CH4.

Slide 1

Molecularly targeted therapies have been developed with these genetic mutations serving as biomarkers. Here, I will be talking about biomarkers.

Slide 2

A biomarker is an index reflecting normal biological processes, the onset of disease, or a pharmacological response as a result of treatment. Biomarkers allow objective measurement and evaluation.

The following 3 types of biomarkers are associated with cancer treatment.

A predictive biomarker is an index to predict the therapeutic effectiveness of a specific drug.

A prognostic biomarker is a determinant of patient prognosis unrelated to drug treatment.

A safety biomarker is an index to predict adverse events caused by a drug.

Slide 3

I will now explain predictive biomarkers.

Slide 4

As I explained a moment ago, driver mutations can serve as biomarkers for molecularly targeted therapy.

A mutation in the EGFR gene is a predictive marker for gefitinib (an EGFR-TKI). An ALK fusion gene is a predictive marker for crizotinib (an ALK inhibitor). A ROS1 fusion gene is a predictive marker for crizotinib. A BCR-ABR fusion gene is a predictive marker for imatinib. A high level of HER2 expression is a predictive marker for trastuzumab. These biomarkers are crucial to genomic medicine for cancer.

Slide 5

I will now describe an example of a mutation in the EGFR gene and a predictive biomarker for an EGFR-TKI.

The IPASS study is a phase III study that compared gefitinib (an EGFR-TKI) and standard treatment (combined therapy with 2 cytotoxic anticancer agents). EGFR mutation-positive patients and EGFR mutation-negative patients all had adenocarcinoma of the lung. As shown in the slide, however, the EGFR-TKI was clearly found to have prolonged survival and to have been more therapeutically effective than chemotherapy in EGFR mutation-positive patients. Chemotherapy was clearly found to have prolonged survival and to have been more therapeutically effective in EGFR mutation-negative patients. Thus, the therapeutic effectiveness of an EGFR-TKI clearly differs as a result of a

biomarker, i.e. a mutation in the EGFR gene.

Slide 6

Results of clinical studies have revealed that cetuximab, an anti-EGFR antibody used to treat colon cancer, is not therapeutically effective in treating a mutation in the KRAS gene. K-ras is 1 of 3 ras oncogenes (H-ras, N-ras, and K-ras), and a mutation in the K-ras gene is the most frequent mutation found in the ras family of oncogenes. A mutation in the KRAS gene currently serves as a biomarker for cetuximab (an anti-EGFR antibody).

Slide 7

RAS is known to be activated by signalling from EGFR. As shown in the slide, RAS is activated when EGF (a ligand) binds to EGFR (a receptor), and RAS is associated with cell proliferation and angiogenesis.

Slide 8

I will now explain cetuximab (an anti-EGFR antibody) and KRAS mutations in colon cancer.
This trial examined the therapeutic effectiveness of cetuximab in combination with another drug in treating patients negative for a KRAS mutation. You can see the survival curve. Satisfactory results were achieved in KRAS mutation-negative patients who also received cetuximab. As you can see from a sub-analysis of the response rate on the right, treatment with chemotherapy alone was highly therapeutically effective in KRAS mutation-positive patients while treatment with chemotherapy and cetuximab was highly therapeutically effective in KRAS mutation-negative patients. Thus, absence of a KRAS mutation is a biomarker with which to predict the therapeutic effectiveness of cetuximab.

Slide 9

A companion diagnostic reagent is a diagnostic reagent used to test whether or not a patient should receive a specific pharmaceutical. I explained the wild-type RAS gene a moment ago. A diagnostic reagent is used to determine whether the RAS gene is the wild-type in “advanced colorectal cancer not amenable to curative surgery.” Companion diagnostic reagents such as a kit to detect R|BRAF V600E in malignant melanoma and a kit to detect ALK in ALK fusion gene-positive lung cancer were initially approved by the FDA.

Slide 10

I explained treatment in accordance with a driver mutation a moment ago. If mutation A is present, then treatment A is administered as shown in the slide. This is genomic medicine. If a mutation is resistant to treatment A, however, A will be inefficacious. The B drug resistance gene was recently discovered, and treatment B will be used to shrink the cancer again. Thus, the genetic mutations A

and B will serve as biomarkers. Treatment sequencing is crucial to genomic medicine, and it can prolong patient survival.

Slide 11

When gefitinib (a first-generation EGFR-TKI) is used to treat EGFR mutation-positive lung cancer, a drug resistance mutation known as EGFR T790M occurs. In such an event, osimertinib (a next-generation EGFR-TKI) has proven to be highly therapeutically effective. This is a typical sequence of treatments as part of genomic medicine. This treatment is possible because of the identification of drug resistance genes and the development of molecularly targeted drugs to treat them.

Slide 12

I will now explain how drug resistance develops when an EGFR-TKI is used to treat lung cancer. I explained the T790M mutation a moment ago. As shown in the slide, the T790M mutation accounts for over 50% of the mechanisms of drug resistance. Other mechanisms of drug resistance include amplification of MET, amplification of HER2, EMT, and transformation into small cell carcinoma.

Slide 13

I will now explain the mechanism of resistance to an EGFR-TKI due to the T790M mutation.
Methionine is substituted for threonine at amino acid 790 of EGFR, causing steric hindrance due to the increased size of the amino acid side chain and increased ATP binding affinity. Inhibitory activity of an EGFR-TKI decreases. When an EGFR mutation is absent, an EGFR-TKI binds to the ATP-binding pocket, signalling is silenced, and cancer cells are killed, as shown in the slide. When, however, a secondary mutation occurs in EGFR (T790M), the structure of the ATP-binding pocket changes. This precludes the binding of an EGFR-TKI. Signalling resumes and cancer cells begin to proliferate.

Slide 14

Thus, secondary mutation in EGFR (T790M) can occur. Osimertinib (a next-generation EGFR-TKI) was designed to readily bind to the altered structure of the ATP-binding pocket. As shown in the slide, it readily binds to the ATP-binding pocket and it silences signalling in patients with the EGFR T790M mutation, so cancer cells will die. In contrast, gefitinib does not fit into the structure of the ATP-binding pocket in T790M mutation-positive patients, so binding is precluded.

Slide 15

A second biopsy of a recurrent tumor and genetic testing are required to check for the T790M drug resistance mutation.
However, a second biopsy of tumor tissue poses the following problems.

- Testing for genetic mutations with a tissue biopsy may overlook a genetic mutation due to the

heterogeneity of tumor tissue.

- Around 15-25% of biopsies results in specimens that are inappropriate or inadequate for testing.
- A biopsy is not possible due to complications in around 20% of patients.

One solution to this problem that has currently garnered attention is a liquid biopsy. As you all well know, this technique ascertains genetic mutations by examining tumor DNA circulating in the blood. However, the sensitivity and specificity of testing for circulating tumor DNA in order to identify *EGFR* mutations in patients with non-small-cell lung cancer are still unclear. Different companies are developing new testing methods.

Slide 16

Cancer cells can sometimes not be readily collected from tumor tissue in routine clinical practice. If samples can be collected to look for genetic mutations in the blood, then genomic medicine for cancer can be provided. Thus, I will now be talking about a liquid biopsy.

Slide 17

Liquid biopsy is a technology using a liquid sample, such as blood, to predict therapeutic effectiveness instead of a conventional biopsy that collects tumor tissue using an endoscope or needle.

Liquid biopsy is less of a burden to patients than a conventional biopsy, and it has garnered attention as a technique lead to appropriate treatment in light of information on cancer-related genes. The main biomarkers measured are circulating tumor cells (CTCs) and cell-free DNA (cfDNA).

Slide 18

I will now explain CTCs.

Cells are released by a primary focus or metastasizing tumor tissue to enter blood vessels. In the initial stages of cancer, just a few cells in in 1 mL of blood can enter the blood stream and travel far. These cancer cells in the blood are called CTCs.

Slide 19

I will now explain cfDNA. cfDNA is genomic DNA from cancer cells that is released into the blood as a result of the apoptosis of those cells. As shown in the figure, this is DNA released by a tumor into the blood.

Slide 20

Thus, liquid biopsy is a promising technology that allows non-invasive genetic analysis. As shown in the figure, CTCs or cfDNA can be collected from 10 ml of blood. These specimens can be used to search for genetic mutations using a sequencer.

Slide 21

An acceptable option would be to detect T790M detected from a recurrent tumor caused by EGFR mutation-positive lung cancer. However, the tumor can recur at a site that is difficult to biopsy again or tumor tissue may not be readily collected. In such an event, cfDNA in the blood can be collected to search for T790M, as I mentioned a moment ago.

A liquid biopsy detects the T790M drug resistance mutation in cfDNA at a lower rate than testing that directly collects DNA from tumor tissue, but a liquid biopsy is minimally invasive to patients and it allows genetic testing, so it is an extremely effective means.

Slide 22

When using gefitinib (an EGFR-TKI) to treat EGFR mutation-positive cancer and resistance develops, checking tumor tissue for T790M may not be possible, but a liquid biopsy can be used to check for T790M based on cfDNA in the blood. If T790M can be detected with this test, it can be targeted for treatment with osimertinib (a next-generation EGFR-TKI).

Slide 23

- The role of molecularly targeted drugs chemotherapy for cancer
- The key role of driver mutations when providing genomic medicine for cancer
- Molecularly targeted drugs with driver mutations as biomarkers have been developed and are highly therapeutically effective.
- The frequency of the same driver mutation can differ depending on the type of cancer, and the effectiveness of therapy to treat it can also differ
- A liquid biopsy collects CTCs or cfDNA in the blood. This allows genetic analysis via minimally invasive means