Genome Medicine for Brain Tumors: Current Status and Future Perspectives

Akitake MUKASA[1]

[1]Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Kumamoto, Japan

Abstract

As a result of rapid progress in genome medicine technologies, such as the evolution of DNA sequencing and the development of molecular targeted drugs, the era of precision cancer medicine has begun. In 2019, a nationwide genome medicine system was established and cancer gene panel sequencing began being covered by national health insurance in Japan. However, patients with brain tumors have not benefited much from genome medicine, even though gliomas contain many potential molecular targets, such as alterations in *EGFR*, *IDH1/2*, *BRAF*, and Histone H3K27. Targeted therapies for these molecules are currently under enthusiastic development; however, such attempts have not yet achieved remarkable success. To date, only a limited number of targeted drugs for brain tumors such as immune checkpoint, neurotrophic tyrosine receptor kinase (NTRK), and Bruton tyrosine kinase (BTK) inhibitors are available, and only in limited cases. Several obstacles remain in the development of drugs to treat brain tumors, including the difficulties in conducting clinical trials because of the relatively rare incidence and in drug delivery through the blood–brain barrier (BBB). Furthermore, general problems for numerous types of cancer, such as tumor heterogeneity, also exist for brain tumors. We hope that overcoming these issues could enable precision genome medicine to be more beneficial for patients with brain tumors such as malignant gliomas. In addition, careful consideration of ethical, legal, and social issues (ELSIs) is important as it is indispensable for maintaining good relationships with patients, which is one of the keys for genome medicine promotion.

Keywords: brain tumor, glioma, gene, targeted therapy, precision medicine

Introduction

As cancer is a disease caused by genetic alterations, gaining a better understanding of the cancer genome is critical for accurate diagnoses and the development of effective treatments. More than 30 years ago, in 1986, Nobel laureate, Professor Dr. Renato Dulbecco declared in the journal *Science* that "if we wish to learn more about cancer, we must now concentrate on the cellular genome," and encouraged cancer scientist to sequence the whole genome.[1] At last, in this twenty-first century, aided by the enormous progress of the so-called next-generation sequencing (NGS) technology, we now can rapidly sequence a large amount of DNA at a reduced cost, and surprisingly detailed genomic and epigenomic profiles of many cancers, as Dr. Dulbecco might have imagined in the last century, have become available. In 2015, in his State of the Union Address, the former president of the United States, Barack Obama, made a memorable announcement regarding the "Precision Medicine Initiative" that sounded like the official beginning of "the era of genome medicine."

When it comes to brain tumors, owing to considerable efforts in cancer genome projects such as The Cancer Genome Atlas Project, the International Cancer Genome Consortium (ICGC),[2] and numerous other elaborate genome profiling projects from a wide range of groups, the molecular profiles of various brain tumors were enthusiastically analyzed, and are now widely publicly accessible. Through these genome profiling studies, the molecular alterations that cause the initiation and progression of brain tumors, especially gliomas, have now been
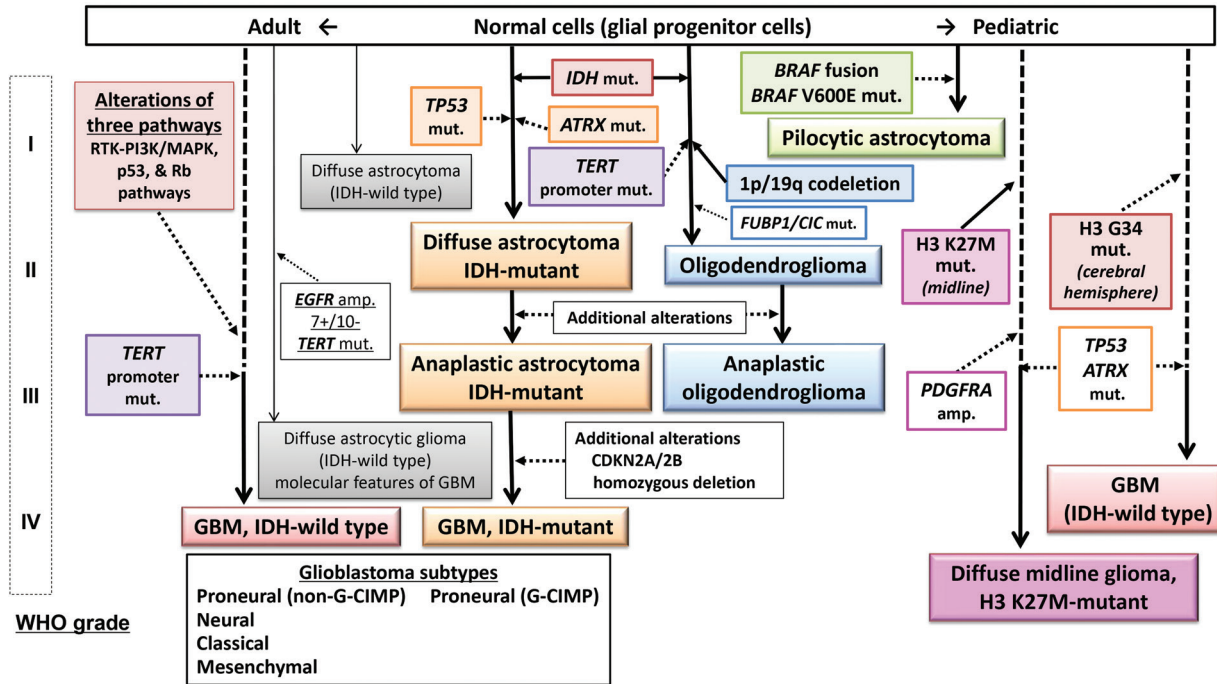
Fig. 1 Current understanding of the initiation and progression of gliomas and related molecular alterations. Various histological types of gliomas are positioned according to their World Health Organization grade. The courses of tumor progression are illustrated with vertical arrows. Gliomas depicted on the left side of the figure tend to arise in adult patients, whereas tumors depicted on the right side tend to arise in pediatric patients. The genetic and genomic alterations, which are thought to drive the initiation and progression of the corresponding tumors, are also depicted beside the vertical arrows. (Reprinted with partial revision with permission from reference 3). amp: amplification, GBM: glioblastoma, G-CIMP: glioma CpG island methylator phenotype, mut: mutation.

extensively characterized (Fig. 1).[3] Based on these data, the neuro-oncology community clearly realized that the classification of at least some brain tumors is more accurate when it is based on information regarding molecular profiles rather than on microscopic observations. Therefore, naturally, an epoch-making transition to a method of pathological diagnosis in which some genetic and genomic alterations, as opposed to observations by a pathologist, are definitive factors for a final diagnosis was made in the 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System, Fourth Edition, Revised (WHO 2016).[4] Primitive neuroectodermal tumors (PNETs), which had been generally used as a pathological classification, was removed from the WHO 2016 classification because molecular analysis recently revealed the possible existence of several molecularly distinct subgroups within tumors previously known as PNETs of the central nervous system (CNS-PNETs), implying that they were the same types of tumor.[5] More recently, it was even demonstrated that the computational algorithm learned through the analysis of accumulated genome-wide methylation profiles from various CNS tumors might provide more accurate

pathological classifications of brain tumors compared with classic pathological observations.[6]

In this review, the future possibilities of genome medicine for brain tumors, especially gliomas, are discussed, focusing mainly on topics surrounding molecular targeted therapy. Despite the evolution of molecular profiling technologies that have led to dramatic progress in the diagnosis of brain tumors, little improvement has been seen in terms of effective treatments for patients. Although numerous molecules have been identified as targetable, even in brain tumors, several obstacles remain before genome medicine can be more successful in the treatment of brain tumors (Fig. 2). The objectives of this review are to increase the familiarity of genome medicine among individuals treating patients with a brain tumor and to facilitate the clinical application of knowledge in precision medicine for treating such intractable tumors.

Current status of molecular targeted therapy for brain tumors

With the rapid technical advancement of genome sequencing and bioinformatics, as well as the nationwide promotion of genome-based personalized
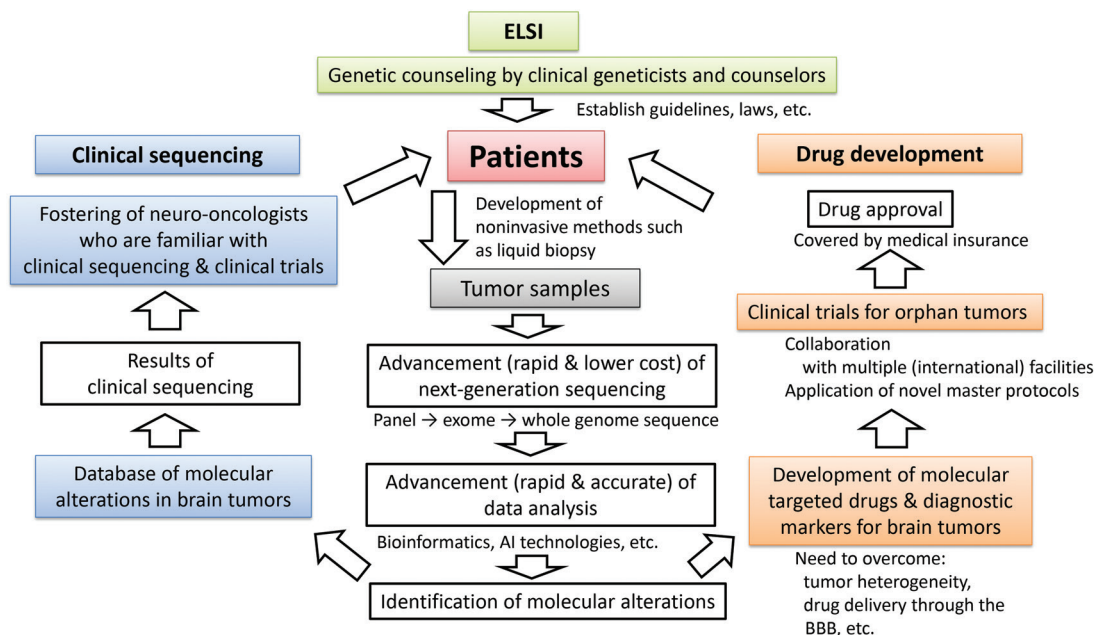
Fig. 2 Multiple obstacles to overcome for promoting genome medicine for brain tumors. This schema illustrates the work flow required for the promotion of genome medicine. Multiple issues needed to be addressed to overcome obstacles in regard to genome medicine for brain tumors. These issues include those related to clinical sequencing, drug development, and ELSIs. ELSLs: ethical, legal, and social issues.

medicine, a medical system for cancer genome medicine was established by the Japanese government, and platforms for cancer gene panel sequencing began to be covered by national health insurance in 2019. When molecular alterations that have a corresponding drug treatment are identified by the cancer gene panel, patients can be treated with the approved drugs under national health insurance or enrolled into clinical trials assessing drugs under development. Therefore, genes that are frequently altered in cancer are typically analyzed by these cancer gene panels. For example, one cancer gene panel, called FoundationOne CDx (Chugai Pharmaceutical, Tokyo, Japan), can analyze alterations such as fusion and mutations in 324 cancer-related genes in a single analysis. FoundationOne CDx is also approved as companion diagnostic for several molecular targeted drugs in Japan. Another approved cancer gene panel is the OncoGuide NCC Oncopanel System (Sysmex, Kobe, Japan), which can analyze alterations in 114 genes, including 12 gene fusions. Since the NCC Oncopanel System analyses DNA from normal tissue, germline mutations can be determined in 13 genes that lead to hereditary cancer syndrome. Considerable numbers of genes analyzed by cancer gene panels are related to brain tumors and have potential usefulness for patients (Table 1). However, to date, only a few of these genes are clinically useful for the treatment of brain tumors, and a very small proportion of patients with brain tumors

tested using cancer gene panel sequencing can be treated with the corresponding molecular targeted drugs. To improve this situation, the development of effective drugs for brain tumors is urgently needed.

Although enthusiastic efforts have been made to develop targeted therapy against brain tumors, especially malignant gliomas, for many years,[7] and gliomas are well known to have numerous potentially targetable molecular alterations, such as the amplification and deletion of epidermal growth factor receptor (EGFR),[8,9] only a few effective molecular targeted drugs are currently available.[10] The development of molecular targeted drugs against commonly observed alterations, such as those targeting EGFR, has been successful for a variety of cancer types; however, these drugs have often only shown minimal efficacy for treating brain tumors. Such a limited clinical response may be explained by several factors, including intratumoral heterogeneity and restricted drug penetration through the blood–brain barrier (BBB). In addition to these obstacles, the relatively rare incidence of brain tumors might also explain the delay in drug development. Further details regarding these issues are discussed later in the paper. Our combined efforts are needed to overcome these problems. The recent discovery of potentially targetable alterations has resulted from extensive molecular profiling, and could be expected to expand opportunities for accelerated drug development.

Table 1 Potential molecular markers and targets of brain tumors that can be identified by the cancer gene panel (FoundationOne CDx)

Category	Tumor types	Cancer-related genes (total of 324 genes represented in the panel)
Clinically useful for diagnosis	Glioma	*ATRX*, *H3F3A*, *IDH1*, *IDH2*, *TERT* promoter
	Pilocytic astrocytoma, others	*BRAF* (mutation & fusion)
Clinically useful for treatment	Pilocytic astrocytoma, others	*BRAF* (mutation & fusion)
	Glioma	*MLH1*, *MSH2*, *MSH6* (MMR genes)
	Various tumors	*NTRK1, 2* and *3* (fusion)
Closely related to brain tumors	Glioma (glioblastoma, etc.)	*CCND1*, *CDK4*, *CDK6*, *CDKN2A*, *CDKN2B*, *CDKN2C*, *EGFR*, *MDM2*, *MDM4*, *MET*, *MYCN*, *PDGFRA*, *PIK3CA*, *PIK3R1*, *PTEN*, *RB1*, *SOX2*
	Oligodendroglioma	*CIC*, *FUBP1*
	Pediatric & cerebellar glioma	*SETD2*
	Primarily CNS lymphoma	*BTG1*, *BTG2*, *MYD88*, *PIM1*, *PIM2*
	Germ cell tumor	*KIT*, *KRAS*, *NRAS*, *MTOR*
	AT/RT	*SMARCB1*(*INI1*)
	Meningioma	*AKT1*, *NF2*, *SMO*
	Other	*FGFR1~3* (fusion), *BCOR*
Related to hereditary cancer syndromes	Various tumors	*APC*, *NF1*, *NF2*, *PTCH1*, *PTEN*, *TP53*,*TSC1*, *TSC2*, *VHL*, *MLH1*, *MSH2*, *MSH6* (MMR genes)
Other molecular features		TMB and MSI

Alterations that can also be analyzed by the NCC Oncopanel System are underlined. AT/RT: atypical teratoid/rhabdoid tumor, CNS: central nervous system, MMR: mismatch repair, MSI: microsatellite instability, TMB: tumor mutation burden.

Targetable molecular alterations in malignant brain tumors

In this section, several approved drugs (Table 2a) and emerging candidates (Table 2b) for targeted therapy of brain tumors, such as glioma and primary CNS lymphoma (PCNSL), are shown. Although the discussion is mostly focused on malignant gliomas in this review, molecular targeted drugs for other types of brain tumors, such as meningioma, are also under development.[11]

Isocitrate dehydrogenase mutation

Frequent mutations of genes encoding isocitrate dehydrogenase (IDH) have been discovered by comprehensive gene sequencing in glioblastomas,[12] and appear to be frequent in lower-grade gliomas (grades II and III) as well as in secondary glioblastomas.[13,14] These mutations are observed in *IDH1*/*IDH2*, and the hotspot mutation, IDH1 R132H, is predominant in gliomas. These mutated IDH1/2 produce oncometabolite 2-hydroxyglutarate, which leads to genome-wide DNA hypermethylation, called glioma CpG island methylator phenotype (G-CIMP).[15] Since

the inhibition of the aberrant enzymatic activity of the mutant IDH has been shown to reduce tumor cell proliferation[16] and these mutations also exist in other tumors, such as acute myeloid leukemia, elaborate drug development targeting these specific mutations is currently underway by several companies. The clinical effectiveness of these inhibitors, especially against non-enhanced IDH-mutant gliomas, has been suggested based on results of phase I clinical trials; therefore, further examination in large-scale phase II/III trials is expected in the future. Since mutant IDH can be a neoantigen, it is also expected to be a novel target for immunotherapy.[17] The aberrant metabolic state of IDH-mutant gliomas might be also targetable since this can lead to metabolic vulnerabilities. For example, an indispensable coenzyme, NAD+, is decreased in IDH-mutant gliomas; thus, the further depletion of NAD+ by inhibiting the NAD+ salvage pathway enzyme can induce tumor cell death.[18]

Histone H3 mutation

Frequent mutations in genes encoding histone H3 variants, especially H3.3 encoded by the *H3F3A*

Table 2 Approved and potential molecular targeted drugs for brain tumors

(a) Currently approved drugs that may be effective for brain tumors

Compound name	Category	Target	Drug type	Indication for brain tumors in Japan (as of May 2020)
Pembrolizumab	Immune checkpoint inhibitor	PD-1	Humanized monoclonal antibody	Microsatellite instability-high-solid tumors (recurrent/refractory)
Entrectinib	NTRK inhibitor	NTRK 1/2/3 fusion	Small molecule	*NTRK* fusion-positive solid tumors (recurrent/refractory)
Tirabrutinib	BTK inhibitor	BTK	Small molecule	PCNSL (recurrent/refractory)

(b) Representative potential targets for molecular targeted therapies for brain tumors

Target	Type of alteration	Representative drug types	Frequently altered brain tumors
IDH1/2 genes	Mutation (hotspot)	IDH inhibitor (small molecule), monoclonal antibody	Lower-grade glioma, glioblastoma IDH-mutant
Histone H3 K27	Mutation (hotspot)	Dopamine receptor D2/3 antagonist, epigenetic modifiers, monoclonal antibody, etc.	Diffuse midline glioma, H3K27M-mutant
TERT promoter	Promoter mutation, etc.	Various types	Glioblastoma and other gliomas
BRAF genes	Mutation (hotspot)	BRAF V600E inhibitor	Pilocytic astrocytoma, PXA,ganglioglioma, epithelioid GBM, craniopharyngioma (papillary)
	Fusion	MAPK pathway inhibitor	pilocytic astrocytoma

GBM: glioblastoma, PCNSL: primary central nervous system lymphoma, PXA: pleomorphic xanthoastrocytoma

gene, are also one of the most important recent discoveries.[19] These mutations occur at a region called the histone tail, which has an important role in the epigenetic regulation of gene transcription. The H3 K27M mutation is mostly found in pediatric diffuse intrinsic pontine glioma (DIPG) but also found in gliomas located at the thalamus and spinal cord in adult patients[20]; therefore, these gliomas are now classified together as "Diffuse midline glioma (DMG), H3 K27M-mutant" in the WHO 2016.[4] By contrast, the H3 G34V/R mutation is frequent in pediatric hemispheric high-grade gliomas (HGGs).[19] In addition to these genes encoding histone H3, a loss-of-function mutation in SETD2, H3K36 trimethyltransferase, has also been reported in pediatric hemispheric high-grade and diffuse cerebellar gliomas.[21,22]

The H3 K27M mutation inhibits the enzymatic activity of polycomb repressive complex 2 (PRC2), which contains an EZH2 subunit, and causes aberrant histone demethylation at lysine 27,[23] and such epigenetic deregulation is thought to lead to tumorigenesis and tumor growth. Therefore, strategies for targeting epigenetic deregulation in H3 K27M-mutant gliomas have been vigorously exploited by numerous research groups,[24–27] and this is expected to be clinically applicable in the near future. Recently, preliminary reports have revealed the efficacy of ONC201, a small molecule selective antagonist of dopamine receptor D2/3 for H3 K27M-mutant DMG, and several clinical trials investigating this drug are ongoing.[28] Similar to mutant IDH, the H3 K27M mutation results in a neoantigen, and therefore, T-cell-based immunotherapy targeting this neopeptide is also under development.[29]

Telomerase reverse transcriptase promoter mutations

The extension of telomeres, which are repetitive nucleotide sequences located at both ends of chromosomes, is essential for the continuous growth of cancer cells because telomeres shorten during replication. The reverse transcriptase enzyme telomerase, which consists of several subunits, including telomerase reverse transcriptase (TERT), plays a key role in the extension of telomeres. Higher telomere activity has been reported in glioblastomas,[30] and recently, frequent *TERT* promoter mutations, which increase TERT mRNA expression, were reported in many tumors, including gliomas.[31,32] Among gliomas, *TERT* promoter mutations are especially frequent in glioblastomas and oligodendrogliomas.

Upregulated TERT is certainly a fascinating candidate for targeted therapy. However, the development of targeting agents against telomerase, such as

imetelstat, a 13-bp RNA antisense oligonucleotide, has not been clinically successful to date.[33)] Indeed, several challenges in regard to targeting telomerase remain. For example, since most cancer cells already have elongated telomeres, cancer cells need to divide multiple times until shortened telomeres cause cell death after telomere inhibition; therefore, immediate response to therapy would not be expected. In addition, telomerase inhibition might lead to the activation of a telomerase-independent elongation mechanism, such as alternative lengthening of telomeres. In Japan, eribulin, which has been shown to inhibit the RNA-dependent RNA polymerase activity of TERT in a preclinical study, has been investigated in a clinical trial to evaluate its safety and efficacy in patients with recurrent glioblastoma.[34)]

BRAF fusion and mutation

KIAA1549–BRAF fusion occurs frequently (66%) in pilocytic astrocytoma.[35)] In addition, recurrent *BRAF* V600E mutations are reported in various brain tumors, such as extracerebellar pilocytic astrocytoma, pleomorphic xanthoastrocytoma (66%), ganglioglioma (18%), epithelioid glioblastoma, and papillary craniopharyngioma (95%).[36,37)] Both fusion and mutation have been shown to activate the mitogen-activated protein (MAP) kinase signaling pathway and enhance tumor cell growth.

A specific inhibitor targeting BRAF V600E is available, and several drugs have already been approved for melanomas. For brain tumors, a dramatic response in papillary craniopharyngioma harboring *BRAF* V600E mutation has been demonstrated.[38)] In gliomas, the antitumor activity of BRAF V600E inhibitor has been shown[39)]; however, the results of a basket-type clinical trial involving a limited number of cases suggested variable efficacy depending on the histologic subtype.[40)] Therefore, a large-scale clinical trial is needed to determine its efficacy in every histological subclass. Furthermore, the activated MAP kinase pathway can be targeted by drugs such as MEK inhibitors. Recently, the efficacy of an MEK1/2 inhibitor, selumetinib, in recurrent, refractory, or progressive pilocytic astrocytoma harboring common BRAF aberrations and NF1-associated pediatric low-grade gliomas was reported in a phase II trial.[41)] In this study, the response rate was not statistically different between tumors harboring *KIAA1549-BRAF* fusion and those harboring *BRAF* V600E mutation. However, it should be noted that patients with pediatric low-grade glioma harboring *BRAF* V600E mutation had shorter progression-free survival, indicating that biological properties might be different between tumors harboring *KIAA1549-BRAF* fusion and those harboring *BRAF*

V600E mutation, in which case, specific treatment strategies would be required depending on the type of alteration. The efficacy of BRAF V600E and MEK inhibitor combination therapy for *BRAF* V600E-mutant epithelioid glioblastoma was also demonstrated.[42)]

Mismatch repair (MMR) gene mutations and immune checkpoint inhibitors

The dramatic response of immune checkpoint inhibitors to formidable cancers such as melanoma has been demonstrated.[43)] Thus, the efficacy of immune checkpoint inhibitors for glioma has been highly expected; however, the results of clinical trials involving checkpoint inhibitors such as the anti-PD1 monoclonal antibody nivolumab for glioblastoma have been disappointing.[44)] This poor responsiveness may be explained by the lower tumor mutation burden (TMB) reported in gliomas,[45)] as immune checkpoint inhibitors have been shown to be more effective for tumors with a higher TMB, which correlates to increased numbers of neoantigens, and these are likely major targets of anticancer cytotoxic T cells.[46,47)] Indeed, rare hypermutated glioblastomas resulting from a germline biallelic MMR deficiency have shown a good response to immune checkpoint inhibitors.[48)]

Mutations in MMR genes such as *MSH2*, *MSH6*, *MLH1*, and *PMS2* are found in various tumors, most notably in colorectal cancers, and deficiencies in MMR genes are known to cause microsatellite instability (MSI), which leads to increased numbers of neoantigens. Since the PD-1 blocker pembrolizumab showed a significant response to solid tumors with an MMR deficiency in a basket-type clinical trial,[49)] the US Food and Drug Administration approved pembrolizumab for MSI-high and MMR-deficient solid tumors in 2017. In Japan, pembrolizumab has also been approved for progressed and refractory MSI-high solid tumors. Although MMR gene mutations can be found in gliomas, it should be noted that most of these mutations are found in gliomas previously treated with the chemotherapeutic agent temozolomide,[50,51)] and that the mutational signatures in these tumors are mostly the hypermutator phenotype and are not MSI-high, which is common in other solid tumors with an MMR deficiency.[51,52)] Therefore, whether checkpoint inhibitors including pembrolizumab are also effective for hypermutated gliomas with an MMR deficiency needs to be investigated in clinical trials targeting this particular cohort.

NTRK fusion

Neurotrophic tyrosine receptor kinase (NTRK) gene fusion involves either *NTRK1*, *NTRK2*, or *NTRK3* encoding the neurotrophin receptors TRKA, TRKB,

or TRKC, respectively. *NTRK* gene fusion also occurs with other genes such as *BCAN* by chromosomal translocation or deletion. Although *NTRK* fusion is rare in cancers, it is known to be oncogenic owing to uncontrolled TRK kinase signaling[53]; therefore, targeted therapy is currently under development. Recently, the ROS1/TRK inhibitor entrectinib was approved for *NTRK* fusion-positive recurrent or refractory solid tumors in Japan based on the efficacy shown in the STARTRK-2 global phase II basket trial for patients with solid tumors harboring *NTRK*, *ROS1*, or *ALK* fusions. Although the efficacy of entrectinib for fusion-positive glioneuronal tumor has been demonstrated,[54] the responsiveness of other types of brain tumors remains largely unknown. Currently, primary brain tumors with *NTRK1/2/3*, *ROS1*, or *ALK* mutations are investigated as part of the STARTREK-NG trial together with other cancers, and the results of this trial are expected soon.

Similar to most other cancers, *NTRK* fusions are rare in brain tumors; mutations are reported in approximately 1% (2/185) of glioblastoma cases,[55] 0.4% (2/461) of low-grade glioma cases,[56] 6% (3/48) of extracerebellar pilocytic astrocytoma cases,[57] 4% (2/54) of DIPG cases, and 10% (6/58) of pediatric non-brainstem HGG (NBS-HGG) cases.[58] Notably, 40% (4/10) of NBS-HGG cases in children younger than 3 years old showed *NTRK* fusion.[58] Considering this rarity, the establishment of an efficient screening method to select candidate patients for further confirmatory testing using the NGS-based in vitro diagnostic, FoundationOne CDx, would be necessary, since it is not easy for most hospitals to perform NGS for all suspected patients. Immunohistochemistry with an anti-pan-TRK monoclonal antibody might be a relatively reliable and cost-efficient way of screening, although not all positive cases can be detected.[59]

BTK inhibitors

Frequent mutations in genes that activate the B-cell antigen receptor (BCR) signaling pathway, such as *CD79B*, or the TLR/MYD88 signaling pathway, such as *MYD88*, are known to occur in PCNSL.[60] These gene mutations drive the NFκB signaling pathway through Bruton tyrosine kinase (BTK) activation and lead to tumor growth; therefore, the inhibition of these pathways by BTK inhibitors such as ibrutinib has been reported to reduce tumor masses in most patients with PCNSL.[61] Monotherapy using a second-generation oral BTK inhibitor, tirabrutinib, which has greater selectivity than ibrutinib, was investigated in phase I/II clinical trials for relapsed/refractory PCNSL, and showed an overall response rate of 64% (28/44) with tolerable toxicity.[62] Based on this favorable outcome,

in 2020, tirabrutinib was approved for relapsed/refractory PCNSL in Japan. Although BTK inhibitor is a molecular targeted drug, companion genetic testing is not necessary prior to the use of tirabrutinib in patients with PCNSL, since most cases of PCNSL are known to show deregulated BTK signal activity, and a majority of patients respond well to this drug.

Issues in precision medicine for brain tumors

Numerous obstacles remain for the promotion of precision cancer genome medicine for brain tumors. Figure 2 shows the work flow for the promotion of genome medicine for brain tumors and associated issues. In this section, some of these key issues that need to be seriously considered are discussed.

Tumor heterogeneity

Both intratumoral and intertumoral heterogeneity are demonstrated in many cancers,[63] and as such, heterogeneity is one of the major obstacles facing precision medicine. A number of studies have reported remarkable spatial and temporal heterogeneity in gliomas.[64,65] For example, the mosaic amplification of multiple receptor tyrosine kinase genes such as *EGFR*, *PDGFRA*, and *MET* was reported in glioblastomas.[64] These observations explain, at least in part, the reason why single anticancer drugs cannot be sufficiently effective. In addition to special heterogeneity, gliomas frequently show temporal heterogeneity by acquiring additional genetic as well as epigenetic changes during the course of observation and treatment, and this temporal heterogeneity can make tumors refractory to therapy.[9,66–68] One such genetic evolution in gliomas involves the emergence of the hypermutator phenotype caused by continuous temozolomide treatment and a resultant deficiency in MMR genes such as *MSH6*.[50,51,69,70]

Due to this temporal heterogeneity, the genetic alterations in recurrent tumors are often dramatically different from those of primary tumors.[50] Therefore, for effective targeted therapy against recurrent tumors, genetic information in regard to recurrent tumor tissues is required. However, surgically resected tissue is not always available from recurrent tumors. To circumvent this problem, a technology known as "liquid biopsy" is greatly expected. Liquid biopsy is a novel diagnostic method that utilizes tumor-derived products such as circulating tumor DNA (ctDNA) and circulating tumor cells in bodily fluids that can be obtained less invasively than can tumor tissue biopsies. Blood plasma is often used for this purpose, but for brain tumors, ctDNA is reported to be more abundant in the

cerebrospinal fluid (CSF).[71] In a previous report, ctDNA was detected in the CSF of about 50% of glioma patients, and genetic tumor profiling using ctDNA can be used to monitor tumor evolution.[72]

To achieve complete regression, the development of strategies to cope with high temporal and special heterogeneity in tumors is necessary. Because multiple driver genes are altered, and several oncogenic pathways are activated simultaneously in most tumors, combinatory administration of molecular targeting agents, as previously reported, might be a possible strategy to treat genetically heterogeneous tumors.[73,74] However, due to their remarkable intratumoral heterogeneity, it is seldom easy to provide a sufficient combination of effective drugs to deal with various oncogenic signals. Another reasonable strategy might be immune enhancing therapy, which is potentially effective for tumors with a high TMB, resulting in increased neoantigens.[46,47] In this context, we recently reported that the reduced transcription of mRNA for neoantigens in recurrent gliomas is a possible mechanism leading to immune evasion.[75] The intrinsic plasticity of malignant gliomas, which is partly exemplified by our report, might be a reason for the generation of heterogeneity and therapeutic resistance.

Drug delivery

The BBB, which prevents drug penetration into the CNS, is certainly an obstacle in the medical treatment of brain tumors. Lipid-soluble or/and low-molecular-weight molecules are known to have a higher chance for penetration; therefore, most anticancer drugs cannot efficiently penetrate the BBB.[76] Consequently, many anticancer agents that are potentially effective for brain tumors cannot achieve a sufficient concentration inside tumors within the CNS, although in malignant brain tumors, a certain degree of BBB disruption is evident. Circumventing inefficient drug delivery is therefore critical for successful anticancer drug treatment.

Multiple strategies to increase drug delivery have been tested. Methods for enhancing the penetration of intravascularly administered drugs include the chemical modification of anticancer agents, intra-arterial administration, and hyperosmotic BBB disruption.[77] For BBB disruption, methods using focused ultrasound[78] or pulsed ultrasound might be also useful.[79] In addition, strategies for efficient local drug administration are under development. Especially, a method called convection-enhanced delivery, which involves the direct infusion of a drug solution through a catheter inserted into the brain, has been enthusiastically tested for brain tumors.[80,81]

Issues in drug development for orphan tumors

Brain tumors such as gliomas are often called "orphan tumors," since the numbers of affected patients are much smaller compared with those with other common tumors, such as lung cancer. Drug development for orphan tumors also faces several issues. For example, owing to smaller number of patients, more effort is usually required to recruit patients for clinical trials necessary for drug approval. In addition, pharmaceutical companies might be reluctant to invest in drug development since the potential number of patients with orphan tumors who can benefit from such drugs is limited. Therefore, the establishment of efficient strategies for conducting clinical trials is urgently needed. The necessity for doctor-initiated clinical trials may be higher for orphan tumors, but planning trials that rely on substantial volunteer efforts by doctors is not easy, especially under the current work environment in Japan. Therefore, fostering more neuro-oncologists who can dedicate efforts to conducting clinical trials for brain tumors might be important in Japan. To compensate for the small numbers of patients, global clinical trials carried out in cooperation with facilities aboard should also be considered.

In the recent era of precision medicine, innovative clinical trial designs, such as basket, umbrella, and platform trials, have become more common (Fig. 3).[82,83] Actually, the immune checkpoint inhibitor pembrolizumab was approved for MMR-deficient tumors through basket-type trials, as mentioned previously.[49] These novel strategies would be helpful when conducting trials for rare cancers such as gliomas, and indeed, several such trials have already been conducted.[84] For example, the efficacy of NTRK and BRAF inhibitors was tested for brain tumors through a basket-type trial, as described in the previous section,[40] and the NCT Neuro Master Match (N²M²) trial is umbrella-type phase I/II trial testing molecular matched targeted therapy plus radiotherapy for patients with primary non-MGMT hypermethylated glioblastoma.[85]

Ethical, legal, and social issues

To promote genome medicine, it is particularly important to foster good doctor–patient relationships. In addition, caregivers need to gain firm trust from patients as well as from society. However, the sequencing of cancer genomes and germline DNA can lead to numerous ethical, legal, and social issues (ELSIs). While technologies for use in genome medicine, such as rapid DNA sequencing, are advancing exponentially, many ELSIs seem to be relatively forgotten. For example, the birth of the world's first gene-edited human babies, twin girls
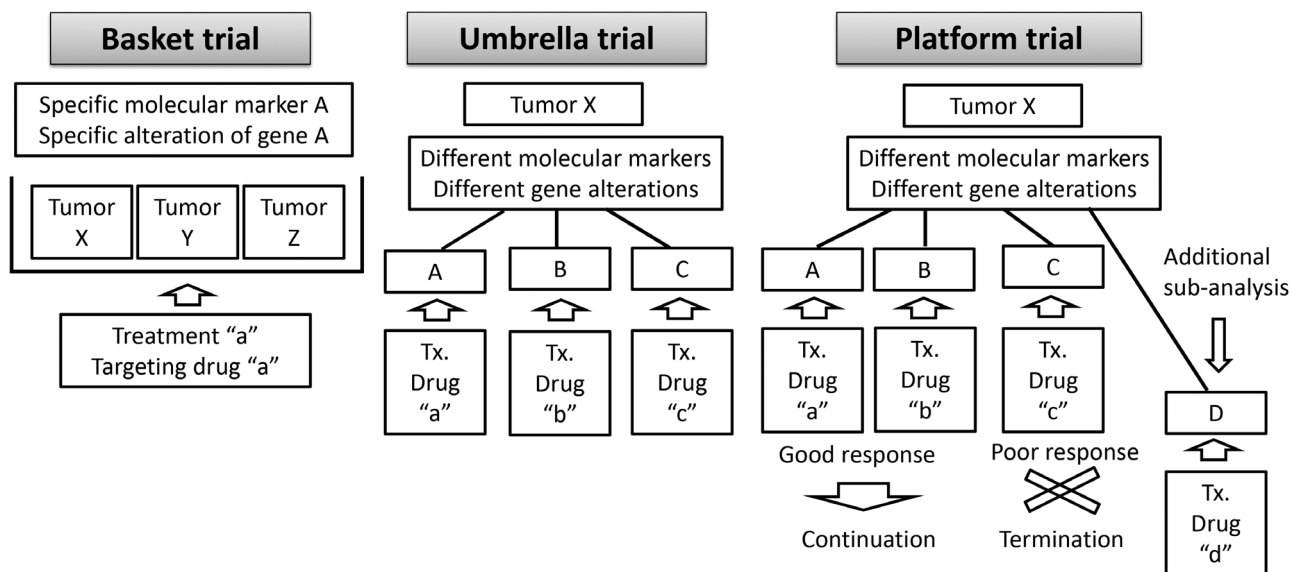
Fig. 3 The types of master protocols that can facilitate clinical trials for molecular targeted drugs. Three types of master protocols (basket, umbrella, and platform trials) are shown. Basket trials test a single treatment or drug for multiple tumors that have a particular molecular marker or genetic alteration in common. Umbrella trials test multiple treatments or drugs according to different identified molecular markers and genetic alterations within the single tumor type. Platform trials are similar to umbrella trials, and are sometimes performed in combination with umbrella and basket trials. Particularly in platform trials, after interim analyses, some sub-analyses with poor outcomes can be terminated, and promising new sub-analyses can be started. Tx.: therapy.

in China, through the use of CRISPR/Cas technologies, raised various ELSIs and led to many protests from the global society; therefore, technical advancements without addressing ELSIs in society can increase the risk of patient refusal and hamper the further advancement of medical innovation. Therefore, to cope with these problems, the continued discussion of and education for ELSIs are needed.

By conducting comprehensive gene sequencing, germline mutations that lead to hereditary diseases such as familial cancers can be incidentally identified. These so-called secondary findings include *BRCA1/2* mutations, which can cause hereditary breast and ovarian cancer (HBOC). Alterations in genes that may cause brain tumors as a part of hereditary cancer syndromes, such as *VHL*, *NF1*, *NF2*, *TSC1/2*, *APC*, *PTEN*, and MMR genes, can be also found. This means that the results of clinical genome sequencing can affect not only patients themselves but also their families. Therefore, the management of ELSIs caused by secondary findings in patients and their families is critical. While care for mental and social affairs, which is partly achieved by genetic counseling, play an important role, such systems have not been well established in many hospitals. There is a serious shortage of counseling specialists, such as board-certified clinical geneticists and genetic counselors, who are accustomed to seeing

cancer patients in Japan. It is therefore important to foster these specialists, and to do so rapidly.

Establishing additional laws that can protect the human rights of patients should also be seriously considered. Patients, and their families, who are diagnosed as having certain genetic diseases may suffer various types of discrimination. For example, they may face disadvantages in obtaining jobs or in being covered by health insurance. In the United States, a law known as the "Genetic Information Nondiscrimination Act (GINA)," which prohibits health insurance- and employment-related discrimination based on genetic information, was enacted in 2008; however, to my knowledge, no such law has been established in Japan. To protect the personal information of patients while facilitating clinical usefulness and promoting medical research, regulations for collecting and utilizing vast amounts of personal genomic data obtained by cancer genome sequencing might also need to be improved.

Concluding Remarks

Recently, rapidly advancing technologies for analyzing cancer genomes have spawned "the era of genome medicine," and cancer patients have begun to enjoy the benefits. However, unfortunately, patients with brain tumors have not been able to obtain the

expected benefits. The hope is that malignant brain tumors such as gliomas will have numerous potential therapeutic targets in genome medicine.[86)] If the brain tumor-specific and nonspecific obstacles described in this review (Fig. 2) can be overcome, there is a good chance that genome medicine could target many brain tumors. To achieve this, further basic and clinical studies, accompanied by patience, are certainly needed. Therefore, efforts should be concentrated on ushering in "the era of genome medicine for brain tumors" for the benefit of patients.

Acknowledgment

Conflicts of Interest Disclosure

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Corresponding author: Akitake Mukasa, MD, PhD
Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto, Kumamoto 860-8556, Japan.
e-mail: mukasa@kumamoto-u.ac.jp