

## Transcriptomic profiling does not refine mastocytosis diagnosis

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# Transcriptomic profiling does not refine mastocytosis diagnosis

The recent 5<sup>th</sup> edition of the World Health Organization (WHO) update on hematopoietic cancers includes relevant changes to the diagnostic criteria of mastocytosis.<sup>1</sup> However, it remains entirely unclear whether transcriptional profiles of the bone marrow of patients with mastocytosis instruct diagnostic, prognostic or predictive information as it has been shown in many other cancer types.<sup>2</sup> Here we show that transcriptional profiling of a large and clinically well-annotated dataset of systemic mastocytosis (SM) patients fails to achieve a further diagnostic refinement of SM above the level of genomic and clinical parameters. Nevertheless, transcriptional differences between clinical and genomic SM subgroups robustly link the expression of certain genes to the mutation-adjusted risk score (MARS) as a surrogate of prognosis.

The advent of next-generation sequencing (NGS), with its increasing availability and reducing cost, has transformed cancer diagnosis and care. Especially in hematological malignancies, seminal breakthroughs showing the potential holistic nature of high throughput sequencing in the diagnosis of malignant disease<sup>1,3</sup> have been achieved. Next to whole genome sequencing (WGS), transcriptomic profiling yields additional layers of complexity linking genetic lesions to signaling consequences and even guiding therapy.<sup>2</sup> Furthermore, the combined usage of WGS and RNA sequencing may benefit the refinement of diagnosis as well as prognosis, bearing large untapped potential in the era of precision medicine.

SM is a heterogeneous group of hematopoietic neoplasms characterized by amplified proliferation of aberrant mast cells in adult patients. Contrary to the predominantly pediatric cutaneous mastocytosis (CM), clonal mast cells in SM expand in the bone marrow and other organs, such as the liver, spleen, gut, and lymph nodes.<sup>4</sup> The 2016 classification of the WHO<sup>5</sup> further subdivides SM into indolent systemic mastocytosis (ISM), smoldering systemic mastocytosis (SSM), SM with associated hematologic neoplasm (SM-AHN), aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL). SM-AHN, ASM, and MCL can be summarized as advanced SM (advSM). Pathognomonic for the disease are somatic gain-of-function mutations in *KIT*, which occur in >90% of all cases, with D816V accounting for >90% of all mutations.<sup>6,7</sup> ISM patients experience mostly mediator-related symptoms such as nausea, flush, and pruritus, whereas advSM patients suffer primarily from symptoms caused by expansion of mast cells leading to bone marrow suppression and altered organ function, namely cytopenia, malabsorption, hepato-splenomegaly or

osteopenia.<sup>4</sup> The severity of symptoms is additionally affected by often allergic comorbidities which can considerably impede oncological management. The clinical complexity translates to prognosis, reaching from near-normal life expectancy in indolent forms to a median survival of 2.9 years and 1.6 years in patients with SM-AHN and MCL, respectively.<sup>8,9</sup> Baseline therapies aim to reduce mediator-related symptoms or serve as prophylaxis and comprise histamine receptor 1 and 2 antagonists, mast cell stabilizers, steroids, bisphosphonates, and vitamin D supplementation. In cases of advSM, systemic cytotoxic treatment with midostaurin,<sup>10</sup> a tyrosine kinase inhibitor, or the recently approved avapritinib<sup>11</sup> is considered the gold standard. However, polychemotherapy treatment analogous to *de novo* AML or cladribine-containing regimen remains the ultima ratio in cases of therapy-refractory or quickly progressive advSM.<sup>12</sup> The majority of SM patients present with additional somatic mutations, predominantly in *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2*, *CBL*, *N/KRAS*, *EZH2*, *IDH1/2*, and *SF3B1* which have a crucial impact on prognosis.<sup>13</sup> A multivariate analysis of risk factors identified age >60 years, anemia (hemoglobin <10 g/dL), thrombocytopenia (platelets <100x10<sup>9</sup>/L), presence of one mutation in *SRSF2*, *ASXL1*, and/or *RUNX1* and presence of two or more mutations in respective genes as associated with overall survival time. The MARS integrates these parameters and was confirmed to be independent of WHO classifications.<sup>14</sup>

Despite the advances in genomic characterization, heterogeneity of the disease remains a challenge to both clinicians and scientists. Additional layers of information to feed the clinical workflow are crucial to improving diagnostics and patient stratification. Therefore, set out to investigate whether transcriptional portraits of different subtypes of SM, for which extensive clinical data were gathered for subsequent analysis, could aid this effort (Table 1; *Online Supplementary Table S1*).

Bone marrow aspirate from 20 male and 10 female patients with SM was taken during routine diagnostic bone marrow punctures. The same material also underwent panel sequencing as part of routine genetic diagnostics (Figure 1A). Healthy control samples were obtained from femoral heads resected during hip joint replacement surgery from two male and two female donors. The cohort comprises cases with ISM (n=5), ASM (n=5), SM-chronic myelomonocytic leukemia (CMML) (n=5), SM-myelodysplastic syndromes/myeloproliferative neoplasm-unclassifiable (SM-MDS/MPNu) (n=6), SM-MDS (n=4), SM-chronic eosinophilic leukemia (SM-CEL) (n=2), SM-MPN (n=1), SM-

**Table 1.** Cohort characteristics and metadata.

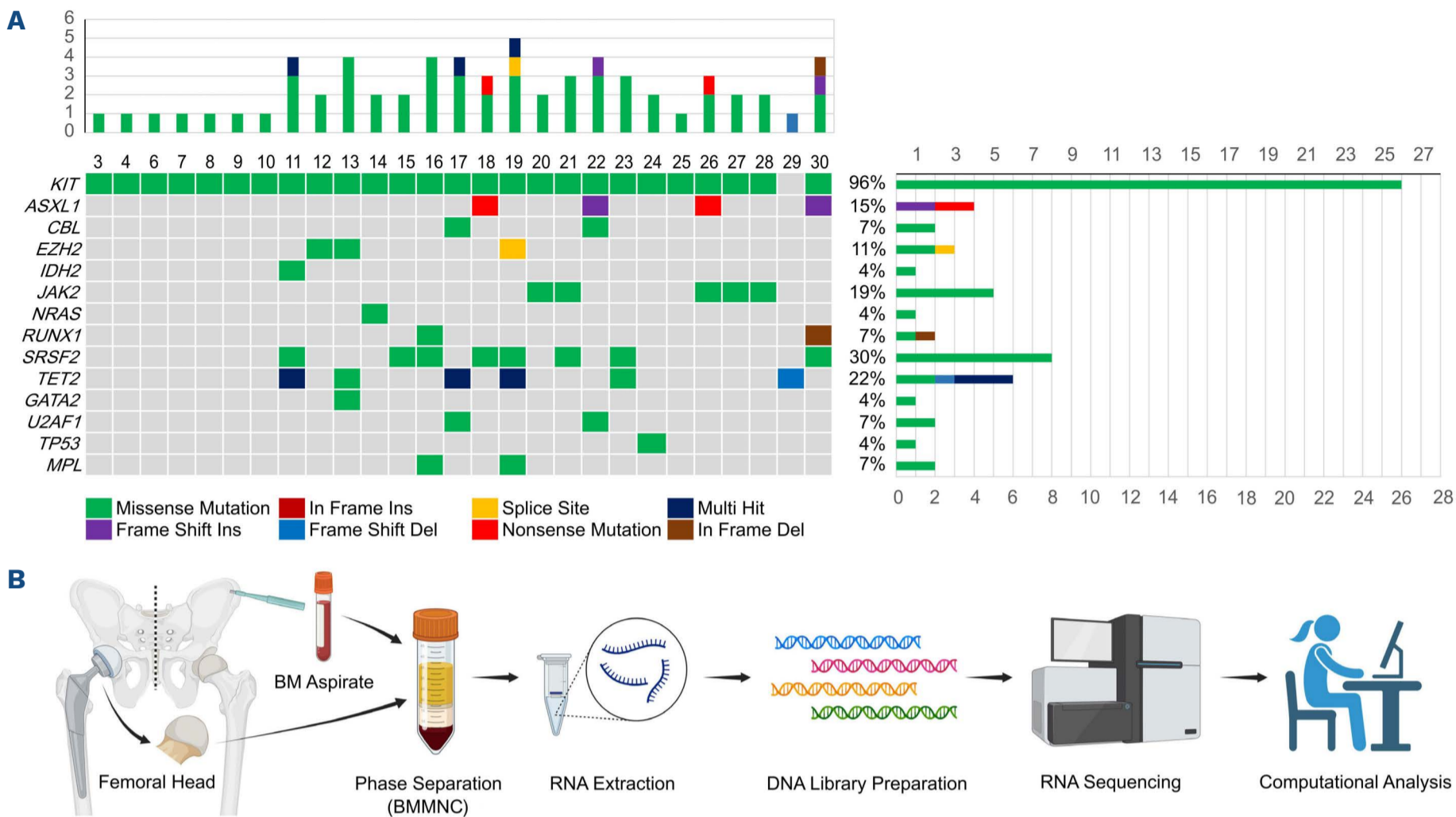
<b>Characteristics</b>		
Age in years, mean (SD)	60.87 (15.22)	
<b>Sex</b>	<b>N</b>	<b>%</b>
Female	10	33.33
Male	20	66.67
<b>WHO subtype</b>	<b>N</b>	<b>%</b>
ISM	5	16.67
ASM	5	16.67
SM-AHN	20	66.67
SM-CMML	5	16.67
SM-MDS/MPNu	6	20
SM-MDS	4	13.33
SM-CEL	2	6.67
SM-MPN	1	3.33
SM-MGUS	1	3.33
SM-AML	1	3.33
<b>Clinical parameters</b>		
Mast cell infiltration in bone marrow (%)	<b>range</b>	<b>mean (SD)</b>
	5-85	27.14 (16.06)
Splenomegaly	<b>N</b>	<b>%</b>
	20	69.00
<b>Laboratory values</b>	<b>range</b>	<b>mean (SD)</b>
Leukocytes/ $\mu$ L	2,580-35,600	10,506.21 (8,294.84)
Hemoglobin, g/dL	6.5-15.8	11.65 (2.43)
Thrombocytes $\times 10^3/\mu$ L	12.0-515.0	196.80 (148.37)
Monocytes/ $\mu$ l	126.0-3,850.0	825.63 (945.56)
Eosinophils/ $\mu$ l	0.0-8,188.0	820.07 (1,624.05)
Tryptase, $\mu$ g/L	23.2-850.0	187.89 (188.75)
Alkaline phosphatase, IU/L	3.0-629.0	217.10 (186.05)
<b>Risk</b>	<b>range</b>	<b>mean (SD)</b>
Mutation-adjusted risk score	0-5	1.76 (1.48)

SD: standard deviation; WHO: world health organization; SM: systemic mastocytosis; ISM: indolent SM; ASM: aggressive SM; SM-AHN: SM with associated hematologic neoplasm; SM-MDS: SM-myelodysplastic syndromes; MPNu: myeloproliferative neoplasm unclassifiable; CEL: chronic eosinophilic leukemia; MPN: myeloproliferative neoplasm; MGUS: monoclonal gammopathy of undetermined significance; AML: acute myeloid leukemia.

monoclonal gammopathy of undetermined significance (SM-MGUS) (n=1) and SM-acute myeloid leukemia (SM-AML) (n=1). RNA was extracted and sequencing was performed using the prime sequencing protocol.<sup>15</sup> Finally, extensive computational analyses were performed (Figure 1B).

Principal component analysis (PCA) did not reveal clustering patterns (Figure 2A). UpSet plots were generated based on differential expression (DE) analysis (*Online Supplementary Table S2*) and gene set enrichment analysis (GSEA) was performed to visualize transcriptional inter- and intra-subtype similarities. Genes differentially expressed in SM of different subtypes are reported compared to healthy controls (Figure 2B). Notably, the most homogenous subgroups ASM and ISM show the highest numbers of (uniquely) differentially ex-

pressed genes. While various deregulated genes are shared between different subgroups, a subset of 26 genes is deregulated in all SM(-AHN) subgroups (Figure 2B; *Online Supplementary Table S2*). The overlap of hallmark gene sets enriched for highly expressed genes (false discovery rate [FDR]  $\leq 0.05$ ) in SM subtypes compared to healthy controls was calculated (Figure 2C). Of note, 18 gene sets are enriched in ASM of which five are unique (*Online Supplementary Table S2*). None of the other subgroups show unique enrichment for any hallmark gene set, except for SM-MDS (*Online Supplementary Table S2*). Six gene sets were enriched across all SM subtypes; if SM-MDS is excluded, three gene sets are overlapping between ASM, ISM, SM-CMML and SM-MDS/MPNu (*Online Supplementary Table S2*). In



**Figure 1. Mutational profile of the analyzed cohort and sample processing workflow.** (A) OncoPrint of patient for which panel sequencing was performed during routine diagnostics. No panel sequencing was performed for patient P01, P02 and P05. (B) Graphical illustration of the sample processing workflow. Ins: insertion; Del: deletion; BM: bone marrow; BMMNC: bone marrow mononuclear cells.

comparison, GSE of lowly expressed genes (FDR  $\leq 0.05$ ) in SM subtypes versus healthy controls was calculated (Figure 2D). The highest number of enriched gene sets (14) is reported for SM-CMML, of which two are unique (Online Supplementary Table S2). Other uniquely enriched gene sets appeared in ISM and ASM. Three gene sets were enriched across all subtypes (Online Supplementary Table S2). In order to link gene expression and prognosis, differential expression of patient samples with a MARS of 5 compared to healthy controls was computed. Additionally, a correlation analysis was performed to extract genes correlating with MARS. In total, 226 genes are differentially expressed in samples with a MARS of 5, while ten genes show a high correlation ( $r > 0.65$  for both Pearson and Spearman) with MARS (Figure 2E). Overall, two genes are both significantly differentially expressed and correlate with MARS simultaneously (Figure 2E, panels 2-4, *FLT3*  $r_{\text{Pearson}} = 0.75$ ,  $r_{\text{Spearman}} = 0.70$ , *IGF2BP2*  $r_{\text{Pearson}} = 0.68$ ,  $r_{\text{Spearman}} = 0.72$ ). Extensive differential expression analysis of WHO subgroups (advSM vs. ISM vs. healthy/SMall vs. healthy), pre- and post-treatment with midostaurin, all mutations detected by the NGS panel, presence/absence of splenomegaly, hemoglobin/thrombocytes below/above cut-offs (hemoglobin  $< 10$  g/dL, platelets  $< 100 \times 10^9$ /L), MARS risk level as well as correlation analyses for mast cell infiltration, levels of leukocytes, hemoglobin,

thrombocytes, monocytes, eosinophils, tryptase, alkaline phosphatase, and albumin were performed (Online Supplementary Table S3). Notably, the cytological parameters did not alter the above-mentioned results, despite the bulk material approach of this work. Additionally, we provide all read counts for the samples analyzed (Online Supplementary Table S3).

Despite advances in the development of novel drugs for SM, targeted therapeutic approaches almost exclusively exploit the canonical *KIT* D816V mutation present in  $> 90\%$  of SM cases. Based on clinical phenotype and histopathological assessment, the new 2022 WHO classification of SM aims to subdivide the disease in a more granular manner but does not include more sophisticated biomarkers. Especially within the particularly heterogeneous subgroup of advanced SM, extensive molecular profiling is of eminent importance to decipher the complexity of the disease to allow an optimized patient and subgroup stratification as this might ultimately lead to patient benefit.

Although our transcriptomic analysis was able to point out individual cases (e.g., AML with stem-like signature, CEL with eosinophilic signature), the approach failed to reveal a distinct pattern in a collection of different SM subtypes. This highlights the potential of RNA sequencing to profile cases *per se*, but also stresses that broad profiling might not yield



aberrant signaling due to oncogenic hits would be less potent in these cells primed for tissue residency and terminal differentiation, which are processes most likely driven by strong inherent transcriptional signatures. Potential limitations of our study include the fact that in total a cohort of only 30 patients was investigated. Future studies with larger cohorts might yield more subtle transcriptional profiles which might refine diagnostic stratification, although the rarity of the disease poses a challenging hurdle. Altogether our data do not endorse transcriptomic approaches to refine molecular stratification of mast cell malignancies. Nevertheless, transcriptomic profiling revealed distinct signatures in individual patients and was able to link gene expression to surrogate risk markers.

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### Disclosures

No conflicts of interest to disclose.

### Contributions

LB and DO performed the clinical and computational analysis, wrote the manuscript and designed the figures. JG performed RNA sequencing. TOO, CW, SJ, UH and AW provided conceptual input. MS supervised the computational analysis. AR and MJ provided the analyzed material. PJJ conceived the analysis and supervised the project. All authors provided critical feedback and helped shape the research, analysis and manuscript.

### Data-sharing statement

The original data as well as protocols will be made available to other investigators without any restrictions. The data can be obtained upon request via email to [lars.buschhorn@med.uni-heidelberg.de](mailto:lars.buschhorn@med.uni-heidelberg.de).

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## LETTER TO THE EDITOR

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