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Trends in Molecular target therapy for Lung Cancer

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1 Introduction

Lung cancer, a refractory cancer, is the most common cause of cancer-related deaths in Japan and developed Western nations, accounts for approximately 20% of overall cancer-related deaths, and has a low 5-year relative survival rate[NOTE 1] of approximately 30% (Figure 1).[1] This is due to the difficulty of early detection and lack of well-established treatment methods that would enable a drastic improvement in the cure rate. Early detection combined with surgical treatment is the classic requirement for it to be cured. However, since lung cancer is often already inoperable and progressive by the time of its detection, up until now, its treatment has been no more than life extension and symptom relief.

With recent progress in molecular biology, the understanding of various disease mechanisms of humans at cellular and molecular levels has improved, resulting in a number of reports identifying molecules involved in the onset and progression of diseases. Accumulated knowledge on various biological molecules and mechanisms involved in the development and progression of cancer is being effectively used for the development of cancer treatments. In particular, in a recent achievement of research and development on molecular target therapy, treatment by blocking the function of targeted biological molecules involved in the abovementioned cancer, has been dramatic. It is hoped that molecular target therapy will become an effective method to treat refractory cancers such as progressive cancer and lung cancer, which are untreatable with traditional anticancer drugs, and that it will bring about personalized medicine, providing the best treatment for individual patients.[NOTE 2]

Here, I will introduce the trends in molecular target therapy, focusing on the treatment for lung cancer. First, I will discuss the importance of lung cancer treatment for Japanese and international healthcare from the epidemiological perspective. Then, I will introduce the status of molecular targeting treatment within overall cancer treatment as well as within lung cancer treatment. Finally I will exemplify the discovery and clinical application of therapeutic target gene *EML4-ALK* for lung cancer, discovered by Dr. Hiroyuki Mano of the University of Tokyo/Jichi Medical University, as a significant achievement of recent years.

2 The importance of lung cancer treatment – an epidemiological perspective

According to an estimation by the World Health Organization (WHO), in 2007, 7.9 million people died of cancer, representing approximately 13% of the total

[NOTE 1] : 5-year relative survival rate

The index of the proportion of people diagnosed with cancer who survive 5 years compared to the proportion of the Japanese population in general who are still alive in 5 years. The general Japanese population in this case indicates the Japanese population adjusted for the distribution of gender, birth year, and age.

[NOTE 2] : Personalized medicine

Treatment planning selecting for the most effective drugs, doses, and administration methods with the fewest side effects based on an investigation of an individual patient's physical characteristics and its relationship to the disease based on genetic testing etc. Since molecular target therapy is provided based on information on genetic abnormalities and excessive expression of its products, it leads to personalized medicine (mentioned later in 3-2).

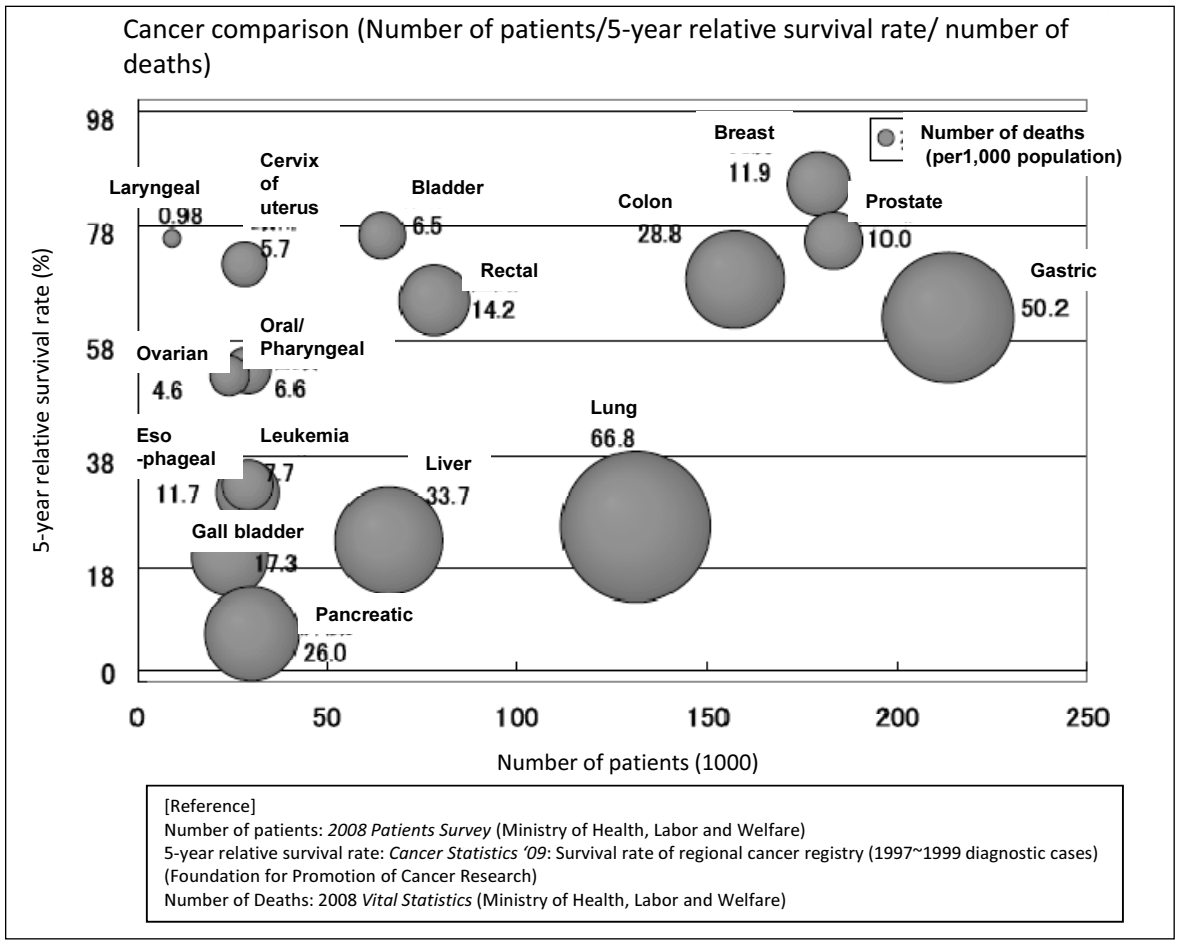


Figure 1 : The number of cancer patients and the survival rate
Source: Cabinet Office, the Second Life Innovation Task Force Data Collection[1]

deaths around the world and confirming cancer as a major cause of death worldwide. In particular, lung cancer contributes to a significant portion of cancer-related deaths. The American Cancer Society's (ACS) *Global Cancer Facts & Figures* 2007 estimated that approximately 975,000 males and 376,000 females died of lung cancer in 2007, and reported that lung cancer was the leading cause of death in men and 2nd leading cause of death in women among all cancer-related deaths.

As for regional factors, according to the ACS, lung cancer is the most common cause of cancer-related death in Central, North, and South Americas, European nations, and Australia. In addition, lung cancer is the most fatal cancer in Asia outside of the South and Central regions. Specifically, taking the U.S. as an example, the U.S. Centers for Disease Control and Prevention (CDC) reported that 89,243 males and 69,356 females died of lung cancer in 2006.

Also in Japan, lung cancer is a significant cause of death. Since cancer replaced cerebral vascular diseases and cardiac diseases as the most common cause of death in 1981, the number of cancer-related death keeps increasing. Within these cancer-related deaths, lung cancer has been the most common since it replaced gastric cancer in 1998. In 2008, 342,963 people died of cancer, within which, 66,849 people, 19.5% of overall cancer-related deaths, died of lung cancer.[2]

Changes in the mortality rate, excluding the effect of age-distribution changes (the age-adjusted death rate[NOTE 3]), show that the overall death rate from cancer is decreasing in Japan. However, the death rate from lung cancer has remained constant (Figure 2). In addition, there is a gender difference in the death rate of lung cancer, which is higher in males by a magnitude of 3 to 4 times.

To summarize, since lung cancer causes a significant

[NOTE 3] : Age-adjusted death rate
Death rate calculated with a standardized population to exclude the effects of the population's age structure. In Japan, the "1985 model population" is often used as the standardized population.

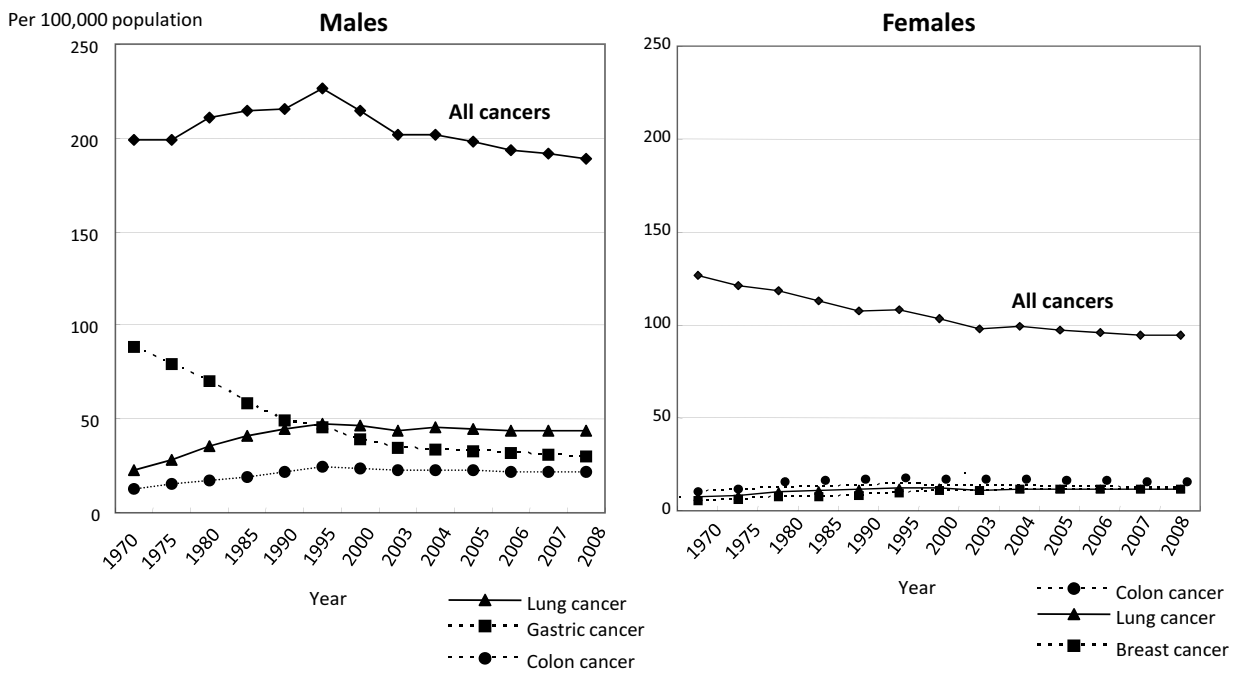


Figure 2 : Transitions of Age-Adjusted Cancer Deaths in Japan
Produced by the STFC based on reference[2]

number of deaths worldwide, the development of effective therapeutic methods to treat lung cancer would be beneficial to Japanese as well as global healthcare.

3 Characteristics of molecular target therapy and its status within cancer treatment

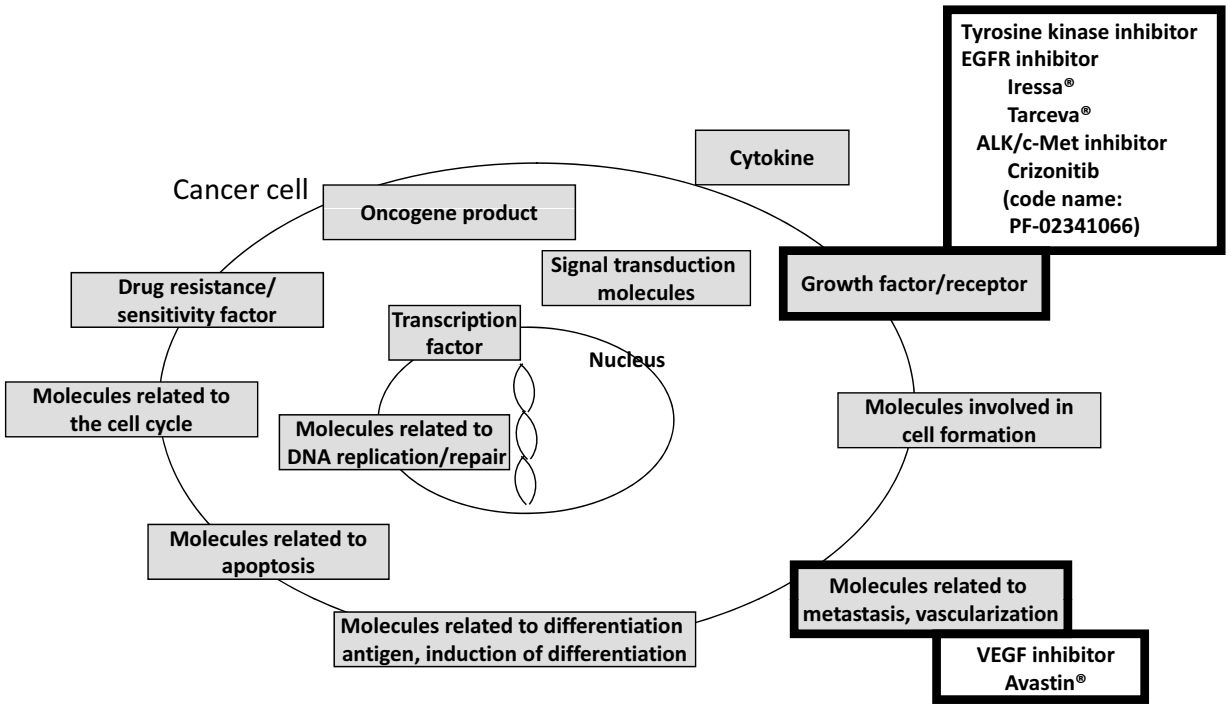
3-1 Characteristics of molecular target therapy

Molecular target therapy aims to improve symptoms and to heal the disease by suppressing specific molecules involved in the development and progression of the disease. It uses medication – molecular target drugs – designed or selected to suppress/ block the functions of specific molecules. Therefore, molecular target drugs are developed by targeting specific molecules based on the assumption that the suppression of those specific molecules will treat the disease. Target molecules of the drug can be a single molecule or a group of molecules with similar molecular structures, and the drugs targeting the latter are called multi-target drugs.

Molecular target drugs include low molecular drugs targeting molecules inside the cell, and antibody drugs targeting protein and/or sugar chains on the cell surface. These drugs are indicated for a wide range of diseases. For example, antibody drugs on the market or in post-phase III clinical trials have a variety of indications for autoimmune disease such as rheumatism, cancer and related diseases, cardiovascular diseases, infectious diseases, neurological diseases, asthma, and osteoporosis.[3]

3-2 Status within cancer treatment

Cancer therapy consists of three major types of treatments: surgical and radiation therapy as a local treatment, chemotherapy as a systemic treatment, and a combination of these treatments, multi-modality therapy, is the common treatment of cancer clinically. In addition to these three major types of treatment, immunological therapy and gene therapy have been developing in recent years and their clinical application is progressing. Each therapy is selected based on the type of cancer (which organ is affected), the stage of cancer (cancer progression), and the histological type (pathological category of cancer), as well as the patient's medical history and general status. Refer to the report on Science & Technology Trends by Dr. Shoji et al. for details on cancer treatment.[4]



EGFR: Epithelial growth factor receptor (tyrosine kinase), VEGF: Vascular epithelial growth factor, ALK/c-Met: Anaplastic lymphoma kinase (tyrosine kinase)/ hepatocyte growth factor receptor (tyrosine kinase)
This is a simplified image, and the positions of each molecule do not reflect their actual site of expression.
Bold frames show drugs introduced in this report.

Figure 3 : Examples of therapeutic target molecules and molecular target drugs for cancer

Produced by the STFC based on reference[5]

Many of traditional anticancer drugs are cytotoxic, and treatment that uses medication is termed chemotherapy. Molecular target therapy is categorized as chemotherapy for its use of medication.

Molecular target therapy for cancer uses molecular target drugs that suppress/ block the function of biological molecules involved in the pathogenesis, progress, and metastasis of cancer. Figure 3 shows candidate biological molecules that are the targets of cancer treatment.[5] Target molecules in cancer treatment varies widely, in other words, cancer pathogenesis and malignant alteration are induced by a complex molecular mechanism, which makes up the "character" of an individual cancer. Therefore, this treatment requires a diagnosis of this character to see if medication targeting specific molecules will be effective. To achieve this, patients need to be clinically screened for abnormalities in the biological molecules involved in cancer or in the genes coding these molecules before treatment is provided. Because of this, molecular target therapy is expected to progress into personalized medicine.

The status of molecular target therapy within cancer treatment becomes clearer when the side effects of molecular target drugs and traditional cytotoxic drugs are compared. General cytotoxic agents kill cancer cells by blocking DNA synthesis or cell division in cells with frequently repeated cell divisions. Since the drug attacks healthy cells as well as cancer cells, continuous treatment often becomes difficult due to a variety of side effects such as the loss of hair, nausea, vomiting, gastrointestinal tract disturbances, and hematotoxicity. However, since molecular target drugs only suppress/block the activity of specific molecules, it is believed that the side effects, like ones caused by cytotoxic drugs, can be reduced. However, the risk of new side effects have been reported with the progress of its clinical use. Gefitinib (Iressa®), a molecular target drug for lung cancer has been reported to have caused severe lung disorder (mentioned in 4-3).

4 Trends in molecular targeting treatment in lung cancer

In this chapter, I will describe molecular target therapy specific to lung cancer.

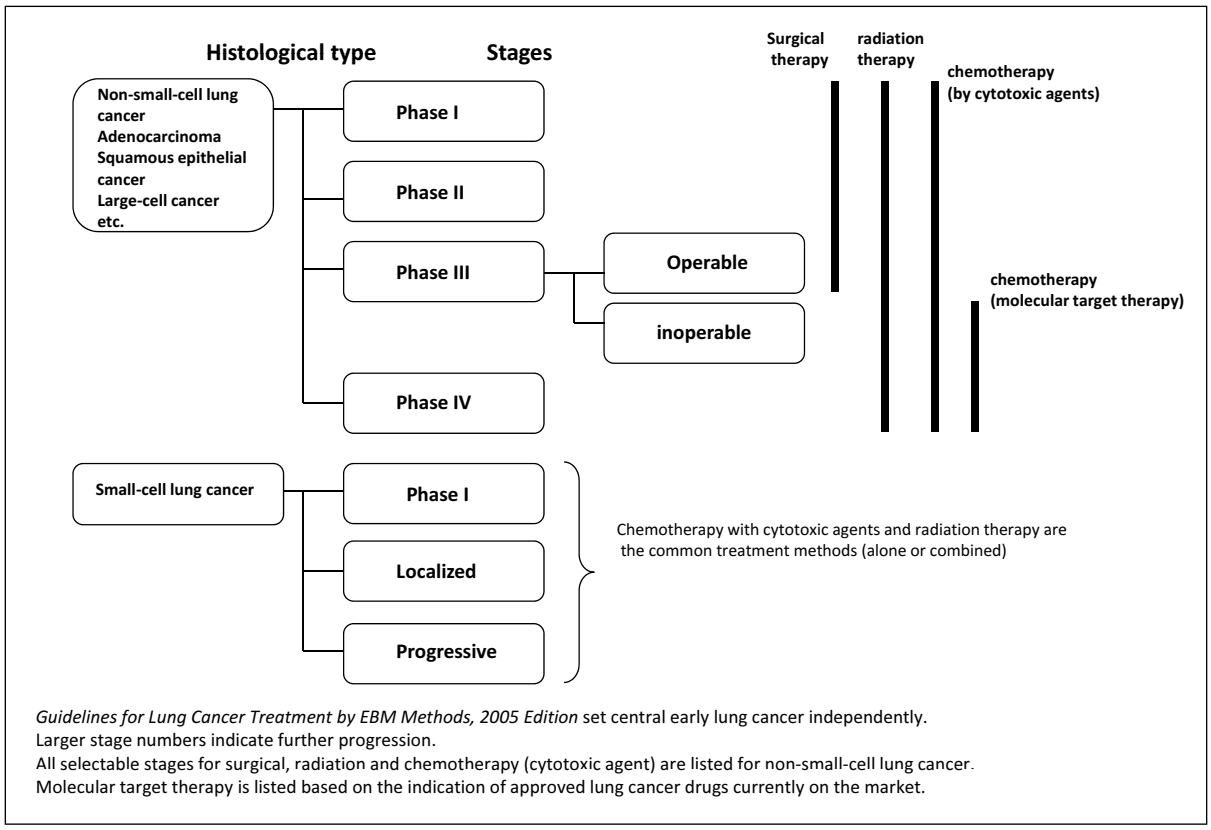


Figure 4 : The relationship between the histological type and stage of lung cancer indicated *in Guidelines for Lung Cancer Diagnosis* (excluding recurrence)

Produced by the STFC based on reference[5]

4-1 General Treatment Strategy for Lung Cancer

As with other cancer treatments, the basic direction of lung cancer treatment is selected based on the histological type and stage of cancer progression. In particular, the two histological types, small-cell lung cancer and non-small-cell lung cancer, have been important factors in the treatment protocol for lung cancer. Histological type can be determined by histopathological examination of expectorated sputum or cells obtained from the focus in the lung. Small-cell lung cancer is named after the small size of cancer cells compared to the cells of other types of lung cancer. Non-small-cell lung cancer indicates lung cancer other than small-cell lung cancer, and is further categorized to adenocarcinoma, which frequently occur in the peripheral of the lungs, squemous cell cancer, which frequently occurs near where bronchia enters the lungs and large-cell cancer, which proliferate rapidly resulting in a large cancer by the time of detection. The latter, non-small-cell lung cancer takes up 85~90% of total lung cancer in Japan. In addition to small-cell lung cancer and non-small-cell lung cancer categorization, the stage of lung cancer is examined before selecting the treatment methods.

Treatment of lung cancer is indicated in *Guidelines for Lung Cancer Treatment by EBM Methods, 2005 Edition* published by the Japan Lung Cancer Society,[6] an NPO. These guidelines indicate the principles of Evidence Based Medicine (EBM) and standard lung cancer treatment methods. The relationship between the histological type and the stage of lung cancer is indicated in Figure 4. Therefore, surgical treatment, radiotherapy, and chemotherapy are not set up on one-on-one basis with each histology-stage relationship but rather, with multiple methods and multiple chemotherapy agents.

Currently, molecular target therapy is practiced only in non-small-cell lung cancer (Figure 4). The three molecular target drugs for lung cancer that have been approved in Japan have indications for inoperable (Stage III~IV in Figure 4) or recurrent non-small-cell lung cancer. On the other hand, molecular target therapy is not practiced for small-cell lung cancer due to lack of effective molecular target drugs. For small-cell lung cancer, chemotherapy using traditional cytotoxic drugs and radiotherapy are used as effective methods.

4-2 Characteristics and Uses of Molecular target drugs for Lung Cancer

Here, I will discuss molecular target therapy for non-small-cell cancer, focusing on the characteristics and the uses of molecular target drugs used for general practice in Japan.

There are three drugs for lung cancer treatment with sales approval in Japan; gefinitib (Iressa®), which was approved in Japan before any other countries in July 2002, erlotinib (Tarceva®), which was approved in October 2007 and bevacizumab (Avastin®) (approved in June 2010). Of these drugs, Iressa® and Tarceva® are low molecular drugs, and had been used to treat over 85,000 lung cancer patients by April 2009 in Japan. Avastin® is an antibody drug which was approved for colon and rectal cancers in April 2007, and its indication was subsequently expanded to include lung cancer in November 2009. All three drugs are used for non-small-cell lung cancers that are inoperable or recurrent. Of the three drugs, Iressa® received sales approval from the U.S. Food and Drug Administration in May 2003, however, its use for new patients was basically banned in June 2007. On the other hand, in the EU, drug developer AstraZeneca of the UK had its application to the European Medicines Evaluation Agency (EMEA) for sales approval in January 2005 turned down, however, the company reapplied in May 2008, and EMEA approved it in July 2009. The reason behind this difference is that the clinical efficacy of Iressa® was reported to vary greatly depending on the background of the patient, in regard to factors such as ethnicity and genetic information, in previous global studies (ISEL, INTEREST, IPASS etc). Clinical efficacy here points to survival benefit indexed by survival period and survival rate, response rate indicating the effect of reducing cancer, and the recurrence suppression effect measured by relapse-free survival period and recurrence rates.

Iressa® and Tarceva® are low molecular drugs targeting the receptors of epidermal growth factor (EGF), known as a cellular proliferation/growth factor (Figures 3 and 5). EGF receptors are transmembrane molecules (glycoprotein), and are a class of receptor tyrosine kinases (EGFR tyrosine kinase) which specifically phosphorylate residues of tyrosine, an amino acid. In some types of lung cancer, *EGFR* tyrosine kinase coding gene (*EGFR* gene) mutation is reported to cause abnormal activation of EGFR tyrosine kinase, and the abovementioned two drugs block the EGFR tyrosine kinase activity.

Abnormal activity of tyrosine kinase, including EGFR, is believed to be one of the causes of various cancers including lung cancer. Tyrosine kinases usually play a central role in the proliferation mechanism of normal cells, taking charge of the regulation of "growth/proliferation factor (extracellular cellular proliferation signals) -> tyrosine kinase -> activation of intracellular proliferation signal transmission" pathway (Figure 5). This pathway is suppressed when there is no stimulation by extracellular cellular proliferation signaling molecules, and is transiently activated only when there is stimulation by cellular proliferation signaling molecules. However, when the pathway becomes constantly active due to the disruption of the pathway regulation caused by amplification, mutation, and/or structural changes in the tyrosine kinase coding gene, there will be a continuous intracellular proliferation stimulus. Subsequently, this is believed to lead to unlimited cellular proliferation.

Since tyrosine kinase is involved in the fundamental life-sustaining process, regulation of cell proliferation, there has been a concern that drugs blocking such molecules may produce some grave side effects. However, since cells of certain types of cancer caused by *EGFR* gene mutation including lung cancer are much more sensitive to tyrosine kinase blocking compared to normal cells, it has been shown that drugs blocking the molecules swiftly produce effects only on cancer cells.

From global clinical trials and clinical experience after marketing, Iressa's® clinical effect for lung cancer is suggested to vary with the background of the patient, including factors such as ethnicity, gender, histological type of cancer and smoking history.[7] In detail, it is more effective in Asians compared to Caucasians, and more effective on adenocarcinoma out of all non-small-cell lung cancers, and also on females and non-smokers. It is also reported that clinical efficacy is high on lung cancer patients with mutation of the *EGFR* gene, the gene coding EGFR tyrosine kinase, the target of Iressa®. *EGFR* gene mutation is found in many lung cancers in Asians, adenocarcinoma and lung cancer in non-smokers, which makes Iressa® effective. In clinical trials with Japanese lung cancer patients with *EGFR* gene mutation as the subjects, which were publicized in

June 2010, the clinical effects of Iressa® were shown to be significantly higher than those of standard chemotherapy using traditional cytotoxic drugs.[8] In this trial, clinical efficacy was measured by a progression-free survival period, which is the amount of time survived without progression. To sum up the report, *EGFR* gene mutation is perceived as a prediction factor for the treatment efficacy of Iressa®. Therefore, it is useful to check the presence of *EGFR* gene mutation before initiating treatment with the drug, and only those with such mutation should be treated. *EGFR* gene mutation screening was approved for coverage by insurance in June 2007 in Japan, and it is clinically practiced as a bench mark to judge an indication for Iressa®. To check the presence of *EGFR* gene mutation, various methods are being developed based on the direct sequence method and PCR (polymerase chain reaction). In addition, the sensitivity/specificity differences and equivalence among the checking methods are currently being examined.[7]

Since Tarceva® targets EGFR tyrosine kinase, its mechanism of efficacy is believed to be similar to that of Iressa®. However, it is different from Iressa® in that it was shown to have a survival benefit for non-small-cell lung cancer patients in clinical trials abroad. The clinical effects of Tarceva®, similar to Iressa®, are higher in lung cancer in Asians, lung adenocarcinoma, lung cancer in non-smokers, and lung cancer patients with *EGFR* gene mutation, however, there is a report claiming that *EGFR* gene mutation and clinical effect do not correlate. In addition, there is a report that Tarceva® is clinically effective for lung cancer with histological type other than adenocarcinomma, lung cancer of smokers, and lung cancer patients without *EGFR* gene mutation. The difference in clinical effects between Tarceva® and Iressa® as well as criteria for the use of Tarceva® will be clarified by the vigorous clinical trials which are currently underway.

Avastin® is an antibody drug (human monoclonal antibody) targeting vascular endothelial growth factor (VEGF). VEGF is a glycoprotein involved in vascularization, which is a critical process for cancer growth and metastasis. VEGF expression increases in various types of cancer including lung cancer and colon cancer, and the correlation between its expression and stages and prognosis of cancer has been reported. Avastin® was approved in the U.S. in February 2004 and in the EU in January 2005 for treating colon cancer as the first vascularization blocker in the world, and its indication was expanded to lung cancer in the U.S. and the EU as well as in Japan. It is approved for breast cancer and kidney cancer in the U.S. and the EU. Domestic clinical trials as well as those abroad with lung cancer patients as their subjects showed that the combination of Avastin® and CP therapy, a combination of carboplatin and paclitaxel used in standard chemotherapy, was affective in both Asian and western lung cancer patients.

4-3 Side Effects of and Drug Resistance to Molecular target drugs for Lung Cancer

As much as the clinical effectiveness of molecular target drugs such as Iressa® in treating lung cancer has been shown, there are still concerns about the risk of new side effects not seen with traditional cytotoxic

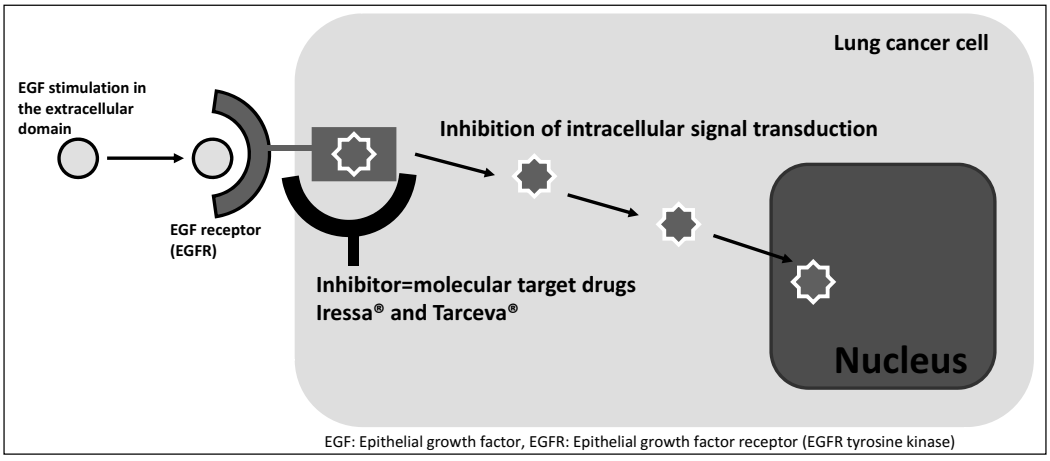


Figure 5 : Effective mechanisms of molecular target drugs for lung cancer, Iressa® and Tarceva®

Produced at the STFC based on the lecture by Dr. Mano

anticancer drugs and about drug resistance.

As for side effects, there have been reports of death caused by acute lung injury and interstitial pneumonia with the use of Iressa®. With consideration to the risk of such side effects and the clinical effects of the drug on lung cancer patients with *EGFR* gene mutation shown in 4-2, the Japan Lung Cancer Society has published its *Guidelines on Gefinitib Use* (revised on July 25, 2005) to increase the benefit/risk ratio. At the same time, death caused by interstitial pneumonia has been reported with the use of Tarceva®. Avastin® carries the risk of lung bleeding, and thus is contraindicated for use on patients with squamous epithelial lung cancer and on patients with a history of hemoptysis.

As for resistance to the drugs, patients clinically suited for Iressa® and Tarceva® acquire resistance within a year to a few years and the cancer recurs in almost all cases.[9] Additional *EGFR* gene mutation has been proposed to lower the drug binding to EGFR tyrosine kinase leading to the drug resistance, and investigation on the detailed mechanisms and drug development to overcome the resistance are underway.

5 New Molecular Target Therapy for Lung Cancer -The discovery of the *EML4-ALK* fusion gene and its clinical application-

The efficacy of molecular target drug Iressa®, which blocks the activity of EGFR tyrosine kinase in lung cancer patients with *EGFR* gene mutation, was introduced in the last chapter. This efficacy indicates that the identification of the molecules causing lung cancer and drugs interfering with the activity of the molecule will result in an effective molecular target therapy. However, as discussed in 3-2, target molecules for cancer treatment vary widely, and there have been a great efforts in basic research inside and outside of the country, such as the search for and analysis of new target molecules in lung cancer treatment. Here, I will introduce a significant achievement in recent years, the discovery of *EML4-ALK*, a therapeutic target gene, by Dr. Hiroyuki Mano of the University of Tokyo/ Jichi Medical University and its clinical application as molecular target therapy.

5-1 The Discovery of a Therapeutic Target Gene -EML4-ALK Fusion Gene-

To discover a new oncogene causing lung cancer, Dr. Mano et al. started with uniquely upgrading and developing the method for screening oncogenes from patients' specimens. This method makes almost all genes contained in a patient's specimen to be forcibly expressed inside fibroblast cells using retrovirus vector (Figure 6). The isolation of oncogene from the transformed focus, in other words, focus formation assay, was one of the methods dominating medical research in the 1980s, however, organ specific oncogenes, expressed only in a specific organ, could not be isolated at the time. In order to achieve this, Dr. Mano et al. established a method to screen oncogenes by forcibly expressing almost all of the genes contained in a patient's specimen based on a report by Dr. Moriuchi and his colleagues[10] at Nagasaki University, and reported this in 2007.[11]

Using this gene screening method, Dr. Mano et al. discovered a new oncogene, a candidate for therapeutic target, the *EML4-ALK* fusion gene, from the specimen of the non-small-cell lung cancer (adenocarcinoma) of a 62-year old smoker, and reported it in Nature on August 2, 2007.[12] The *ALK* gene codes for a tyrosine kinase named anaplastic lymphoma kinase (ALK), and the *EML4* gene codes for microtubule associated protein EML4, and normally, their products, ALK tyrosine kinase and EML4 protein, are present independently inside normal cells. However, when the *ALK* gene and the *EML4* gene fuse, abnormally active EML4-ALK tyrosine kinase is produced by the *EML4-ALK* fusion gene (Figure 7) and is believed to cause lung cancer. In fact, *EML4-ALK* fusion gene has been shown to be present only in lung cancer cells. Please refer to the reference[12] as well as open patent information from Japan and the U.S. (Japanese Published Unexamined Application No.2008-295444, U.S. Patent application publication No.2009/099193 etc) for details of *EML4-ALK* fusion gene.

Lung cancer caused by EML4-ALK tyrosine kinase has been confirmed in animal studies. In transgenic mice with the *EML4-ALK* fusion gene expressed specifically in alveolar epithelium, making the lungs produce EML4-ALK tyrosine kinase, and developed a few hundred lung adenocarcinoma simultaneously in both lungs within a few weeks after birth.[13] The abnormal activity of tyrosine kinase had been already known to be carcinogenic in the case of EGFR (refer

to 4-2), and EML4-ALK tyrosine kinase further confirmed it.

In normal human cells, since the *ALK* gene and the *EML4* gene are located close to each other on chromosome 2 in opposite orientations, fusion of these genes observed in lung cancer cells indicates a structural abnormality of the chromosome. Using the genomic DNA of lung cancer patients, Dr. Mano et al revealed that the chromosome sandwiched between *ALK* gene and *EML4* gene gets excised and subsequently the *ALK* gene and the *EML4* gene are bound in revered orientation and become fused.

Examples of cancer caused by abnormally active tyrosine kinase production triggered by its gene fusing with another gene due to chromosomal structural abnormality are hematological cancers such as chronic myelocytic leukemia and anaplastic large cell lymphoma. Chronic myelocytic leukemia is believed to be caused by production of abnormally activated BCR-ABL tyrosine kinase due to an Abelson leukemia tyrosine kinase coding gene, the *ABL* gene, fusing with the *BCR* gene at the breakpoint cluster region (the *BCR-ABL* fusion gene) triggered by the structural abnormality of the chromosome. This structural abnormality in the chromosome is called Philadelphia chromosome. Currently, low molecular drug imanitib (Glivec®), which blocks abnormally activated BCR-ABL tyrosine kinase, is used as a first-line therapy in chronic myelocytic leukemia. In addition, anaplastic large cell lymphoma is believed to be caused by the production of abnormally activated NPM-ALK tyrosine kinase due to abovementioned *ALK* gene fusing with the nucleophosmin-anaplastic lymphoma kinase (NPM) coding *NPM* gene, triggered by a chromosomal structural abnormality.

On the other hand, fusion of genes caused by chromosomal abnormality was generally believed to cause hematological cancers, but not solid cancers such as lung cancer. Dr. Mitelman et al. reported in 2004 that there was a possibility that chromosomal structural abnormality maybe a major cause of solid cancer,[14] however, it was not until the reports by Dr. Mano et al. in 2007 on lung cancer[12] and by Dr. Tomlin et al. on prostate cancer[15] that this was verified. The discovery of fusion gene *EML4-ALK*, which caused lung cancer, had a great academic impact, and was selected as one of 10 most important medical discoveries of the year in the December 2007 issue of Nature Medicine.

There are two important matters of clinical significance regarding the discovery of *EML4-ALK* fusion gene. One of them is the implementation of early lung cancer detection and the other is a potential for new molecular target drug development for lung cancer. Diagnosis and drug development are discussed below.

5-2 Development and Clinical Application of New Lung Cancer Diagnostic Methods

As mentioned in 4-1, lung cancer has been traditionally diagnosed based on pathohistological testing using specimens such as expectorated sputum. However, the sensitivity of such examinations is low. For example, expectorated sputum has to contain at least a few percent of cancer cells in 1ml of sample for a diagnosis. This means that in many cases, lung cancer has already progressed by the time of its diagnosis, and a method for detecting lung cancer with a higher level of sensitivity was long been awaited.

Dr. Mano et al. have developed a molecular diagnostic method for lung cancer using reverse transcription polymerase chain reaction (RT-PCR).[12] This method detects the mRNA of *EML4-ALK* fusion gene which is only present in lung cancer cells. Its sensitivity is much higher than the traditional sputum examination, and it is able to detect lung cancer with only 10 cancer cells within 1ml of sputum sample.

In March 2009, Dr. Mano et al. founded the ALK Lung Cancer Study Group (ALCAS) in order to develop a nation-wide diagnostic network structure for lung cancer patients in Japan. ALCAS is carrying forward the abovementioned multiplex RT-PCR with improved sensitivity for *EML4-ALK* mRNA detection[16] as well as the intercalating antibody-enhanced polymer method (iAEP method), which detects abnormally activated EML4-ALK tyrosine kinase with a high sensitivity[17] as a lung cancer diagnostic screening. Multiplex RT-PCR is an exhaustive detection method able to detect even the mRNA of *EML4-ALK* fusion genes variants, enabling early detection of lung cancer from samples such as pleural effusion, bronchial lavage fluid, and frozen specimen as well as sputum.

ALCAS has investigated the presence of the *EML4-ALK* fusion gene in 220 patients with non-small-cell lung cancer using multiplex RT-PCR. As a result, the *EML4-ALK* fusion gene was detected in 11 patients, all of whom had adenocarcinoma, accounting for 5% of

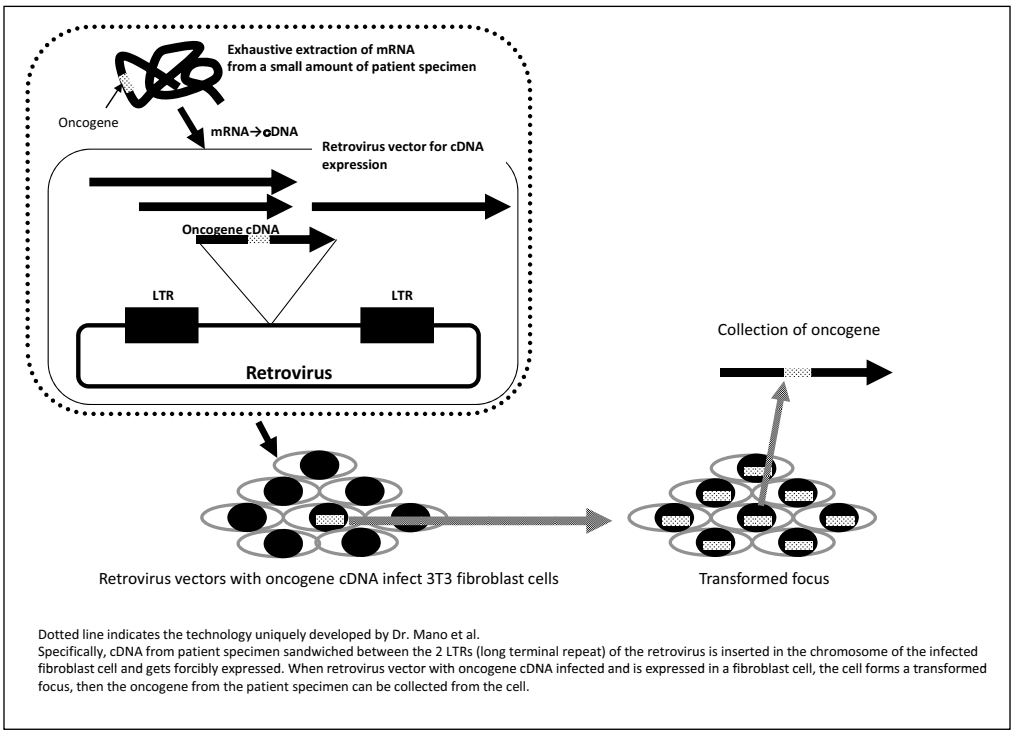


Figure 6 : Method of oncogene screening from patient specimen independently improved and developed by Dr. Mano and et al.

Produced by the STFC based on the lecture by Dr. Mano

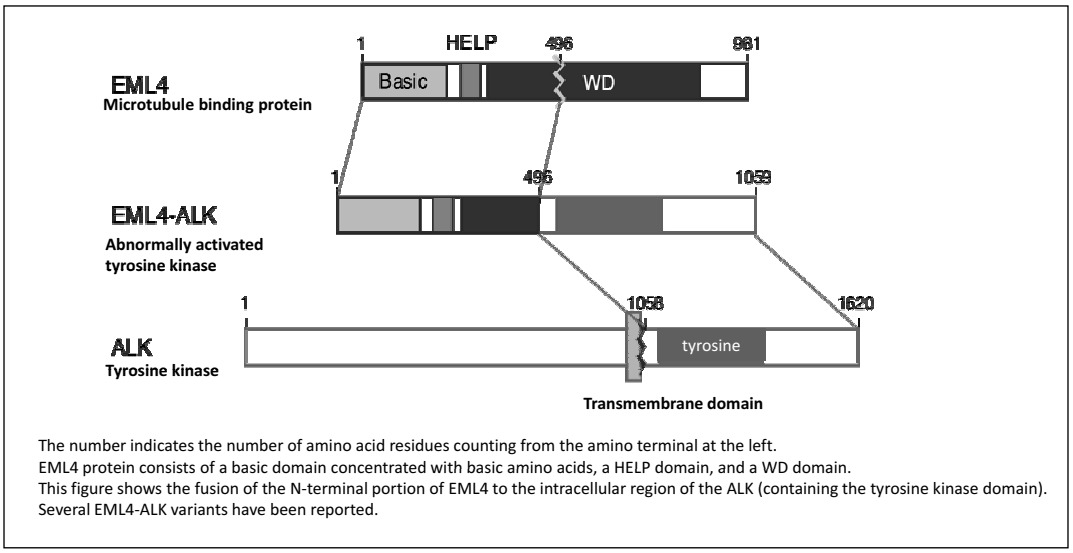


Figure 7 : Structure of EML4-ALK tyrosine kinase produced by *EML4-ALK* fusion gene

Produced by the STFC based on the lecture by Dr. Mano

the subjects of non-small-cell lung cancer. In addition, the detection rate of the *EML4-ALK* fusion gene was higher in patients with non-small-cell lung cancer below the age of 50, accounting for 35% of the total.[18] Meta-analysis of research data obtained inside and outside the country up to date, including past research done by Dr. Mano et al., showed that the *EML4-ALK* fusion gene was present in 3% to 7% of non-small-cell lung cancer in Asians (Japanese, Korean, Chinese).[19] As mentioned before, approximately 63,000 Japanese patients die from lung cancer annually, 85% to 90% of which are from non-small-cell lung cancer, and early detection of 5% of these patients and provision of molecular target therapy would save over 2500 patients annually, thus its clinical value is extremely high. In particular, the diagnosis of patients with non-small-cell lung cancer who are under the age of 50 is significant considering the high detection rate of the *EML4-ALK* fusion gene in this age group.

In addition, it is shown that patients with the *EML4-ALK* fusion gene and those with *EGFR* gene mutation do not overlap.[12] In other words, *EGFR* gene mutation

and the *EML4-ALK* fusion gene have a mutually exclusive relationship. Therefore, it is necessary to conduct testing to detect the presence of *EGFR* gene mutation and the *EML4-ALK* fusion gene before starting a molecular target therapy, in order to judge the appropriate indication for Iressa®, Tarceva®, and *ALK* inhibitors shown in 5-3.

5-3 Development of New Drugs for Lung Cancer

Molecular target drugs for lung cancer, Iressa® and Tarceva®, and Glivec® for chronic myelocytic leukemia all block abnormally activated tyrosine kinase. This means that blocking the central cause of carcinogenesis, abnormal tyrosine kinase, is at the basis of its effective molecular targeting treatment. Therefore, EML4-ALK tyrosine kinase that has been abnormally activated by the fusion of ALK tyrosine kinase and EML4 protein can be a therapeutic target molecule.

Dr. Mano et al. reported, in December 2008, that EML4-ALK tyrosine kinase could be a possible candidate as a therapeutic target molecule for lung cancer treatment.[13] Transgenic mice expressing EML4-ALK tyrosine kinase specifically on the lung alveolar epithelium develop severe lung cancer. When 2,4-pyrimidinediamide, a ALK tyrosine kinase specific blocker, was administered orally to these mice, most of the cancer disappeared within 25 days. Lung cancer caused by EML4-ALK tyrosine kinase was experimentally verified to be treatable by specific inhibition of ALK tyrosine kinase.

Taking the above experimental results, molecular target drug inhibiting ALK tyrosine kinase was developed and is currently undergoing clinical trials. Among the drugs being developed by multiple companies, crizotinib (code name: PF-02341066) developed by Pfizer Inc. is at the forefront of clinical trials. Crizotinib is a multi target drug (refer to 3-1) which targets the molecules ALK tyrosine kinase and c-Met. c-Met is a receptor for hepatocyte growth factor (HGF), and is also a tyrosine kinase like ALK. In the phase I of clinical trials conducted in the U.S., Australia, and Korea in 2008, tests were conducted on various types of cancer, but subsequently, expansion tests were conducted in the same countries with non-small-cell lung cancer as the subject at the recommended dose achieved in the phase I of the clinical trials.

At the American Society for Clinical Oncology 2010 conference held June 4 to 8, 2010, the results of clinical trials of crizotinib which had as its subjects 82 patients with non-small-cell lung cancer with *EML4-ALK* fusion gene in the U.S., Australia, and Korea were presented. Disease control rate of crizotinib was reported to be extremely high at 87%, adding together complete response (the drug was completely responded to), partial response (the drug was effective) and no change (the drug blocked the progression).[20] Reference[20] is the abstract of the presentation, and the actual presentation included additional patients and revised data.

Global Phase III of clinical trials has already started for crizotinib (scheduled for September 2009 to September 2012).[21] This Phase III of the clinical trials is aiming to evaluate the efficacy and safety of crizotinib by comparing the drug with standard chemotherapy agents (cytopathic drug pemetrexed or docetaxel). Countries conducting trials are the U.S., Australia, Bulgaria, Canada, Germany, Hong Kong, Hungary, Italy, Japan, Korea, Poland, Russia, Spain, and England, and within Japan, trials is scheduled to be conducted in Tokyo, Osaka, Aichi, Chiba, Hokkaido, Hyogo, Shizuoka, Okayama, and Fukuoka (as of July 10, 2010).

6 Future Research and Development in Japan and Around the World

Development of molecular target therapy for lung cancer started with the low molecular drug Iressa® targeting EGFR. Another low molecular drug targeting EGFR, Tarceva® and antibody drug Avastin® targeting VEGF are now used for lung cancer treatment. In addition, while the development of new molecular target drugs for lung cancer has undertaken, ALK tyrosine kinase inhibitor, which is currently undergoing clinical trials, should begin to be used in clinical practice in the near future. Though problems seen with Iressa® such as side effects and drug resistance should be resolved in the early stages, clinical selection of molecular target drugs for lung cancer is widening.

While the actual application of molecular target drugs for lung cancer is progressing, researchers around the world are making a great effort to find new target molecules and develop molecular target drugs. In the search of therapeutic target molecule for lung cancer, the Cancer Genome Atlas Project

(TCGA project), which started as a pilot project at the U.S. National Institutes of Health (NIH) in 2005, is receiving much attention. This project aims to identify the important genes responsible for lung cancer by screening the entire genome of cancer cells using squamous cell cancer of non-small-cell lung cancers and by evaluating the samples from patients (in their pilot project, they were targeting only 3 types of cancers: brain cancer, ovarian cancer, and lung cancer; however, the NIH reported that it would expand the targets to cover over 20 types of cancer in 2009).

While discovery of new therapeutic target molecules is anticipated from large-scale cancer genome analysis such as the TCGA project and currently ongoing projects, there has been an effort to expand the indication of molecular target drugs approved for treating other types of cancers to include lung cancer. Since there is an expansive range of basic and applied research as well as clinical trials, I have discussed the research by Dr. Mano et al. as a successful example. Dr. Mano et al. discovered the *EML4-ALK* fusion gene responsible for lung cancer by a focus formation assay, achieved by breaking through its limitations to isolate an organ-specific oncogenes. It is believed that this new technology will be extremely useful in the search for other oncogenes other than the *EMK4-ALK* fusion gene. The acceleration project, New Cancer Gene Identification Project by the Japan Science and Technology Agency (JST)'s Core Research for Evolutional Science and Technology (CREST) will use the methods developed by Dr. Mano et al. (scheduled for January 2009~March 2014).[22]

In order for molecular target therapy for lung cancer to progress, it is urgent for the mechanisms and risks of side effects of and drug resistance to currently used drugs to be analyzed and for preventative measures for the risk to be developed. In addition, continuous efforts should be made to find new therapeutic molecules for lung cancer and to develop drugs and diagnostic methods based on the molecular information. In addition to these scientific and technological tasks, issues of the medical system should be resolved as well. Taking the research of Dr. Mano et al. as an example, since its clinical value has already been accepted, molecular diagnosis using the *EML4-ALK* fusion gene should be made more clinically accessible by opening it up to insurance coverage like EGFR gene testing. Early discovery of cancer by *EML4-ALK* fusion gene diagnosis enables the early start of treatment, which subsequently reduces treatment costs. In addition, in such cases, the indication of molecular target theraphy should be set earlier than stage III to IV of lung cancer. Guidelines on lung cancer treatment should be reconstructed, and physicians should be clearly notified.

Cancer research in Japan has been conducted centering on the Comprehensive 10-year Strategy for Cancer Control (fiscal years 1984 to 1993), the New Comprehensive 10-year Strategy for Cancer Control (fiscal years 1994 to 2003), and the Third Comprehensive 10-year Strategy for Cancer Control (fiscal years 2004 to 2013).[23] From fiscal year 2011, cancer research is likely to progress even further with the general governmental action plan for science and technology policies. To achieve "life innovation," the important goal set by the New Growth Strategy (cabinet approved on June 18, 2010), the action plan aims to actualize a "physically and mentally healthy and vigorous" society and "independence for the elderly and handicapped" by 2020, and promotes three priority tasks.[24] One of the three is the "improvement of the cure rate" of cancer "through innovative development of diagnosis and treatment," and one of the directions to achieve this is the development of molecular target therapy. The development includes basic research such as "finding new targets by revealing the properties of cancer (proliferative inhibition, differentiation control, prevention of metastasis and cell death)," and extends to applied research as "research and development of drugs (low-molecular and antibody drugs)," showing that the development of molecular target theraphy will be promoted as a national strategy.

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References

[1] Cabinet Office, The Second Life Innovation Task Force Data Collection, April 13, 2010: http://www8.cao.go.jp/cstp/budget/aptf/life2/siryo2.pdf

[2] Vital and Health Statistics Division, Statistics and Information Department, Ministry of Health, Labor and Welfare, Summary of Vital Statistics 2008 (determinate number): http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei08/index.html

[3] Susumu Sekine, The current state and the issues of antibody drugs, Science & Technology Trends, No.103, October 2009

[4] Mariko Shoji and Shinichi Mogi, Recent Trend of Cancer Research -molecular target therapy and translational research-, Science & Technology Trends, No.13, April 2002

[5] Takashi Tsuruo, Molecular target therapy for cancer, Overview, *Gendai Iryo*, 32:10, 20-25, 20004

[6] Medical Information Services Minds, lung cancer, The Japanese Lung Cancer Society, 2005 edition, Guideline: http://minds.jcqhc.or.jp/stc/0007/1/0007_G0000073_GL.html

[7] The Japan Lung Cancer Society, EGFR manual committee, Manual for EGFR gene mutation test in lung cancer patients, 1.7 edition: http://www.haigan.gr.jp/uploads/photos/148.pdf

[8] Maemondo M et al.. Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR. N Engl J Med, 362: 2380 — 2388, 2010.

[9] Seiji Yano, EGFR – Strategy to overcome TKI resistance, *Saishin Igaku* vol.65 (3), 36-42, 2010

[10] Yoshizuka N et al.. An alternative transcript derived from the trio locus encodes a guanosine nucleotide exchange factor with mouse cells — transforming potential. J Biol Chem 279: 43998 — 44004, 2004.

[11] Hatanaka H et al.. Transforming activity of purinergic receptor P2Y, G — protein coupled, 2 revealed by retroviral expression screening. Biochem Biophys Res Commun 356: 723 — 726, 2007

[12] Soda M et al.. Identification of the transforming EML - ALK fusion gene in non-small cell lung cancer. Nature 448: 561-566, 2007.

[13] Soda M et al.. A mouse model for EML — ALK — positive lung cancer. Proc Natl Acad Sci USA 105: 19893-19897, 2008.

[14] Mitelman F et al.. Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer. Nat. Genet 2004 36: 331- 334.

[15] Tomlins SA et al.. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007: 448: 595- 599.

[16] Takeuchi K et al.. Multiplex reverse transcription — PCR screening for EML4 — ALK fusion transcripts. Clin Cancer Res 14: 6618- 6624, 2008.

[17] Takeuchi K et al.. KIF5B — ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 15: 3143-3149, 2009

[18] Soda et al. Multiplex RT –ALK fusion cancer gene diagnosis by PCR- ALK-lung cancer study group: (ALCAS), interim report of diagnostic network, The 50th Annual Meeting of the Japanese Respiratory Society Mini Symposium, April 25, 2010

[19] Horn L and Pao W. EML4- ALK: Honing in on a new target in non-small cell lung cancer. J Clin Oncol 27: 4232-4235, 2009.

[20] Bang Y et al.. Clinical activity of the oral ALK inhibitor PF- 02341066 in ALK-positive patients with non-small cell lung cancer (NSCLC) 2010 ASCO Annual Meeting Abstract No.3.: http://abstract.asco.org/AbstView_74_50854.html

[21] U.S. National Institutes of Health, ClinicalTrials.gov. An Investigational Drug, PF-02341066 is being studied versus standard of care in patients with advanced non — small cell lung cancer with a specific gene profile involving the anaplastic lymphoma kinase (ALK) gene (processed on June 6, 2010): http://clinicaltrials.gov/ct2/show/NCT00932893?term=PF — 02341066&rank=5

[22] The acceleration project, "New Cancer Gene Identification Project" of Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology Agency (JST), January 27, 2009:

http://www.jst.go.jp/pr/info/info605/index.html
[23] Ministry of Education, Culture, Sports, Science and Technology (MEXT), Council for Science and Technology, Life Science Committee (The 57th), held on June 25, 2010
[24] Minister of State for Science and Technology Policy, Council for Science and Technology Policy Expert Member, Action Plan for Science and Technology Policy for 2011 fiscal year, July 8, 2010
http://www8.cao.go.jp/cstp/output/20100708ap.pdf

* URLs are as of July 10, 2010.

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