**The Clonal Origin of Lymph Node Metastasis in Multifocal Prostate Cancer: Defining the Biologically Dominant Nodule**

**Simpa S. Salami, MD, MPH**[1]; Daniel H. Hovelson, MS[2]; Romain Mathieu, MD[3,4]; Martin Susani, MD[5]; Nathalie Rioux-Leclercq, MD[6]; Shahrokh F. Shariat, MD[3]; Scott A. Tomlins, MD, PhD[2]; Ganesh S. Palapattu, MD[1]

[1]Department of Urology, University of Michigan, Ann Arbor, Michigan
[2]Department of Pathology, University of Michigan, Ann Arbor, Michigan
[3]Department of Urology, Medical University Vienna, Vienna, Austria
[4]Department of Urology, Rennes University Hospital, Rennes, France
[5]Department of Pathology, Medical University Vienna, Vienna, Austria
[6]Department of Pathology, Rennes University Hospital, Rennes, France

**Background:** The clonal origin of lymph node metastasis in multi-focal prostate cancer is unknown. In this feasibility study, we sought to analyze and compare the genetic profiles of multifocal areas of cancer within the prostate with concordant lymph node metastasis to better define cancer foci capable of metastasis.

**Methods:** Patients who underwent radical prostatectomy and lymph node dissection that revealed node positive disease were identified for this IRB-approved study. All pathology slides were re-reviewed by a dedicated genitourinary pathologist. RNA/DNA samples were co-isolated from whole mount prostate samples with each tumor focus and normal tissue (control) pre-identified on formalin fixed paraffin embedded (FFPE) specimens. High depth, targeted DNA next generation sequencing (NGS) was performed to characterize the genetic profile of each sample, prioritized using the oncomine comprehensive panel (4 patients) or the comprehensive cancer panel (3 patients) and in house developed pipelines.

**Results:** A total of 27 primary tumor and 10 lymph node metastatic foci from 7 patients were analyzed (along with matched normal tissue for each patient). The median number of primary tumor foci and metastatic tumor foci were 4 and 1, respectively (See Table 1). In three of the seven patients, we observed significant shared genetic mutations between some primary tumor foci and matched lymph node metastasis. In patient #1, while all primary tumors showed concordant *TP53* and *TPR* non-synonymous mutations and broad copy number alterations (CNAs) with two lymph node metastasis foci, only two of the primary tumors showed concordant complex high level CNAs present in both lymph node metastasis foci. In patient #4, all tumor and lymph node metastasis foci shared a large number of somatic mutations, including a frameshift mutation in *PTEN*, with no high level CNA identified, consistent with a hypermutated genotype. The last patient's (ID #7) tumor samples and lymph node metastasis all shared a focal, high level *BIRC2* (chr 11) deletion. These specific mutations were not seen in the matched control samples of respective patients. RNA targeted NGS is currently underway to further characterize the clonal origin of each primary or metastatic tumor foci.

**Conclusions:** In this ongoing study, we demonstrate that molecular studies to better characterize the biologically dominant lesion in multi-focal prostate cancer may inform on mechanisms of tumor progression and hold promise for the development of improved prognostic biomarkers as well as identification of novel therapeutic targets.

**Conflict of Interest:** None

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**Table 1. Clinical and Pathologic Characteristics of the Cohort**

| Patient ID | Age | PSA (ng/mL) | Overall stage | Overall GS | Primary tumor foci | Metastatic lymph node foci |
|---|---|---|---|---|---|---|
| **01** | 50 | 43.4 | T3a+ | 5+4 | 5 | 2 |
| **02** | 66 | 170 | T3a | 5+5 | 4 | 2 |
| **03** | 70 | 53.5 | T2 | 5+5 | 4 | 1 |
| **04** | 68 | 22.9 | T3a+ | 5+5 | 4 | 2 |
| **05** | 64 | 29 | T2 | 4+4 | 3 | 1 |
| **06** | 72 | 7.4 | T2+ | 4+4 | 4 | 1 |
| **07** | 56 | 12.4 | T2 | 3+4 | 3 | 1 |

*Abbreviation: ID – Identity; PSA – Prostate specific antigen; GS – Gleason score*