Article published online: 2024-07-03

Biomarkers

Evaluation of Applicability of Tumor Budding and Poorly Differentiated Clusters as Additional Prognostic Markers in Colorectal Cancers

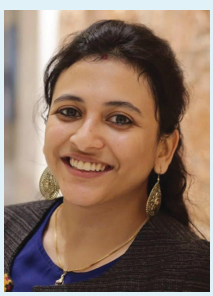
Sagarika Sarkar[1] Rena Guha[2] Sudipta Chakrabarti[3]

[1] Department of Pathology, Employees State Insurance Post Graduate Institute of Medical Sciences and Research (ESI PGIMSR), Manicktala, Kolkata, West Bengal, India
[2] Department of Pathology, Prafulla Chandra Sen Government Medical College and Hospital, Arambagh, West Bengal, India
[3] Department of Pathology, Employees State Insurance Corporation (ESIC) Medical College, Bihta, Patna, Bihar, India

Address for correspondence Sudipta Chakrabarti, MD, Department of Pathology, Employees State Insurance Corporation (ESIC) Medical College, Bihta, Patna 801103, Bihar, India (e-mail: sudiptach@gmail.com).

South Asian J Cancer 2024;13(4):315–319.

Abstract



Sagarika Sarkar

Purpose Very few studies have assessed tumor budding (TB) and poorly differentiated cell clusters (PDCs) simultaneously in colorectal cancers (CRCs). The goal of this study was to establish a correlation between these two pertinent histological features and to reinforce the importance of their incorporation in routine histopathological reporting of CRC cases as a means to predict clinical outcome.

Methods Resection specimens of colorectal carcinoma were included in the study. Patients who received presurgical therapy, or refused consent were excluded. PDC and TB were evaluated in routine hematoxylin and eosin-stained histopathological sections taken from the advancing edge of the tumor. TB and PDC were reported by selecting a "hotspot" chosen after review of all available slides with invasive tumor. It was then followed by their correlation with other known prognostic factors.

Results Spearman's rho calculator for strength of association between TB and PDC as well as association of TB and PDC individually with known prognostic factors revealed statistical significance. Correlation of TB and PDC with histologic grade, primary tumor (pT), and regional lymph node (pN) stage was done based on one-way analysis of variance calculator, which yielded statistically significant results.

Conclusion Evaluation of these two histological parameters in the same hotspot field at the tumor invasive front plays a fundamental role in the definition of cancer aggressiveness and prediction of tumor behavior.

Keywords

- colorectal cancers (CRC)
- morphology
- tumor budding (TB)
- poorly differentiated cell cluster (PDC)
- prognostic markers

Introduction

The tumor invasive front is a significant interface of tumor host interactions which regulates tumor progression. Tumor budding (TB) is a histological hallmark at the tumor invasive front which supposedly initiates invasion and eventually metastasis.[1] According to the International Tumor Budding

DOI https://doi.org/10.1055/s-0044-1788050 ISSN 2278-330X

How to cite this article: Sarkar S, Guha R, Chakrabarti S Evaluation of Applicability of Tumor Budding and Poorly Differentiated Clusters as Additional Prognostic Markers in Colorectal Cancers. South Asian J Cancer 2024;13(4):315–319.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Consensus Conference (ITBCC) criteria, TB is defined as a single tumor cell or a cluster of fewer than five tumor cells detached from the main tumor at the invasive front, while clusters of five or more tumor cells lacking gland formation are termed as poorly differentiated clusters (PDCs).[2,3]

TB has been designated as an adverse prognostic factor in colorectal cancers (CRCs) by the International Union against Cancer (UICC). The UICC has included TB under "additional prognostic markers," besides histologic grade, perineural invasion (PNI), and tumor border.[4–6]

Tumor grade based on glandular differentiation is an important predictive factor of CRC aggressiveness. However, this system poses a significant interobserver variability due to the absence of objectivity in assessment of glandular component. A novel histological grading system based on PDCs has been highlighted that is expected to achieve an objective assessment of tumor differentiation.[7] Besides, PDC is strongly predictive of unfavorable histological features, namely, infiltrating tumor borders, TB, lymph vascular, and PNI in CRCs.[8–10]

PDCs and tumor buds form a continuum of morphological features with a randomly set cut-point of 5 tumor cells to distinguish between the two.[2,3] PDCs possibly represent the evolution of tumor buds, which acquire proliferative and aggregative potential. Thus, they have common molecular, clinical, and pathological features of CRC cases.[3]

Very few studies have assessed the development of TB and PDC simultaneously in CRCs. Evaluation of these two histological parameters in the same hotspot field at the tumor invasive front is expected to assume a fundamental role in the definition of cancer aggressiveness and prediction of tumor behavior.

PDC and TB can be evaluated in routine hematoxylin and eosin-stained histopathological sections taken from the advancing edge of the tumor.

The present study aims to evaluate TB and PDC simultaneously in CRC cases by morphological assessment. The study further strives to establish a correlation of these two parameters with other known prognostic factors of CRCs.

The goal of our study was to establish a correlation between these two pertinent histological features and to reinforce the importance of their incorporation in routine histopathological reporting of CRC cases as a means to predict clinical outcome.

Materials and Methods

The study was conducted in the department of pathology over a period of 1 year (May 2020 to May 2021). Resection specimens of colorectal carcinoma received in the department of pathology were included in the study. Patients who received presurgical therapy, or refused consent were excluded from the study. After applying the exclusion criteria, 54 cases were included in the study. The current study was a single institutional, noninterventional, observational, prospective study. Clinical parameters were analyzed followed by histomorphological assessment of colonic resection specimens. Pertinent macroscopic findings of each specimen were tabulated after diligent assessment. Formalin-fixed paraffin-embedded tissue blocks were cut at 4 µm thickness and stained with hematoxylin and eosin. Histopathological reporting was done according to the College of American Pathologists protocol for the Examination of Resection Specimens from Patients with Primary Carcinoma of the Colon and Rectum (Version: Colon and Rectum Resection 4.1.0.0; Protocol Posting Date: February 2020). TB was reported by selecting a "hotspot" chosen after review of all available slides with invasive tumor. The total number of buds were reported in an area measuring 0.785 sq. mm, which corresponded to 20× field in LM-52-6000 Mega, LYNX Penta Head Microscope (based on the ITBCC, 2016 recommendation).[2,3] Both total number of buds and a three-tier score (based on 0.785 sq.mm field area) were reported: low (0–4 buds), intermediate (5–9 buds), and high (10 or more buds). PDC was evaluated in the advancing edge of the tumor in the same hotspot field as TB. The cases were categorized into three grades based on the highest PDC count: grade 1 (G1) count less than 5, grade 2 (G2) range between 5 and 9, and grade 3 (G3) 10 or more. TB and PDC data of each CRC case was assessed simultaneously. It was then followed by their correlation with other known prognostic factors, namely, tumor border, radial margin involvement, lymphovascular (LVI) and PNI, dirty tumor necrosis, histological grade, and pathological stage. All cases were initially assessed by a single pathologist blinded to other data followed by review of the cases by a second pathologist. Discordant findings, if any, were finally assessed by a third pathologist and the consensus opinion was incorporated in the final tabulation. Statistical analysis of the data was performed using the "social science statistics" Web site and the statistical tests employed were one-way analysis of variance (ANOVA) calculator, including Tukey's honest significant difference (HSD) and Spearman's rho calculator (https://www.socscistatistics.com/).

Results and Analysis

A total of 54 CRC cases were studied. The patients belonged to a wide age range spanning from 3rd to the 6th decades, majority being in the range of 50 to 54 years (20.4%). Fifty-six percent patients were females. Sixty-one percent of the cases presented with involvement of the left colon. Rectum was the most common tumor location. Bulk of the cases (57.4%) was of histologic grade 2. Note that 81.5% of the cases belonged to pT3. Resection margins were uninvolved in 70.4% of the cases (►Table 1).

TB score was done according to the ITBCC guidelines and the study comprised 37% low TB score, and 31.5% each of intermediate and high scores. PDC was evaluated and graded in the advancing edge of the tumor in the same hotspot field as TB. Low, intermediate, and high PDC scores were noted in 9.3, 66.6, and 24.1% cases, respectively, in the present study (►Fig. 1).

Spearman's rho calculator for strength of association between TB and PDC as well as association of TB and PDC individually with known prognostic factors, namely, lymph node metastasis, LVI, and PNI, revealed statistical significance. The correlation of TB with LVI and PNI was found to be statistically significant ($p = 0.008$ for TB vs. LVI, $p = 0.005$ for

Table 1 Distribution of cases based on demographic/pathologic characteristics and TNM staging

Sex	
Female	30 (55.6%)
Male	24 (44.4%)
Tumor location	
Caecum	12 (22.2%)
Ascending colon	04 (7.4%)
Hepatic flexure	04 (7.4%)
Splenic flexure	02 (3.7%)
Descending colon	02 (3.7%)
Sigmoid colon	03 (5.6%)
Rectosigmoid	02 (3.7%)
Rectum	15 (27.8%)
Anal canal	01 (1.8%)
Caecum and IC junction	02 (3.7%)
Rectum and anal canal	05 (9.3%)
Left colic flexure and part of descending colon	02 (3.7%)
Histologic grade	
G1	19 (35.2%)
G2	31 (57.4%)
G3	04 (7.4%)
Primary tumor (pT)	
pT1	01 (1.8%)
pT2	03 (5.6%)
pT3	44 (81.5%)
pT4	10 (18.5%)
Regional lymph nodes (pN)	
pN0	23 (42.6%)
pN1	15 (27.8%)
pN2	16 (29.6%)
TNM stage	
Stage I	4 (7.4%)
Stage II	40 (74.1%)
Stage III	10 (18.5%)

Abbreviation: IC, ileocecal.

TB vs. PNI); however, the correlation of PDC with LVI and PNI was not statistically significant. Correlation of TB and PDC with histologic grade, primary tumor (pT), and regional lymph node (pN) stage was done based on one-way ANOVA calculator, including Tukey's HSD, which yielded statistically significant results (►Table 2). Also, the correlation of TB and PDC individually with overall TNM stage was statistically significant. TB was found to be better correlated to TNM stage compared to PDC ($p = 0.004$ for TB vs. TNM, $p = 0.008$ for PDC vs. TNM). Hence, the present study establishes the prognostic significance of TB and PDC as well as their relation with known prognostic factors in CRCs, thereby reinforcing the importance of their incorporation in routine reporting. Furthermore, these two parameters when used in conjunction provide objectivity in grading the CRC cases and strengthens the histologic grading system for better risk stratification.

Discussion

TB is one of the preliminary steps of cancer progression, as TB cells invade extracellular matrix and lymphovascular spaces, thereby giving rise to metastatic deposits in lymph nodes and distant organs. PDCs were first defined in 2008 and are a predictive and prognostic factor in CRCs. Tumor grading by TB and PDC scoring has been used with a view to achieving greater objectivity compared to histologic grading alone.[11–14] Our present study was conducted to reinforce the prognostic value of TB when used simultaneously with PDC in CRCs.

According Lugli et al and Marx et al, in CRC, pTB (peritumoral TB) and iTB (intratumoral TB) are important indicators of higher TNM stage and tumor grade, LVI, or nodal and distant metastases.[15,16] Resemblances in adverse prognostic roles of pTB and iTB were established in a study by Lugli et al.[16] The present study also revealed that the TB positively correlated with histologic grade and pTNM stage (p-value is < 0.00001).

In a study by Zlobec et al, it was found that iTB could also identify the patients who might show aggressive behavior and benefit from adjuvant therapy in a subgroup of node-negative CRC.[17] Hence, routine reporting of TB will help in identifying a subgroup of patients who will require adjuvant therapy.

Ueno et al in their study delineated that pPDC (peritumoral PDC) was a more accurate prognostic indicator than pTB.[3,11]

Archilla et al, in their study delineated the fact that lymph nodal tumor burden significantly correlates with newer prognostic indicators, namely, higher TB and PDC scores in CRCs.[18] The current study has revealed similar findings.

As per the study by Mohan et al, statistically significant association was found between TB and LVI ($p < 0.01$) as well as TB vs. PNI ($p = 0.002$). The current study also showed similar statistically significant results.[19]

Study on PDC in colorectal carcinoma by Maurya et al failed to show any significant association between PDC and depth of invasion (pT) with p-value of 0.136.[20] While the study by Shah et al revealed significant correlation between TB with T stage, N stage, and TNM stage.[21] This was in concordance with the present study in which the correlation between PDC and pT was found to be statistically significant with a p-value of < 0.05.

The current study reinforced the fact that the correlation of TB and PDC individually with overall TNM stage was statistically significant. However, TB was found to be better correlated to TNM stage compared to PDC ($p = 0.004$ for TB vs. TNM, $p = 0.008$ for PDC vs. TNM).

TB and PDC are associated with the epithelial-mesenchymal transition phenomena, which is triggered by the Wnt/beta-catenin signaling pathway.[2,22,23] Transition of TB to a mesenchymal phenotype is mediated by nuclear translocation of beta-catenin and simultaneous loss of membranous E-cadherin

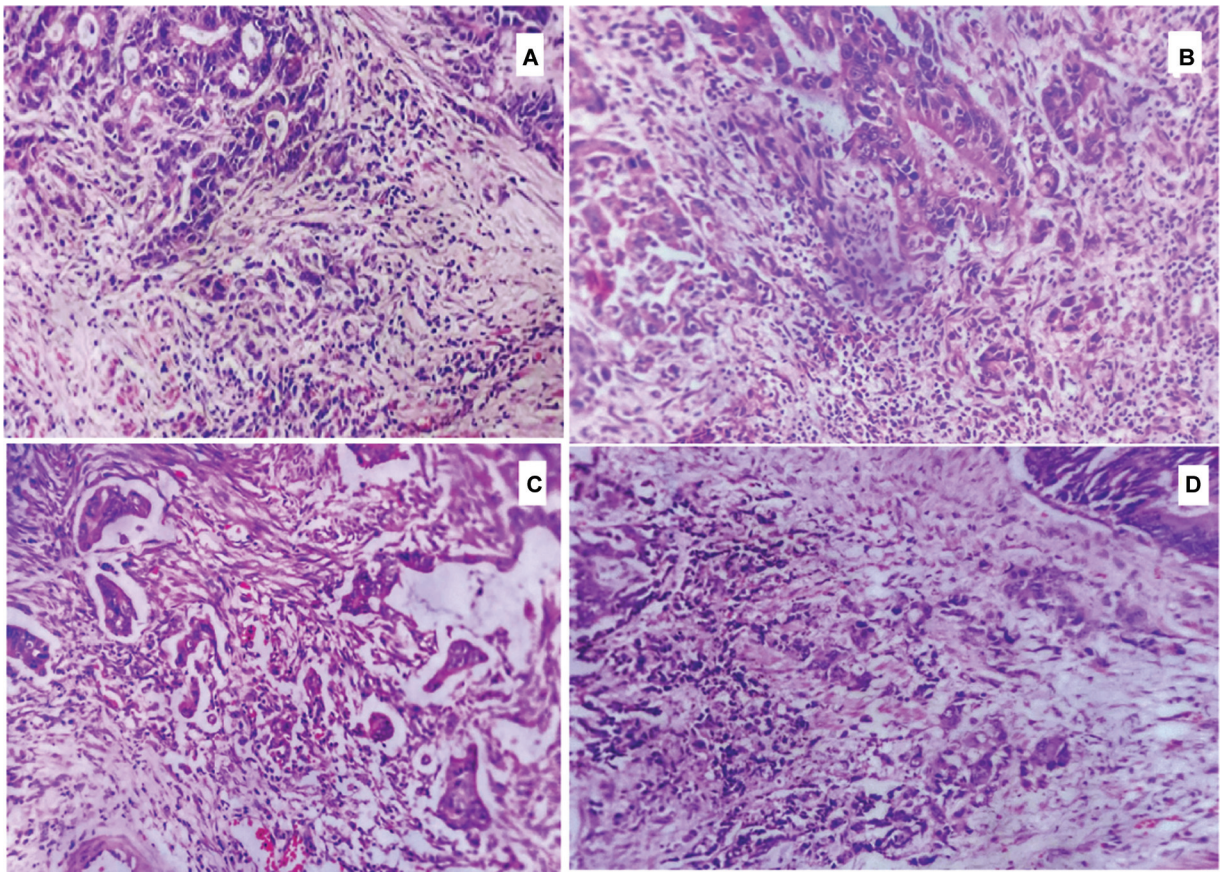


Fig. 1 Morphology of tumor budding (TB) and poorly differentiated cluster (PDC) scores in different histologic grade of tumor (hematoxylin and eosin [H&E], 200×). (**A**) TB score: Low (04) and PDC: G2 (05). (**B**) TB score: Intermediate (06) and PDC: G3 (10). (**C**) TB score: High (13) and PDC: G3 (10). (**D**) TB score: High (10) and PDC: G2 (08).

Table 2 Correlation of TB and PDC with histologic grade, primary tumor (pT), and regional lymph node (pN) stage (based on one-way ANOVA calculator, including Tukey's HSD)

TB: PDC	M1 = 7.19 M2 = 7.35	0.17	Q = 0.38 (p = 0.96152)
TB: G	M_1 = 7.19 M_3 = 1.72	5.46	Q = 12.37 (p = 0.00000)
PDC: G	M_2 = 7.35 M_3 = 1.72	5.63	Q = 12.74 (p = 0.00000)
The f-ratio value is 52.58697. The p-value is < 0.00001. (M1: mean of TB, M2: mean of PDC, M3: mean of histologic grade)			
TB: pN	M_1 = 7.19 M_3 = 0.85	6.33	Q = 14.26 (p = 0.00000)
PDC: pN	M_2 = 7.35 M_3 = 0.85	6.50	Q = 14.64 (p = 0.00000)
The f-ratio value is 69.62738. The p-value is < 0.00001. (M1: mean of TB, M2: mean of PDC, M3: mean of pN stage)			
TB: pT stage	M_1 = 7.19 M_3 = 3.07	4.11	Q = 9.32 (p = 0.00000)
PDC: pT stage	M_2 = 7.35 M_3 = 3.07	4.28	Q = 9.69 (p = 0.00000)
The f-ratio value is 30.14761. The p-value is < 0.00001. (M1: mean of TB, M2: mean of PDC, M3: mean of pT stage)			

Abbreviations: ANOVA, analysis of variance; G, histologic grade; HSD, honest significant difference; PDC, poorly differentiated cell clusters; pN, regional lymph nodes; pT, primary tumor; TB, tumor budding.
Note: The result is significant at $p < 0.05$. A blue value for Q (below) indicates a significant result.

expression. The development of PDC is associated with the upregulation of L1 cell adhesion molecule (L1CAM), which mediates epithelial cell migration and is one of the target factors of the Wnt signaling pathway.[22,23]

The present study strives to establish the importance of simultaneous assessment of TB and PDC in the prognostication of CRCs since both the parameters have significant positive correlation with histologic grade and pTNM stage. However, one weakness of the study is that the survival data of the study population could not be incorporated.

Conclusion

The study reinforces the fact that even in a resource poor setup, morphological assessment of TB and PDC will help achieve a greater objectivity in prognostication of CRCs than histologic grade alone. Identification of cases with higher TB score will in turn help to select subgroups who have a more aggressive behavior and likely to benefit from adjuvant chemotherapy. This current study also emphasizes the importance of scoring PDC in the same hotspot field that is used in the assessment of TB to strengthen the prognostic and predictive significance of TB in CRCs.

Authors' Contributions

S.S. helped in study conception and design, material preparation, data collection, and analysis, written first draft of the manuscript; whereas R.G. and S.C. was involved in study conception and design, material preparation, data collection, and analysis.

Informed Consent

Informed consent was obtained from all participants included in the study.

Ethical Approval

The Institutional Ethics Committee has approved the study.

Conflict of Interest

None declared.

References

1 Zlobec I, Lugli A. Tumour budding in colorectal cancer: molecular rationale for clinical translation. Nat Rev Cancer 2018;18(04): 203–204
2 Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 2017;30(09):1299–1311
3 Ueno H, Kajiwara Y, Shimazaki H, et al. New criteria for histologic grading of colorectal cancer. Am J Surg Pathol 2012;36(02):193–201
4 Hase K, Shatney C, Johnson D, Trollope M, Vierra M. Prognostic value of tumor "budding" in patients with colorectal cancer. Dis Colon Rectum 1993;36(07):627–635
5 Mitrovic B, Schaeffer DF, Riddell RH, Kirsch R. Tumor budding in colorectal carcinoma: time to take notice. Mod Pathol 2012;25 (10):1315–1325
6 Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. Postgrad Med J 2008;84(994):403–11
7 Jass JR, Barker M, Fraser L, et al. APC mutation and tumour budding in colorectal cancer. J Clin Pathol 2003;56(01):69–73
8 Okuyama T, Nakamura T, Yamaguchi M. Budding is useful to select high-risk patients in stage II well-differentiated or moderately differentiated colon adenocarcinoma. Dis Colon Rectum 2003;46 (10):1400–1406
9 Ueno H, Price AB, Wilkinson KH, Jass JR, Mochizuki H, Talbot IC. A new prognostic staging system for rectal cancer. Ann Surg 2004; 240(05):832–839
10 Zlobec I, Minoo P, Terracciano L, Baker K, Lugli A. Characterization of the immunological microenvironment of tumour buds and its impact on prognosis in mismatch repair-proficient and -deficient colorectal cancers. Histopathology 2011;59(03):482–495
11 Ueno H, Hase K, Hashiguchi Y, et al. Site-specific tumor grading system in colorectal cancer: multicenter pathologic review of the value of quantifying poorly differentiated clusters. Am J Surg Pathol 2014;38(02):197–204
12 Barresi V, Bonetti LR, Ieni A, Branca G, Baron L, Tuccari G. Histologic grading based on counting poorly differentiated clusters in preoperative biopsy predicts nodal involvement and pTNM stage in colorectal cancer patients. Hum Pathol 2014;45(02):268–275
13 Barresi V, Branca G, Ieni A, et al. Poorly differentiated clusters (PDCs) as a novel histological predictor of nodal metastases in pT1 colorectal cancer. Virchows Arch 2014;464(06):655–662
14 Barresi V, Reggiani Bonetti L, Branca G, Di Gregorio C, Ponz de Leon M, Tuccari G. Colorectal carcinoma grading by quantifying poorly differentiated cell clusters is more reproducible and provides more robust prognostic information than conventional grading. Virchows Arch 2012;461(06):621–628
15 Marx AH, Mickler C, Sauter G, et al. High-grade intratumoral tumor budding is a predictor for lymphovascular invasion and adverse outcome in stage II colorectal cancer. Int J Colorectal Dis 2020;35(02):259–268
16 Lugli A, Vlajnic T, Giger O, et al. Intratumoral budding as a potential parameter of tumor progression in mismatch repair-proficient and mismatch repair-deficient colorectal cancer patients. Hum Pathol 2011;42(12):1833–1840
17 Zlobec I, Hädrich M, Dawson H, et al. Intratumoural budding (ITB) in preoperative biopsies predicts the presence of lymph node and distant metastases in colon and rectal cancer patients. Br J Cancer 2014;110(04):1008–1013
18 Archilla I, Díaz-Mercedes S, Aguirre JJ, et al. Lymph node tumor burden correlates with tumor budding and poorly differentiated clusters: a new prognostic factor in colorectal carcinoma? Clin Transl Gastroenterol 2021;12(03):e00303
19 Mohan N, Kalaranjini KV, Mohandas L. Assessment of tumour budding in colorectal carcinoma and its correlation with pathological staging among patients undergoing resection at a tertiary care hospital in Kerala, India. J Clin Diagn Res 2023;17(10): EC01–EC07
20 Maurya S, Patel S, Srikantegowda H. A study of significance of poorly differentiated clusters in colorectal carcinomas: association with histopathological prognostic factors. J Clin Diagn Res 2020;14(11):EC28–EC32
21 Shah AH, Gami AJ, Desai NH, Gandhi JS, Trivedi PP. Tumor budding as a prognostic indicator in colorectal carcinoma: a retrospective study of primary colorectal carcinoma cases in a tertiary care center. Indian J Surg Oncol 2022;13(03):459–467
22 Terry S, Savagner P, Ortiz-Cuaran S, et al. New insights into the role of EMT in tumor immune escape. Mol Oncol 2017;11(07): 824–846
23 Chae YK, Chang S, Ko T, et al. Epithelial-mesenchymal transition (EMT) signature is inversely associated with T-cell infiltration in non-small cell lung cancer (NSCLC). Sci Rep 2018;8(01):2918

