Chapter3

Slide 1

Next, I will talk about ALK fusion genes.

Slide 2

ALK is an abbreviation for anaplastic lymphoma kinase. ALK is a gene that codes for a kinase that was identified in lymphoma.

ALK forms a fusion gene with another gene such as EML4 or KIF5B, causing lung cancer.

The gene was discovered by a group including Drs. Mano and Dr. Soda, who were at Jichi Medical University at the time, and it was reported in Nature in 2007.

The protein that is transcribed and translated from the EML4-ALK fusion gene forms a dimer and it facilitates powerful signaling, thus causing lung cancer.

EML4-ALK accounts for most ALK fusion genes. EML4-ALK is detected almost exclusively in adenocarcinoma of the lung, and it is present in 3-5% of adenocarcinomas of the lung.

Slide 3

Methods of detecting an ALK fusion gene are shown.

The EML4 gene and the ALK gene are both encoded on the short arm of chromosome 2.

As indicated in the figure, a portion of the EML4 gene and a portion of the ALK gene fuse, forming the EML4-ALK fusion gene.

The ALK gene is detected when it is part of a fusion gene, i.e. it is rearranged. Probes enclosing the cleavage and attachment sites are labeled with different fluorescent dyes (e.g. green and red) and fluorescence in situ hybridization (FISH) is performed.

When the gene is not rearranged, green and red will be adjacent and overlap, revealing a yellow signal.

When the gene is rearranged, green and red will be distinct. As shown in the photo on the lower right, green and red dots (spots) are evident in the nucleus, so an ALK fusion gene is present.

This test is performed on tumor tissues or cells.

This test is covered by National Health Insurance.

If an ALK fusion gene is present, ALK inhibitors are efficacious at a rate of 80%.

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In cancer cells that are positive for an ALK fusion gene, an ALK fusion protein can be detected with immunostaining.

The ALK protein is not expressed in normal cells for the most part.

In cancer cells that are positive for an ALK fusion gene, ALK protein will be weakly expressed, so it

can be detected with more sensitive immunostaining.
Currently, lung tissue with an adenocarcinoma is examined for expression ALK protein using immunostaining. Positive results are confirmed with FISH. If FISH is also positive, then treatment with an ALK inhibitor is recommended.
FISH is performed as necessary even if immunostaining for ALK is negative. If FISH is positive, then treatment with an ALK inhibitor can be provided.
Immunostaining is initially recommended because it can be performed at less cost and faster than FISH.

Slide 5
Shown here is the therapeutic effectiveness of ALK-TKIs in treating ALK fusion gene-positive lung cancer.
When an anticancer agent was administered for secondary treatment of ALK fusion gene-positive lung cancer, the response rate was around 20%.
However, crizotinib (an ALK-TKI) had a response rate of 65%.
Given these results, crizotinib was approved for the treatment of ALK fusion gene-positive lung cancer at the fastest pace in history, i.e. 4 years after the identification of an ALK fusion gene.

Slide 6
Alectinib (a second-generation ALK-TKI) was subsequently approved for treatment of ALK fusion gene-positive lung cancer.
Compared to the first-generation ALK-TKI crizotinib, alectinib is highly selective for ALK, effective against mutations that induce resistance to crizotinib, and highly safe.
A trial directly compared the first-generation ALK-TKI crizotinib and the second-generation ALK-TKI alectinib for primary treatment of ALK fusion gene-positive lung cancer.
Results of that trial indicated that alectinib (a second-generation ALK-TKI) was clearly superior to crizotinib (a first-generation ALK-TKI) in terms of progression-free survival. Although the data on overall survival are still tentative, overall survival also tended to be longer.
The second-generation ALK-TKI alectinib will presumably be used for primary treatment of ALK fusion gene-positive lung cancer in the future.

Slide 7
Shown here is a patient with adenocarcinoma of the lung who responded to alectinib.
Details have been omitted, but FISH confirmed that tumor tissue specimens were positive for the EML4-ALK fusion gene.
After the primary focus in the lung was surgically resected, multiple bone metastases and

carcinomatous pleurisy on the right recurred.
You can see uptake at the sites of recurrence on PET/CT images.
Uptake at foci disappeared as a result of therapy with alectinib, and pleural effusion on the right also disappeared.
The level of CEA (a tumor marker) returned to normal, decreasing from 23.9 to 2.4.
Thus, alectinib is highly efficacious in treating ALK fusion gene-positive lung cancer.

Slide 8
Next, I will talk about ROS1 fusion genes.

Slide 9
ROS1 is known to be an oncogene and is a member of the subfamily of tyrosine kinase insulin receptors.
Like ALK, ROS1 forms a fusion gene with other genes and is known to cause lung cancer.
A ROS1 fusion gene is rarer than an ALK fusion gene and is detected in 1% of adenocarcinomas of the lung.

Slide 10
Crizotinib has been approved for treatment of ALK lung cancer, and its indications were expanded to treating ROS1 fusion gene-positive lung cancer. Therapy with crizotinib is covered by National Health Insurance.
Crizotinib inhibits both ALK and ROS1 and is approved for treatment of both ALK fusion gene-positive lung cancer and ROS1 fusion gene-positive lung cancer.
This figure is a waterfall plot indicating its effectiveness against ROS1 fusion gene-positive lung cancer. A bar indicates the rate of tumor shrinkage in a single cancer patient.
Therapy with crizotinib causes tumors to shrink in most patients.
When a tumor shrinks by more than 30%, i.e. the bar extends below -30%, then the patient responded.
Crizotinib had a high response rate (72%) in the treatment of ROS1 fusion gene-positive lung cancer.

Slide 11
Here is a figure indicating progression-free survival for patients with ROS1 fusion gene-positive lung cancer who received crizotinib therapy. The median progression-free survival was long (19.2 months), and crizotinib was efficacious in treating ROS1 fusion gene-positive lung cancer.

Slide 12
Here are FDG-PET images. On the left, uptake by multiple metastases in the right lung was noted

prior to treatment. However, crizotinib therapy was started and uptake disappeared in week 7, indicating that crizotinib therapy was highly efficacious.
Thus, ROS1 fusion gene-positive lung cancer is identified and genomic medicine is provided in the form of personalized medicine (therapy with crizotinib).

Slide 13
Next, I will talk about BRAF mutations.

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Here is BRAF. BRAF is a serine/threonine kinase in the RAF family. There are 3 forms of RAF: ARAF, BRAF, and CRAF. BRAF is a component of MAP kinase pathways (denoted here as MAPK pathways) that consist of RAS, RAF, MEK, ERK and other molecules downstream of the receptor.
When MAPK pathways are activated, cell growth is promoted, so BRAF is a molecule that is responsible for signaling needed for cell growth.

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Various mutations can occur in BRAF. A quintessential one is a mutation known as V600E. This is a mutation in which glutamic acid is substituted for valine at codon 600 of BRAF.
The V600E mutation accounts for the vast majority of BRAF mutations.
The BRAF V600E mutation is known to be detected in various types of cancer. It is found in 20-30% of melanomas, 30-70% of papillary adenocarcinomas of the thyroid, 3-5% of colorectal cancers, and around 1% of non-small cell lung cancers. Its frequency in other types of cancer shown here is unclear, but it is detected.

Slide 16
A combination of a BRAF inhibitor and an MEK inhibitor is more therapeutically effective in treating cancer cells with the BRAF V600E mutation than a BRAF inhibitor alone, and combination therapy is efficacious in treating melanoma and lung cancer as well.
I will now describe the theoretical grounds for this.
As I mentioned a moment ago, BRAF is a component of MAPK pathways downstream of the receptor. MAPK pathways are important pathways that facilitate growth stimulation. When they are overactivated, cells become cancerous and can die. Thus, BRAF acts as a brake by providing negative feedback to the receptor expressed on the cell membrane via ERK.
When a mutation occurs in BRAF, it is constantly activated, and signaling of downstream MEK/ERK is facilitated. Cells become cancerous and proliferate.

When BRAF is inhibited with a BRAF inhibitor

Slide
Downstream MEK and ERK are temporarily inhibited, but

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Negative feedback pathways disappear…

Slide
And the receptor on the cell membrane is activated.
As a result, MAPK pathways are reactivated by signaling from the activated receptor, and

Slide 18-19
This can no longer be halted by a BRAF inhibitor at a concentration that the body can tolerate.
Cells survive, i.e. BRAF-mutant cancer cells develop drug resistance to the BRAF inhibitor.
Or if BRAF is inhibited in BRAF-mutant cancer cells, ARAF or CRAF will instead aid in signaling by activating MEK, resulting in drug resistance.
However, combined use of a BRAF inhibitor and an MEK inhibitor allows the efficient inhibition of MAPK pathways and can kill cancer cells.

Slide 20
This is a waterfall plot indicating the efficacy of combined therapy with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib in treating advanced BRAFV600E mutation-positive treatment-naïve lung cancer. When a tumor shrinks by more than 30%, i.e. the bar extends below –30%, then the patient responded. The response rate was 64%, progression-free survival was 14.6 months, and median overall survival was 24.6 months, indicating a substantial response.
A characteristic adverse event, i.e. a fever developing several days after treatment started, was noted in 53% of patients, but it was treated via administration of an antipyretic analgesic.
This combination therapy was approved for treatment of V600 mutation-positive melanoma as of December 2017, but its indications will presumably be expanded to include BRAFV600 mutation-positive lung cancer in the near future.