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REVIEW ARTICLE **OPEN ACCESS**

Management of Non-Small cell Lung Cancer by genetic studies: A review

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ABSTRACT
Lung cancer is the leading cause of mortality worldwide. There are multiple cellular, local and genetic alterations that are involved in lung carcinogenesis. According to *genetic profiling and mutational analysis the most relevant genes that are responsible for lung cancer are RET, ROS1, EGFR, KRAS, BRAF, MET, PIK3CA, LKB1, CyclinD1, BCL2, MYC,* EML4-ALK fusion gene, *and ALK. Immunotherapeutic strategies such as monoclonal antibodies are emerging as efficacious treatment to curb the risk of lung cancer. In this review we highlighted the genetic bases of lung cancer. Current status of diagnosis and treatment has also been discussed. It is anticipated that this effort will provide baseline data for future research.*
Key Words: *Carcinogenesis, Monoclonal Antibodies, Biopsy, Ganciclovir drug, Glycosylation.*

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INTRODUCTION

Lung cancer is the number one killer among all cancers. It starts when lung cells become abnormal and grow in an uncontrolled manner. It may form a tumor or spread to other parts of body. Significant global bioburden of lung cancer has been observed during recent years**.** (1,350,000 new cases and 12.4% of total new cancer cases) and (1,180,000 deaths and 17.6% of total cancer deaths). (Figure 1) Almost as many Americans die of lung cancer every year than die of breast, prostate, colon cancer combined [1,2].
There are two different types of lung cancers. About 10% to 15% are small cell lung cancer (SCLC) and 80% to 85% of lung cancers are non-small cell lung cancer (NSCLC). About 40% of lung cancers are adenocarcinomas, 25-30% are squamous cell carcinomas, and large cell (undifferentiated) carcinoma are 10% to 15%. Other subtypes such as sarcomatoid carcinoma, adenosquamous carcinoma are less common [4]. Besides genetic alterations, smoking is the leading cause of lung cancer it has been reported that smokers who give up smoking for about 15 years have 80-90% in their risk for lung cancer [5].
This review summarizes comprehensive overview of current status of lung cancer in areas of its genetics, diagnosis, and treatment.

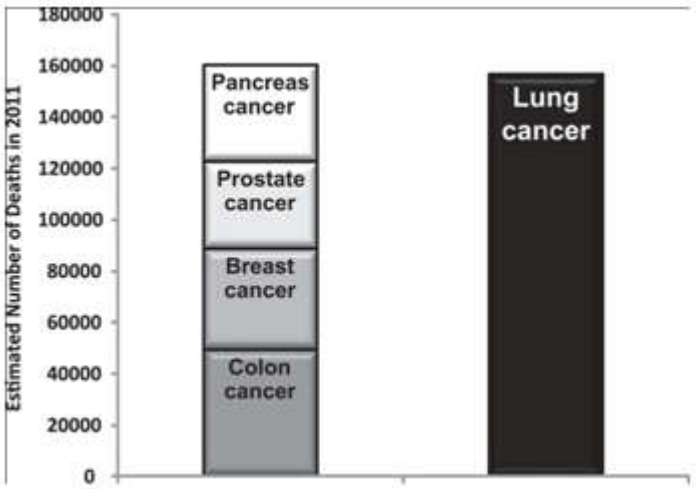


Fig 1: Estimated deaths caused by lung cancer are equal to combined deaths caused by breast, prostate, colon, and pancreas cancer [3].

Genetics of lung cancer

Carcinogen related diverse changes in genome of susceptible lung cells lead to lung cancer. In lung cancer sequential accumulation of number of morphologic and genetic changes occurs in normal epithelial cells of lung. Unchecked cell growth and proliferation occurs as a result of negative signallers mediated by tumor suppressor RB protein and positive signallers mediated by oncogene RAS. Other possible molecular derangements that cause cancer are angiogenesis, metastases, tissue invasion, senescence and evasion of apoptosis.

Genetic changes responsible for lung cancer are multiple, complex, and heterogenous both in mechanistic pathways and chronology. Activation of proto-oncogene MYC through copy number amplification and homogeneously amplified region of chromosome 8q are present in cancer cells. Accumulating evidence suggest that molecular lesions are specific to both subtypes of lung cancers SCLC (type with small cell lung cancer neuroendocrine phenotype) and NSCLC (non-small cell lung cancer specifically squamous cell carcinoma, large cell carcinoma, and adenocarcinoma). Some genes can be targeted for both subtypes e.g, p53 mutations whereas, other genes are subtype specific e.g, in cases of NSCLC frequent alterations are observed in p16INK4a inactivation and ras activation whereas, in SCLC changes are identified in RB inactivation and myc activation [6].

Number of somatic mutations including oncogenes and tumor-suppressor genes has been identified in last 30-40 years. DNA damage is unable to repair in lung and cause mutations which form the basis of genomic instability and tumor growth. Depending on the type of polymerase, reported rate of spontaneous errors of replication is 1/10,000 to 1/100,000 base pairs. Three classical examples of mutations are p16, p53, and k-ras [7].

p16, is component of Rb pathway and this tumor suppressor gene is inactivated in more than 40% of NSCLCs. It has previously been reported that hypermethylation of the gene, loss of heterozygosity, and point mutations on 9p21 modifies inactivation mechanism in 30–50% of NSCLCs [8]. Homozygous deletions or point mutations as loss of p16 function has been observed in smokers with tumors because relationship between loss of p16 mutations and tobacco plays an important role in pathogenesis of lung cancer [9].

Most of genetic lesions in human cancers are caused by prototype tumor suppressor gene p53 [10]. P53 mutations are commonly observed in small-cell carcinoma and squamous carcinoma affecting more than two-thirds of lung cancers. Mutated p53 acts as an oncogene and can be found over-expressed in about 50% of lung cancers because of prolonged half-life. Most frequent mutations found in the lungs of smokers are G to T transversions [11].

About 30% of adenocarcinomas of lung are caused by K-ras mutations. Mutated K-ras activates ERK-MAP kinase pathway that transform airway epithelial cells [12].

Multiple genetic loci are behind sporadic lung cancers. Molecular genetic studies have shown alterations in both growth suppressing tumor-suppressing genes and growth-promoting oncogenes. Tumor suppressor gene locus is indicated by loss of heterozygosity similar to oncogene that is indicated by DNA amplification related to increased copy number. About 30% of victims of lung adenocarcinoma have mutation in KRAS oncogene. EGFR, Cyclin D1*, and* MYC are over-expressed and amplified in 6%, 5%, and 2.5-10% of non-small cell lung cancer. Over-expression of BCL2 or C-erbB2 (Her-2/neu) is observed in 25% patients [13-15].

Novel mutations such as BRAF have been identified in 2% of adenocarcinoma patients through systematic resequencing of oncogenes. *BRAF mutations are observed in tumors in which KRAS mutations are absent* [16]. Mutations in EGFR have also been detected recently. Individuals infected with NSCLC have found to express transforming EML4-ALK fusion gene. Approaches that combine expression with genomic data and resolution screens for copy number changes are revealing novel candidate oncogenes [17]. Recent evidences have suggested oncogenic changes in transcription factors as other cause of lung cancer. Several deletions such as loss of heterozygosity or homozygosity have been identified at several regions of chromosomes but candidate tumor suppressor genes belonging to these regions have been detected in only subset [18]. Loss of heterozygosity is considered as one of two 'hits' necessary to inactivate tumor suppressor gene such as 9p21 for p14ARF, 13q14, p16INK4a for RB, multiple loci of 3p for RASSF1A, FHIT, or unidentified genes, and 17p for p53. About 40% of NSCLC patients face abnormal transcription of FHIT gene that plays an important role in proapoptic signaling. This gene is present on 3p14.2 and cloned by positional cloning in 1992 [19]. Abberant transcription or no transcription of DLC1 gene (deleted in lung cancer 1) was observed in 10% and 27% of NSCLC patients respectively [20]. Other tumor suppressor genes p34 and CYGB have been rendered responsible for lung cancer. Cell line studies revealed many specific genetic alterations that occur in NSCLC and SCLC [21]. Novel proto-oncogene (*NK2 homeobox 1* or *NKX2-1*) detected in amplified region of chromosome 14q13.3 has been observed in about 12% of lung cancer samples [22].

Genome Instability

Another basic characteristic of cancer progression and cancer is genome instability. However, mechanism leading to instability, rate of instability, and time of instability is not completely understood. Different pathways cause instability. Microsatellite instability occurred as a result of mismatch repair deficiency and is observed in small fraction of lung tumors whereas, aneuploidy is observed in tumors. Appearance of aneusomy, loss of regulation of cell proliferation and apoptotic control, accumulation of mutations are associated with dysplasia phenotype. Specific defects in DNA repair may also cause lung cancer. Mutated DNA repair genes XPD (codon 312 Asp/Asp vs Asp/Asn) have been found to cause impairment of DNA repair efficiency and apoptotic function in lung cancer [23]. New techniques enable scientists to understand changes in small numbers of preneoplastic cells and individuals. FISH probes are used to assess copy number changes in single cells. Microdissection of dysplastic epithelium have allowed analysis of microsatellite instability, chromosomal deletions, DNA methylation patterns, and point mutations [24].

Epigenetics of lung cancer

In case of lung cancer, tumor suppressor genes can easily be silenced by DNA methylation of promoter sequence and this epigenetic change works in coordination with histone tail modification that changes the chromatin condensation status. Besides SNPs or chromosomal changes, aberrant DNA methylation inactivates tumor suppressor genes as demonstrated in Knudson's two hit hypothesis. Several different aberrantly methylated genes are known to cause lung cancer. Protein complexes which contain histone deacetylases and CpG-binding proteins as eminent components can easily bind methylated promoters and trigger deacetylation of histones which lead to production of transcription-repressing chromatin [25, 26]. To achieve re-expression in *in vitro* experiments, DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine or specific inhibitor of histone deacetylases, trichostatin A (TSA) is added. Promoter hypermethylation also play an active role in lung cancer [27].

Aberrant promoter methylation of another tumor suppressor gene p16 has also been studied. Other *examples are* death-associated protein (DAP) kinase *1 (*DAPK1*)* [28], H-cadherin [29], 14-3-3 sigma [30], and *tumor suppressor gene* RASSF1A [31]. According to *Zochbauer-Muller* et al*. s*everal genes such as tissue inhibitor of metalloproteinase 3 *(*TIMP3*),* retinoic acid receptor β-2 *(*RARβ*),* glutathione S-transferase P1 *(*GSTP1*),* p14ARF, E-cadherin *(*ECAD*),* DAPK1, and retinoic acid receptor β-2 *(*RARβ*) were methylated in 107 primary NSCLC* [32].

DNA methylation events also act as diagnostic and molecular marker of tumors e.g, p16 promoter methylation acts as biomarker of lung cancer. PCR-based methylation analysis reveals methylation of MGMT or p16 promoter in sputum of smoker with squamous cell lung carcinoma. Many other epigenetic biomarkers for lung cancer are currently under investigation [33].

Gene silencing of *SEMA3B, FUS1, FHIT, C/EBPα, RIZ1 RASSF1A,* has been identified in lung tumors. According to Palmisano *et al.* the promoter methylation of MGMT or p16 detects development of squamous cell carcinoma about three years earlier than clinical diagnosis [34].

Novel genes responsible for lung cancer that have been reported recently are present on chromosome 5p15.33, 6p21, 6q23-25, 13q31.3and 15q24-25.1. There are three nicotine acetylcholine receptor subunit genes present in 15q25 region and their polymorphisms have been associated with nicotine dependence. Chromosomal region 5p15.33 has been linked with lung adenocarcinoma [35]. The future of lung cancer related research is based on integrative oncology (Figure 2).

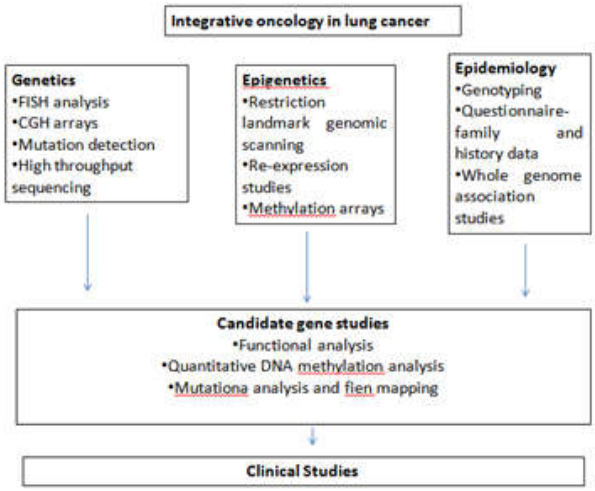


Fig 2: Employing epigenetics, genetics and epidemiology in an integrative oncology approach will develop understanding towards lung carcinogenesis and form the basis of identification of candidate gene loci for future research.

Diagnosis

As first investigative step Chest Radiograph is performed for early detection cancer of lungs, consequently mass of cells of varied thickness, widening of mediastinum (spread to lymph nodes), atelectasis (collapse), consolidation (pneumonia) [36] may reveals, CT imaging gives exact information about type and spread of disease. Bronchoscopy is used for sampling the tumor for Histopathology [37]. There are some techniques that can be used for diagnosis of lung cancer:

- **Computed Tomography (CT) Scan**
  A usual X-ray and a longer X-ray called a Computed tomography (CT) scan. It is performed to create a picture of our lungs to reveal is there any part of our lungs that do not seem to be normal.
- **Biopsy**
  For the detection of lung cancer the tissues of lungs are taken by different techniques (as it depends upon the part of our lung the biopsy is taken from). After observing under microscope results are drawn [38].
- **Percutaneous fine-needle aspiration (FNA) biopsy**
  To remove small fragments of lung tissue a needle is guided through our chest cavity by Ultrasound or CT scan. For this type of diagnosis overnight stay is expected and is usually done when edges of lungs are affected.
- **Core biopsy**
  Core biopsy doesn't require surgical incision so a larger needle is used to remove tissue larger than FNA biopsies. The drawback of biopsy (especially FNA biopsy) is that some time the amount is not sufficient for diagnosis the DNA changes (for choosing anticancer drugs).
- **Transthoracic needle biopsy**
  A small area is numbed with anesthesia and needle is inserted and guided through skin on chest wall. Lungs are observed by fluoroscopy (a type of X-ray that generate a moving image) or CT scan. The problem of this procedure is leakage of air in space between lungs and chest wall (pneumothorax) cause breathing problem. Small air leak heals automatically but large heal is treated by putting tube in chest space to draw air for one or more day then it heals.
- **Bronchoscopy**
  Firstly numbing medicine is sprayed for feeling relaxed then a thin optic tube (bronchoscope) is inserted in lungs via mouth or nose by trained nurse to take biopsy samples, this uncomfortable procedure takes hardly 20 minutes [39]. Bronchoscopy is done if the inner part of our lung is affected or tumor is suspected in larger airways. Sampling can also be done by bronchial brushing or washing and observed under microscope.
- **Sputum cytology**
  To find the cancer in airways such as squamous cell lung cancer, sputum sample is taken and observed under microscope to observe if there are cancer cells. This test is not supportive for finding types of non-small lung cancer.

- **Thoracentesis**
  This test is performed if it is suspected that cancer spread to lining of lungs (fluid form around lungs i.e. pleural effusion). As the skin is numbed the fluid or tissue sample is taken by inserting a hollow needle between ribs called Thoracoscopy. A chemical test of the fluids to reveals the malignant (cancerous) pleural effusion. The accumulation of fluid result in keeping the lungs from filling with air, so thoracentesis can assist a person breathe better. The fluid formation might also be caused by heart failure or an infection.

Spread of cancer in chest?

If cancer spread to other parts of body it affect the treatment of lung cancer so some test are performed to test its spread are:

- **Endobronchial ultrasound**

An ultrasound transducer (Generate sound waves and picks those echoes that spring back off body tissues) is fixed with a bronchoscope at its tip and moved into the windpipe the help of numbing medicine (local anesthesia) and slight sedation. To view the lymph nodes transducer is pointed in different orders and if there is an enlarged lymph node a hollow needle is moved through the bronchoscope and biopsy is done for examination.

- **Endoscopic esophageal ultrasound**

Just like Endobronchial ultrasound, except endoscope (flexible scope) inserted downwards throat and then into the esophagus and biopsy of enlarged lymph nodes is done.

- **Mediastinoscopy and Mediastinotomy**

These kinds of actions are performed to view the samples from the structures in the mediastinum (the part among the lungs). The size of the incision and locality is main difference in these procedures.

- **Mediastinoscopy:**

By giving general anesthetic a tube is inserted surgically through a cut over breast bone to see the space between the lymph nodes and lungs near it.

- **Mediastinotomy:**

A large incision (i.e, 2 inches long) is made next to the breast bone between the second and third ribs on left side. This help the surgeon to reach those lymph nodes that can't be reached by mediastinoscopy.

- **Blood tests**

Blood tests are not sufficient to diagnose lung cancer, like **CBC** that determines either our blood has normal numbers of various types of blood cells. Blood forming cells in bone marrow are affected by chemotherapy. **Blood chemistry tests** mark malfunctioning in liver or kidneys. For instance, if cancer has spread to the liver and bones, high level of lactate dehydrogenase (LDH) is observed.

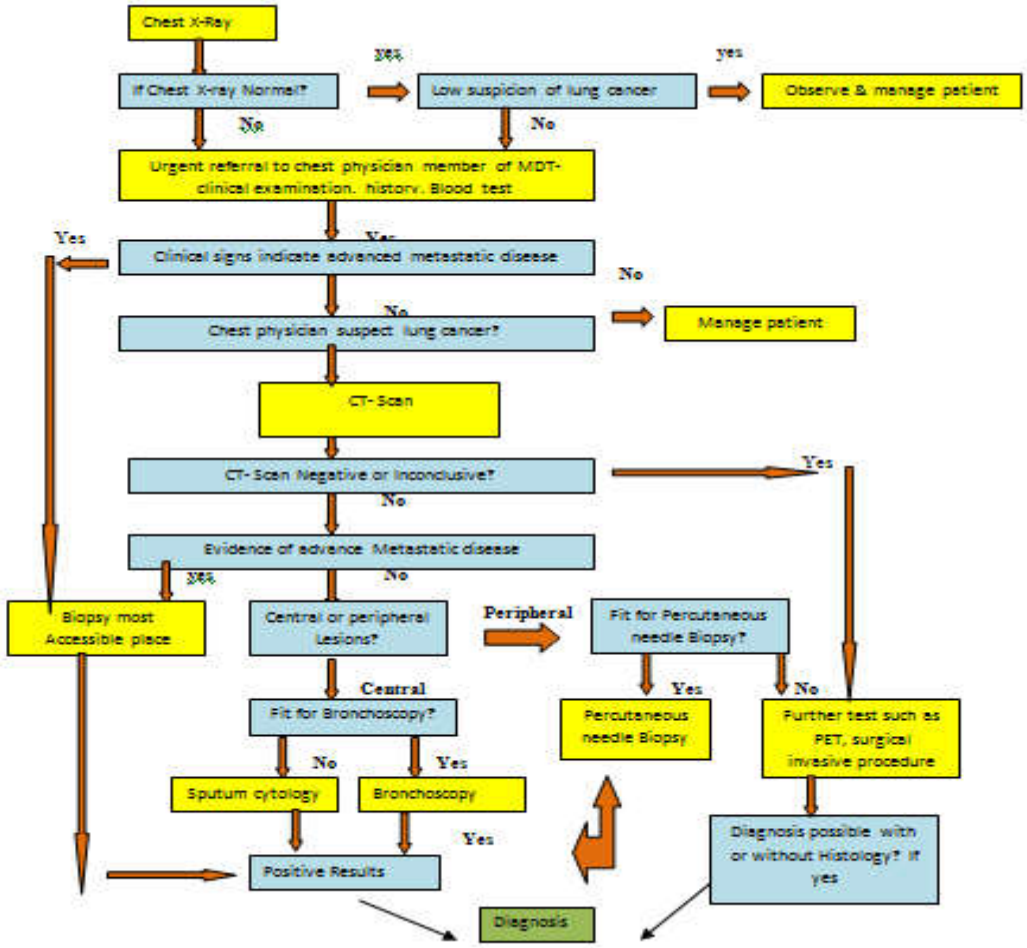
- **Pulmonary function tests**

If lung cancer is diagnosed pulmonary function tests (PFTs) are performed to observe the working of lungs, in a case if surgery is only option in treating cancer. Even people with poor lung function (smokers) can't survive removing even part of a lung; it actually gives a clue that how much part of lung can easily be removed.Sometimes a test known as arterial blood gas is coupled with PFTs. In order to perform it, blood is taken from an artery (not vein, like in other blood tests) to assess the amount of oxygen and carbon dioxide that it contains. After confirmation of cancer cells **staging of lung cancer** is done to choose treatment, and for this different test are performed [40-42] (**Figure. 3).**

Table1. Staging of lung cancer

CT scan	X-rays are use to create a picture of our lungs. It indicates the location of cancer.
Ultrasound	To create a picture of our body for detecting location of cancer sound waves are used.
Magnetic resonance imaging (MRI)	This kind of scanning includes magnetism to reveal the tumor location.
Bone scan	If harmless radioactive material is injected in our blood. They tends to assemble in an area where the cancer is, and detected by our camera.
Positron Emission Tomography (PET)	To trace the tumor location minute amount of radioactive drug is injected into blood.
Thoracoscopy	In order to take a view inside chest for cancer detection a thin tube with camera is used.

Fig: 3 Access to Diagnosis [42]



Treatment of Lung Cancer

According to cancer.org, several kinds of treatments are available for lung cancer; some of them have been in medical practice for years while some of them are still in clinical trials. The usual treatments include surgery, chemotherapy, radiation therapy, immune therapy or a combination of these as maintenance therapy. Latest treatments include targeted therapy, drugs and vaccines which are vigorously researched upon in labs and tested in clinical trials.

Targeted therapy

In lung adenocarcinomas, several somatic mutations have been sequenced in epidermal growth factor receptor EGFR tyrosine kinase gene. Such mutations have been discovered in about 20% of Caucasians and 50% of East Asians suffering from adenocarcinomas in lung. Based on these mutations, drugs have been designed to specifically target the mutated regions. Some of the mutations are uncommon in different populations which cannot be targeted easily but some mutations are highly common in lung cancer patients of different populations such as deletion of Exon 19 (Del 19) or L858R. Tyrosine kinase inhibitors (TKI) account for the targeted drugs such as erlotinib, afatinib or gefitinib that specifically target the mutated EGFR receptor tyrosine kinase. All these targeted therapy drugs have shown approximately 60% of objective response rates (ORR) in patients which is quite high. But the mutations vary in their response to drugs. The choice of most appropriate TKI drug depends on the response of the mutations whether it is sensitive or resistant to it. DEL 19 as many studies indicated has shown to be sensitive as compared to L858R mutation. Certain other mutations like L861Q, S768I, G719X , E709K, Del 18 and exon 19 insertion (Ins19) have shown ORR of 30 to 50% (moderate sensitivity) to erlotin or gefitinib. Certain others such as Ins 20 mutations are considered to be fairy resistant but osimertinib may show an effect on a few rare subtypes of these resistant mutations and nazartinib brings a promising response to a majority of these. [43]

In addition, anti EGFR monoclonal antibodies are also available. They serve as an alternative to inhibit the EGFR activation and signaling. These can act via two pathways. Either they act as competitive inhibitors to ligands that bind to activate the EGFR receptors or they form a receptor-monoclonal antibody complexes that get endocytosed and degraded. In this way, they inhibit the tyrosine kinase receptor

signaling. Some of the mAbs available are matuzumab, necitumumab, panitumumab and cetuximab. Phase II trials have been carried out on NSCLC patients in which mAbs are given to patients in combination with a chemo drug. Cetuximab, given in combination with platinum doublet chemo therapy in phase II trial showed did show a marginal increase in overall survival by 11.3 months as compared to 10.1 months by chemo alone. But in another BMS099 trial, it failed to show results. The phase II trials are yet still being carried out on other anti-EGFR mAbs [44].

Other than EGFR mutations, other mutations can be targeted as well. Genes of ALK kinase and EML 4 rearrange and fuse to from an addictive oncogene that contributes to progression of about 4% NSCLC. Crizotinib was the first generation drug, approved for use in 2011, that specifically targeted this fusion and resulted in considerable tumor regression and improved the quality of life. But the multiple mutations in ALK gene overtime resulted in buildup of resistance against Crizotinib. This problem can fortunately be overcome by the use of second generation ALK inhibitors Alectinib and Ceritinib although these are still in clinical trials but have shown effective treatment to crizotinib resistant tumors. The issue of mutations evolving may still pose resistance to even the second generation drugs. These drugs may be given to patients on a biomarker based approach [45].

Immune Therapy

Although lung cancer does not involve immunogenic malignancy yet high amount of $CD4^{+ve}$ and $CD8^{+ve}$ T cells during early stage and large number of macrophages $CD8^{+ve}$ T cells encountered in tumors nest at stage IV suggest that immune system can be manipulated to generate anti-tumor effects. There can be three possibilities by which immune therapy can be carried out. First approach is to use the tumor specific monoclonal antibodies. Second therapeutic approach is cancer vaccination in which specific tumor antigens can be used to induce the production of antibodies by host, Third approach is non-antigenic specific modulation in which immune system can be triggered by immunomodulatory agents such as modulating the T cell activity by mAbs.

Previously interleukins and interferons were used in cancer among the immune modulators, but were not much effective in lung cancer. But research is going on and some of the clinical trials have also been carried out. More recently, two Phase III trials were carried out with ProMune® (PF-3512676). This works similarly to TLR9 in human body that improves the maturation of dendritic cells. Unfortunately, the trial showed negative results. Talactoferrin alpha is another example of an oral immune-modulatory agent that was tested in pphase II and phase III clinical trials. It was a recombinant human lactoferrin that played its role in recruitment and activation of dendritic cells in gut associated lymphoid tissue. Results were identical to that of placebo in lung cancer patients. Its effectiveness is still being investigated by administering it to patients along with paclitaxel and carboplatin.

Monoclonal antibodies against certain immune system targets have also been developed. CTLA-4 is an immune modulator in immune system that negatively regulates the T cell mediated responses by interaction with its ligands CD80/CD86. Anti-CTLA-4 antibody (Ipilimumab) is a humanized IgG1 monoclonal antibody that has been designed to prevent the interaction of CTLA-4 with its ligands so to block the inhibitory signal that negatively regulates the T cell mediated response. Once the signal is blocked, it may lead to enhance the activation and proliferation of tumor specific T cells accounting for an effective anti-tumor response. Currently, Ipilimumab is undergoing Phase III trials in combination with chemo-therapy drugs.

Another immune system target is PD-1 which is a T cell surface receptor, a member of B7-CD28 superfamily. However it can also be expressed on B cells, natural killer cells, dendritic cells and activated monocytes and interacts with two common ligands PD-L1 (B7-H1) and PD-L2 (B7-DC). Normally PD-1 interacts with its ligands in case of an inflammatory response that may result in autoimmunity. Its role is to reduce the activity of T cells in peripheral tissues. However in case of tumor cells, $CD8^{+ve}$T lymphocytes are required for killing tumor cells while activation of PD-1 impeded the effective anti-tumor response. Several humanized monoclonal antibodies have been designed to target PD-1such as MK-3475 (humanized IgG4), CT-011 (humanized IgG1) etc. BMS-936558 (Nivolumab) is a fully human IgG4 mAb designed to target that has been investigated in trials. European Society of Medical Oncology Annual Meeting in 2012 reported that tumor responses were evaluated at all three dose levels and response rates were 6, 27 and 17% respectively. [46]

On the contrary, it is also possible to target certain immune system receptors by designing a mimic of their actual ligands. IL-11 receptor (IL-11 R) has found to be expressed more in cytoplasm of tumor cells as compared to non-malignant cells in patients. For this purpose, [47] isolated a peptide motif CGRRAGGSC that mimics a motif of IL 11 and showed the ability to bind to IL-11R in-vitro as well as to IL-11R expressing tumors in-vivo. This synthetic peptide ligand led to the design of a potential drug candidate BMPT-11 that can target the tumor moieties. Currently, BMPT11 is in clinical trials. BMPT-11 showed in vitro cell death in human lung cancer cell lines, growth attenuation of subcutaneous xenografts and

reduction in number of pulmonary tumors after injection of human lung cancer cells in mice. The drug can show its potential in patient derived tumors and preclinical lung cancer models.

Vaccines

Due to metastasis in lung cancer, there was a need of more direct molecular targeting therapies. The human lung adenocarcinoma cells over express a protein called surfactant protein B (SP-B). Lung adenocarcinoma cells have also been shown to be susceptible to the infection by the JC-polyomavirus (JCPyV). [48] designed the JCPyV virus like particles VLP's a molecular targeted gene therapy. Within these VLP's, they introduced SP-B promoter–driven thymidine kinase suicide gene (pSPB-tk). First they designed plasmid as vector constructs consisting of SP-B promotor with either Green fluorescent protein (pSPB-gfp) or thymidine kinase gene (pSPB-tk). At first, the lung carcinoma cell lines (A549, CH27, and H460) and non-lung cancer cell lines were transfected with this plasmid in order to determine the tissue specificity of the promotor. The GFP protein expression of the designed plasmid was successfully observed after transfection in H460 and A549 cells. The GFP reporter gene was successfully delivered via CPyV VLPs in A549 cell. As far is cytotoxicity of these suicide gene packaged VLP's is concerned, these proved to be cytotoxic in A549 carcinoma cell line but not in non-lung cells. Mice injected with pSPB-tk–carrying JCPyV VLPs followed by treatment of ganciclovir drug, showed 80% growth inhibition of lung carcinoma nodules of humans. This is a gene therapy treatments that holds potential for treatment during upcoming years.

Vaccines may either be full protein vaccines, peptide vaccines, viral vector vaccines, ganglioside vaccines, full tumor vaccines, recombinant vaccines etc.

MAGE-A3 is exclusively expressed on surface of tumor cells. Its function is yet unknown but it contributes to prognosis of lung cancer. MAGE-A3 expression has been reported in 35% of early stage and 55% of late stage NSCLC patients. The vaccine design is based on a recombinant fusion protein created by fusion of MAGE-A3 with protein D isolated from Haemophilus influenza. The vaccine is given in combination with an adjuvant for enhanced immunogenicity. High therapy compliance with well tolerated treatment was reported in MAGE-A3 phase II randomized clinical trials.[46]

TG4010 is a viral vector vaccine that targets MUC 1. MUC 1 is expressed on apical surfaces of epithelial cells as a glycosylated transmembrane protein but in cancerous cells, MUC1 is overly expressed due to which it is aberrantly glycosylated. Its peptide epitopes are an ideal target for immunotherapy. MUC 1 has oncogenic properties such as resistance to chemotherapy, suppression of immune response, impaired apoptosis etc. TG4010 was designed by attenuating Ankara virus and genetically modifying it to express MUC 1 and IL-2 as an immune adjuvant so that it reverses the suppression of T cell response caused by tumor associated MUC 1 as a counter effect. TG4010 is still under clinical trials and did not show a significant difference due to different reasons. Its side effects were mild such as fever, abdominal pain and reactions at injection sites. [46]

L-BLP25 is a peptide based vaccine (Stimuvax®) that is a 25 amino acid sequence derived from MUC 1 protein conjugated with monophosphoryl lipid A as an adjuvant. The vaccine has a liposome based delivery mechanism. It showed a good safety profile and showed some benefits in patients receiving chemotherapy and radiotherapy. Based on these findings, a large Phase III trial was initiated at international level. It did not reach its objective but some treatment effects were observed to some extent. Currently, INSPIRE, a phase III trial is running on stage III NSCLC Asian patients [46].

Epidermal growth factor receptor (EGFR) is a target validated for drugs and vaccines because EGFR strongly contributes to progression and angiogenesis of non-small cell lung cancer. More recently, a recombinant vaccine has been designed that targets EGFR and stimulates the production of antibodies that block the interaction between EGF and EGFR. It is called CIMAvax-EGF, It consists of a recombinant EGF molecule that is conjugated to Montanide ISA51 as an adjuvant and a carrier protein. Its overall safety, survival and immunogenicity were tested in phase III trials and it did show increased MST in patients and was well tolerated by them [49].

To design peptide based vaccines, it is essential to have immunogenic peptides that could be recognized as non self by patient's immune system. More recently, neo antigens were discovered as a new class of tumor associated antigens (TAA) that could be used as vaccines but neo antigens vary from patient to patient and considered non self by immune system. Therefore, optimized cryptic peptides that resemble neo antigens have been used in vaccine design. Optimized cryptic peptides are recognized as non self antigens because they escape self tolerance and also highly immunogenic because their sequence has been optimized to increase the affinity to be recognized by HLA molecules. Vx-001 is the first vaccine based on the optimized cryptic peptide approach that has been designed to target tumor antigen telomerase reverse transcriptase (TERT) that is widely expressed in patients. Vx-001 has completed its phase I clinical trial and is now being tested in phase II clinical trial in a randomized fashion in non-small cell lung carcinoma patients [50].

NY-ESO1 is a potential cancerous antigen which could be targeted for a vaccine design and the same vaccine could be used to stimulate immune responses against others antigens as well due to epitome spreading. Based on this idea,[51] developed dendritic cells (DC) based vaccine in which DCs were sensitized to NY-ESO1. The trials showed the induction of an efficient anti-tumor response by NY-ESO1 antigenic peptide sensitized DC vaccines as it triggered the activation of antigen specific cytotoxic T cells and the Natural Killer subgroup with cytotoxicity and prolong PFS of NSCLC patients.

Racotumomab is an anti-idiotype murine IgG1, previously also known as 1E10 mAb that was designed to target the glycol-conjugated molecules that express on membranes of solid tumors. It was originally designed to be a mirror image of idiotype of another antibody that targeted the NeuGcGM3 ganglioside and other N-glycolyl-containing molecules. It showed a successful phase I/phase II trial and was conditionally approved in Latin American countries to be used in combination with chemo therapy and other traditional therapies. It is a ganglioside vaccine. Gangliosides are expressed on surface of tumor cells and involved in cell differentiation, matrix adhesion and cell-cell recognition [51].

CONCLUSION

Lung cancer is the leading death causing cancer all over the world. Other than smoke induced lung cancer, it may also be caused due to complex genetic mutations and epigenetic variations. Advances in molecular biology have enabled scientists to identify many such underlying mutations at genetic and epigenetic level which could be diagnosed and targeted for therapy. With real time tumor imaging and use of biomarkers, diagnosis of lung carcinoma has improved to a great extent. Due to current knowledge of disease genetics and extensive research facilities, scientists have been able to develop novel treatment strategies for lung cancer, in particular, drugs that specifically target only the tumors without harming normal cells. The list is increasing and beyond the scope of this review. Several trials have been conducted and yet others are still going on. More recently, nanoparticle based drug delivery systems have been developed that will take this success one step ahead and will show effective results in treatment of non-small cell lung carcinoma.

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