Chaptre2

Slide 1

When effective molecularly targeted drugs exist and genetic abnormalities in cancer cells that can be targeted with those drugs are identified, then patients who are likely to respond are selected.

This applies to personalized medicine for lung cancer, breast cancer, and gastric cancer.

The genes EGFR, ALK, ROS1, and BRAF are detected in lung cancer, and HER2 is detected in breast cancer and gastric cancer.

Personalized medicine based on the identification of genetic abnormalities is most advanced in treating lung cancer, so I will start by explaining about lung cancer.

Slide 2

Originally, lung cancer was histologically categorized as small cell cancer and non-small cell cancer, and non-small cell cancer was further divided into forms such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

The most frequent is adenocarcinoma, which accounts for about half of lung cancers.

Small cell cancer has recently been categorized as neuroendocrine carcinoma.

Nevertheless, the standard treatment for advanced lung cancer as of 2002 or so was combined therapy with 2 cytotoxic agents, regardless of the histologic type.

EGFR mutations were identified in 2004. In addition to EGFR mutations in adenocarcinomas, which account for about half of lung cancers, numerous abnormalities in driver genes such as ALK fusion genes, ROS1 fusion genes, and BRAF mutations were successively identified. Here, abnormalities in driver genes are gene abnormalities that cause lung cancer to develop. In other words, these gene abnormalities cause lung cancer.

If there are abnormalities in the driver genes EGFR, ALK, or ROS1, then they are currently treated with molecularly targeted drugs. This is personalized medicine, which is genomic medicine in a broad sense.

Slide 3

This slide summarizes abnormalities in driver genes involved in lung cancer, their frequency in adenocarcinoma of the lung, and molecularly targeted drugs available for treatment.

These gene abnormalities are detected in adenocarcinomas, which account for most lung cancers. The most frequent are EGFR mutations, which are found in around 50% of adenocarcinomas of the lung.

About half of lung cancers are adenocarcinomas, so this means EGFR mutations are found in 1/4 of all lung cancers. There are 4 molecularly targeted drugs that are approved for treatment of EGFR

mutation-positive lung cancer: gefitinib, erlotinib, afatinib, and osimertinib.
An ALK fusion gene is detected in around 5% of adenocarcinomas of the lung, and it can be treated with 3 molecularly targeted drugs: crizotinib, alectinib, and ceritinib.
An ROS1 fusion gene is rare and found in around 1% of adenocarcinomas of the lung. Crizotinib can also be used to treat ALK fusion gene-positive lung cancer.
A BRAF mutation is rare and is found in 1% of adenocarcinomas of the lung. Combined therapy with trametinib and dabrafenib is likely to be approved in the near future.
Thus, there are molecularly targeted drugs for treatment of gene abnormalities in lung cancer. Personalized medicine, i.e. genomic medicine, is provided based on the identification of gene abnormalities, as I hope you now understand.

Slide 4
The Japan Lung Cancer Society has issued EBM-based guidelines for management of lung cancer, and they include information like this treatment algorithm for Stage IV non-small cell lung cancer.
Histologically, lung cancer is categorized as squamous cell carcinoma or non-squamous cell cancer. Abnormalities in driver genes are likely to be identified non-squamous cell cancers, which are predominantly adenocarcinomas.
Thus, we will examine mutations in the EGFR gene, ALK fusion genes, and ROS1 fusion genes.
If these gene abnormalities are present, then they can be treated with the appropriate molecularly targeted drug.
Immune checkpoint inhibitors, which have recently garnered attention, have been approved for treatment of non-small cell lung cancer. If immunostaining is used to check for PD-L1 expression by tumor cells and over 50% of cancer cells express PD-L1, then the immune checkpoint inhibitor pembrolizumab can be used for primary treatment of squamous cell carcinoma or non-squamous cell cancer.
Now, I will explain about gene abnormalities in detail.

Slide 5
I will start by talking about EGFR mutations.

Slide 6
EGFR, or epidermal growth factor receptor, is a molecule that plays an important role in normal cells.
EGFR is expressed in the cell membrane of normal cells. When a molecule called a ligand binds to EGFR, 2 receptor molecules come together to form a dimer.
Other ligands for EGFR besides EGF include transforming growth factor α (TGF-α), amphiregulin, and HB-EGF.

Once EGFR forms a dimer, ATP binds to EGFR at a site known as an intracellular domain. Tyrosine is phosphorylated, various downstream pathways are activated, and cell growth or cell survival is stimulated, promoting organ formation and development. Thus, stimulation from EGFR is essential to normal cells and the body.

Slide 7

When a mutation in EGFR occurs, exactly how does it occur?

When deletion of exon 19 occurs or a point mutation (L858R) occurs in exon 21 of EGFR, the tyrosine kinase domain of EGFR in cells is constantly activated without the binding of ligands to EGFR, and MAPK pathways via downstream RAS/MEK/ERK and PI3K/AKT/mTOR pathways are activated. As a result, cancer develops.

Slide 8

This figure depicts the type of mutations that occur in EGFR in more detail.

The EGFR gene is encoded on chromosome 7 and consists of 28 exons.

The most frequent EGFR mutation is a deletion of exon 19, which accounts for around 45% of all EGFR mutations.

The next most frequent mutation is a point mutation (L858R of exon 21) in which arginine is substituted for leucine at codon 858. This mutation accounts for around 40% of all EGFR mutations.

Therefore, these 2 mutations account for around 95% of all EGFR mutations.

Another mutation is G719X, where X represents a residue such as alanine, serine, or cysteine that substitutes for glycine at codon 719 of exon 18. This mutation accounts for around 3% of all EGFR mutations.

These mutations are activating mutations that respond to EGFR inhibitors.

ins20 is a mutation in which several amino acids are added at exon 20, and this mutation accounts for around 5% of all EGFR mutations. This mutation does not respond to EGFR inhibitors, so caution is required.

T790M is a mutation in exon 20 in which methionine is substituted for threonine at codon 790. A T790M mutation confers resistance to EGFR-TKIs, so please keep that fact in mind.

Slide 9

EGFR tyrosine kinase inhibitors (EGFR-TKIs) are molecularly targeted drugs. Next, I will talk about how EGFR-TKIs inhibit the proliferation of EGFR-mutant lung cancer and their mechanism of action.

Mutated EGFR hampers the binding of ATP to the tyrosine kinase domain in cells and is constantly activated despite the absence of a ligand. Cells receive survival or growth signaling, and cancer cells

proliferate.
An EGFR-TKI dislodges ATP and binds to the tyrosine kinase domain, halting signaling. This is referred to as competitive inhibition. It causes the apoptosis of cancer cells, causing them to die.

Slide 10
These are chest X-rays and CT images from a patient with EGFR mutation-positive adenocarcinoma of the lung who responded well to gefitinib, which is an EGFR-TKI.
Prior to treatment, a large mass with a long axis greater than 5 cm was noted in the middle lobe of the right lung. An EGFR mutation, L858R, was detected in tumor cells.
Hepatic metastases were also present, so the patient took gefitinib. The images in the middle are 2 months later, indicating that the mass shrank markedly.
The patient continued to receive gefitinib therapy. Over a year later, the tumor remained shrunken.
CEA is a tumor marker, and its level improved markedly, as indicated at the bottom.
If patients who are positive for an EGFR mutation can be accurately identified, then EGFR-TKIs (molecularly targeted drugs) can yield a substantial clinical benefit.

Slide 11
So how are mutations in the EGFR gene identified?
Tumor tissues and cells are collected from patients with lung cancer, cancer cells are identified pathologically, and lung cancer is definitely diagnosed.
Remaining specimens are submitted for testing, and mutations in the EGFR gene are identified.
A typical method of testing is real-time PCR.
Identification of mutations in the EGFR gene is covered by National Health Insurance. If a patient has advanced adenocarcinoma of the lung, EGFR mutations are actively identified.

Slide 12
As one would expect, molecularly targeted drugs only act on tumors expressing the target molecule.
Therefore, an EGFR-TKI will only act on patients who are positive for an EGFR mutation.
Here are results of the IPASS study. This study compared gefitinib and anticancer agents when administered for primary treatment of non-small cell lung cancers.
Data on progression-free survival are shown. In patients who were positive for an EGFR mutation on the left, gefitinib significantly prolonged progression-free survival compared to anticancer agents.
Conversely, gefitinib performed worse than anticancer agents in terms of progression-free survival when administered to patients without an EGFR mutation.
These results show that molecularly targeted drugs are only effective against the tumors they target.

Slide 13

Next, I will talk about the T790M mutation in the EGFR gene and drug resistance.

Slide 14

As I have mentioned thus far, EGFR mutation-positive lung cancer responds well (70-80%) to EGFR-TKIs. As treatment continues, however, cancer cells develop drug resistance, and cancer will almost certainly recur within several years.

Slide 15

Numerous causes of resistance to EGFR-TKIs have been reported with regard to EGFR-mutant lung cancer.

The quintessential factor for drug resistance is the T790M mutation in EGFR. T790M is detected in around 60% of patients who develop drug resistance, so it presumably induces drug resistance.

The T790M mutation is a mutation where methionine is substituted for threonine at position 790 of EGFR.

This is precisely the place where ATP and an EGFR-TKI bind to EGFR. In EGFR with a T790M mutation, methionine juts out because it is a large amino acid, preventing an EGFR-TKI from readily entering the pocket to bind to EGFR.

Moreover, EGFR with a T790M mutation has an increased affinity for ATP, so competitive inhibition by EGFR-TKIs is hampered. These 2 mechanisms preclude the binding of an EGFR-TKI to EGFR with a T790M mutation, and cancer cells become drug-resistant.

Slide 16

This information can be presented again as a diagram. EGFR harboring an activating mutation produces a mutated EGFR protein. Intense survival and growth signaling is facilitated, and cells become cancerous and proliferate.

An EGFR-TKI binds to mutated EGFR and silences signaling and induces cell death.

When, however, a T790M mutation (a secondary mutation) occurs in mutated EGFR, an EGFR-TKI is unable to bind to EGFR. Survival signaling cannot be silenced, and cancer cells become drug-resistant.

Slide 17

Recently, third-generation EGFR-TKIs that can even bind to the drug-resistant EGFR T790M mutation have been developed and used in clinical settings.

Gefitinib and erlotinib (first-generation EGFR-TKIs) and afatinib (a second-generation EGFR-TKI)

inhibit EGFR proteins with deletion of exon 19 or L858R (activating mutations), but they also inhibit wild-type EGFR protein. However, a drug-resistant EGFR protein with T790M cannot be inhibited. In contrast, osimertinib (a third-generation EGFR-TKI) inhibits activated EGFR protein as well as drug-resistant EGFR protein but does not inhibit wild-type EGFR protein for the most part.

Slide 18

This information is shown in diagram again.

A first-generation EGFR-TKI inhibits EGFR with an activating mutation as well as wild-type EGFR. However, it does not inhibit drug-resistant EGFR with a T790M mutation.

A third-generation EGFR-TKI inhibits activated EGFR as well as drug-resistant EGFR, but it does not inhibit wild-type EGFR for the most part.

In other words, third-generation EGFR-TKIs have an ideal target inhibition profile: they inhibit mutated EGFR expressed in cancer cells but they do not inhibit wild-type EGFR expressed in normal cells for the most part, and they display antitumor action while causing only slight adverse reactions.

Slide 19

Shown here is a patient with non-small cell lung cancer who responded to osimertinib.

The patient was a woman in her 60s with an activating mutation (L858R) in EGFR. Therapy with afatinib was temporarily effective. However, she developed drug resistance after about a year.

Lymphangitic carcinomatosis and malignant pleural effusion developed in the right lung, and hepatic metastases developed. When hepatic metastases were biopsied again, the T790M mutation was detected, so the patient with treated with osimertinib (a third-generation EGFR-TKI).

The patient responded well to osimertinib. Lymphangitic carcinomatosis in the right lung and hepatic metastases disappeared, and malignant pleural effusion on the right was also alleviated.

Thus, third-generation EGFR-TKIs are efficacious for many patients in whom the T790M mutation is detected.

Slide 20

So just how is the EGFR-T790M mutation detected?

Conventionally, T790M would be detected using tumor tissue specimens or cytology specimens. It can also be detected in plasma from collected blood.

Plasma contains circulating DNA from cancer cells, and T790M can be detected via amplification of that DNA.

An advantage of using tumor tissues or cells is that T790M is likely to be detected if a specimen contains cancer cells.

A disadvantage is that specimen collection is highly invasive for the patient.

An advantage of using plasma is that it is minimally invasive for a patient from whom specimens are being collected, but disadvantages are false-negatives and a low rate of detection of T790M.
Regardless of the specimen used when T790M is detected, the false-positive rate is low and patients are likely to respond to osimertinib.
At the current point in time, testing using tumor tissues or cells is repeated, but only 1 round of testing for T790M in plasma per patient is covered by National Health Insurance, so when to test plasma for the mutation and the state of the tumor need to be carefully considered.