

## Strategies of Uniplex and Multiplex IHC Staining in Limited Tissue Biopsies

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### Introduction

A paradigm shift occurred in the past decade for the treatment of lung cancer as targeted therapies emerged for tumors based on their histologic type and molecular alteration status. Accurate histologic tumor classification drives further ancillary testing and, ultimately, appropriate therapeutic selection. With current efforts to conserve tissue for potential molecular testing, small sample size is often a limiting factor. As such, the ability to perform more than one IHC marker on a single tissue section could be advantageous and may be required when limited tissue is presented.

Non-small cell lung cancer can be classified into several histologic types, most commonly lung adenocarcinoma (LADC) or squamous cell carcinoma (SCC). With the introduction of targeted therapies that can result in dramatically different outcomes, the importance of accurate classification has been amplified. Antibodies such as p63, CK5, p40, Desmoglein 3 (DSG3), TTF-1 and Napsin A have been used to phenotype LADC and SCC.<sup>1-7</sup> Several of these key lung cancer panel antibodies are mouse monoclonal (MM), thus making it much more difficult to utilize two-color multiplex technology that incorporates a combination of a mouse and a rabbit antibody. Studies have shown that the combination of the following three MM antibodies, DSG3 [BC11] (membrane/cytoplasmic), p40 [BC28] (nuclear) and TTF-1 [SPT24] (nuclear), are the most specific and most sensitive lung marker panel to distinguish LADC vs SCC.<sup>1-7</sup> Therefore, we have developed an immunohistochemistry (IHC) assay using a novel uniplex MM antibody cocktail of DSG3 + p40 (DAB) followed sequentially by a multiplex TTF-1 (Fast Red) on a single tissue section.

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### Materials and Methods

75 cases of previously diagnosed archival whole tissue lung cancer cases were evaluated. In LADC cases, 19 cases were poorly differentiated and 28 cases were moderately differentiated. In lung SCC cases, 16 cases were poorly differentiated and eight cases were moderately differentiated. There were four additional cases later determined to be poorly differentiated lung carcinoma, currently classified as (unknown).

### Uniplex + Multiplex IHC Procedure

All cases were initially stained with H&E. A single tissue section from each case was then stained as follows: MM cocktail of DSG3 + p40 (uniplex) was incubated for 30 minutes followed by a universal HRP-detection system and then visualized with DAB. A sequential stain was then added to the same specimen, beginning with the application of an acid elution step for 10 minutes. TTF-1 [SPT24] was incubated for 30 minutes followed by a mouse polymer alkaline-phosphatase detection system and visualized with fast red chromogen (multiplex). To verify that the elution step did not degrade SPT24 staining, single stained tissue sections (elution step removed) with SPT24 were compared (n=47). All tissues were stained on an IntelliPATH FLX<sup>®</sup> automated IHC platform (Biocare Medical).

## Figures 1-9

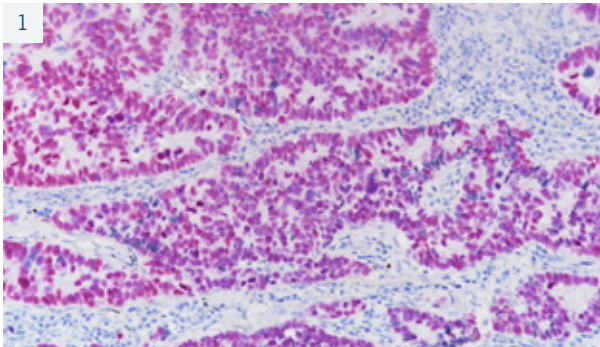


Figure 1: Poorly differentiated LADC stained with TTF-1.

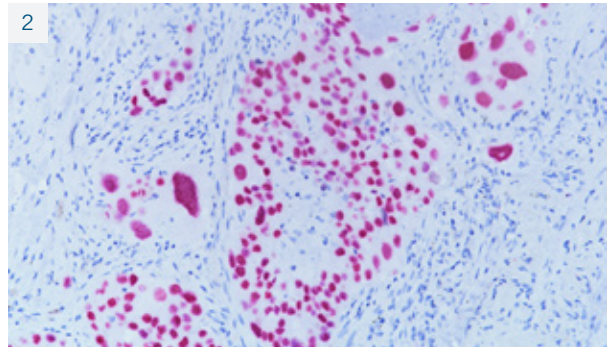


Figure 2: Poorly differentiated LADC stained with TTF-1.

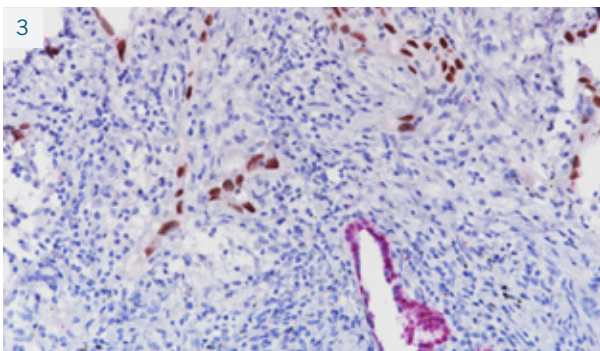


Figure 3: Poorly differentiated LADC negative for TTF-1 and positive for p40 reclassified by IHC to lung SCC.

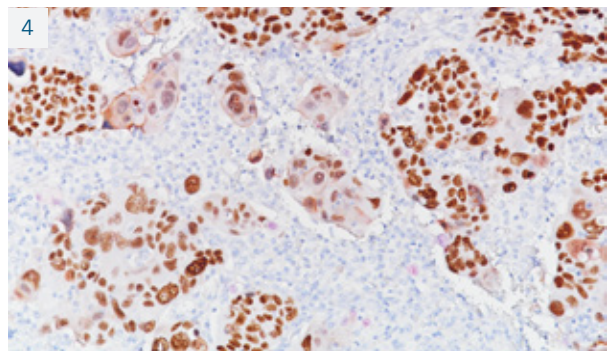


Figure 4: Poorly differentiated lung SCC stained with DSG3 and p40.

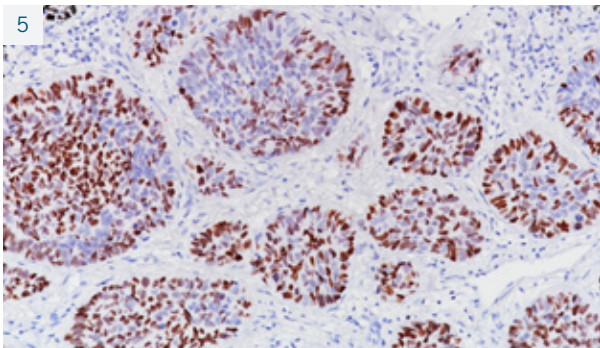


Figure 5: Poorly differentiated lung SCC stained with p40 and negative for DSG3.

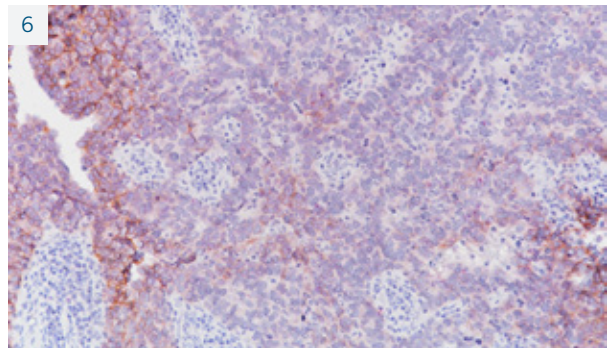


Figure 6: Poorly differentiated lung SCC stained with DSG3 and negative for p40.

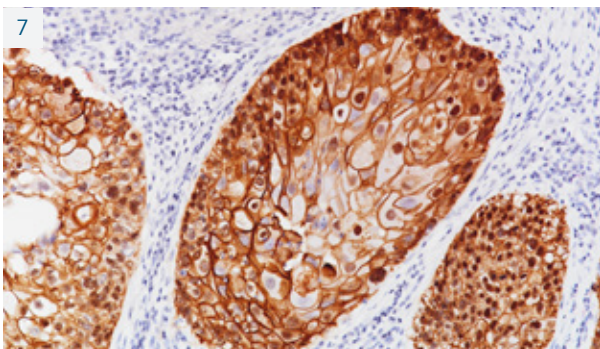


Figure 7: Moderately differentiated lung SCC stained with DSG3 and p40.

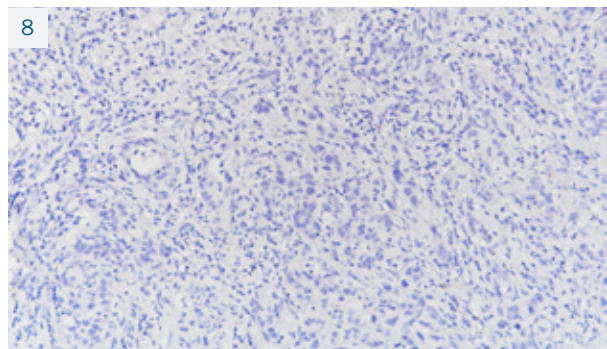


Figure 8: Poorly differentiated lung carcinoma of unknown phenotype. All three markers were negative.

## Figures 1-9

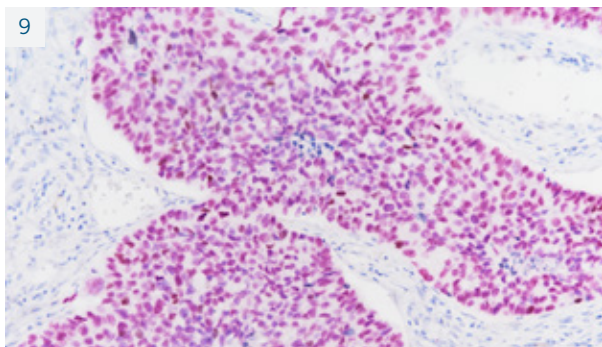


Figure 9: Poorly differentiated lung carcinoma of unknown phenotype stained predominantly with TTF-1 and also expressed a low percentage of p40. Case reclassified by IHC as adenosquamous lung carcinoma.

## Results

### *Lung Adenocarcinoma*

In TTF-1 positive cases, SPT24 demonstrated equal staining sensitivity before and after the elusion step. Among the poorly differentiated cases, 17/19 (90%) stained with SPT24 (fast red) (Figures 1-2). In moderately differentiated cases, 26/28 (93%) stained with SPT24. In all cases of LADC, 43/47 (92%) stained positive for TTF-1. In poorly differentiated LADC cases, 1/19 (5.3%) was negative for TTF-1 and DSG3 and positive for p40, and therefore reclassified by IHC as lung SCC (Figure 3). All other LADC cases were negative for DSG3 and p40. In moderately differentiated cases, 2/28 (7.1%) favored an LADC diagnosis; however, one case stained positive for DSG3 and p40 and negative for TTF-1 and the other case stained strongly positive for p40 and negative for DSG3 and TTF-1. Both cases were re-evaluated, and IHC results favored lung SCC. Consequently, 45/47 (96%) LADC cases were classified with the IHC uniplex/multiplex assay.

### *Lung Squamous Cell Carcinoma*

Within the poorly differentiated lung SCC cases, 15/16 (94%) expressed DSG3 (cytoplasmic/membrane) and/or p40 (nuclear) (DAB) (Figures 4-6). DSG3 and p40 were co-expressed in 7/16 (44%) cases; 2/16 (13%) cases expressed DSG3 only and 6/16 (38%) cases expressed p40 only. 1/16 (6.3%) cases was negative for all three markers. In the moderately differentiated category of lung SCC (Figure 7), 8/8 (100%) cases were positive for DSG3 and/or p40, 7/8 (88%) cases co-expressed DSG3 and p40 and 1/8 (13%) expressed p40 only.

### *Lung Carcinoma of Unknown Phenotype*

In the four cases of poorly differentiated lung carcinomas that were previously classified as unknown, three of the four cases were negative for all three antibodies (Figure 8), and the remaining case stained with TTF-1 and p40 which would favor adenosquamous lung carcinoma. In the combined cases of both LADC and lung SCC, 70/75 (93%) were classified by IHC, three cases (3/75, 4.0%) were re-evaluated and/or reclassified (Figure 9) and five cases (5/75, 6.7%) were negative for all three markers (DSG3, p40 and TTF-1).

## Conclusion

The uniplex IHC cocktail incorporated with a sequential multiplex application allows three key mouse monoclonal antibodies to be used on a single tissue section. This will improve the ability to phenotype lung tumors more accurately while conserving tissue for molecular or target therapeutic testing such as DNA assays, PD-L1, ALK or ROS1.



## References

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