

# An Immunohistochemical Analysis of p40 Mouse Monoclonal [BC28] in Lung, Skin, Head and Neck, Esophageal, Cervix, Bladder, Breast, Cervical, Prostate, Renal Cell and Colon Cancers

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## Introduction

p63 [4A4] has been a frequently used marker for lung, head and neck, bladder, breast, and prostate cancers.<sup>1</sup> In particular, p63 has proven to be a sensitive marker for lung squamous cell carcinoma (SqCC); however, it suffers from specificity limitations due to reactivity of a subset of lung adenocarcinomas (LADC). In contrast,  $\Delta$ Np63, a p40 variant, recognizes an epitope selectively expressed in lung SqCC, offering an opportunity for improved specificity. Considering the importance of accurately differentiating LADC vs. lung SqCC, p40 may be valuable antibody and could be a potential replacement for p63.

In recent studies, a rabbit polyclonal p40 (P) antibody exhibited equivalent staining sensitivity vs. p63 in lung SqCC; however, p40 (P) exhibited superior specificity due to staining fewer cases of lung ADC, and its specificity in other lung phenotypes.<sup>2, 3</sup> Recently, a p40 mouse monoclonal (M) antibody [BC28] has been developed. In this study, a comprehensive evaluation of p40 (M) [BC28] will be evaluated in normal tissues, as well as neoplastic tissues where p63 has traditionally been used, including: lung, skin, head and neck, esophageal, cervix, bladder, breast, cervical, prostate, renal cell and colon cancers.

## Design

Formalin-fixed paraffin-embedded (FFPE) tissue microarrays (TMAs) were constructed in-house or purchased commercially (U.S. Biomax, Rockville, MD, and Pantomics, Richmond, CA). All tissue sections were deparaffinized in the usual manner and hydrated to water. Slides were placed in a modified citrate buffer and heated in a pressure cooker (Decloaking Chamber) at 110°C for 15 minutes. Antibodies p63 [4A4], p40 (M) [BC28] and p40 (P) (Biocare Medical, Concord, CA) were titrated for optimum dilutions, using a 30 minute incubation time for all antibodies. Slides were rinsed in tris-buffer saline (TBS) and incubated for 30 minutes with a biotin-free, goat anti-mouse or goat anti-rabbit horseradish peroxidase (HRP) polymer detection system. Tissue sections were visualized with diaminobenzidine (DAB) and briefly counterstained in a modified Mayer's hematoxylin.

## Tissue Microarrays

Sets of lung TMAs were evaluated for sensitivity and specificity with p63 [4A4], p40 (M) and p40 (P) in both LADC (n=50) and lung SqCC (n=40). Additional TMAs consisting of 34 normal tissues (n=98) and

lung cancers (n=191), including LADC (n=57) lung SqCC (n=81), large cell neuroendocrine carcinoma (n=10), small cell carcinoma (n=20), and malignant mesothelioma (n=23) were evaluated with p40 (M). p40 (M) was further evaluated in various cancer phenotypes (n=431), including skin (n=20), head and neck (n=67), esophageal (n=10), cervical (n=18), bladder (n=48), breast (n=65), prostate (n=84), renal cell (n=70) and colon cancers (n=49).

## Results

Normal tissues stained with p40 (M) included bladder, breast, esophagus, placenta, ureter and cervix (Table 1). p40 (M) demonstrated equal staining sensitivity in lung SqCC (n=40) compared to p63 and p40 (P) (Table 2). p40 (M) was negative in 98% of cases of LADC (n=50) (Figure 1A) and positive in 92.5% of lung SqCC (Figure 1B). Both p40 (M) and p40 (P) provided higher specificity in LADC vs. p63 (Table 2), as p63 stained a higher percentage of cases of LADC (10%) vs. 2% LADC staining for both p40 (M) and p40 (P). A noticeable difference between p40 (M) vs. p40 (P) was high non-specific background staining observed in macrophages with p40 (P), which was absent with p40 (M) (Figure 2 A, B); and non-specific background staining with p40 (P) in lung SqCC, also absent with p40 (M) (Figure 2C, 2D).

Immunohistochemical staining for p40 (M) was observed in a high percentage of urothelial carcinomas (Figure 3A), but not bladder adenocarcinomas (Figure 3B). It was also observed in squamous and basal cell skin carcinomas, squamous cell carcinomas of the head and neck, esophagus, and cervix. (Table 3, Figure 4A-D).

## Discussion

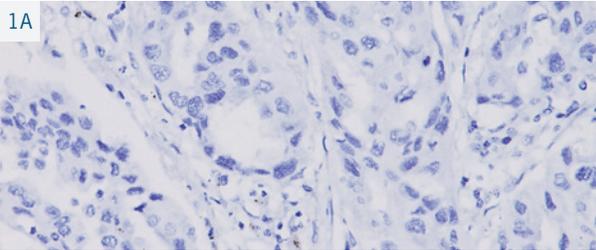
A total of 281 lung cancers, 438 various neoplastic tissues, and 34 different types of normal tissues were evaluated. In this study, the newly developed mouse monoclonal p40 (M) [BC28] provided equal sensitivity to p40 (P) and p63 for lung SqCC; however, p40 (M) was more specific than p63 staining only 2% of LADC (Table 2), vs. 10% LADC staining with p63. Furthermore, p40 (M) showed sharper staining, lacked non-specific background staining, and did not stain macrophages, unlike p40 (P) (Figure 2A-D). It should also be noted that lot-to-lot variability is common with rabbit polyclonal antibodies, and thus, mouse monoclonal antibodies is recommended.

# Discussion (continued)

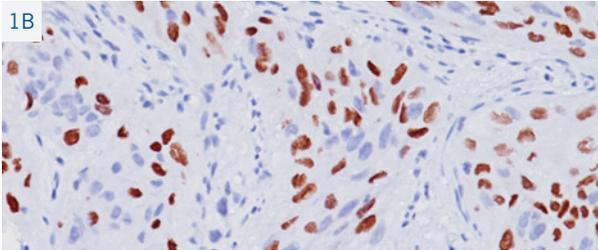
In all lung cancers tested, p40 (M) stained 99/107 (92.5%) of lung SqCC cases, and 1/121 (0.8%) cases of LADC, thus confirming the high specificity of p40 (M) (Table 3) as observed in previous studies.<sup>2,3</sup> p40 (M) was also evaluated in other various types of squamous cell carcinomas, including skin, head and neck, esophageal and cervical cancers (Table 3, Figure 4A-D). Past studies have shown p63 expression in these types of squamous cell carcinomas, and thus, similar results were expected for p40 (M). The high percentage of p40 (M) staining in these targeted tissues demonstrated its utility in cancers of squamous cell origin (Table 3). Notably, p40 (M) was negative in 98% of prostate cancers, and was negative in all breast, renal cell and colon cancers examined, thus establishing its high specificity in squamous cell carcinomas vs. various types of adenocarcinoma.

In past studies, staining of p63 and its variants, such as p40, have also been demonstrated in urothelial carcinomas.<sup>4,5</sup> In this study, p40 (M) stained 85.4% of urothelial carcinomas (Table 3, Figure 3); in poorly differentiated urothelial carcinomas, 70% of cases showed positive staining with p40 (M) (data not shown). Used in combination with other bladder carcinoma markers such as GATA-3 and Uroplakin II, and combined with the lack of p40 (M) staining in prostate and renal cell carcinomas, p40 (M) may be an important marker in the differential diagnosis of primary urothelial carcinoma vs. other primary genitourinary tract (ie, kidney, prostate, ovarian, and endometrial) carcinomas.

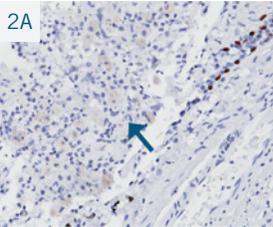
## Figures



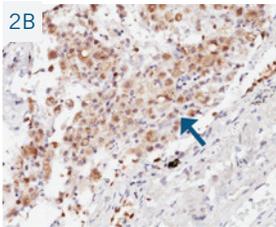
LADC is negative for p40 (M)



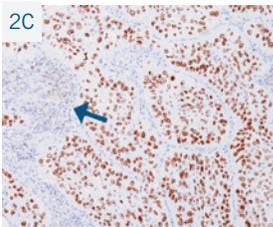
Lung SqCC is positive for p40 (M)



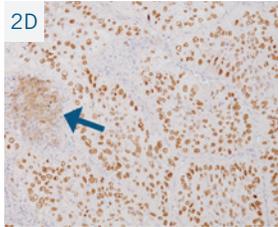
p40 (M) was (-) for lung macrophages (arrow)



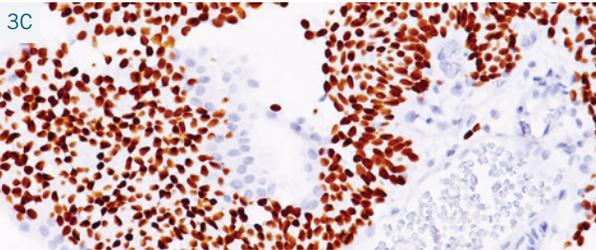
p40 (P) stained macrophages (arrow)



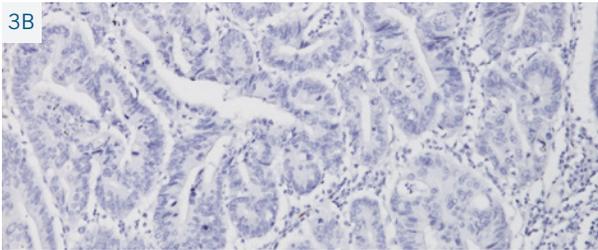
Lung SqCC stained with p40 (M) (arrow)



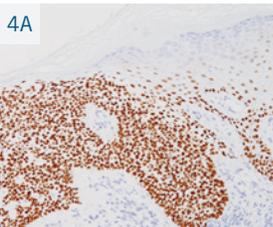
Lung SqCC stained with p40 (P) (Note: Non-specific staining vs. p40 (M)) (arrow)



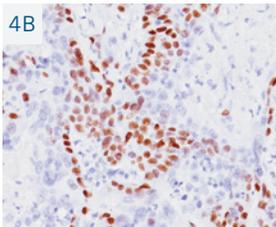
Urothelial carcinoma stained with p40 (M)



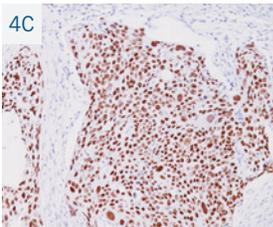
Bladder adenocarcinoma is negative with p40



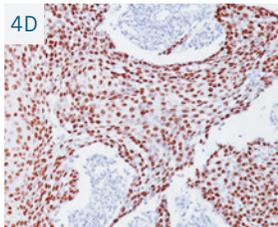
Skin BCC stained with p40 (M)



SqCC of the Larynx stained with p40 (M) (Head and Neck)



SqCC of the Esophagus stained with p40 (M)



SqCC of the Cervix stained with p40 (M)

Table 1: 34 Normal Tissue Types (n=98)

Normal Tissue	Positive Cases / Total Cases	Normal Tissue	Positive Cases / Total Cases
Adrenal gland	0/3	Ovary	0/3
Bladder (transitional cells)	2/3	Pancreas	0/3
Bone marrow	0/1	Parathyroid	0/3
Eye	0/1	Pituitary gland	0/2
Breast (myoepithelial cells)	3/3	Placenta (trophoblasts)	1/3
Brain (cerebellum)	0/3	Prostate (basal cells)	3/3
Brain (cerebral cortex)	0/3	Skin (basal cells)	3/3
Fallopian tube	0/3	Spinal cord	0/2
Esophagus (squamous cells)	3/3	Spleen*	2/2
Stomach	0/3	Skeletal muscle	0/3
Small intestine	0/3	Testis	0/3
Colon	0/3	Thymus*	3/3
Rectum	0/3	Thyroid	0/3
Heart	0/3	Tonsil**	3/3
Kidney	0/6	Ureter	3/3
Liver	0/3	Cervix (squamous cells)	3/3
Lung	0/3	Uterus (endometrium)	0/3

\*p40 did not stain B or T cells. Only epithelial cells were observed staining (<1%). \*\*Nuclear staining was only observed in normal squamous epithelium.

Table 2: Comparison of p40 (M), p40 (P) and p63 Positive Staining in Lung Cancer

Normal Tissue	Positive Cases / Total Cases	Normal Tissue	Positive Cases / Total Cases
Adrenal gland	0/3	Ovary	0/3
Bladder (transitional cells)	2/3	Pancreas	0/3

Table 3: All Lung Cancer Cases and Various Neoplastic Tissues stained with p40 (M)

Cancer Type	Positive Specimens	Number of Specimens	% Positive
Lung squamous cell carcinoma	99	107	92.5%
Lung adenocarcinoma	1	121	0.8%
Lung large cell neuroendocrine	0	10	0%
Lung small cell carcinoma	0	20	0%
Mesothelioma (various phenotypes)	0	23	0%
Urothelial carcinoma	41	48	85.4%
Skin cancer (squamous/basal cell carcinoma (BCC))	19	20	95.0%
Breast cancers (infiltrating ductal and DCIS)	*0	65	0%
Head & neck SqCC (various types)	54	67	80.6%
Esophageal cancer (squamous cell)	9	10	90.0%
Cervical cancers (squamous cell)	16	18	88.9%
Prostate adenocarcinoma	2	84	2.4%
Renal cell carcinoma (clear cell, papillary and others)	0	70	0%

\*p40 staining was observed in myoepithelial cells in breast ductal carcinoma *in situ* (DCIS) and/or as DCIS components in breast infiltrating ductal carcinoma (18/65).

## Conclusion

p40 (M) appears to be an important advancement in the differential diagnosis of lung SqCC vs. LADC, due to its increased specificity when compared to p63. In addition, p40 (M) may also be valuable for determining squamous origin in various other cancers including skin, head and neck, esophageal and cervical cancers. The utilization of p40 (M) may also be an important marker for identifying urothelial carcinomas.

## References

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