# BONE MARROW MICROENVIRONMENT OVERLAP BETWEEN VEXAS AND MYELODYSPLASTIC SYNDROME DEMONSTRATED BY TARGETED TRANSCRIPTOMIC AND ARTIFICIAL INTELLIGENCE

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## INTRODUCTION

VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is an inflammatory process that involves bone marrow (BM). Active inflammation is also a major feature of the microenvironment in the BM of patients with myelodysplastic syndrome (MDS). Patients with VEXAS frequently have signs and symptoms similar to MDS. Some of VEXAS patients develop overt MDS and some may develop acute myeloid leukemia. Exploring the biological basis for this overlap may help in developing therapeutic approaches for treating this disease.

## AIM

We explored using artificial intelligence (AI) approach and compared the transcriptomic profile of the BM from VEXAS patients (N=59) with the transcriptional profile of BM from MDS patients (N=1021) and from the BM of individuals without specific diagnostic abnormality or were presenting with CHIP (clonal hematopoiesis of indeterminate potential) only (N=1030) (Normal).

## METHOD

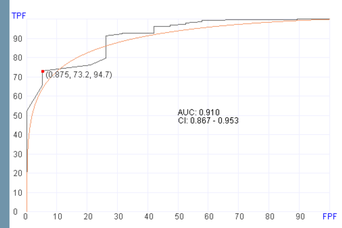
RNA was extracted from the bone marrow samples from patients with VEXAS (N=59), MDS (N=1030), and Normal (1030). Most of samples considered normal had clinical reason for testing bone marrow including staging or history of hematologic neoplasm or cytopenia but molecularly were classified as CHIP. The RNA was sequenced by next generation sequencing (NGS) using a targeted RNA panel of 1600 genes. Hybrid capture sequencing library preparation was used, and RNA was quantified using transcript per million (TPM). To compare between the three groups, we used the RNA expression levels in an AI model based on Bayesian statistics and random forest. Bayesian statistics were used to rank the genes that distinguish between groups, then random forest was used to establish the signatures that distinguish between the groups. Two thirds of the samples were used for training and one third was used for testing the models.

## RESULTS

***Developing a Transcription signature for VEXASb Vs Normal:***

*8 genes:* SF3A1, SMAD5, PRPF8, MCM3AP, NFYC, FGFR1OP, PPM1D, THRA.

*Testing set*

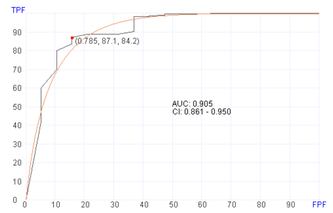



- Splicing : SF3A1, PRPF8, MCM3AP
- Transcription / signaling : NFYC, THRA, SMAD5, PPM1D
- Centrosomal gene involved in various cellular functions including cell cycle regulation: FGFR1OP
- All these genes are overexpressed in VEXAS and in MDS

***Developing a Transcription signature for VEXASb Vs MDS:***

*40 genes*

*Testing set*




- Inflammatory and immune response : IL1R1, ITGAM, MMP9, MAP2K6, LCP1, WDR1
- Cell differentiation: BCOR, MN1, DNTT, TG
- Cell growth and proliferation: CDKN1C, WRN, PKM, RANBP17, ANPEP, NEURL1
- Transcription factors and epigenetic regulators: MN1, BCOR, NUTM2A, BCL7A

453 genes showed significant (LogFDR <-3) difference in the level of expression between VEXAS and normal.

*Example: Top genes*

| Gene | Median VEXAS | Median Normal | LogFDR |
|---|---|---|---|
| AFF3 | 2.3225 | 3.3393 | -Infinity |
| BCOR | 4.7956 | 5.3032 | -Infinity |
| CD79B | 2.9984 | 4.226 | -Infinity |
| DNTT | 0.5058 | 2.1748 | -Infinity |
| DTX1 | 1.4097 | 2.6487 | -Infinity |
| IRF4 | 2.7095 | 3.6212 | -Infinity |
| NEURL1 | 1.0149 | 1.6881 | -Infinity |
| PAX5 | 0.9699 | 1.9901 | -Infinity |
| PPM1D | 4.1785 | 4.5994 | -Infinity |
| TCL1A | 0.4985 | 1.8131 | -Infinity |
| MRE11 | 4.6428 | 5.1056 | -12.7785 |
| NAMPT | 6.743 | 1.4074 | -12.7785 |
| LUC7L2 | 4.8163 | 0.7856 | -12.4775 |
| MCM3AP | 4.1358 | 0.6439 | -12.4775 |
| NFYC | 3.8048 | 0.5655 | -12.4775 |
| PRPF8 | 4.9629 | 0.7807 | -12.4775 |
| SF3A1 | 4.9241 | 0.7736 | -12.4775 |
| AKAP9 | 5.4141 | 5.9079 | -12.3014 |
| THRA | 3.754 | 0.5899 | -12.3014 |
| SMAD5 | 2.7991 | 0.4458 | -12.1764 |
| EVI2A | 4.1187 | 0.6973 | -12.0003 |
| RGS7 | 0.2838 | 0.6581 | -11.8243 |

All genes that were significantly abnormal in VEXAS as compared with normal were also significantly abnormal in MDS VS Normal

| Gene | Median MDS | Median Normal | LogFDR |
|---|---|---|---|
| AFF3 | 2.8539 | 3.3393 | -Infinity |
| BCOR | 5.1925 | 5.3032 | -2.29533 |
| CD79B | 3.639 | 4.226 | -Infinity |
| DNTT | 1.9184 | 2.1748 | -0.15185 |
| DTX1 | 2.0435 | 2.6487 | -Infinity |
| IRF4 | 3.2481 | 3.6212 | -Infinity |
| NEURL1 | 1.4997 | 1.6881 | -4.10901 |
| PAX5 | 1.315 | 1.9901 | -Infinity |
| PPM1D | 4.4383 | 4.5994 | -10.5771 |
| TCL1A | 1.0745 | 1.8131 | -Infinity |
| MRE11 | 4.9703 | 5.1056 | -6.94341 |
| NAMPT | 3.8449 | 1.4074 | -Infinity |
| LUC7L2 | 2.9065 | 0.7856 | -Infinity |
| MCM3AP | 2.4994 | 0.6439 | -Infinity |
| NFYC | 2.0881 | 0.5655 | -Infinity |
| PRPF8 | 2.9506 | 0.7807 | -Infinity |
| SF3A1 | 2.8446 | 0.7736 | -Infinity |
| AKAP9 | 5.7507 | 5.9079 | -9.75814 |
| THRA | 2.2289 | 0.5899 | -Infinity |
| SMAD5 | 1.7201 | 0.4458 | -Infinity |
| EVI2A | 2.486 | 0.6973 | -Infinity |
| RGS7 | 0.5376 | 0.6581 | -2.76276 |

152 genes were significantly different (LogFDR <-3) between MDS and VEXAS.

*Example: Top genes*

| Gene | Median VEXAS | Median MDS | LogFDR |
|---|---|---|---|
| DNTT | 0.5058 | 1.9184 | -Infinity |
| RANBP17 | 1.469 | 2.2263 | -Infinity |
| MN1 | 1.2671 | 2.2151 | -12.1764 |
| BCOR | 4.7956 | 5.1925 | -9.83057 |
| CDKN1C | 2.3364 | 3.1365 | -8.75607 |
| IL1R1 | 4.4487 | 3.5507 | -8.72994 |
| MAP2K6 | 4.6377 | 3.9082 | -8.61839 |
| NEURL1 | 1.0149 | 1.4997 | -8.4564 |
| NUTM2A | 2.4177 | 2.9505 | -8.26836 |
| TG | 2.4293 | 1.6449 | -8.00891 |
| ITGAM | 7.2563 | 6.4354 | -7.7228 |
| TENM1 | 3.6896 | 2.8373 | -7.70597 |
| WDR1 | 7.1181 | 6.7755 | -7.68773 |
| LCP1 | 8.1054 | 7.4678 | -7.61619 |
| BCL7A | 2.0765 | 2.5381 | -7.49879 |
| CAPZB | 6.8975 | 6.5675 | -7.4709 |
| WRN | 4.4629 | 4.8281 | -7.39126 |
| MMP9 | 8.0862 | 6.874 | -7.28458 |
| ANPEP | 7.0019 | 6.3312 | -7.20205 |
| MRE11 | 4.6428 | 4.9703 | -6.88366 |
| ITGB4 | 3.1064 | 2.1481 | -6.77037 |
| LAMP2 | 6.3407 | 5.8468 | -6.64824 |
| PKM | 7.8837 | 7.4864 | -6.60453 |
| GSN | 6.9577 | 6.4272 | -6.48027 |
| NUMA1 | 5.8068 | 6.0605 | -6.45865 |
| TCEA1 | 5.6267 | 5.9469 | -6.34848 |
| PRDM16 | 0.4391 | 0.9418 | -6.27372 |
| CD79B | 2.9984 | 3.639 | -6.25244 |
| MME | 5.144 | 4.1383 | -6.24183 |
| ZFTA(C11orf95) | 1.4075 | 1.876 | -6.22212 |
| AKAP9 | 5.4141 | 5.7507 | -6.19586 |
| DCLK2 | 0.6196 | 1.169 | -6.03253 |
| IRF4 | 2.7095 | 3.2481 | -5.93576 |
| ARG1 | 7.2101 | 6.2849 | -5.88229 |
| TCL1A | 0.4985 | 1.0745 | -5.74914 |

## CONCLUSIONS

- Bone marrow microenvironment in patients with VEXAS is significantly abnormal despite the clinical manifestation of VEXAS are more related lungs, skin, blood vessels and joints
- The bone marrow microenvironment in VEXAS mimics that seen in myelodysplastic syndrome
- VEXAS and MDS share similar abnormalities inflammatory and immune response, cell proliferation and differentiation and various transcription factors.
- Changes and interaction between 8 genes involved in splicing and transcription regulation provide highly reliable biomarkers for the diagnosis of VEXAS when used in machine learning algorithm.
- Therapeutic approaches targeting these abnormalities specific abnormalities may help in ameliorating the clinical manifestation of VEXAS

## CONTACT INFORMATION

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