# Tumor infiltrating neutrophils are enriched in basal-type urothelial bladder cancer

**Giulio Eugenio Mandelli [1,†], Francesco Missale [1,2,3,†], Debora Bresciani [1], Luisa Benerini Gatta [1,4], Patrizia Scapini [5], Elena Caveggion [5], Elisa Roca [4,6], Mattia Bugatti [1,6], Matilde Monti [1], Luca Cristinelli [4,6], Sandra Belotti [4,6], Claudio Simeone [4,6], Stefano Calza [1], Laura Melocchi [7] and William Vermi [1,6,8*]**

[1] Department of Molecular and Translational Medicine, School of Medicine, University of Brescia, 25100 Brescia, Italy
[2] IRCCS Ospedale Policlinico San Martino, 16121 Genova, Italy
[3] Department of Otorhinolaryngology, Head and Neck Surgery—University of Genoa, 16121 Genova, Italy
[4] Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, 25100 Brescia, Italy
[5] Section of General Pathology, Department of Medicine, University of Verona, Verona, Italy
[6] ASST Spedali Civili di Brescia, 25100 Brescia, Italy
[7] Department of Pathology, Fondazione Poliambulanza, 25100 Brescia, Italy
[8] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63130, USA
* Correspondence: william.vermi@unibs.it; Tel.:+39-030-399-8425
† These authors equally contributed to the work.

## Supplementary Tables

**Supplementary Table S1** reports the details of clinical and pathology data of the retrospective MIBC cohort.

**Supplementary Table S2** reports the details of reagents for the Gene Expression Assay on UBC lines.

**Supplementary Table S3:** reports the neutrophil and chemokine signatures used for Analysis of the TCGA, GSE32894 and GSE124305 Datasets.

**Supplementary Table S4** reports summary statistics of CD3 and CD66b as continuous variable, p values by Shapiro-Wilk normality test.

**Supplementary Table S1.** Details of clinical data of the retrospective MIBC cohort.

| ID | Density CD66B (cell/mm^2) | Density CD3 (cell/mm^2) | Immunoscore | Age | Gender | pT | pN | CIS | STAGE | PFS cens (1=yes 0=no) | PFS time (months) | Status: (1=Died 0=Alive) | OS time | pSTAT3 | pSTAT3 HiLo | Subtype | FOSL1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 148,9 | 1376,0 | 3_HiHi | 51 | M | T2a | N0 | 0 | 2 | 0 | 108 | 0 | 108 | 2 | Hi | Luminal | |
| 2 | 732,3 | 248,9 | 1_3LoHi | 68 | M | T2b | N0 | 0 | 2 | 0 | 92 | 1 | 92 | 3 | Hi | Basal | 3 |
| 3 | 282,5 | 788,4 | 3_HiHi | 82 | M | T4 | N0 | 0 | 3 | 0 | 89 | 1 | 89 | 3 | Hi | Basal | 3 |
| 4 | 975,6 | 2020,8 | 3_HiHi | 73 | M | T3b | N0 | 0 | 3 | 1 | 84 | 0 | 108 | 3 | Hi | Basal | 2 |
| 5 | 86,2 | 788,2 | 3_HiHi | 68 | M | T3b | N1 | 1 | 4 | 0 | 108 | 0 | 108 | 1 | Lo | Luminal | |
| 6 | 60,2 | 288,8 | 2_3HiLo | 57 | M | T2b | N0 | 1 | 2 | 0 | 96 | 0 | 96 | 2 | Hi | Non-type | |
| 7 | 225,4 | 1385,3 | 3_HiHi | 52 | M | T2b | N0 | 0 | 2 | 0 | 101 | 0 | 101 | 1 | Lo | Basal | 3 |
| 8 | 32,7 | 1557,8 | 2_3HiLo | 78 | M | T3a | N0 | 1 | 3 | 0 | 96 | 0 | 96 | 3 | Hi | Non-type | |
| 9 | 143,5 | 400,2 | 3_HiHi | 73 | M | T3a | N0 | 1 | 3 | 0 | 96 | 0 | 96 | 3 | Hi | basal | 2 |
| 10 | 105,9 | 60,9 | 1_3LoHi | 54 | F | T2b | N0 | 1 | 2 | 0 | 84 | 0 | 84 | 2 | Hi | luminal | 1 |
| 11 | 1048,9 | 467,9 | 3_HiHi | 78 | M | T3a | N0 | 1 | 3 | 0 | 84 | 0 | 84 | 2 | Hi | Non-type | |
| 12 | 156,3 | 536,7 | 3_HiHi | 70 | F | T2b | N0 | 1 | 2 | 0 | 84 | 0 | 84 | 2 | Hi | Luminal | 1 |
| 13 | 139,9 | 327,1 | 3_HiHi | 61 | M | T2b | N0 | 1 | 2 | 0 | 72 | 0 | 72 | 3 | Hi | Basal | 3 |
| 14 | 155,2 | 638,2 | 3_HiHi | 69 | M | T2 | N0 | 1 | 2 | 0 | 72 | 0 | 72 | 2 | Hi | Basal | 3 |
| 15 | 31,7 | 400,6 | 2_3HiLo | 71 | M | T2b | N0 | 0 | 2 | 0 | 54 | 0 | 54 | 1 | Lo | Luminal | 2 |
| 16 | 86,0 | 1745,1 | 3_HiHi | 76 | M | T3 | N0 | 0 | 3 | 0 | 60 | 0 | 60 | 1 | Lo | Non-type | |
| 17 | 17,3 | 453,1 | 2_3HiLo | 69 | M | T3a | N0 | 0 | 3 | 0 | 36 | 0 | 36 | 0 | Lo | Non-type | |
| 18 | 117,2 | 348,8 | 3_HiHi | 50 | M | T4a | N0 | 1 | 3 | 0 | 36 | 0 | 36 | 1 | Lo | Non-type | 1 |
| 19 | 356,0 | 263,5 | 1_3LoHi | 65 | M | T4 | N0 | 0 | 3 | 1 | 3 | 1 | 14 | 3 | Hi | Basal | 3 |
| 20 | 12,1 | 27,3 | 0_LoLo | 73 | M | T3b | N2 | 1 | 4 | 1 | 1 | 1 | 50 | 1 | Lo | Non-type | |
| 21 | 172,6 | 892,8 | 3_HiHi | 90 | F | T2b | N0 | 0 | 2 | 0 | 65 | 1 | 65 | 3 | Hi | Basal | 3 |
| 22 | 11,2 | 214,4 | 0_LoLo | 57 | M | T3a | N0 | 0 | 3 | 1 | 20 | 1 | 25 | 2 | Hi | Non-type | |
| 23 | 9,2 | 671,7 | 2_3HiLo | 75 | M | T3a | N1 | 1 | 4 | 1 | 18 | 0 | 132 | 1 | Lo | Luminal | 0 |
| 24 | 47,9 | 461,5 | 2_3HiLo | 80 | F | T2b | N1 | 1 | 4 | 1 | 2 | 1 | 4 | 2 | Hi | Luminal | 0 |
| 25 | 10,1 | 283,4 | 0_LoLo | 60 | M | T3a | N0 | 1 | 3 | 1 | 9 | 1 | 10 | 2 | Hi | Non-type | |
| 26 | 25,6 | 564,3 | 2_3HiLo | 65 | M | T2b | N0 | 1 | 2 | 0 | 85 | 1 | 85 | 1 | Lo | Luminal | |
| 27 | 127,4 | 436,0 | 3_HiHi | 76 | M | T3a | N1 | 1 | 4 | 1 | 51 | 1 | 92 | 1 | Lo | Luminal | 1 |
| 28 | 223,7 | 357,5 | 3_HiHi | 65 | M | T2a | N0 | 1 | 2 | 1 | 38 | 0 | 91 | 1 | Lo | Luminal | |
| 29 | 18,3 | 98,2 | 0_LoLo | 58 | M | T3a | N0 | 0 | 3 | 1 | 5 | 1 | 7 | 3 | Hi | Basal | 3 |
| 30 | 60,0 | 464,3 | 2_3HiLo | 86 | F | T3b | N0 | 0 | 3 | 1 | 16 | 1 | 27 | 3 | Hi | Basal | 0 |
| 31 | 565,8 | 418,2 | 3_HiHi | 62 | M | T4 | N0 | 1 | 3 | 1 | 9 | 0 | 72 | 1 | Lo | Luminal | 0 |
| 32 | 48,7 | 484,2 | 2_3HiLo | 70 | M | T2b | N0 | 1 | 2 | 1 | 4 | 0 | 68 | 1 | Lo | Luminal | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 24,8 | 397,2 | 2_3HiLo | 68 | M | T3a | N2 | 0 | 4 | 1 | 1 | 1 | 11 | 2 | Hi | Non-type | |
| 34 | 15,2 | 196,8 | 0_LoLo | 59 | M | T2a | N0 | 0 | 2 | 1 | 4 | 0 | 47 | 1 | Lo | Luminal | 1 |
| 35 | 307,5 | 462,8 | 3_HiHi | 66 | M | T3a | N0 | 0 | 3 | 1 | 4 | 1 | 11 | 3 | Hi | Basal | 3 |
| 36 | 71,7 | 975,7 | 2_3HiLo | 61 | M | T2b | N0 | 1 | 2 | 1 | 24 | 1 | 46 | 3 | Hi | Non-type | |
| 37 | 154,9 | 507,7 | 3_HiHi | 75 | M | T3b | N2 | 1 | 4 | 1 | 19 | 1 | 33 | 1 | Lo | Luminal | |
| 38 | 33,3 | 500,6 | 2_3HiLo | 81 | F | T3a | N3 | 1 | 4 | 1 | 10 | 1 | 35 | 1 | Lo | Luminal | 0 |
| 39 | 32,8 | 63,8 | 0_LoLo | 74 | M | T3a | N0 | 0 | 3 | 1 | 3 | 1 | 10 | 3 | Hi | Non-type | |
| 40 | 40,5 | 230,3 | 0_LoLo | 64 | M | T4b | N0 | 1 | 4 | 0 | 3 | 1 | 3 | 1 | Lo | Luminal | |
| 41 | 13,6 | 18,1 | 0_LoLo | 73 | M | T4b | N0 | 1 | 4 | 0 | 7 | 1 | 7 | 0 | Lo | Non-type | |
| 42 | 45,0 | 255,8 | 0_LoLo | 82 | F | T4 | N1 | 1 | 4 | 1 | 3 | 1 | 11 | 1 | Lo | Luminal | |
| 43 | 28,3 | 274,3 | 0_LoLo | 74 | M | T3a | N0 | 1 | 3 | 1 | 6 | 0 | 36 | 0 | Lo | Luminal | 2 |
| 44 | 538,5 | 211,1 | 1_3LoHi | 70 | F | T3b | N2 | 0 | 4 | 0 | 2 | 1 | 2 | 2 | Hi | Non-type | |
| 45 | 51,4 | 21,1 | 0_LoLo | 61 | F | T3b | N1 | 1 | 4 | 1 | 4 | 1 | 9 | 1 | Lo | Luminal | 0 |
| 46 | 537,3 | 544,1 | 3_HiHi | 67 | M | T2 | N0 | 1 | 2 | 0 | 20 | 0 | 20 | 2 | Hi | Non-type | |
| 47 | 57,9 | 801,2 | 2_3HiLo | 77 | M | T2a | N0 | 0 | 2 | 1 | 2 | 0 | 6 | 1 | Lo | Luminal | 1 |
| 48 | 223,5 | 403,3 | 3_HiHi | 84 | M | T3a | N0 | 1 | 3 | 0 | 24 | 0 | 24 | 1 | Lo | Non-type | |
| 49 | 537,0 | 395,1 | 3_HiHi | 65 | M | T2b | N1 | 0 | 4 | 0 | 24 | 0 | 24 | 2 | Hi | Basal | 3 |
| 50 | 126,0 | 415,2 | 3_HiHi | 81 | M | T4 | N0 | 1 | 3 | 1 | 6 | 1 | 11 | 3 | Hi | Non-type | |
| 51 | 72,8 | 184,0 | 0_LoLo | 85 | M | T3a | N2 | 1 | 4 | 1 | 3 | 1 | 6 | 2 | Hi | Basal | 3 |
| 52 | 128,0 | 133,3 | 1_3LoHi | 76 | M | T3a | N1 | 0 | 4 | 1 | 6 | 1 | 19 | 1 | Lo | Non-type | |
| 53 | 225,0 | 294,3 | 3_HiHi | 82 | M | T3a | N0 | 1 | 3 | 1 | 4 | 0 | 9 | 1 | Lo | Luminal | |
| 54 | 28,1 | 756,3 | 2_3HiLo | 81 | F | T3a | N1 | 0 | 4 | 1 | 5 | 1 | 14 | 1 | Lo | Non-type | |
| 55 | 25,8 | 110,3 | 0_LoLo | 85 | M | T3a | N0 | 1 | 3 | 1 | 3 | 1 | 8 | 1 | Lo | Luminal | |
| 56 | 82,8 | 609,1 | 3_HiHi | 65 | M | T2b | N0 | 0 | 2 | 0 | 16 | 0 | 16 | 2 | Hi | Luminal | |
| 57 | 29,2 | 654,4 | 2_3HiLo | 72 | M | T3a | N0 | 1 | 3 | 0 | 9 | 0 | 9 | 2 | Hi | Luminal | |
| 58 | 51,1 | 285,5 | 0_LoLo | 47 | F | T4 | N0 | 0 | 3 | 1 | 1 | 1 | 4 | 3 | Hi | Basal | 3 |
| 59 | 182,8 | 55,3 | 1_3LoHi | 85 | M | T4 | N1 | 1 | 4 | 0 | 10 | 0 | 10 | 2 | Hi | Basal | 1 |
| 60 | 116,1 | 98,7 | 1_3LoHi | 81 | M | T4 | N0 | 1 | 3 | 0 | 9 | 0 | 9 | 1 | Lo | Non-type | |
| 61 | 206,1 | 1686,7 | 3_HiHi | 77 | M | T2a | N0 | 1 | 2 | 0 | 9 | 0 | 9 | 2 | Hi | Luminal | |
| 62 | 87,5 | 955,3 | 3_HiHi | 83 | M | T2a | N0 | 1 | 2 | 0 | 7 | 0 | 7 | 1 | Lo | Luminal | |
| 63 | 887,3 | 1405,5 | 3_HiHi | 87 | M | T2b | N0 | 1 | 2 | 0 | 7 | 0 | 7 | 2 | Hi | Non-type | |
| 64 | 151,1 | 865,4 | 3_HiHi | 74 | M | T3a | N0 | 0 | 3 | 0 | 5 | 0 | 5 | 3 | Hi | Basal | 3 |
| 65 | 33,0 | 243,3 | 0_LoLo | 72 | M | T2a | N1 | 1 | 4 | 0 | 4 | 0 | 4 | 3 | Hi | Luminal | 1 |
| 66 | 52,1 | 319,6 | 2_3HiLo | 69 | M | T3a | N2 | 1 | 4 | 0 | 18 | 1 | 18 | 2 | Hi | Luminal | 2 |
| 67 | 45,7 | 366,5 | 2_3HiLo | 79 | M | T3a | N1 | 0 | 4 | 0 | 1 | 1 | 1 | 1 | Lo | Non-type | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 27,1 | 1083,2 | 2_3HiLo | 72 | M | T2 | N0 | 1 | 2 | 0 | 8 | 0 | 8 | 0 | Lo | Luminal | 1 |
| 69 | 9,0 | 298,1 | 2_3HiLo | 41 | F | T4a | N1 | 0 | 4 | 1 | 27 | 0 | 31 | 3 | Hi | Basal | 2 |
| 70 | 442,5 | 1385,0 | 3_HiHi | 85 | M | T4a | N0 | 0 | 3 | 0 | 8 | 1 | 8 | 1 | Lo | Basal | 3 |
| 71 | 142,4 | 176,1 | 1_3LoHi | 80 | M | T3a | N1 | 0 | 4 | 0 | 8 | 1 | 8 | 1 | Lo | Luminal | 0 |
| 72 | 192,7 | 356,9 | 3_HiHi | 82 | F | T3a | N1 | 0 | 4 | 0 | 4 | 1 | 4 | 0 | Lo | Non-type | |
| 73 | 647,1 | 106,3 | 1_3LoHi | 54 | M | T3a | N0 | 1 | 3 | 1 | 3 | 0 | 24 | 0 | Lo | Luminal | 1 |
| 74 | 155,8 | 1011,9 | 3_HiHi | 66 | M | T3a | N2 | 1 | 4 | 1 | 3 | 0 | 17 | 0 | Lo | Luminal | 2 |
| 75 | 23,8 | 174,0 | 0_LoLo | 82 | M | T4 | N1 | 1 | 4 | 0 | 9 | 0 | 9 | 1 | Lo | Non-type | |
| 76 | 516,7 | 195,8 | 1_3LoHi | 79 | F | T3a | N0 | 0 | 3 | 1 | 2 | 1 | 3 | 3 | Hi | Basal | 3 |
| 77 | 184,1 | 304,3 | 3_HiHi | 50 | M | T4 | N0 | 0 | 3 | 1 | 1 | 0 | 9 | 1 | Lo | Luminal | |
| 78 | 36,2 | 70,1 | 0_LoLo | 82 | F | T3a | N0 | 1 | 3 | 0 | 1 | 1 | 1 | 2 | Hi | Luminal | 0 |
| 79 | 9,4 | 126,9 | 0_LoLo | 79 | M | T3a | N0 | 1 | 3 | 0 | 6 | 0 | 6 | 1 | Lo | Luminal | |
| 80 | 18,7 | 157,0 | 0_LoLo | 65 | M | T4 | N2 | 0 | 4 | 0 | 4 | 0 | 4 | 1 | Lo | Luminal | |
| 81 | 42,5 | 420,3 | 2_3HiLo | 65 | M | T4a | N2 | 1 | 4 | 1 | 1 | 1 | 4 | 2 | Hi | Luminal | 1 |
| 82 | 29,1 | 130,0 | 0_LoLo | 79 | M | T3a | N0 | 0 | 3 | 0 | 4 | 0 | 4 | 1 | Lo | Luminal | |
| 83 | 64,7 | 77,2 | 0_LoLo | 71 | M | T4 | N0 | 1 | 3 | 0 | 3 | 1 | 3 | 0 | Lo | Luminal | |
| 84 | 37,3 | 837,0 | 2_3HiLo | 75 | F | T3a | N2 | 1 | 4 | 1 | 4 | 0 | 4 | 1 | Lo | Luminal | |

**Supplementary Table S2.** Reagents for gene expression assay on UBC lines.

| Gene | Cat. no. | Company |
|---|---|---|
| **CXCL1** | qHsaCEP0053391 | Bio-Rad |
| **CXCL2** | qHsaCEP0058163 | Bio-Rad |
| **CX3CL1** | qHsaCEP0049180 | Bio-Rad |
| **CXCL8** | qHsaCEP0053894 | Bio-Rad |
| **HPRT1** | 4333768T | Applied Biosystems, Thermo Fisher Scientific |

**Supplementary Table S3:** genes defining the PMN and Chemokine signatures.

| Neutrophil signature | | Chemokine signature | |
|---|---|---|---|
| | | **Chemokine** | **Inflammatory functions** |
| ARG1 | | CCL14 | No |
| CEACAM3 | | CCL15 | No |
| CEACAM8 | | CCL16 | No |
| CHI3L1 | | CCL18 | No |
| CXCR1 | | CCL19 | No |
| CXCR2 | | CCL21 | No |
| CYP4F3 | | CCL23 | No |
| FCGR3B | | CCL24 | No |
| FPR2 | | CCL25 | No |
| HSPA6 | | CCL27 | No |
| IL1R2 | | CCL28 | No |
| KCNJ15 | | CCRL2 | No |
| MME | | CXCL12 | No |
| MMP9 | | CXCL13 | No |
| MMP25 | | CXCL14 | No |
| OLR1 | | CXCL17 | No |
| PGLYRP1 | | CCL1 | Yes |
| S100A8 | | CCL11 | Yes |
| S100A9 | | CCL13 | Yes |
| S100P | | CCL17 | Yes |
| TNFAIP6 | | CCL2 | Yes |
| TNFRSF10C | | CCL20 | Yes |
| TREM1 | | CCL22 | Yes |
| VNN3 | | CCL26 | Yes |
| | | CCL3 | Yes |
| | | CCL3L1 | Yes |
| | | CCL4 | Yes |
| | | CCL4L2 | Yes |
| | | CCL5 | Yes |
| | | CCL7 | Yes |
| | | CCL8 | Yes |
| | | CKLF | Yes |
| | | CX3CL1 | Yes |
| | | CXCL1 | Yes |
| | | CXCL10 | Yes |
| | | CXCL11 | Yes |
| | | CXCL16 | Yes |
| | | CXCL2 | Yes |
| | | CXCL3 | Yes |
| | | CXCL5 | Yes |
| | | CXCL6 | Yes |
| | | CXCL8 | Yes |
| | | CXCL9 | Yes |
| | | XCL1 | Yes |
| | | XCL2 | Yes |

**Supplementary Table S4.** Summary statistics of the CD3 and CD66b as continuous variable, p values by Shapiro-Wilk normality test

| | Min. | 1st Q | Median | Mean | 3rd Q | Max. | p |
|---|---|---|---|---|---|---|---|
| **CD3 area (mm2)** | 7.50 | 73.45 | 144.26 | 156.07 | 233.54 | 392.99 | 0.015 |
| **CD3 density (cells/mm2)** | 18 | 204 | 396 | 501 | 646 | 2020 | <0.001 |
| **CD66b area (mm2)** | 7.39 | 72.55 | 137.37 | 151.29 | 222.19 | 420.72 | 0.015 |
| **CD66b density (cells/mm2)** | 9 | 32 | 78 | 167 | 183 | 1049 | <0.001 |