The Realities of Genomic Medicine for Cancer 2　Somatic mutations

Seiji Yano

Chapter1

Slide 1

I am Seiji Yano of the Division of Medical Oncology at Kanazawa University's Cancer Research Institute.

The Realities of Genomic Medicine for Cancer 2　I would like to start by talking about somatic mutations.

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A genome is a neologism from the German word for gene, which is also gene, and the Greek suffix -ome, meaning body, that means a body (or set) of genes.　A genome refers to all genetic information from DNA.

Genomic medicine for cancer is the use of genomic information to provide medical care, i.e. diagnosis or treatment, to cancer patients and to prevent cancer.

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The most practical clinical setting for genomic medicine for cancer is treatment selection based on genomic information.

There are generally 2 types of treatment selection based on genomic information.

The first is when an approved treatment is available for use in routine practice.

In such an event, specific genetic abnormalities evident in cancer cells are identified.　Based on the genetic abnormalities identified, treatment that is covered by National Health Insurance is provided.

The second type of treatment selection is when no approved treatment exists.

In such an event, genetic abnormalities in cancer cells are comprehensively analyzed.　Based on the genetic abnormalities found, drugs that should be efficacious are identified.　If potential drugs are identified, treatment not covered by National Health Insurance, e.g. that provided in a clinical trial, is provided.　Here, a comprehensive analysis includes whole-genome analysis or whole-exome sequencing using next-generation sequencing or panel testing to analyze 100–200 genes.

In this lecture, I will provide information of use in a practical clinical setting, so I will describe approved treatments.

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Genomic medicine for cancer that is provided in routine practice, i.e. care that is covered by National

Health Insurance, can largely be divided into 3 types.

The first involves effective molecularly targeted drugs. Genetic abnormalities in cancer cells that will be targeted with a drug are identified and patients who are likely to respond are selected.

The second involves the identification of genetic abnormalities in cancer cells for which molecularly targeted drugs are ineffective. Patients who are unlikely to respond are excluded.

The third involves the identification of genetic abnormalities in normal cells that are associated with serious adverse reactions to anticancer agents. This information will be used to determine whether or not an anticancer agent is administered and the dose.

The theme of this lecture is Cancer Arising from Somatic Mutations, so the first and second types of genomic medicine are relevant. The third concerns changes in germ cells, so it is closely related to treatment with an anticancer agent. I will briefly touch on it in this lecture.

So let me explain each type in order.

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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.

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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.

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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.

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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.

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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.

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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.

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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.

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

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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.

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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.

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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.

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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.

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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.

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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.

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%.

Slide 4

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.

Slide 14
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.

Slide 15
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

Slide 17
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.

Chapter4

Slide 1

Next, I will talk about genomic medicine for breast cancer.

Based on expression of the estrogen receptor and the progesterone receptor (hormone receptors) and HER2, breast cancer is categorized as shown in this table.

In routine clinical practice, expression of a hormone receptor is detected using immunostaining, but expression of HER2 is detected via gene amplification with FISH in addition to immunostaining.

In other words, this falls under genomic medicine, as I will explain.

The HER2 gene is amplified in around 20% of patients with breast cancer.

Slide 2

HER2 is an abbreviation for human epidermal growth factor receptor 2.

HER2 is also called erbB2 or neu.

As its name indicates, HER2 is a receptor tyrosine kinase belonging to the EGFR family. The gene is encoded on the long arm of chromosome 17.

HER2 is thought to be associated with the development and maintenance of the heart and nerves, but there are no known ligands that bind to HER2.

HER2 forms a homodimer, in which HER2 binds to HER2, or a heterodimer, in which HER2 binds to another protein in the EGFR family, facilitating signaling downstream.

When the HER2 is amplified, HER2 protein is overexpressed. Overexpressed HER2 protein facilitates growth signaling, and breast cancer grows.

Slide 3

There are 2 methods of assessing HER2 amplification: immunostaining and FISH. In immunostaining, the intensity with which the cell membrane of tumor cells is stained is assessed and scored.

In FISH, 20 tumor cells are assessed, and the ratio of HER2 signaling to endogenous control signaling is calculated. If the ratio is greater than 2.2, then the HER2 gene is deemed to be amplified.

Slide 4

If immunostaining reveals the staining of the cell membrane of more than 30% of invasive cancer cells with an even intensity, then a score of 3+ is given. If only immunostaining is performed, administration of Herceptin (trastuzumab) is indicated. If staining is weaker (a score of 2+), then FISH is performed. If the HER2/CEP17 ratio is 2.2≥, then the gene is amplified and Herceptin is indicated.

Slide 5

The efficacy of trastuzumab, an anti-HER2 antibody, is correlated with HER2 expression by breast cancer cells.

The response rate to trastuzumab alone is around 5% in patients who test negative according to FISH but 35% in patients with a score of 3+ in immunostaining or who test positive according to FISH.

In addition, adding trastuzumab to an anticancer agent to treat HER2-expressing breast cancer significantly increased the progression-free survival rate compared to an anticancer agent alone.

Thus, breast cancer highly expressing HER2 is identified and genomic medicine is provided in the form of personalized medicine using an anti-HER2 antibody.

Slide 6

Personalized medicine is similarly provided to treat expression of HER2 in gastric cancer.

Gastric cancer is categorized as an intestinal type or a diffuse/mixed type based on mucin expression.

Overexpression of HER2 is noted at a frequency of 15–50% in the intestinal type and 2–25% in the diffuse/mixed type.

HER2 expression in gastric cancer, like that in breast cancer, is detected using immunostaining and ISH. If immunostaining yields a score of 3+, then an anti-HER2 molecularly targeted therapy is indicated. If the score is 2+, then the specimen is retested using ISH. Caution is required since the cutoff for positivity according to ISH is 2.0, unlike the cutoff of 2.2 for breast cancer. If results are positive according to ISH, then anti-HER2 molecularly targeted therapy is indicated.

Slide 7

Here are images obtained using immunostaining (immunohistochemistry or IHC) and dual color in situ hybridization (DISH) to test actual gastric cancer tissue for HER2 expression.

Immunostaining revealed a high level of HER2 protein expression on the cell membrane in gastric cancer tissue on the left and DISH revealed HER2 gene amplification in the nucleus (black dots), so the HER2 gene was amplified from cancer cells at those sites, and the HER2 protein was overexpressed.

Immunostaining and DISH revealed no expression of the HER2 protein or gene in the gastric cancer tissue on the right.

In other words, HER2 expression in gastric cancer can be assessed using immunostaining and ISH. With HER2-positive breast cancer, almost every cancer cell will test positive, but heterogeneity in HER2 expression will be present in gastric cancer, so caution is required. If @@% of cells are positive, then the patient is diagnosed with HER2-positive gastric cancer, and therapy targeting HER2 is indicated.

Slide 8

Shown here are the standards for detecting HER2 with immunostaining.

Slide 9

Here are the results of the ToGA trial, which compared the efficacy of standard chemotherapy as initial treatment of HER2-positive advanced gastric cancer and a combination of trastuzumab (an anti-HER2 antibody) and standard chemotherapy. A group receiving cisplatin+5FU as standard chemotherapy had a median survival of 11.1 months while a group receiving trastuzumab in addition to cisplatin+5FU had a median survival of 13.8 months, so median survival was prolonged 2.7 months. Based on a hazard ratio of 0.74 and significant differences as indicated by a P value of 0.0046, results indicated that trastuzumab should be added.

Thus, HER2 expression and gene amplification are assessed in breast cancer as well as gastric cancer, and genomic medicine is provided in the form of personalized medicine.

Chapter5

Slide 1

Next, I will describe an instance where a gene abnormality that will not respond to molecularly targeted drugs is identified in cancer cells and, conversely, the selection of patients who are likely to respond.

The gene abnormality in question is found in KRAS.

Slide 2

The name Ras comes from rat sarcoma. RAS was identified as the product of an oncogene in the Harvey sarcoma virus and the Kirsten sarcoma virus, which are retroviruses that cause sarcoma in rats.

HRAS, NRAS, and KRAS are members of the RAS family.

The most frequent mutations are KRAS mutations, so I will explain about KRAS.

KRAS is protein consisting of 186 amino acids with a molecular weight of 21 kDa. There are 3 hotspots where a mutation is likely to occur in KRAS: codons 12, 13, and 61.

Mutations in these 3 codons account for 99% of all KRAS mutations.

The most prevalent is a mutation in codon 12.

Slide 3

As I mentioned in the chapter on BRAF, the RAS family includes KRAS. RAS is located downstream of receptors such as EGFR, and it is an important molecule that facilitates stimulation of various signaling pathways, such as MAPK pathways with downstream RAF/MEK/ERK and PI3K/AKT/mTOR. When a mutation occurs in KRAS, downstream signaling is constantly activated despite the lack of signaling from receptors such as EGFR that are located upstream. The mutation promotes cell growth and survival.

Slide 4

As shown here, a KRAS mutation is detected in various types of cancer. The mutation is detected in over 60% of pancreatic cancers, over 40% of colon cancers, and over 25% of adenocarcinomas of the lung.

Because of their relationship to treatment with molecularly targeted drugs, KRAS mutations must be assessed in colon cancer.

Slide 5

Anti-EGFR antibodies are approved for treatment of colon cancer. Two, cetuximab (human-mouse chimeric antibody) and panitumumab (a human antibody), are used in Japan.

Slide 6

These anti-EGFR antibodies only act on colon cancer cells with a KRAS mutation. I will now explain the mechanism by which they act.

In cancer cells with wild-type KRAS, ligands such as EGF, TGF-α, or amphiregulin bind to EGFR expressed on the cell membrane. As a result, downstream KRAS/RAF/MEK/ERK, i.e. MAPK pathways, are activated, and cells survive and proliferate. When anti-EGFR antibodies bind to EGFR under these conditions, they inhibit binding of a ligand to EGFR, so downstream signaling is inhibited, cancer cells die, and the efficacy of an anticancer agent is enhanced.

If, however, a KRAS mutation is present, then downstream MAPK pathways are constantly activated. Even if anti-EGFR antibodies are administered and bind to EGFR, downstream signaling is constantly activated, so an anticancer agent will have no effect.

Slide 7

This fact was indicated clinically in the CRYSTAL trial.

The therapeutic effectiveness of standard chemotherapy alone was compared to that of standard chemotherapy with the anti-EGFR antibody cetuximab added for initial treatment of metastatic colorectal cancer (i.e. colon cancer).

A combination of anticancer agents known as FOLFIRI was used as standard chemotherapy.

A stratified analysis as part of that trial indicated that progression-free survival was significantly prolonged for patients with wild-type KRAS when cetuximab was added to standard chemotherapy compared to standard chemotherapy alone.

However, adding cetuximab had no benefit whatsoever for patients who were positive for a KRAS mutation.

Based on these results, adding anti-EGFR antibodies to standard chemotherapy resulted in no benefit for patients with KRAS mutation-positive colon cancer. Anti-EGFR antibodies are not used to treat patients with a KRAS mutation.

Slide 8

Right now, there are no drugs that can treat a KRAS mutation, so a KRAS mutation is a biomarker of drug inefficacy.

Drugs to treat a KRAS mutation will be developed in the future, and research needs to continue so that a KRAS mutation can become a biomarker of drug response.

Chapter 6

Slide1

Last, I will explain about the identification of genetic abnormalities in normal cells that are associated with serious adverse reactions to anticancer agents. I will explain how this information is used to determine whether or not an anticancer agent is administered and the dose.

The gene abnormalities in question are UGT1A1 polymorphisms.

Slide 2-3

Let us review the term gene polymorphism.

Roughly speaking, the gene abnormalities I have talked about thus far are extremely rare changes (i.e. a frequency of less than 1 in 100 people). These genetic changes harm the body, like an EGFR mutation that causes lung cancer and BRCA1 that causes breast cancer and ovarian cancer.

In contrast, a gene polymorphism is noted at a considerable frequency since it occurs in more than 1 in 100 people. This genetic change will pose no problems in terms of normal life. Although some gene polymorphisms will have absolutely no effect on the survival and evolution of humanity, some unexpectedly might affect the survival and evolution of humanity. In an easily explainable example, some people can tolerate alcohol while others cannot. Gene polymorphisms are one factor that determines a person's tolerance. Polymorphism in a gene coding for an enzyme that breaks down alcohol causes diminished enzyme activity in some people, making them less able to tolerate alcohol. Alcohol is consumed and turns into a toxin known as acetaldehyde in the body. Acetaldehyde is converted into acetic acid by an enzyme known as ALDH in the liver, where acetic acid is then broken down into water and carbon dioxide.

If, however, there is a polymorphism in the gene for the enzyme ALDH, then acetaldehyde cannot be converted into acetic acid. ALDH will accumulate in the body, causing a person to immediately become drunk and suffer a hungover later.

However, this genetic change poses no problem to the survival and evolution of humanity whatsoever if people do not consume alcohol.

When a polymorphism occurs in an enzyme that breaks down an anticancer agent, it can cause a more severe adverse reaction to occur.

Slide 4

That anticancer agent is irinotecan.

Irinotecan is a semisynthetic derivative of an anticancer drug called camptothecin that is extracted from the fruit and roots of a tree known as the Happy Tree or Cancer Tree (Camptotheca acuminata

Decne).

When irinotecan is hydrolyzed, its active form is known as SN-38. Irinotecan exhibits antitumor action by inhibiting topoisomerase 1.

Irinotecan is approved for the treatment of numerous types of cancer, including small cell lung cancer, non-small cell lung cancer, cervical cancer, ovarian cancer, gastric cancer, colon cancer, and breast cancer.

Slide 5

Irinotecan is infused intravenously. Carboxylesterase in the liver converts it into its active form, SN-38, as I mentioned a moment ago. SN-38 had anticancer action, but it can also cause adverse reactions.

In the body, SN-38 is inactivated and detoxified by UDP-glucuronosyl transferases (UGTs).

The UGT1A1 gene codes for a UGT that is involved in that detoxification. A certain percentage of people are born with a polymorphism in that gene. If people with the polymorphism develop cancer and they receive irinotecan, it cannot be fully detoxified and they will suffer severe adverse reactions.

Slide 6

Patients with the polymorphism *28 or *6 in UGT1A1 have a high risk of serious adverse reaction to irinotecan.

In specific terms, patients who are homozygous for UGT1A1 *28 have a 7-fold higher risk of a serious adverse reaction, such as neutropenia, to irinotecan.

Around 10% of Japanese are homozygous for *28, homozygous for *6, or heterozygous for *28 and *6, so these polymorphisms are found in 1 in 10 people. People with these polymorphisms have a high risk of a serious adverse reaction to irinotecan.

A UGT1A1 polymorphism can be detected in normal cells as well, so in routine clinical practice blood is collected prior to treatment, and a kit is used to identify UGT1A1 gene polymorphisms *28 and *6. Results are received in about 2 weeks.

If a polymorphism is found, then the irinotecan will be roughly halved or another anticancer agent will be used.

Thus, genomic medicine for cancer helps to identify gene polymorphisms and to predict adverse reactions to anticancer agents.

Slide 7

Last is a depiction of genomic medicine for cancer in the future.

One could envision a shift from a diagnosis based on a conventional histologic classification by organ to the identification of specific gene abnormalities and a diagnosis by genetic mutation.

One could envision panel testing that identifies around 100 gene abnormalities and a comprehensive genetic analysis using next-generation sequencers that identifies 20,000–30,000 gene abnormalities. Treatment will increasingly be provided by selecting drugs that should be efficacious.
Now, I'd like to start brining my lecture to a close.

Slide 8
Here is a summary of what we have covered.
With this, I conclude my lecture.