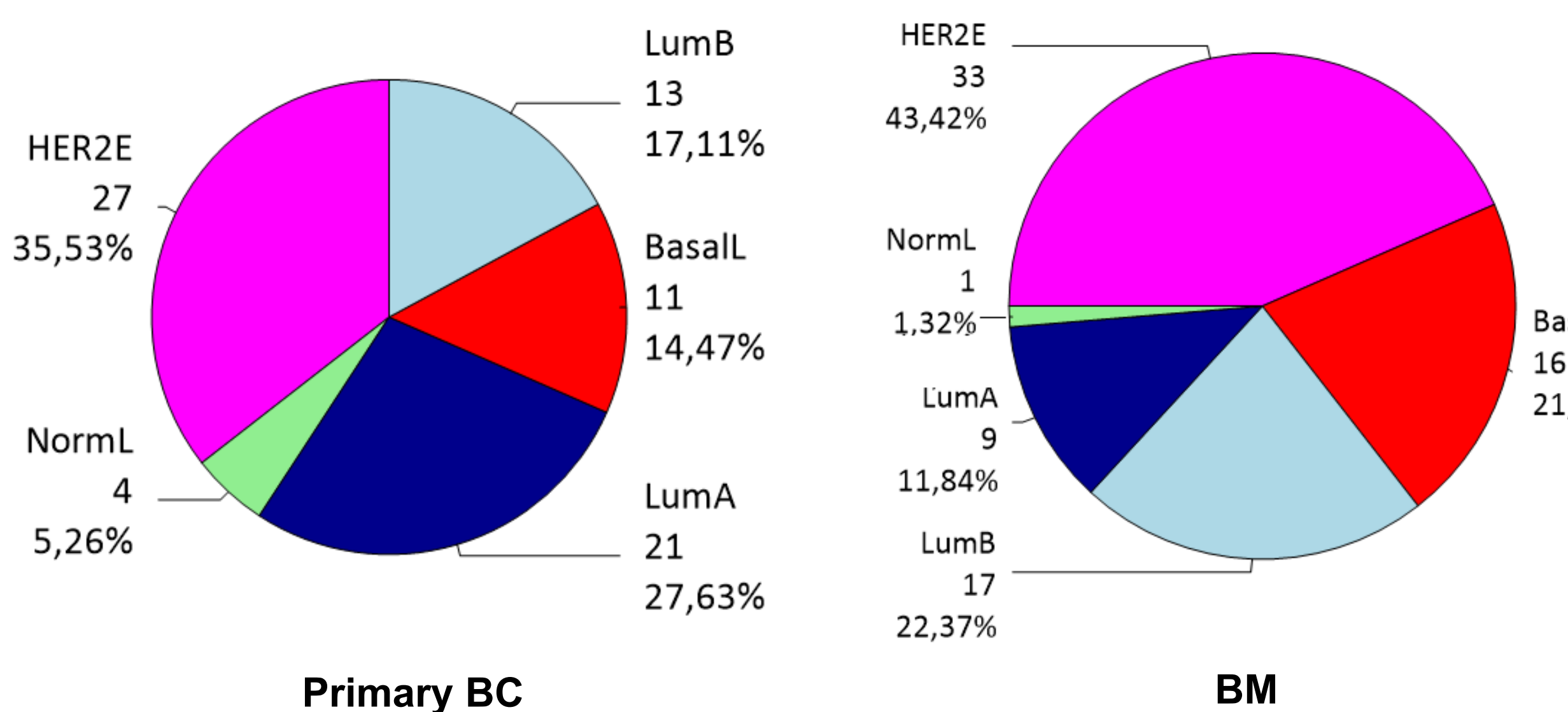
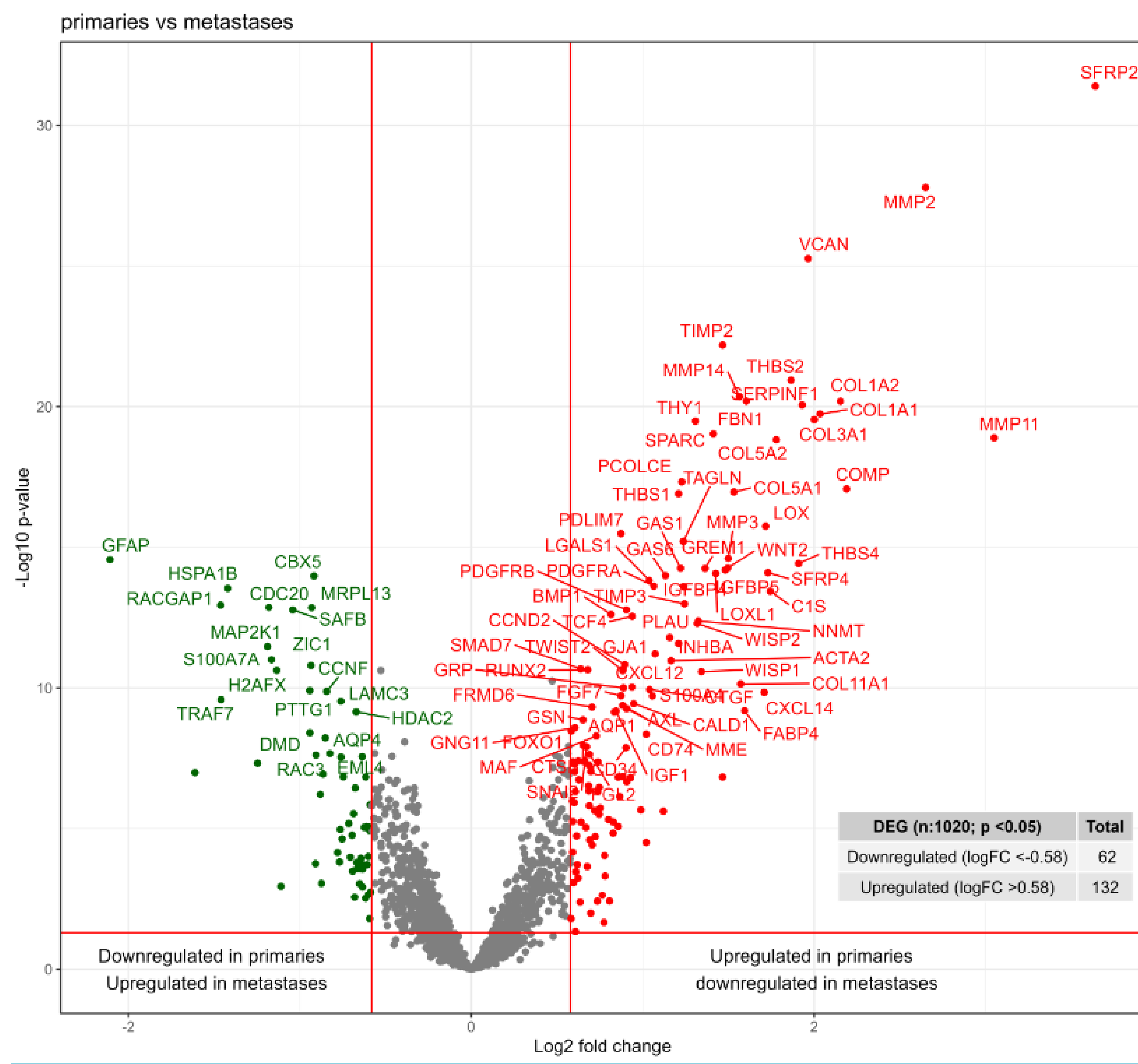


Table 2: HER2-status according to IHC in primary BC and BM

HER2 IHC	Overall in primary BC n=76 (%)	Overall in BM n=76 (%)
Ultralow	4 (7.1)	7 (12.5)
IHC 0	25 (44.6)	21 (37.5)
IHC 1+	9 (16.1)	8 (14.3)
IHC 2+	0 (0)	3 (5.4)
IHC 3+	18 (32.1)	17 (30.4)
missing	20	20

Figure 3: Volcano Plot gene expression analysis



- Following AIMS changes could be identified in BC and corresponding BM:

- Lower proportion of Luminal A-like subtype in BM (BC n=21, 27.6% vs. BM n=9, 11.8%)
- Higher proportion of HER2-positive subtype in BM (BC n=27, 35.5% vs. BM n=33, 43.4%)
- AIMS basal-like BC also gained numbers towards BM (BC n=11, 14.5% vs. BM n=16, 21.1%), detecting switches mainly from HER2-positive and normal-like BC (see figure 1a/b for AIMS distribution in BC and BM and figure 1c for AIMS subtype switches figure 1c).
- Following status of HER2 IHC could be detected
 - Comparable proportion of HER2 3+ IHC status in primary BC and BM: 32.1% (n=18) BC pts. had HER2 IHC 3+ and 30.4% (n=17) of BM patients
 - HER2 switches from primary BC to BM were more commonly in IHC 0 BC (n=25 (44.6%) IHC 0 BC, n=21 (37.5%) IHC 0 BM), resulting in IHC 1+ or ultralow BM (see figure 2 for IHC HER2-class switches).
 - Higher proportion of HER2 ultralow status in BM vs. primary BC (primary BC n=4, 7.1% vs. n=7 12.5% in BM).

Figure 1c: AIMS class switch from primary BC to BM

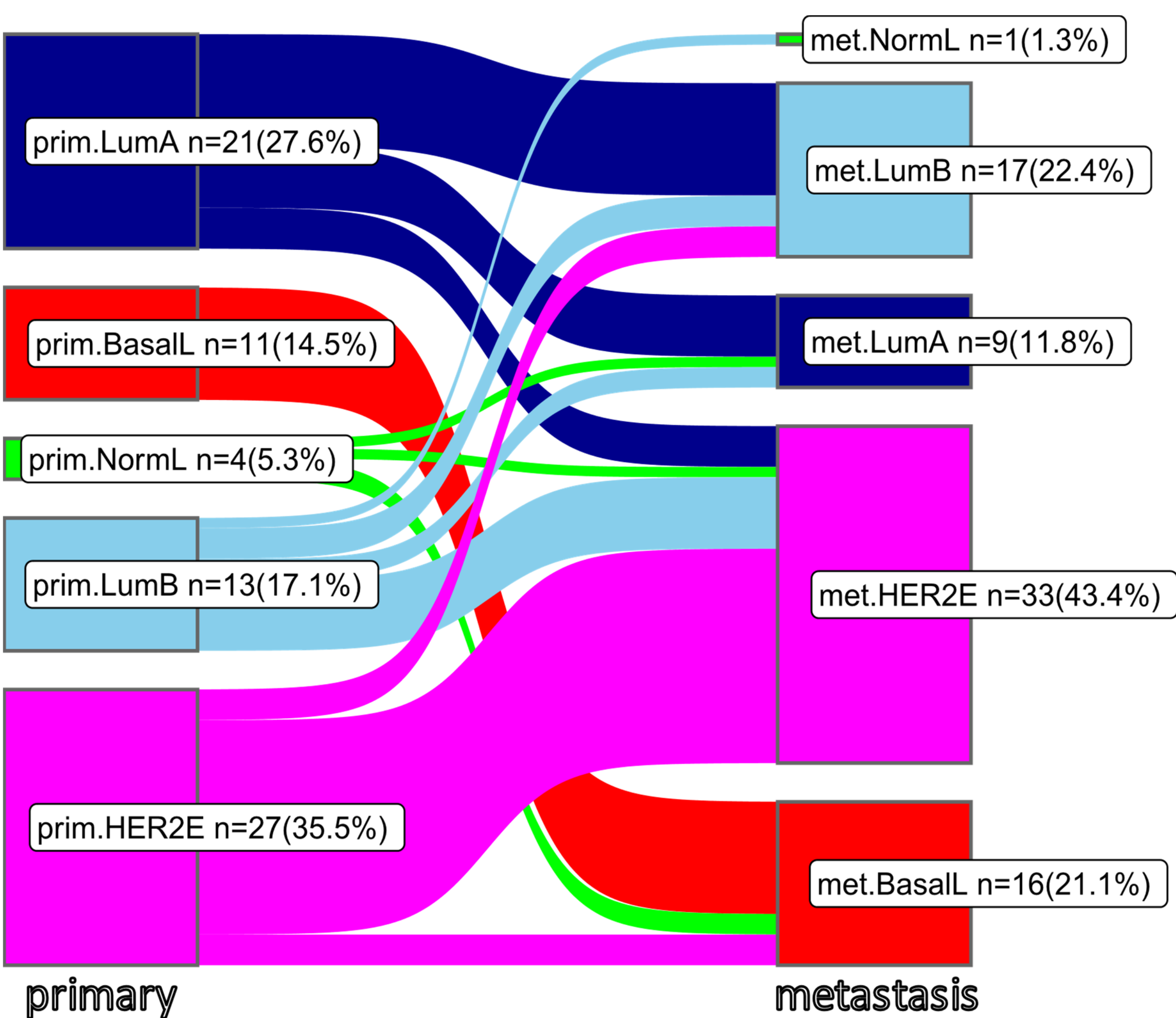
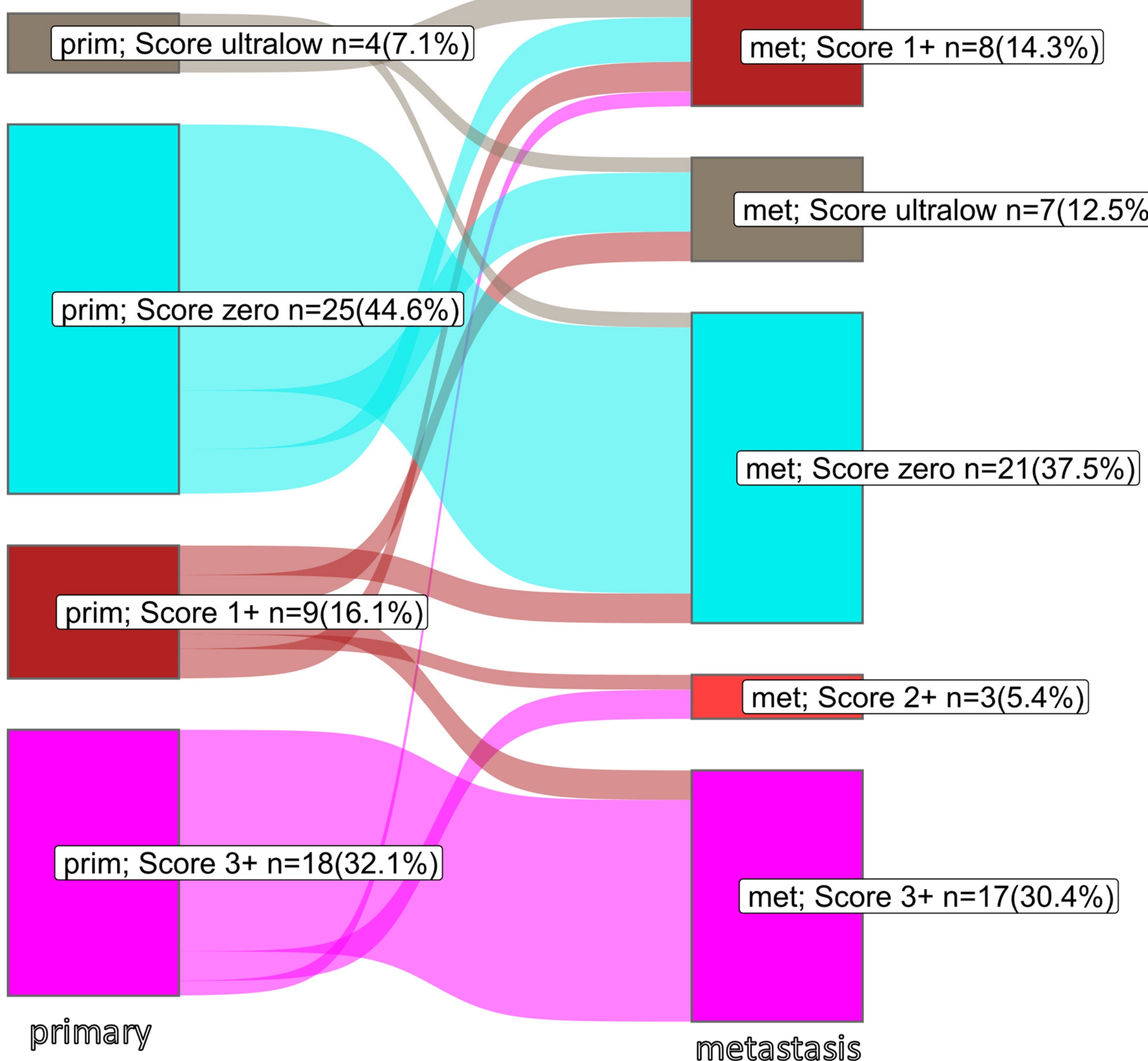


Figure 2: HER2-class switch according to IHC



Conclusions

Our analysis identified AIMS subtype and performed HER2 IHC analysis in primary BC and BM in matched pairs that could be relevant for prognosis assessment and treatment decisions. In addition, our preliminary differential gene expression analyses showed clear differences in various cellular processes between BC and BM tissues.