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Novel prognostic molecular markers in lung cancer (Review)

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Abstract. Lung carcinoma, especially in its most commonly diagnosed non-small cell histological form, is a challenge to diagnose and treat worldwide, due to the prognosis in patients with this type of cancer being poor and mortality rates being high. However, a number of patients with this type of lung carcinoma exhibit a longer than average overall survival. The specific molecular background of non-small-cell lung cancer that favors longer survival has not yet been determined. The aim of the current study was to review articles published in the years 2017-2018 and create a list of the most important and strongest non-conventional factors that could be used in the future assessment of the prognosis of patients with adenocarcinoma and squamous cell carcinoma of the lung who cannot undergo current targeted therapy. Analysis identified multiple prognostic factors in non-small cell lung carcinoma, including tumor mutational burden, which was revealed to be independent of the tumor stage or grade as well as other factors, including age, sex or targeted therapy effects. The selected molecular factors exhibit the potential to be used in the treatment of patients with specific problematic lung cancer, and may contribute to setting recommendations for the diagnosis, prognosis and treatment of individual patients with lung cancer.

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1. Introduction

Lung carcinoma (LC) is regarded as the most common, lethal, malignant neoplasm. It is also the leading cause of cancer-related death worldwide, with a 5-year overall survival slightly exceeding 16% (1). Classically, lung carcinoma is divided into 2 main clinical types: Small-cell (SCLC) and non-small cell (NSCLC). The latter is also subdivided into 3 main histological types, adenocarcinoma (ADC), squamous cell carcinoma (SCC) and large cell carcinoma (LCC). The last type, LCC, is considered to be a poorly differentiated variation of the other two types (2). Theoretically, only approximately 6 mutations in the essential genes are necessary and sufficient to cause a malignant neoplasm, especially solid

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Abbreviations: ADC, adenocarcinoma; AKT1, akt serine/threonine kinase 1; ARHGEF39, rho guanine nucleotide exchange factor 39; ASPM, abnormal spindle microtubule assembly; BCAR1, Breast cancer anti-estrogen resistant protein 1; BRCA1, Breast cancer type 1 susceptibility protein; C3ORF18, chromosome 3 open reading frame 18; Cav 3.1, caveolin; CCNA2, cyclin a2; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen related cell adhesion molecule 5, ceacam5; CERS4, ceramide synthase 4; CHK1, checkpoint kinase 1; circRNA, circular rnas; CK20, keratin 20; CREG2, cellular repressor of e1a stimulated genes 2; c-SRC, non-receptor tyrosine kinase; CX3CL1, c-x3-c motif chemokine ligand 1; CYP17A1, cytochrome p450 family 17 subfamily a member 1; DAAM2, dishevelled associated activator of morphogenesis 2; DENND1C, denn domain containing 1c; EGFL6, egf like domain multiple 6; EGFR, epidermal growth factor receptor; EMAF, entropy-based maf; FOXM1, forkhead box m1; FUT4, fucosyltransferase 4; Girdin, also known as ccdc88a, coiled-coil domain containing 88a; H2Bub1, histone h2b monoubiquitination; HJURP, holliday junction recognition protein; HNK-1, human natural killer 1; HR, hazard ratio; HSF, heat shock protein; HURP, hepatoma up-regulated protein; IGFBP1, insulin like growth factor binding protein 1; IHC, immunohistochemistry; IL-33, interleukin 33; JNK1, also known as mapk1, mitogen-activated protein kinase 8; Ki67, kiel-67; KLHDC8B, kelch domain containing 8b; KRAS, kirsten rat sarcoma 2 viral homolog; LC, lung carcinoma; LCC, large cell carcinoma; LDHA, lactate dehydrogenase a; LDLRAD3, low density lipoprotein receptor class a domain containing 3; LINC00152, also known as cytor-cytoskeleton regulator rna; lncRNA, long non-coding RNAs; MAF, mutant allele fraction; MFI2, melanotransferrin; NSCLC, non-small-cell lung; OS, overall survival; P38alfa, also known as mitogen-activated protein kinase 14; PD-L1, Programmed death ligand 1; PFS, progression-free survival; PINK1, pten induced kinase 1; PTPRA, protein tyrosine phosphatase, receptor type a; RGS20, regulator of g protein signaling 20; RRM1, ribonucleoside-diphosphate reductase large subunit; SCC, squamous cell carcinoma; SLC25A42, solute carrier family 25 member 42; SLC2A1, solute carrier family 2 member 1; SOX2, sry-box 2; SPATA6, spermatogenesis associated 6; ST-2, suppression of tumorigenicity 2; STAT-3, signal transducer and activator of transcription 3; TIMP1, tissue inhibitor of metalloproteinases 1; TMB, tumor mutation burden; TNM, classification of malignant tumors; TTF1, transcription termination factor 1

Key words: non-small cell lung cancer, prognosis, immunohistochemistry, genetic factors

tumors. Most of them arise as chance mutations. However, it has been well documented (3) that in order for lung cancer to develop, only three sequential mutations are required. Yet, LC may be an exception as it is more frequently diagnosed in smokers. In the light of this, it appears that a behavioral factor plays the most important role in the LC development. Nevertheless, the number of non-smokers affected by lung cancer is increasing. Thus, it is possible that along with the 'driving mutations' playing a key role in cancer development as well as in its progress and invasion, there may exist certain 'favorable passenger' gene mutations or gene expression alterations which could explain a longer overall survival of some LC patients.

Even though immunological and targeted therapies have been in use, the currently provided treatment to lung cancer patients with no specific mutations is still insufficient and (more often than not) unsuccessful. Chemotherapy and radiation therapy are commonly used but their effectiveness is relatively low. Due to severe side effects, they may drastically compromise the LC patients' quality of life or even contribute to their premature death and shorter survival. Therefore, in order to be able to anticipate the natural history of the disease in individual patients, it is mandatory that the nature of lung cancer in each patient should be clearly understood since only individually tailored molecular profiles and markers could spare the patients from undergoing a potentially more harmful, aggressive chemical therapy or even leave them untreated.

The new and promising molecular markers, brought to light thanks to the use of advanced technology, appear to have the potential to help predict the natural history, treatment targets and outcomes, thereby contributing to the improvement of the patients' overall survival.

Having studied both experimental and review articles published in 2017-2018, we have selected and described the most important and strongest non-conventional prognostic factors in adenocarcinoma and squamous cell carcinoma of the lung.

2. Revision of novel prognostic factors in lung cancer

Use of immunohistochemistry in the assessment of lung cancer prognosis. Among many techniques, immunohistochemistry (IHC) appears to be the most reliable, routinely tested and reported method. It serves as a prognostic and predictive tool and enables conducting a semiquantitative analysis of specific proteins in the context of tissue architecture. The IHC examination is performed with the use of a light microscope. After certain antibodies are bound to the color markers, they are visualized as brown or red spots in the blue background (4). The advantage of this way of testing is that, since it uses the very same sample previously collected for the regular histopathological examination, the LC patients are spared from undergoing additional invasive procedures, thus, most of them have either a surgical specimen or a biopsy sample examined. What is more, IHC is relatively inexpensive. On the other hand, a semiquantitative evaluation, adequate for diagnostic purposes, is not always sufficient for the prognostic or predictive assessment.

However, despite its shortcomings, IHC's numerous and undisputed advantages make it an adequate method to be a routine part of the histopathological examination performed in LC patients.

In order to shed more light on the survival in ADC and SCC lung cancer patients, the signaling proteins as well as those engaged in different biological processes were studied. Out of 22 immunohistochemically studied proteins, Jin *et al* successfully distinguished two prognostic six- and five-protein sets characteristic of ADC and SCC, respectively (5). They are as follows: For ADC: c-SRC, Cyclin E, transcription termination factor 1 (TTF1), p65, checkpoint kinase 1 (CHK1), mitogen-activated protein kinase 8 (JNK1 also known as Mapk1) and for SCC: epidermal growth factor receptor (EGFR), Sry-Box 2 (SOX2), E-cadherin, Akt Serine/Threonine Kinase 1 (AKT1), mitogen-activated protein kinase 14 (also known as P38alfa). Each of the proteins had its individual role in the LC development, e.g. c-SRC activation by protein tyrosine phosphatase, receptor type A (PTPRA) was reported to be a poor prognostic factor in SCC (6). TTF1 is known to show both anti- and protumoral activity in LC (7). JNK1 plays a role in the induction of apoptosis and has been shown to be the key contributor to the tumor-promoting activity of tobacco smoke in LC (8-10). An increased expression of cyclin E1 is a useful marker of poor prognosis in LC (11). AKT1, involved in the cell motility, invasion and metastasis, has been shown to have an anti-metastatic role in the NSCLC cells with Kirsten Rat Sarcoma 2 Viral Homolog (KRAS) or EGFR mutations (12), whereas SOX2 deregulation has proved to cause bronchial dysplasia (13). The prognostic value of these proteins was assessed as a set that provided grounds for dividing the ADC and SCC patients into two groups: One group consisted of patients with assumed good prognosis and the other included those with poor prognosis. Expression of the six- and five-signaling proteins set turned out to be a powerful prognostic factor, both in ADC and SCC (HR=7.67 and 3.51, respectively). The 5-year survival in the good prognosis ADC group was ~3-5 times higher than in the group of patients with poor prognosis, varying in different study cohorts (coming from different hospitals). In SCC, the differences in the 5-year survival were ~30% for the poor prognosis group to 90% in the good prognosis group (5). Most importantly, both protein sets proved to be independent prognostic indicators and outperformed the TNM staging. It should be noted that administering chemotherapy did not change the prognosis in the good prognosis group, while it had only some impact on the survival in the poor prognosis group (5).

Yang *et al* reported girdin (also known as Ccdc88a, Coiled-Coil Domain Containing 88a) and Signal Transducer And Activator Of Transcription 3 (STAT-3) as essential proteins in the prognosis of NSCLC patients (14). Girdin plays an important role in the cell migration and angiogenesis, while STAT-3 belongs to the Janus kinases and is suggested to be engaged in tumor metastasizing. Their expression level in the IHC study strongly correlated with the progression-free survival (PFS) and overall survival (OS). Girdin and STAT3 expressions were positive and negative for the LC tissue and normal surrounding tissue, respectively. Their expression level also correlated positively with the TNM stage. In those lung cancer patients who presented low Girdin and STAT-3 expressions, the chances for survival longer than five years were three times higher than in those with high Girdin and STAT-3 expressions (14).

Heat Shock protein (HSF), engaged in regulation of the cellular response to stress and playing an important role in protecting the cell against unfavorable conditions (hypoxia, low glucose), is known to be impaired in the cancerous cells, which leads to its high expression (15). High expression of HSF is an adverse prognostic factor in many malignancies, including hepatocellular carcinoma, breast carcinoma or esophageal squamous cell carcinoma (16-18). Wan *et al* proved it had an impact on both OS and PFS (HR=2.19) and correlated with the TNM stage and tumor grade (15).

A signaling molecule, C-X3-C Motif Chemokine Ligand 1 (CX3CL1), acts in a paracrine fashion triggering the recruitment of the inflammatory cells. CX3CL1 has also been suggested to activate a surface tyrosine kinase receptor, EGFR, to stimulate cellular proliferation (19). EGFR appears to affect the natural history of many types of malignant neoplasms (20-22). A high level of expression of this protein causes activation of T $CD8^+$ lymphocytes and NK cells within the tumor, which leads to a more effective elimination of the transformed cells (21). A more detailed study into the role of CX3CL1 in the lung tumors revealed that ADC patients with a smoking history and high expression of CX3CL1 had a shorter OS (HR=3.01) (23). Moreover, in the same study, a high CX3CL1 expression in the smoking AD patients happened half as often as in the SCC and ADC non-smoking patients. In the entire NSCLC cohort, no correlation between the expression of the protein and clinical course of the disease was observed. Such an unexpected result may be explained by different pathways of carcinogenesis in the smoking and non-smoking patients as well as in SCC and ADC patients (23). High mitotic activity is a known histopathological feature of malignant neoplasms. Classically, the higher mitotic activity, the more aggressive the tumor. In their NSCLC study, Folescu *et al* used the Kiel-67 (Ki-67) antibody (IHC marker expressed in all the cells except those in the G0 cell cycle stage) and observed that in the group with a high Ki67 expression the TNM stage was higher. In the same patients the probability of recurrence was also higher (24). The level of expression of Ki67 affected the overall prognosis as well. Due to the small size of the tested group, the results were not statistically significant, although considering the theoretical background, they reflect an important tendency.

Pten Induced Kinase 1 (PINK1) is a molecule that, due to its effect on mitophagy (genetically programmed removal of damaged mitochondria), may facilitate the cell survival under cellular stress conditions. In NSCLC and other types of cancer, PINK1 is also known to cause chemoresistance (25) and its expression is believed to have an impact on the clinical course of NSCLC (26). In ADC of the lung, the expression of PINK1 was found to be a strong and bad prognostic factor (HR=2.14), whereas in SCC this correlation was statistically insignificant. In SCC, the PINK1 expression was not an independent risk factor since it strongly correlated with TNM. Therefore, the immunohistochemical examination of PINK1 expression in the lung ADC might be helpful in assessing the prognosis in individual patients (27).

Egf Like Domain Multiple 6 (EGFL6) is another potential molecular prognostic factor measured by IHC. This protein participates in the growth regulation of many cancer types (28,29). In ADC of the lung, a high EGFL6 expression is reported to have correlated with the clinical course of the disease as well as with the patients' overall survival (HR=1.52), it also heralded a worse, more dismal prognosis, which was especially relevant for patients under 63 years of age (30). Among the IHC measured proteins, there was an expression of the Forkhead Box M1transcription factor 1 (FOXM1), the protein known to play a crucial role in the cell proliferation and differentiation. An abnormal FOXM1 expression correlated strongly with the clinical course of NSCLC. An average 5-year overall survival after a surgery was 25 and 70% in the FOXM1 positive and negative group, respectively (HR=2.88) (31). The results were confirmed by Sun *et al* who concluded that a high expression level of FOXM1 is an independent risk factor in NSCLC (HR=1.73) (32). Further *in vitro* studies on the human cancer cell lines explained this relationship as FOXM1 was proved to promote vascular invasion (31). Down-regulation of interleukin-33 (IL-33) and its receptor in the tumor tissue appears to affect the tumor growth. This cytokine plays an important, dual role in oncogenesis via acting both as an inhibitor and facilitator of the tumor growth. It activates not only the anti-tumor NK-cells and T cells mediated-immune response but the suppressor T-lymphocytes as well. A low concentration of the serum IL-33 correlates with the progression of LC (33). Yang *et al* discovered that the IL-33 expression was generally lower in cancer than in the surrounding healthy tissue. The same applies to Suppression of tumorigenicity 2 (ST-2), the receptor for IL-33. Its expression correlated negatively with both the tumor stage in SCC and tumor grade in ADC. A higher level of IL-33 was a good prognosis factor as the lung ADC patients with a high expression of this cytokine were characterized by a longer overall survival (34).

Flotillins are another type of potentially important proteins in the clinical course of NSCLC. Flotillin-1 is a protein engaged in endocytosis, intracellular signal transduction and intercellular adhesion. Flotillin-2 plays a role in the axonal growth, cell differentiation and endocytosis. In the literature there are numerous reports on the role of these molecules in cancer development, however, the presented results are dissimilar. A meta-analysis performed by Deng *et al* reviewed the current knowledge about the link between the expression of these molecules and human cancers. Over-expression of both Flotillin-1 and -2 was significantly associated with poor prognosis in LC (HR=1.99 and HR=1.76) (35). The prognostic value of the Flotillin 2 expression in NSCLC was even higher when measured on the mRNA level (HR=2.67) (36). A combined analysis of both high Flotillin and EGFR expression was a poor prognostic factor in NSCLC as well (37).

Feng *et al* studied the role of several immunohistochemical markers, i.e. (Keratin 20 (CK20), Caudal Type Homeobox 2 (CDX2), Carcinoembryonic Antigen Related Cell Adhesion Molecule 5 (CEA), villin and mucin-2 in prognosing the lung ADC. CK20 and CDX2 proved to play a crucial role in this respect (38). The mean survival time was 36 and 14 months in the CK20 positive and the CK20 negative group, respectively. The CDX2 positive group had a mean survival of 25 months, while the survival in the CDX2 negative group was only 7 months. In the case of CEA, the negative group had a mean survival of 36 months, while the positive group's survival was 18 months. There was no association between the expression of villin and overall survival (38).

The voltage-gated calcium channels (VGCCs) expression in cancers had an influence on the prognosis in NSCLC. This protein plays an important role in the cell adhesion, migration and control of the cell cycle. Silencing the calcium channels expression resulted in suppression of the tumor proliferation (39,40).

In a study by Suo *et al*, a specific type of molecule, Caveolin-Cav 3.1, was analyzed. It was observed that tumors with overexpression of Cav.3.1 tend to be larger, more advanced in stage and more likely to be a case of SCC. The mean survival of the overexpressing and non-overexpressing patients was 48.6 to 106.7 months, respectively (41).

Rho Guanine Nucleotide Exchange Factor 39 (ARHGEF39) was proven to facilitate the NSCLC cells proliferation, migration and invasion. An IHC low expression of ARHGEF39 showed a negative correlation with the overall survival. The mean postoperative survival in the patients with overexpression was 52.5 months and in those without overexpression-64.3 months (42).

The expression of another protein, Hepatoma Up-Regulated Protein (HURP), is significantly higher in the neoplastic tissue of NSCLC patients. An *in vitro* study found that silencing the HURP expression led to inhibition of the human lung cancer cell lines' proliferation but did not affect apoptosis. What is more, its high level of expression had a negative impact on the progression-free survival (HR=2.71) as well as on the overall survival (HR=1.75) in NSCLC. High levels of HURP expression in the IHC studies also correlated with a higher clinical stage and grade (43).

The PTPRA protein is another factor affecting LC patients' survival. Its role in carcinogenesis is believed to be linked to the activation of the SRC family kinase pathway. A study by Gu *et al* revealed that the level of expression of this molecule correlates with the TNM stage in lung cancer as well as with PFS (HR=1.7) and OS (HR=2.3) in lung SCC (6).

Another interesting target for IHC are the cells that infiltrate the tumor and are part of the neoplastic environment. Due to the nature of the disease and the host's reaction to it, the immune cells seem to be a natural and obligatory target of the research into the prognosis in neoplasms. Abundance of infiltrate should signify both a strong reaction to the ongoing neoplastic process and recognition of the tumor cells as abnormal by the immune system. The CD57-positive cells are a group of potential tumor-infiltrating immune system cells. They are also called Human Natural Killer 1 (HNK-1) cells. The density of these cells in the tumor micro-environment was found to affect the clinical course of many malignant neoplasms (44,45). It influenced the survival in gastric, esophageal, colorectal or hepatocellular cancer. Similarly, a meta-analysis showed that a high level of the CD57+ cells improved the OS in NSCLC (HR=0.48) (44). Lymphocytes are another group of tumor infiltrating cells. They might be localized in the stroma or within the tumor nests themselves. Frequently, there are more immune system cells in the tumor area than in the surrounding, healthy tissues. There is evidence that the CD8+ T cells infiltrating the tumor stroma and epithelium inhibited progression and metastasis (46).

Genomic features. Apart from the immunohistochemically rated proteins, RNA molecules are another significant group of molecular factors with the prognostic potential in malignant neoplasms.

miRNA are 18-22-nt-long, non-coding RNA of low-molecular-weight that play a superior role in the gene expression regulation in both physiological and pathological processes. They are involved in modulating the key biological processes, including cancer initiation and progression. Apart from that, they are believed to play an oncogenic and suppressive role, and their expression profile is characteristic of specific types of cancer. In many cancers, the miRNA profile is a diagnostic, prognostic or predictive factor (47).

The miR-590 expression, known to play the key role in the cellular proliferation, differentiation and apoptosis (48), is associated with the clinical course of lung cancer. In a study by Ma *et al*, a low level of miR-590 was qualified as a bad prognostic factor (HR=2.15) (49).

Another miRNA, i.e. miR155, frequently deregulated in hematopoietic malignancies and considered an oncomiR, plays multiple roles in the control of the innate and adaptive immune processes (50). It is also known to have an impact on the clinicopathological features and OS in ADC (HR=3.03) (51).

miR-340 is another microRNA molecule playing an important role in LC. It is involved in the induction of apoptosis, inhibition of invasion and proliferation in many malignant neoplasms (52,53). A recent study has found that expression of this microRNA in the tumor tissue is related to the clinical course of NSCLC, thereby correlating with the TNM and influencing PFS and OS dramatically (the authors do not provide HR). Patients with a low miR-340 expression had a significantly reduced 5-year overall survival resulting from the miR-340 influence on the lung cell growth and proliferation (54).

There is evidence that down-regulation of Tissue Inhibitor of Metalloproteinases 1 (TIMP1) affects the tumor growth due to its association with miRNA-125a-5p. TIMP1 is known to play a role in the miR125-5p synthesis. On the other hand, the latter molecule promotes apoptosis, which leads to suppression of the tumor growth. Its expression in cancer is usually lower than in the adjacent healthy tissues. The studies *in vitro* have been confirmed by a clinical analysis. The patients with a high TIMP1 level had a significantly lower OS (HR=3.17) and PFS (HR=1.71) (55). It is worth noting that a low level of miRNA-125a-5p itself also correlates with a poor prognosis in this disease (HR=0,134) (56).

miR-494-3p is also a type of miRNA and its role in the LC prognosis is of great importance for it is known to be involved in the maintenance of the tumor cells' population and their invasiveness. LC patients with a higher expression of miR-494-3p had a significantly shorter OS (HR=2.75) (57).

miR-148a is another molecule known to affect the LC prognosis and correlate with OS (HR=1.6). It acts as a regulator of the cell cycle via *WNT1* gene. In the literature, miR-148a has been reported to be significantly down-regulated in the NSCLC tissue, thereby reducing apoptosis, increasing invasion, causing higher tumor grade and lymph node metastases, all of which resulted in a shorter OS (HR=1.6) (58).

Circular RNAs (circRNA), the transcriptional product in thousands of the human genes, are specific non-coding RNA species with multiple functional capacities. They are modulators of the miRNA activity and regulators of cancer development.

Table I. Simplified table showing influence of miRNAs and genes on different signaling pathways.

miRNA	Potential genes	Signaling pathways
mir-590-5p	RECK, FOXO1, TGFBR2, SMAD7, PITX2, YAP1, FGF18 and CREB5	AKT/ERK and STAT3 signaling pathways (SPs), NF90/VEGFA signaling pathway (SP) TGF-βSP, Wnt-β-catenin SP, Hippo SP, ATM SP, CREB1/CREB5-NF-κB SP
mir-155-5p	PDK1, SOCS1, FOXO3, TAB2, BACH1, ZNF652, JARID2, APC, SDCBP, CEBPB, IKBKE, SPI1, ETS1, SMAD2, CCND1 and E2F2	mTOR pathway MAPK SP Hedgehog SP, Neurotrophin SP, VEGF SP, PTEN/PI3K/AKT Insulin/IGF SP, STAT3 SP, IL-13/TAB2 SP, PKC/NF-κB SP, JAK/STAT SP, WNT/β-CATENIN SP
miR-340-5p	LGR5, NRAS, CCND1, MEKK1, MEKK2, MEKKK3, MDM4, TP53, XIAP, CCND2 and SKP2	Wnt/β-catenin SP, MAPK SP, TP53 SP
miR-125a-5p	LIN28A, TP53, VEGFA, ERBB3, ERBB2, TAZ, KLF13, TNFAIP3 and EIF4EBP1	Stat3 SP, TP53 SP, VEGFA/VEGFR2 SP, ERBB2 and ERBB3 SP, BCL2, BCL2L12 and Mcl-1 SP, EGFR SP, Hippo SP, PI3K/AKT and Ras-ERK SPs, KRAS and NF-κB SPs
miR-494-3p	PTEN, BMI1, MYC, RAD23B, HOXA10 and IGF1R	PTEN/PI3K/AKT SP, mTOR/RICTOR SP, SDF-1/CXCR4 SP
mir-148a-3p	DNMT1, CCKBR, DNMT3B, PBXIP1, CDKN1B, ITGB8, ROCK1, SMADR and S1PR1	Notchsignaling TGF-β/Smad SP, FcγRIIA SP, Wnt/B-catenin SP, NF-κB SP
Circ_001569	WNT1 and PI3K	Wnt/β-catenin SP, Akt/mTORSP, Notch1 SPs
LINC00152	P38a, STAT1, STAT3, CCNE1, CREB1 and c-MYC	

The table is based on miRTarBase, (available online at: http://mirtarbase.mbc.nctu.edu.tw/php/index.php, accessed 20.10.2019) and Pathway Commons [available online: (http://www.pathwaycommons.org, accessed 20.10.2019)]. The genes and pathways were selected based on strength of evidence and number of papers supporting them. miR, microRNA.

They can also support tumor suppressors in control of cancer cell growth, and engage in tumor-promoting inflammation, invasion and metastasis activation (59). One of the significant circRNAs, correlating with clinicopathological features of NSCLC, is circ_001569. The expression of circ_1569 was found to be significantly higher in the NSCLC tissue than in the adjacent healthy tissue. Its level was connected with the tumor differentiation as well as with the T and N features of the TNM staging. Patients with higher circ_001569 levels had a significantly poorer clinical outcome and overall survival. In studies *in vitro*, inhibition of circ_001569 had an impact on the cell proliferation via WNT/beta-catenin pathway, which resulted in the tumor growth inhibition (60).

Non-coding RNA-Cytor (Cytoskeleton Regulator Rna also known as LINC00152), called a tumor propellant in pan-cancer, is involved in the promotion of carcinogenesis (61). The LINC00152 overexpression in the lung ADC cells was reported to be accompanied by stimulation of proliferation, tumor cell invasion and migration. Linc00152 has proved to be an independent risk factor for OS as approximately 2.5 times more patients with a low expression of the molecule are likely to survive 5 years after the surgery. The level of LINC00152 was also observed to correlate tightly with the lymph node metastasis, tumor size and TNM staging (62).

The non-coding RNAs as well as their target genes were grouped in terms of molecular pathways in which they seem to play an important role. As single non-coding RNA molecule has impact on many different genes and pathways, the most important ones were listed and grouped in Table I. The list is based on MiRTarBase and Pathway Commons database. Table I.

Apart from circRNA, long non-coding RNAs (lncRNA) were also studied with regard to their prognostic value. Chen *et al* investigated a set of seven lncRNAs, i.e. FLJ30679, LINC00511, LINC01127, MIF-AS1, RP11-278J6.4, RP11-25K19.1, CTC-429P9.1 in LC. The expression of five out of seven (FLJ30679, LINC00511, LINC01127, MIF-AS1, RP11-278J6.4) turned out to be a bad prognostic factor and the latter two proved to be a good prognostic factor. The study also revealed that the set was a strong prognostic factor of overall survival, with HR for OS varying between 1.47 and 2.72, depending on the group chosen from separate datasets (63).

Zheng *et al* studied a set of eight lncRNAs: LINC00857, RP11-284F21.7, TMPOAS1, RP11-284F21.9, LINC01137 and RP11-253E3.3, which proved to be poor prognostic factors, as well as RP11-344B5.2 and CTC-429P9.1, which turned out to be good prognostic factors. The median survival difference between the poor and good prognostic groups was

approximately 38.5 to 86 (64). On the other hand, Liu *et al* linked two lncRNAs, i.e. IGF2BP2-AS1 and DGCR5, with a good prognosis in the lung SCC (60 months survival-35 to 55%) and MIR31HG, CDKN2A-AS1 and LINC01600 with a poor prognosis in the lung ADC (60 months survival 25% to 45%) (65).

Analysis of the lung ADC gene databases has revealed certain genes that may play an important role in the prognosis of this disease. In a study by Zhao *et al*, almost 1,500 candidate genes were found and expressions of twenty of them were proven to be useful in the prediction of clinical course of the LC, they are as follows: *CERS4*, *FUT4*, *C3ORF18*, *CYP17A1*, *ASPM*, *HJURP*, *LOC645166*, *DENND1C*, *SLC25A42*, *CCNA2*, *LDHA*, *IGFBP1*, *SLC2A1*, *DAAM2*, *RGS20*, *MFI2*, *LDLRAD3*, *KLHDC8B*, *CREG2* and *SPATA6*. On the basis of the prognosis and status of gene expression, the LC patients were divided into two groups. Analysis of the two groups, divided according to the gene expression profile, showed that the overall survival in both of them was significantly different. A 5-year overall survival in the high risk group was less than 10%, while in the low risk group it was over 60%. Moreover, the TNM stage was found to have a less significant impact on the overall survival than the genetic profile. On the basis of the prognostic score, the authors compared the 20-gene set to a 4-gene set (*FUT4*, *SLC25A42*, *IGFBP1* and *KLHDC8B*) and the compared 4-gene set turned out to be effective, yet, its capacity to predict the clinical course was much lower than in the 20-gene set. The 5-year survival in the high and low group was ~20 and 50%, respectively (66).

Apart from specific gene expression and mutations, also the number of mutations and their intratumoral heterogeneity, called mutant allele fraction (MAF) heterogeneity, have an influence on the clinical course of NSCLC. Shen *et al* analyzed the correlation between the MAF and LC course and prognosis. The patients were divided into two groups according to their high and low entropy-based MAF (EMAF) heterogeneity. The high EMAF group had lower OS (HR=1.5) in both SCC and ADC. Moreover, the EMAF analysis, together with other factors, e.g. staging, grading, age, gender, and smoking, significantly improved the accuracy of predicting a 3-year survival (67).

Tumor Mutation Burden (TMB), a factor similar to MAF, is defined as a total number of non-synonymous mutations of the genome's coding regions (68). Its correlation with OS survival was tested by Owada-Ozaki *et al* who found out that high TMB was a very poor prognostic factor both for OS (HR=12.31) and PFS (HR=6.07) (69). On the other hand, Devarakonda *et al* proved that high non-synonymous TMB (>8/Mb) had a good impact on the overall survival, however, low TMB (<4/Mb) was a positive predictor of response to adjuvant chemotheraphy (70). For the moment, the results are contradictory and further studies are required to shed more light on the role of TMB in lung cancer. Nevertheless, TBM does seem to be a promising prognostic factor.

The genetic factors that influence clinicopathological features of NSCLC are not limited to changes in the quantity and structure of nucleic acids. Histones, the proteins that play a crucial role in the DNA folding, were also included in the study. Any change in their structure and function alters the ability of the cell to reproduce, which, theoretically, seems to be very important in the development of various types of cancer. There is evidence that the expression of a specific form of a Histone H2b Monoubiquitination (H2Bub1) may play a role in the prognosis of lung cancer. Tests *in vitro* showed that H2Bub1 had an impact on migration, proliferation, invasion and resistance to cisplatine, while correlational studies *in vivo* revealed that H2Bub1-negative cancers were characterized by poorer overall survival in ADC (71).

3. Discussion

The conducted literature review has brought to light multiple prognostic factors in the non-small cell lung carcinoma which appear to be independent of the tumor's stage, grade or other factors, such as age, sex or possibility of targeted therapy. Most of them change the prognosis slightly but there are many that have a great impact on the overall survival of LC patients (Table II).

However, it is worth noting that the correlation between the TNM stage and overall survival varies from study to study and the values of HR range from 1.997 (30) to 2.43 (5), which means that it is not a strong prognostic factor.

It is also worth mentioning that known prognostic molecular factors do not seem to have more impact on the OS than the TNM system. High expression of PD-L1 is associated with shorter survival with the HR of 1.75 in ADC (72). Low BRCA1 expression has even lower impact on OS in lung cancer with the HR of ~1.6 (73). Patients with low RRM1 expression tend to have OS only less than 4 months longer in comparison to patients with high expression of the molecule (74). High expression of BCAR1 is a slightly more powerful prognostic factor of OS in NSCLC, as the HR in this case is ~2.5 (75). This data indicates that the novel markers are more powerful in predicting the course of LC.

Immunohistochemistry has a great potential and, as a part of histological diagnosis, it can spare the patients from undergoing additional invasive procedures since a single sample collection is sufficient for conducting the IHC examination. Moreover, the cost of IHC is relatively low and does not require extra equipment or personnel, while histopathological laboratories should have credentials to perform this kind of examination. Even though further research is required in order to introduce specific recommendations for different types of LC patients' treatment in specific situations, some of the above mentioned factors may be helpful in individual, problematic cases of lung cancer, especially in the older patients, in poor general condition who are suspected not to be able to survive chemotherapy or radiation therapy.

The reviewed study results show that there is no single marker that can predict the prognosis. There are plenty of them, yet, when analyzed individually, they did not turn out to be predictive factors of much importance. However, the reviewed literature provides evidence that combining two or three variables might bring promising results. Moreover, the review also shows that not many of these markers can work as independent risk factors since in the reviewed studies they correlated very strongly with classical factors, such as TNM.

In order to assess the usefulness of the above mentioned methods in making an individual treatment plan for NSCLC patients, some variables must be considered. The impact on

Table II. Quantitative Assessment of prognostic factors.

Prognostic factor	Type	Quantitative change in prognosis (OS)
20 gene set	ADC	6 (66)
CDX2 (positive)	ADC	3,5 (38)
CX3CL1 (low)	ADC (smokers)	3 (23)
Signaling proteins: c-SRC, cyclin E1, TTF1, p65, CHK1, and JNK1	ADC	2,75 (5)
4 gene set	ADC	2,5 (66)
LINC00152 (low)	ADC	2,5 (62)
CK20 (positive)	ADC	2,5 (38)
CEA (negative)	ADC	2 (38)
PINK1	ADC	2 (25)(26)(27)
Ki-67 (low)	ADC	2 (24)
MIR31HG, CDKN2A-AS1, LINC01600 (as set)	ADC	1.5 (65)
IL-33 (high)	ADC	1,5 (34)
EGFL6 (low)	ADC	1,5 (30)
Flotillin 1 (low)	LC	2 (35)
Flotillin 2 (low)	LC	1.75 (35)
TMB (high)	NSCLC	12.25 (69)
miRNA-340 (high)	NSCLC	4 (54)
TIMP1/miRNA-125a-5p (low)	NSCLC	3.25 (55)
FOXM1 (negative)	NSCLC	3 (31)
MiRNA 494-3p (low)	NSCLC	2.75 (57)
Girdin (low)	NSCLC	2.75 (14)
STAT3 (low)	NSCLC	2.5 (14)
Girdin and STAT3 (low)	NSCLC	2.5 (14)
Cav3.1 (low)	NSCLC	2.25 (41)
HSF1 (low)	NSCLC	2.25 (15)
CD57 (high)	NSCLC	2 (44)
HURP (low)	NSCLC	1.75 (43)
EMAF (low)	NSCLC	1.5 (67)
miRNA 148a (high)	NSCLC	1.5 (58)
ARHGEF39 (negative)	NSCLC	1.25 (42)
Signaling proteins: EGFR, p38α, AKT1, SOX2, and E-cadherin	SCC	10 (5)
miRNA-590 (high)	SCC	2.25 (49)
IGF2BP2-AS1 and DGCR5 (as set)	SCC	1.5 (65)

The quantitative change in prognosis is to some extent a subjective value. In cases where authors of the original study calculated the HR, The prediction value is HR rounded to the nearest quarter. When it was not calculated in a previous study, it is mean survival of two groups compared, or a value based on a Kaplan-Meier curve analysis. miRNA, microRNA; OS, overall survival; ADC, adenocarcinoma; SCC, squamous cell carcinoma; NSCLC, non-small cell carcinoma; LC, lung carcinoma.

OS, which has to be at least as high as in the case of TNM classification to provide the physician with crucial information, appears to be the most important of them all. Availability and cost of the method are also of importance.

On the basis of the presented criteria, it can be hypothesized that the five- to six-protein sets (5) should be an effective marker, especially when it is known that a good prognosis ADC group does not respond very well to chemotherapy. It is essential to emphasize that the diagnostic value of 5/6 antibodies is relatively high in comparison to its moderate cost, which, under the circumstances, makes it a most desired solution.

For institutions with poorer access to antibodies, a combination of three essentials, i.e. CK20, CDX2 and CEA, seems to be a good solution in terms of the cost-effect relationship as well as availability (38). IHC staining for girdin and STAT3 also appears to meet the above described criteria of an effective examination with regard to the prognosis in NSCLC. Another antibody that may be taken into consideration when planning individual treatment in problematic cases is Cav3.1. It is also important that information about the amount of the inflammatory infiltrate within the tumor should be provided as it correlates with the overall survival (44,75).

It must be remembered that the results of the studies are relevant for certain populations and may not apply to patients from different geographical regions, which is why further research is still needed.

Analysis of non-coding RNAs may also help in obtaining important information about the patients' prognosis and, since the cost of such an examination is relatively low, it is recommended that a set of miRNAs that gives precise information about the clinical course of the disease should be identified. For the present moment, miRNA 494-3p, 125a-5p, 340, 590 are the most interesting recently found markers. Earlier literature reports pointed to let-7e as a candidate molecule for LC prognosis (56).

Genetic expression analysis strongly correlates with prognosis, especially the 20-gene set (66), therefore, it may become the main prognostic asset in the nearest future requiring well equipped genetic laboratories and cost-effective procedures so that it can become a part of routine testing. If the cost of the procedure was reduced, for instance by devising a standardized kit, it could be successfully used to predict the clinical course of NSCLC.

Lastly, the cost of IHC and genetic analysis should be in opposition to the cost of chemotherapy, hospitalization, treatment and adverse effects. After all, under the circumstances, the cost of genetic testing appears to be relatively low. On the other hand, the absence of good prognostic factors or the lack of bad prognostic factors may be an indication for a more aggressive therapy, which, in this case, is believed to increase the patient's overall survival.

4. Conclusion

In the literature, there are a lot of data and reports on the correlation of certain factors and prognosis in NSCLC. However, there is not sufficient information to set specific recommendations for the diagnosis, prognosis and treatment of patients who present specific features. Most information comes from the analysis of small patient cohorts and different geographical areas. Therefore, further research is required to test the effectiveness and the relative cost of individually adjusted treatment based on the described factors. Apart from the economical aspect, the reduction in the patient's quality of life should be taken into consideration. However, it must be emphasized that there is no effective treatment for most cases of NSCLC and the routinely administered chemotherapy may be more harmful than the disease itself. It is never any guarantee that the intended chemotherapy will bring desirable effects. Information about an individual patient as well as a more detailed prognosis would be beneficial as it could shed more light on to the case and help in taking better-thought-out decisions regarding starting, forsaking or continuing a specific treatment.

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Authors' contributions

MP designed the study, searched, analyzed and interpreted the literature and was a major contributor in writing the manuscript. JS revised the manuscript and BMK prepared and revised the manuscript.

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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