The Realities of Genomic Medicine for Cancer: 1 Somatic mutations and cancer (1)
-Genomic abnormalities in non-hereditary tumors and their relationship to medicine-

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CH1.

Slide 1

I will be talking about the Realities of Genomic Medicine for Cancer: Somatic mutations and cancer. In specific terms, I will be talking about genomic abnormalities in non-hereditary tumors and their relationship to medicine.

Slide 2

I will start off by providing an overview of chemotherapy for cancer.　I will then explain the general realities of genomic medicine for cancer.　I will mainly be talking about driver mutations, molecularly targeted drugs, biomarkers, and liquid biopsy.

Slide 3

Cancer treatment consists of 3 pillars: surgery, radiation therapy, and chemotherapy.　However, immunotherapy has come to play an important role in cancer treatment as a result of recent advances in immunotherapy.

Slide 4

We use anticancer agents in chemotherapy, so I will now briefly explain anticancer agents.　An anticancer agent refers to a drug that inhibits the proliferation of cancer cells or that kills cancer cells. Altogether, there are around 100 oral and infused anticancer agents.　There are differences between anticancer agents and generics.　Anticancer agents have a narrower therapeutic range than generics and can readily cause adverse reactions or unexpected adverse events.　Moreover, there are vast individual differences in their efficacy and toxicity.

Slide 5

I will now explain the differences between anticancer agents and generics from the perspective of their therapeutic effectiveness and incidence of toxicities in accordance with the dose.　The figure on the left shows the efficacy and toxic response in accordance with the dose of a generic, but it clearly has a wider safety margin compared to the anticancer agent in the figure on the right.　Thus, generics have a wide safety margin while anticancer agents have a narrow safety margin.

Slide 6

Shown here is a classification of organ disorders by the therapeutic effectiveness of anticancer agents. A is a group who can be cured with chemotherapy (e.g. patients with hematologic malignancies), B is

a group whose life can be prolonged (e.g. patients with small cell lung cancer or breast cancer), C is a group whose symptoms can be alleviated and whose QOL can be improved (e.g. patients with non-small-cell lung cancer, head and neck cancer, or a gastrointestinal tumor), and D is a group that is expected to benefit little (e.g. patients with thyroid cancer).

Slide 7

Shown here are the general types of anticancer agents. Agents are mainly classified into 3 categories: chemotherapy agents, molecularly targeted drugs, and hormone therapies. Chemotherapy has cytotoxic action and causes intense adverse reactions.

Molecularly targeted therapies specifically target molecules associated with the infiltration, proliferation, or metastasis of cancer cells. Hormone therapy is used to treat forms of cancer such as breast cancer, endometrial cancer, and prostate cancer, and it causes relatively few adverse reactions.

Slide 8-9

I will now explain chemotherapy, which involves cytotoxic anticancer agents. As shown in the slide, these agents damage DNA in cancer cells and they inhibit cancer cell division. However, they also damage normal cells, so they pose a problem in terms of adverse reactions such as disordered hematopoiesis.

Slide 10

I will now explain the types of cytotoxic anticancer agents.

Alkylating agents such as cyclophosphamide alkylate DNA and inhibit DNA synthesis. Platinum-based drugs such as cisplatin bind to double-stranded DNA and inhibit DNA synthesis. Antimetabolites such as 5-FU act on the phase of the cell cycle when DNA synthesis occurs and inhibit DNA synthesis. Microtubule inhibitors such as paclitaxel and docetaxel inhibit microtubules involved in cell division. Topoisomerase inhibitors such as irinotecan and etoposide bind to DNA fragments during cell division.

Slide 11

I will now explain the thinking behind treatment with chemotherapy.

When a cytotoxic anticancer agent is administered, normal cells decrease but the cell count will recover over time. However, tumor cells recover slower than normal cells, so tumor cells will gradually decrease with repeated administration of an anticancer agent, and normal cells that decreased will recover. Thus, repeated sessions of chemotherapy reduce the damage to normal cells and readily reduce tumor cells.

Slide 12

The best treatment involves the use of a combination of cytotoxic anticancer agents with different

mechanisms of action. A cell cycle-specific drug is often combined with a cell cycle-non-specific drug. The standard treatment for lung cancer involves a combination of 2 agents, e.g. a platinum-based drug and a taxane, a topoisomerase inhibitor, or an antimetabolite. This is more therapeutically effective than monotherapy.

Slide 13

I will now explain molecularly targeted drugs. As I just explained, cytotoxic anticancer agents damage normal cells as well as cancer cells, as shown in the slide. Thus, they can cause intense adverse reactions. In contrast, molecularly targeted drugs stop the action of cancer cells by specifically attacking cancer cells. Thus, these drugs do little damage to normal cells and they efficiently kill cancer cells.

Slide 14

Shown here are examples of molecularly targeted drugs. ATRA has proven to be efficacious in treating acute myelogenous leukemia and imatinib has proven to be efficacious in treating chronic myeloid leukemia. Imatinib has also proven to be efficacious in treating a GIST. Rituximab is used to treat malignant lymphoma, and bortezomib is used to treat multiple myeloma. Gefitinib and erlotinib (EGFR-TKIs) are used to treat lung cancer, and particularly adenocarcinoma of the lung. Trastuzumab (a HER2 inhibitor) is used to treat breast cancer. Cetuximab (an anti-EGFR antibody) is used to treat colon cancer. Bevacizumab (an angiogenesis inhibitor) is used chemotherapy for colon cancer and lung cancer.

Slide 15

I will now briefly explain signalling by epidermal growth factor receptor (EGFR) while using EGFR as an example of a molecular target. A ligand known as epidermal growth factor (EGF) binds to a receptor known as EGFR. Afterwards, EGFR forms a dimer. Phosphorylation of a given tyrosine kinase in cells occurs, and RAS-RAF-MAPK is activated, causing cell proliferation. The PI3K-AKT pathway is activated, leading to the proliferation of cancer cells. The process by which a stimulating agent known as a ligand binds to receptors on the cell membrane and conveys information to cells is referred to as signalling.

Slide 16-17

I just explained EGFR. In 2004, mutations in EGFR were found to respond to an EGFR tyrosine kinase inhibitor (TKI). This finding was announced in the world's leading scientific journals such as the NEJM and Science. As shown in the slide, phosphorylation of tyrosine kinases must occur when signalling information is conveyed. When mutations occur in these tyrosine kinases, an EGFR-TKI can readily bind to them, block signalling, and inhibit the proliferation of cancer cells.

Slide 18-19

In EGFR mutation-positive patients, an EGFR-TKI readily binds to the ATP-binding site of tyrosine kinases, as I briefly explained a moment ago. As shown in the slide, a TKI binds to a tyrosine kinase and signalling is silenced. In EGFR mutation-negative patients, the ATP-binding site of tyrosine kinases precludes the binding of an EGFR-TKI. A TKI is unable to bind to a tyrosine kinase. Signalling cannot be disrupted, so cancer cells continue to proliferate. A mutation in the EGFR gene can be identified by subjecting tumor cell DNA to PCR.

Slide 20-22

This slide shows an EGFR-TKI blocking cancer signalling.

A ligand binds to EGFR and a signal is transmitted. Signalling is blocked by an EGFR-TKI and proliferation of cancer cells is inhibited. Thus, the EGFR-TKI is therapeutically effective.

Slide 23

When EGFR mutation-positive lung cancer was actually treated with gefitinib (an EGFR-TKI), the response rate was about 70%. When it was treated with a cytotoxic anticancer agent, the response rate was about 30%. When a molecule (i.e. EGFR) was targeted, therapeutic effectiveness was found to have at least doubled compared to chemotherapy.

Slide 24

A phase 3 trial compared an EGFR-TKI and standard treatment of EGFR mutation-positive lung cancer with a cytotoxic anticancer agent. Survival after each treatment was compared. As shown in the slide, the survival curve for patients receiving the EGFR-TKI (indicated in red) surpassed that for patients receiving chemotherapy (indicated in blue). Statistical analysis revealed that the EGFR-TKI significantly improved patient prognosis after treatment of EGFR mutation-positive lung cancer.

Slide 25

Shown here are chest X-ray findings when a patient actually received gefitinib (an EGFR-TKI).

On the left is an X-ray prior to treatment. Lung cancer is evident in the right lung and ascites due to carcinomatous pleurisy was noted. In the center is an X-ray 10 days after treatment. The opacity in the lung field has markedly diminished. On the right is an X-ray 10 days after treatment. The opacity has disappeared. Thus, patients who respond to an EGFR-TKI are often encountered in routine clinical practice.

Slide 26-27

This slide summarizes advances in chemotherapy for lung cancer.

In the 1970s, survival was 2 to 5 months with supportive therapy alone. Prognosis improved to 6 to 8 months with a platinum-based drug alone and to 8 to 14 months with combined therapy with a

platinum-based drug and a cytotoxic anticancer agent. I mentioned EGFR a moment ago. When a mutation in that molecule was detected and it was treated with an EGFR-TKI, prognosis improved dramatically to 2 to 3 years. Thus, genomic medicine for cancer is considered to be a promising treatment to substantially improve patient prognosis as well as QOL in routine clinical practice.

CH2.

Slide 1

To provide genomic medicine for cancer, tumor tissue must first be biopsied and cancer cells must be collected. There are times when cells cannot be collected from tumor tissue, as I will explain in the latter half of this lecture. Recently, a method of directly collecting DNA released by tumor cells or cancer cells into the blood has been developed. This is known as liquid biopsy. Once cancer cells are collected, DNA can be extracted and a genetic analysis can be performed with a next-generation sequencer to determine whether a genetic mutation is present. Let us now look at driver mutations, which are genetic mutations associated with the growth of cancer. As an example, if gene A is present, then cancer may respond to treatment A. If gene B is present, it may respond to treatment B. If gene C is present, it may respond to treatment C. Treatment targeting genes A to C is genomic medicine for cancer. The target genes are biomarkers of treatment. Treatment can be chosen by looking for those genes with a companion diagnostic reagent or via genetic panel testing.

Slide 2

I will start by talking about driver mutations.

Slide 3

Before that, I will briefly explain cell proliferation and signalling.

Cell proliferation (cell division) occurs as a result of an extracellular growth factor (a ligand) binding to a receptor, stimulating signalling. That signal travels from a receptor on the cell membrane through the cytoplasmic matrix to the nucleus. Genes that facilitate proliferation are known as oncogenes, and genes that inhibit proliferation are known as tumor supressor genes. As shown in the slide, a growth factor binds to a receptor and signalling occurs. This causes apoptosis or it causes cancer to grow, e.g. angiogenesis, proliferation, differentiation, and metastasis. A mutation in an oncogene or tumor supressor gene results in the synthesis of an abnormal protein associated with cell proliferation or dysfunction in a protein, causing “oncogenesis.”

Slide 4

I will now explain ligands and growth factors.

A ligand is a molecule that binds to a large molecule, like a receptor, and that is responsible for transmitting information from cells to cells. It is also referred to as a first messenger. In simple terms, a ligand is a “key” that conveys information and a receptor is the “keyhole” receiving that information.

Typical ligands are as shown in the slide. As I explained earlier, EGF is a ligand that is crucial to the growth of cancer.

Slide 5

I will now explain receptors .

A receptor is the general term for proteins located on the surface of the cell membrane, in the cytoplasm, and in the nucleus. These proteins specifically recognize and bind to various physiologically active substances from outside cells and they convey information about physiologically active substances to cells and DNA. Receptor tyrosine kinases play a central role in cellular differentiation and proliferation signaling. Cell surface receptors include ion channel receptors, tyrosine kinase receptors, and G protein-coupled receptors. I will now explain tyrosine kinase receptors.

Adenosine triphosphate (ATP) is a phosphate compound that releases energy in cells for metabolic reactions such as protein synthesis.

Slide 6

I will now explain tyrosine kinases (TKs). There are around 90 TKs in humans (of those, 58 are receptor tyrosine kinases, 32 are non-receptor tyrosine kinases, and the rest are neither receptor tyrosine kinases nor non-receptor tyrosine kinases). TKs are all related to cell proliferation. TKs that are often mutated in cancer cells are shown in the slide. These TKs are targets of tyrosine kinase inhibitors. EGFR is a TK. A mutation in that TK is noted in around 20-30% of all Japanese with non-small-cell lung cancer. In contrast, gefitinib (Iressa) and erlotinib (Tarceva) are highly efficacious EGFR tyrosine kinase inhibitors (EGFR-TKIs) to treat those mutations.

VEGFR is abundantly located on the cell membrane of vascular endothelium, and it is a major pathway for growth signalling. VEGFR does not actually undergo mutation. Rather, vast amounts of VEGF (a growth factor) are secreted by cancer cells. VEGF binds to the VEGFR of vascular endothelial cells and it promotes cell proliferation, i.e. angiogenesis. Thus, a TK inhibitor is used to suppress it. Drugs targeting VEGFR and PDGFR are known as “angiogenesis inhibitors” and are currently used in routine clinical practice.

Slide 7

Shown here is a schematic diagram of phosphorylation of a typical tyrosine kinase.

As shown at the bottom of the slide, a ligand binds to a tyrosine kinase receptor to form a dimer. This causes structural changes in the tyrosine kinase receptor. The ATP-binding pocket of the intracellular kinase domain opens and ATP binds to it, causing phosphorylation. The signal from the ligand is ultimately transmitted to the nucleus.

Slide 8

I will now explain driver mutations. There are 2 types of genetic mutations in cancer. A driver mutation is a mutation in a gene that directly contributes to the development of cancer and a passenger mutation is a mutation caused by instability in a gene and is unrelated to its expression. Oncogenes and tumor supressor genes are driver mutations. The EGFR and ALK genes that I will explain later

are oncogenes that are essential to oncogenesis. In contrast, the PIK3CA gene is not essential to oncogenesis.

Slide 9

I will now explain driver mutations and various types of cancer.

EGFR, ALK, ROS1, and RET are well-known genes associated with lung cancer, and adenocarcinoma of the lung in particular. Treatment of mutations in genes other than RET is covered by National Health Insurance and is provided as part of routine clinical practice.

Slide 10

I will now explain the role of the EGFR, ALK, ROS1, and RET genes in adenocarcinoma of the lung.

Slide 11

A mutation in the EGFR gene is noted in about 50% of adenocarcinomas of the lung. An ALK fusion gene is noted in 3.8%, and a ROS1 fusion gene is noted in 0.9%. Molecularly targeted inhibitors of these 3 mutations can be used in routine clinical practice and they are highly therapeutically effective. Treatments for the BRAF and RET fusion genes are not available as part of routine clinical practice, but molecularly targeted drugs have been developed and may be used in the future.

Slide 12

There are racial differences in the incidence of driver mutations. Data for Japanese are on the left and data for Westerners are on the right. A mutation in the EGFR gene is noted in 53% of Japanese versus 11.3% of Westerners, so its incidence clearly differs.

Slide 13

Differences in the expression of driver mutations are also noted in terms of smoking history. An EGFR mutation has been detected in 58.6% of non-smokers and in 47.5% of smokers.

Slide 14

There are also sex differences in the expression of driver mutations. An EGFR mutation has been detected in 62.7% of women and in 43.0% of men.

Slide 15

The frequency of a mutation in the EGFR gene differs depending on the type of cancer. As was indicated a moment ago, a mutation has been noted at a frequency of about 50% in adenocarcinomas of the lung. As shown in the slide, a mutation has been noted in 4% of pancreatic cancers, 14% of

cholangiocarcinomas, 12% of esophageal cancers, and 1-16% of head and neck cancers, but it has not been noted in hepatocellular carcinoma, colon cancer, breast cancer, gastric cancer, or leukemia.

Slide 16

I will now explain about the EGFR gene. The EGFR gene is located on the short arm of chromosome 7 (7p12) and it consists of 28 exons and 27 introns. Exons 1-16 code for extracellular domains, exon 17 codes for a transmembrane domain, and exons 18-28 code for intracellular domains.

Slide 17

Exons 18, 19, 20, and 21 are located in the intracellular domain. Deletion of 15 bases in exon 19 results in the deletion of 5 amino acids. L858R is a point mutation where arginine is substituted for leucine at amino acid 858 in exon 21. Treatments for these mutations are highly effective. The mutation in exon 19 is noted in 48.2% of patients and the mutation in exon 21 is noted in 42.7%. The figure on the right shows therapeutic effectiveness, which ranges from 70 to 80%. These treatments clearly have a higher level of antitumor action than treatments for a mutation in exon 18 or exon 20.

Slide 18-19

If a patient is positive for a mutation in the EGFR gene, why is treatment effective? This is a crucial clinical issue. EGF (a ligand) binds to EGFR (a receptor), and that stimulus is conveyed to a tyrosine kinase domain in cells. TP binds to the ATP-binding pocket of the tyrosine kinase domain, so the signal is transmitted to the nucleus of cells and cancer grows.

Slide 20-22

As I explained a moment ago, a ligand binds to a tyrosine kinase receptor. As shown in the slide, a dimer is formed and ATP binds to the ATP-binding pocket of the intracellular kinase domain. When, however, a mutation occurs in the EGFR gene, the structure of the ATP-binding pocket changes, and an EGFR-TKI readily binds to it. ATP is precluded from binding, and phosphorylation does not occur. The signal is not transmitted to the nucleus, and the growth of cancer is inhibited.

Slide 23

This slide shows a patient with EGFR mutation-positive adenocarcinoma of the lung who was treated with gefitinib (an EGFR-TKI). As a result of treatment, the primary foci and brain metastases indicated by the white arrows shrank markedly. A drug has difficulty reaching the brain because of the brain-blood barrier (BBB), but the drug proved to be efficacious even against brain metastases.

CH3.

Slide 1

We have recently become aware than a gene can fuse with another gene to become a powerful oncogene. As shown in the slide, gene A and gene B fuse to create a fusion gene consisting of gene A+gene B. This fusion gene can become a powerful oncogene, as recent studies have demonstrated.

Slide 2

I will now explain the role of the ALK, ROS1, and RET fusion genes in lung cancer and the role of the BCR-ABL fusion gene in chronic myeloid leukemia.

Slide 3

I will now explain ALK fusion genes.

In 2007, the research group led by Tatsuo Mano of the University of Tokyo discovered an ALK fusion gene that could serve as a therapeutic target for lung cancer. As shown in the slide, its frequency in lung cancer is 3.8%, so it is extremely rare. EML4 and ALK kinase on chromosome 2 fuse, as shown in the slide. This creates activated EML4-ALK fusion kinase, which heavily promotes oncogenesis. This gene is heavily involved in the growth of cancer to the extent that it is known as “the champion oncogene.”

Slide 4

Crizotinib (an ALK inhibitor) has proven to be efficacious in treating lung cancer with an ALK fusion gene, and it was approved in Japan in 2010. Crizotinib was therapeutically effective in this patient. The tumor that was present in the left lung prior to treatment clearly disappeared after crizotinib was administered. Thus, an ALK inhibitor was found to be highly therapeutically effective in treating ALK fusion gene-positive lung cancer.

Slide 5

Crizotinib (an ALK inhibitor) significantly improved survival for patients with ALK fusion gene-positive lung cancer compared to a conventional cytotoxic anticancer agent.

This slide shows the progression-free survival curve from a phase III trial comparing crizotinib and chemotherapy for initial treatment of ALK fusion gene-positive lung cancer. The group receiving crizotinib (indicated in blue) had a median progression-free survival of 10.9 months, so crizotinib significantly prolonged survival compared to a median progression-free survival of 7.0 months for patients receiving chemotherapy. As a result of this clinical trial, crizotinib (an ALK inhibitor) became the standard treatment for ALK fusion gene-positive lung cancer, and it is used in routine clinical practice. Recently, highly therapeutically effective ALK inhibitors such as alectinib and ceritinib have been developed and used.

Slide 6

Shown here is the antitumor action of crizotinib (an ALK inhibitor) and chemotherapy.

This figure is a waterfall plot. The further down the bar extends, the greater the rate of tumor shrinkage. The further up the bar extends, the more the tumor is likely to grow.

The response rate to an ALK inhibitor was 74%, extending far down the bar. In most patients, the tumor shrank. In contrast, the response rate to chemotherapy was 45%. The bar only extended slightly downwards compared to that for an ALK inhibitor. This indicates that an ALK inhibitor clearly shrinks a tumor substantially.

Slide 7

Cancers with an abnormal ALK gene besides lung cancer have been reported.

As shown in the slide, ALK and EML4 fuse in lung cancer, ALK and NPM1 fuse in lymphoma, ALK and TPM3 fuse in an inflammatory myofibroblastic tumor, and ALK and VCL fuse in kidney cancer to become powerful oncogenes. Studies reported that inhibiting the ALK protein is therapeutically effective in treating cancer with these fusion genes, which is why those cancers have recently been referred to as ALKoma.

Slide 8

I will now explain ROS1 fusion genes.

A mutation in ROS1 is a rare mutation found in about 1% of lung cancers. ROS1 is located on the long arm of chromosome 6, and 14 genes can fuse with it. CD74, EZR, SLC34A2, and SDC4 account for 70% of those mutations.

ROS1 has a high level of affinity for the tyrosine kinase domain of ALK, and an ALK inhibitor has the ability to inhibit ROS1 tyrosine kinase, so crizotinib (an ALK inhibitor) is now used as a treatment, as I explained a moment ago.

Slide 9

Shown here are results of a clinical trial of crizotinib to treat ROS1 fusion gene-positive lung cancer and patients who benefited it. The therapeutic effectiveness of monotherapy was already reported in the NEJM. In 36 of 50 patients (72%), the cancer shrank. The bar in the waterfall plot, which I explained a moment ago, extends far down for almost all of the patients, indicating that the tumor shrank substantially. On the right are FDG-PET findings. On the left are intrapulmonary metastases and lymph node metastases, which appeared as dark spots prior to treatment. The tumors almost disappeared 7 weeks after crizotinib therapy.

Slide 10

I will now explain RET fusion genes. These genes are a rare genetic mutation noted in 1.9% of lung cancers. Facilities such as the National Cancer Center and the Japanese Foundation for Cancer Research identified these genes as a new target in lung cancer in 2012. RET fuses with another gene on chromosome 10 to create a driver gene.

Slide 11

RET-positive lung cancer was examined during genomic testing for lung cancer at the national level primarily at the National Cancer Center's Eastern Hospital. An organization known as LC-SCRUM-Japan screened 1,536 patients and identified 34 patients with RET-positive lung cancer. A clinical trial was conducted to determine whether or not vandetanib, a molecularly targeted drug that inhibits RET tyrosine kinase, could treat RET-positive lung cancer, and 17 eligible patients were enrolled.

Slide 12

Shown here is the therapeutic effective of vandetanib in treating RET-positive lung cancer. On the right is a waterfall plot, and in 9 of 17 patients (53%) the cancer shrank. CT findings before and after treatment are shown on the far right. A tumor adjacent to the heart was clearly found to have shrunk. Vandetanib is not available for treatment of RET-positive lung cancer in routine clinical practice, but it likely could be in the future.

Slide 13

I will now explain BCR-ABL. As shown in the slide, chromosomal translocation of the ABL gene on chromosome 9 and the BCR gene on chromosome 22 results in the BCR-ABL fusion gene, which causes cancer cells to proliferate, leading ot chronic myeloid leukemia. In previous clinical trials, imatinib caused the Philadelphia chromosome to mostly disappear compared to standard treatment, and it is currently used as standard treatment.

Slide 14

I will now explain gastrointestinal stromal tumors (GISTs).

Slide 15

I will now explain KIT. Studies have reported that KIT-activating gene mutations are a major cause of GIST. A KIT mutation is noted in exons 9, 11, 13, and 17 at a frequency of about 90%. Imatinib (an ABL kinase inhibitor) has potent action as a KIT kinase inhibitor and has become a treatment for a GIST. A clinical trial used imatinib to treat a GIST in 147 patients, and the tumor shrank in 79 (53.7%). Imaging findings on the right indicate that a massive GIST in the abdomen generally shrank as a result of treatment. The fact that imatinib was so therapeutically effective in treating a GIST that did not respond to chemotherapy was a major finding, and imatinib is currently used in routine clinical

practice.

Slide 16
I will now explain HER2.

Slide 17-18
HER2 is a transmembrane tyrosine kinase receptor in the EGFR family. It is a major oncogene, and its amplification and overexpression are noted in many types of cancer. As shown in the slide, the HER family consists of HER1 (EGFR), HER2, HER3, and HER4. HER1, HER2, and HER3 are known to be involved in the proliferation, survival, and differentiation of tumor cells, but the role of HER4 in breast cancer is unclear. HER1 is an EGFR, as I explained a moment ago.

Slide 19
A humanized anti-HER2 monoclonal antibody, trastuzumab is known to be a drug that specifically binds to HER2 protein (a product of the HER2 gene), and trastuzumab is used to treat HER2-positive breast cancer and gastric cancer.

Slide 20
Shown here are results of a clinical trial on trastuzumab to treat breast cancer. This trial compared chemotherapy alone and combined therapy with chemotherapy and trastuzumab in 234 patients with breast cancer with a high level of HER2 expression.
You can see the curve for progression-free survival. Survival significantly improved in the group receiving trastuzumab in addition to chemotherapy.

Slide 21
Shown here are results of a clinical trial on trastuzumab to treat gastric cancer.
3,807 patients were screened, and 810 with HER2-positive cancer were identified. 584 patients were eligible for this clinical trial. Its design compared survival. Two hundred and ninety patients were treated with capecitabine, 5FU, and cisplatin while 294 were also treated with trastuzumab.

As shown in the slide, the survival curve for the group that also received trastuzumab surpassed that for the group that did not receive trastuzumab. Median survival was 13.8 months for the group that also received trastuzumab and 11.1 months for the group that did not receive trastuzumab, so trastuzumab significantly improved survival. Based on these findings, trastuzumab is now used in routine clinical practice to treat HER2-positive gastric cancer.

Slide 22
I will now explain mutations in the BRAF gene.

Slide 23

BRAF is located on chromosome 7 (7q34) and consists of 18 exons. When a mutation occurs in the codon for valine at amino acid 600 (V600) of BRAF, BRAF kinase is constantly activated.

V600E accounts for about 91% of BRAF mutations, and other mutations are extremely rare.

The frequency of a mutation in the BRAF gene in different types of cancer is shown in the slide. As you can see, it is most frequent in malignant melanoma (43%), followed by thyroid cancer (27%), ovarian cancer (15%), and colon cancer (14%). Its frequency in lung cancer is rare (3%). A BRAF inhibitor (vemurafenib) that selectively inhibits BRAF kinase and that inhibits the proliferation of cancer cells is known to be efficacious in treating BRAF mutation-positive patients.

Slide 24

Shown here are data from a clinical trial on a BRAF inhibitor (vemurafenib) to treat malignant melanoma.

When overall survival after dacarbazine and vemurafenib therapy (standard treatment) to treat BRAF V600E-positive malignant melanoma was compared, vemurafenib significantly improved survival, and it is currently used as standard treatment.

Slide 25

Also shown here are progression-free survival and antitumor action. Vemurafenib significantly improved progression-free survival. As shown in the waterfall plot, the response rate to vemurafenib was 40% while the response rate to dacarbazine was 5%. Vemurafenib yielded satisfactory results in terms of antitumor action.

Slide 26

The therapeutic effectiveness of a BRAF inhibitor (vemurafenib) in treating cancer other than malignant melanoma is shown in the table. There were few patients with each type of cancer, but its antitumor action against different types of cancer was 42% for non-small-cell lung cancer, 0% for colon cancer, 12% for cholangiocarcinoma, and 29% for thyroid cancer. The therapeutic effectiveness of vemurafenib was found to have differed depending on the type of cancer. That said, the response rate of BRAF V600E-positive malignant melanoma and non-small-cell lung cancer to vemurafenib was about 40%, and the tumors were found to have similarly shrunk.

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