LOGGIC Core BioClinical Data Bank: Added clinical value of RNA-Seq in an international molecular diagnostic registry for pediatric low-grade glioma patients

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Abstract

Background. The international, multicenter registry LOGGIC Core BioClinical Data Bank aims to enhance the un- derstanding of tumor biology in pediatric low-grade glioma (pLGG) and provide clinical and molecular data to sup- port treatment decisions and interventional trial participation. Hence, the question arises whether implementation of RNA sequencing (RNA-Seq) using fresh frozen (FrFr) tumor tissue in addition to gene panel and DNA methylation analysis improves diagnostic accuracy and provides additional clinical benefit.

Methods. Analysis of patients aged 0 to 21 years, enrolled in Germany between April 2019 and February 2021, and for whom FrFr tissue was available. Central reference histopathology, immunohistochemistry, 850k DNA methylation analysis, gene panel sequencing, and RNA-Seq were performed.

Results. FrFr tissue was available in 178/379 enrolled cases. RNA-Seq was performed on 125 of these samples. We confirmed KIAA1549::BRAF-fusion (n = 71), BRAF V600E-mutation (n = 12), and alterations in FGFR1 (n = 14) as the most frequent alterations, among other common molecular drivers (n = 12). N = 16 cases (13%) presented rare gene fusions (eg, TPM3::NTRK1, EWSR1::VGLL1, SH3PXD2A::HTRA1, PDGFB::LRP1, GOPC::ROS1). In n = 27 cases (22%), RNA-Seq detected a driver alteration not otherwise identified (22/27 actionable). The rate of driver alteration detection was hereby increased from 75% to 97%. Furthermore, FGFR1 internal tandem duplications (n = 6) were only detected by RNA-Seq using current bioinformatics pipelines, leading to a change in analysis protocols.

Conclusions. The addition of RNA-Seq to current diagnostic methods improves diagnostic accuracy, making precision oncology treatments (MEKi/RAFi/ERKi/NTRKi/FGFRi/ROSi) more accessible. We propose to include RNA-Seq as part of routine diagnostics for all pLGG patients, especially when no common pLGG alteration was identified.

- 81% of drivers found by RNA-Seq alone were actionable targets.
- Improving pLGG molecular diagnostics supports accessibility of targeted therapies.

Importance of the Study

Patients with pediatric low-grade glioma (pLGG) frequently suffer from recurrence or tumor progression, requiring multiple treatments. The international molecular and clinical registry LOGGIC Core BioClinical Data Bank, established in 2019, aims to identify the underlying genetic alteration of each registered patient as precisely as possible. This is the first report analyzing the added value of RNA sequencing using fresh frozen (FrFr) tumor tissue in this prospective cohort. When compared to current diagnostic methods (eg, gene panel sequencing and DNA methylation analysis), we demonstrated an increased detection of clinically relevant rare gene fusions, hereby making targeted therapies more accessible. This improves diagnostic accuracy and clinical patient benefit, underlining the importance of implementation of RNA sequencing for pLGG patients without detectable MAPK alteration.

Pediatric low-grade glioma (pLGG) are the most common CNS tumors in childhood and adolescence, accounting for 25–30% of pediatric CNS tumors and resulting in 1200– 1500 new cases in the USA per year.1,2 pLGG are classified as WHO grade 1 and 2 based on their low-grade, slow- growing characteristics, rarely showing infiltrating growth or progression to higher grades.2 This heterogeneous group of tumors comprises various histo-molecular diagnoses, including numerous subgroups of astrocytoma, glioneuronal tumors, and ganglioglioma. In recent years, compelling evidence has mounted that the oncogenesis of pLGG lies in activating alterations within the mitogen- activated protein kinase (MAPK) pathway, and as such, can be considered a single-pathway disease.3–6 Most commonly, pLGG will harbor BRAF alterations, specifically KIAA1549::BRAF fusion and BRAF V600E mutation.6,7 About 20% of pLGG are NF1 related.8,9 These patients frequently present with a pilocytic astrocytoma of the optic nerve (optic pathway glioma),10 making biopsies in this lo- cation difficult and rare (and thus the frequency of this sub- group may be underestimated in molecular cohorts).

Treatment Challenges

pLGG are characterized by a high overall survival of 94% but a PFS of only 45% after 10 years when patients that have an indication for further therapy are treated with standard of care (surgery and chemotherapy).11 Despite the good chance of survival, extensive late effects and permanent consequences of treatment pose challenges in addition to lifelong struggles with treatment and relapse.12–16 Loss of visual function and other CNS-related impairments are only a few of the many long-term burdens of this often chronic disease.4,17 While complete surgical resection achieves a cure in nearly 40% of all pLGG patients,18 one in three patients require nonsurgical therapy, either in cases of nonresectable tumors at diagnosis, or as treatment for clinically symptomatic patients and/or those with radio- logical progression. Since pLGG patients frequently suffer from chronic progressive disease,15 they undergo several lines of treatment. In conjunction with the observed long-lasting overall survival, this places great emphasis on the lifelong impact of treatments on the quality of survival and age-appropriate participation. In the hope of improving treatment precision and progression-free survival as well as reducing adverse effects caused by therapy, novel drugs are being incorporated increasingly into individual treatment plans as targeted therapy options.19,20

LOGGIC Core BioClinical Data Bank

As it currently remains enigmatic which pLGG patients will be treatable with surgical resection alone, and who will present with progressive disease requiring multiple lines of treatment, the international, multicenter registry LOGGIC Core BioClinical Data Bank (LOGGIC Core) aims to enhance the understanding of tumor biology in pLGG by prospectively gathering high-quality molecular and clinical follow-up data of pLGG patients. As a molecular matching platform, it provides an integrated diagnosis based on ref- erence neuropathology and precise determination of the driver alteration, aiming to increase accessibility and par- ticipation in subsequent interventional trials. This is the first report showing the feasibility and diagnostic benefit of LOGGIC Core. By evaluating the first two operational years and establishing the necessary logistical and ana- lytical pipelines, this analysis demonstrates improvement of diagnostic accuracy for pLGG patients through addition of RNA sequencing using fresh frozen (FrFr) tumor tissue to current diagnostic methods (eg, gene panel sequencing and DNA methylation analysis).

Methods

Study Design, Eligibility, and Patients

LOGGIC Core is an international, prospective, noninterventional multicenter registry collecting histo-pathological, molecular, and clinical data. Key eligibility criteria of LOGGIC

Core include children and adolescents below the age of 21 years, with all histologically verified subtypes of pLGG, at primary diagnosis, progression sub- sequent to initial observation, or at progression/relapse following a previous treatment. The patient selection criterion for this analysis was determined as enrollment in Germany within the first two operational years; between April 2019 and February 2021.The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients or their legally acceptable representative, or both (if possible), provided written informed consent. Approvals for the study protocol (and any modifications thereof) were obtained from independent ethics committees and the institutional review board at each participating center. The study was registered with the German Clinical Trial Register, number DRKS00019035.

Procedures

After initial pLGG diagnosis, primary histopathology evaluation was performed at the local neuropathology departments. Following local verification of the diagnosis and written informed consent obtained by the treating physicians, the patients were registered into a globally accessible web portal (MARVIN, XClinical) in a pseudonymized fashion in parallel to registration in the German HIT-LOGGIC-Registry for pLGG. Formalin-fixed paraffin-embedded (FFPE) tissue, FrFr tissue, and blood were shipped for molecular diagnostics (Figure 1A) as part of the German referral network for pLGG. FFPE samples were used for first-level molecular diagnostic procedures at the Department of Neuropathology, Charité, Berlin, or according to the national hub for any of the participating international centers, respectively. This includes reference histopathology for verification of histological diagnosis, primary molecular diagnostics, and DNA methylation array analysis. By combining both histopathology and DNA methylation classification results, a central refer- ence integrated diagnosis was obtained. At the second- level molecular diagnostic facility (Heidelberg University Hospital and German Cancer Research Center [DKFZ]), molecular profiling was completed as part of the German referral network for pLGG. FrFr tumor tissue of each pa- tient was used for DNA and RNA extraction to perform gene panel sequencing and RNA sequencing. Prior to RNA sequencing, FrFr tumor tissue passed through a histolog- ical tumor verification procedure with exclusion of sam- ples showing low tumor cell content. This was followed by two-stage quality control after DNA and RNA extraction, resulting in RNA sequencing solely of samples meeting a sufficient RNA concentration and RNA integrity number (RIN). Finally, a diagnostic result was communicated to the local physicians.

Immunohistochemistry and Molecular Profiling

Covering common pLGG alterations, the tumors underwent a diagnostic workup applying standard immunohistochemistry markers (GFAP, MAP2, neurofilament, synaptophysin, p53,

IDH1 R132H, H3 K27M, CD45, Ki67), KIAA1549::BRAF fusion analysis from 850k methylation data in search of a primary MAPK alteration, BRAF pyrosequencing for detection of BRAF V600E mutations, and detection of CDKN2A/B deletion based on DNA methylation array copy number results.

As previously described,21,22 DNA methylation analysis was performed using Illumina Human Methylation EPIC (850k) Array and internal classifier V11b4, mapping the results to methylation patterns of over 2800 reference cases in 82 CNS tumor classes. Copy number variants (CNVs) were identified from EPIC array data by manual inspection of the methylation profiles. After enrichment with an Agilent SureSelectXT kit applying the custom panel NPHD2019A for 160 CNS tumor-related genes,22 the mutational status of tumor DNA was analyzed using next-generation sequencing on an Illumina NextSeq plat- form. Sequencing reads were matched with the 1000 Genomes phase 2 human reference assembly (NCBI build 37.1) using BWA (version 0.6.2). Sequences from periph- eral leucocyte DNA were subtracted, providing a filter for nonsomatic alterations. In addition, exonic alterations not reported in the 1000-genome database were selected from the data. Custom pipelines previously developed were used for detection of single-nucleotide variants and small insertions/deletions (InDels). RNA sequencing of FrFr tumor tissue was run using IlluminaTruSeq RNA Access re- agents, with sequencing on the Illumina NextSeq platform, followed by analysis of gene fusions based on deFuse and Arriba 2.0.23,24

Results

Patients and Baseline Characteristics

Between April 1, 2019 and February 17, 2021, 379 patients from 44 pediatric oncology centers (range of patients per center: 1–43) in Germany were enrolled in LOGGIC Core BioClinical Data Bank and the HIT-LOGGIC-Registry (Figure 1B). From these, 178 FrFr tumor samples arrived at the second-level molecular diagnostic facility. For the remaining 201 patients, no sample was available, either be- cause no FrFr tissue was preserved during surgery or the respective sample was not submitted for analysis. After three-stage quality control, n = 125 (70%) submitted samples fulfilled the criteria for further analysis (sufficient tumor cell content, tissue amount, RNA concentration, and RIN). Of the 53 samples not analyzed, 3 could not be as- signed to a patient upon tissue arrival, 33 did not contain tumor tissue within the submitted sample, 7 did not meet the required tissue amount for RNA sequencing, and 10 showed poor sample quality (eg, RNA concentration too low, insufficient RIN; Figure 1B). All 125 patients included in the subsequent analyses fulfilled the criteria of primary diagnosis or progression following initial observation and had not received any previous systemic nonsurgical treatment. Subject demographics are described in Table 1. Half of the analyzed tumors were localized infratentorially, whereas both hemispheric/cortical and supratentorial midline tumors were each found in about 25% of pa-

tients (Table 1 and Figure 2A). Of all 379 enrolled pa- tients, 92 patients had supratentorial midline tumors. Of these, 29 tumors were ultimately successfully analyzed (32%). Hemispherical/cortical tumors were detected in 103 patients (31 of 103 successfully analyzed; 30%), while infratentorial location occurred in 161 patients (63 of 161 successfully analyzed; 39%). As expected, central reference integrated diagnosis revealed a vast majority of pilocytic astrocytoma (n = 82, 66%), followed by dysembryoplastic neuroepithelial tumor (n = 9, 7%), ganglioglioma (n = 6, 5%), and pleomorphic xanthoastrocytoma (n = 5, 4%), among other entities (Table 1 and Figure 2B).

Molecular Drivers

In this prospective cohort, the application of RNA sequencing using FrFr tissue samples allowed us to detect a driver alteration not identified by current diagnostic methods (immunohistochemistry, gene panel, CNV analysis derived from methylation profiling) in 27 cases (22%) (Supplementary Data S1A). While a molecular driver was detected by those diagnostic techniques alone in 94 of 125 samples (75%), the addition of RNA sequencing improved the overall rate of driver alteration detection to 97% (121 of 125) (29% increase). In 21 of these 27 additionally identified samples, gene panel and CNV analysis failed to detect the underlying molecular driver. For the remaining six samples, routine analysis was incomplete (eg, gene panel/CNV were not performed due to unavailability of DNA/FFPE material or insufficient sample quality), resulting in diagnostic precision relying solely on the avail- ability of the RNA sequencing result. Twenty-two of the 27 additionally identified driver alterations (81%) were action- able drug targets, for example, BRAF, FGFR1, NTRK1, and ROS1 inhibitors, thereby illustrating the clinical relevance of the additional use of RNA sequencing. In n = 6 (5%), RNA sequencing results were inconclusive and inferior to cur- rent diagnostic methods in finding the relevant molecular driver, meaning that RNA sequencing missed to detect the driver alteration (not detected at all, neither by the pipeline algorithm nor by manual identification. It is assumed that this was due to poor RNA sample quality, low tumor cell content, or low expression of the fusion). Based on CNV and gene panel sequencing data, three of these six tumor samples showed KIAA1549::BRAF fusions, while rare gene fusions including one SLC44A1::BRAF, SRGAP3::RAF1, and CCDC6::BRAF fusion each were found in the remaining three cases (Supplementary Data S1A).

When omitting the cases with detected driver point mutations and only taking the samples with underlying gene rearrangements (n = 94) into account, the impact of RNA sequencing from FrFr tumor tissue is even more substantial, now revealing a diagnostic benefit in n = 24 patients (26%) (Supplementary Data S1B). Most rare gene fusions as well as all FGFR1 internal tandem duplications (ITD) fall into this category, relying on detection using FrFr RNA

sequencing when utilizing current pipelines. This finding led to a change in analysis protocols for future cases.

We confirmed KIAA1549::BRAF fusion (n = 71), BRAF V600E mutation (n = 12), and alterations in FGFR1 (n = 14) as the most frequent driver alterations in pLGG (Figure 3). The FGFR1 alterations were further subdivided into FGFR1::FGFR1 ITD (n = 6), FGFR1 point mutations (n = 7), and FGFR1::TACC1 fusion (n = 1). Other identified drivers include mutations of NF1, NF2, TSC1, IDH1 as well as SMARCB1. (Initially, this sample had been considered as desmoplastic infantile ganglioglioma through central histopathological reference evaluation and registered in LOGGIC Core as pLGG. Paired with a SMARCB1 deletion detected by RNA sequencing and inconclusive CNV and gene panel sequencing results, it was ultimately referred to as "SMARCB1 deficient tumor, not elsewhere classified," not meeting a category according to the WHO classification. The diagnostic findings were communicated to the local physicians.) N = 16 cases (13%) presented rare gene fusions (TPM3::NTRK1 [n = 1], EWSR1::VGLL1 [n = 1], SH3PXD2A::HTRA1 [n = 1], PDGFB::LRP1 [n = 1], EML4::ALK [n = 1], MYBL1::RP11-89A16 [n = 1], GOPC::ROS1 [n = 1], RAF1 fusions [n = 3], MYB fusions [n = 2], and other BRAF fusions [n = 4]; Figure 3 and Supplementary Data S2). As depicted in Figure 4B, 11 of the 16 rare gene fusions found in this sample cohort were solely identified by FrFr RNA sequencing, being more sensitive in detection compared to the other ap- plied molecular diagnostic methods. Furthermore, all FGFR1 ITD (n = 6) remained undetected by gene panel/ CNV analysis but were revealed by the additional application of RNA sequencing from FrFr tumor tissue. The correlation of the integrated diagnosis and corresponding alteration underlined the strong association of pilocytic astrocytoma to the prevalent KIAA1549::BRAF fusion, found in n = 67 pilocytic astrocytoma (82%) (Figure 4A). ITD of FGFR1 were almost exclusively detected in samples identified as dysembryoplastic neuroepithelial tumor (DNET) (83% of FGFR1::FGFR1 ITD). Tumor samples with a BRAF V600E mutation were evenly distributed between pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and ganglioglioma, while conversely 50% and 60%, respectively, of ganglioglioma and PXA harbored a BRAF V600E mutation. The remaining samples showed no ap- parent correlation.

Discussion

The first analysis of LOGGIC Core revealed clinically relevant targets including rare gene fusions that were identified through routine application of RNA sequencing from frozen tumor tissue while not being detectable by current diagnostic methods. We showed an improvement of diagnostic accuracy in 22% of cases, in which the underlying molecular alteration was detected solely through RNA sequencing. N = 16 cases (13%) harbored rare gene fusions (eg, TPM3::NTRK1, EWSR1::VGLL1, SH3PXD2A::HTRA1, PDGFB::LRP1, MYBL1::RP11-89A16, GOPC::ROS1), 11 of which had not been detected by gene panel sequencing and CNV analysis derived from methylation profiling, ultimately being identified

solely by RNA sequencing from FrFr tumor material. These results highlight the added value of RNA sequencing especially for patients with rarer driver alterations, as 81% of samples uncovered by RNA sequencing alone harbor druggable alterations. This also applies to the six FGFR1 ITDs, all of which were exclusively found in RNA sequencing, exposing a diagnostic gap in gene panel analysis for this important target in DNETs. The advantage of RNA sequencing for FGFR1 ITD detection in the respective n = 6 cases is based mainly on an analytical problem of the gene panel analysis. While it is possible to detect these alterations in DNA samples by manual search, our bioinformatic tools routinely used for diagnostics did not identify them. Thus, this led to a change in analysis protocols for future cases without driver detection.

As the strength of RNA sequencing lies in the detection of gene rearrangements rather than mutations, we evaluated the benefit of RNA sequencing from FrFr tumor tissue when only considering samples with underlying gene rearrangements. This revealed an even higher impact compared to gene panel and CNV analysis derived from methylation profiling, as 26% of the 94 samples relied on RNA sequencing for driver detection, further highlighting the strength of this diagnostic method. Hehir-Kwa et al. recently showed that RNA sequencing can significantly increase the diagnostic yield of gene fusion detection, with the same specificity as current diagnostic methods and a higher sensitivity.25 While the rate of sample suitability for RNA sequencing (97%) is better compared to our cohort (70%), an important methodological difference lies in the inclusion of a variety of pediatric tumor entities (eg, hematologic tumors, solid tumors, but only a low number of CNS tumors; 17%), and the use of both fresh (frozen) tissue or bone marrow for RNA sequencing. On the one hand, this illustrates the challenges with CNS tumor biopsies. On the other hand, we detected 27 molecular drivers by RNA sequencing alone, 81% of these being actionable drug targets, while Hehir-Kwa et al. identified five (21%) actionable alterations among the 24 RNA sequencing-specific gene fusions.25 This underlines the importance of the additional RNA sequencing specifically for children with pLGG. In six samples of our cohort, RNA sequencing results did not align with current diagnostic methods or were inconclusive. We hypothesize that the group of predominantly common KIAA1549::BRAF fusions (n = 3) remained undetected likely due to poor RNA sample quality, low tumor cell content, or low expression of the fusion, and not as a result of an insufficiency of the RNA sequencing itself.26 Furthermore, we noticed that the proportion of samples harboring NF1 alterations appeared very small, that is, 2% of our cohort, whereas other literature describes NF1 relation in 20% of pLGG cases.8 It is important to mention that this underrepresentation of NF1 cases can be explained by specific tumor location. As most NF1-related pLGG tumors within the brain occur along the optic pathway or other midline structures, only few patients will receive biopsies or resections. Therefore, the requirement of FrFr tumor tissue availability for inclusion in our cohort will have favored patients with tumors in locations that are more easily accessible for biopsy/resection, not representing the true prevalence of NF1-associated pLGG. The correlation of tumor pathology and underlying molecular driver

(Figure 4) reveals a strong concurrence of FGFR1 ITD and dysembryoplastic neuroepithelial histology, con- sidering that 5 of 6 FGFR1::FGFR1 ITD (83%) found in our cohort occurred in DNET, and 5 of 9 DNET (56%) harbored FGFR1::FGFR1 ITD. This high fraction carrying the FGFR1 ITD confirms the description of enrichment in DNET.27,28

Regarding the distribution of molecular divers (Figure 3), the proportion of samples with undetermined molecular driver (3%) appears low, considering that previous findings in the literature have suggested a higher percentage of around 16%, in which no driver alteration could be identified by molecular diagnostics.8 This coincides with only nine samples (7%) of not otherwise specified diagnosis (NOS) in our cohort after stating an integrated histo-molecular diagnosis, whereas previous analysis found 35% NOS in non-NF1 cases.8 By adding RNA sequencing using FrFr tumor material to routine di- agnostic methods, currently actionable targets for LGG become more reliably detectable. RNA sequencing in- creased the overall rate of driver alteration detection by 29%, from 75% to 97% (121 of 125). This can lead to participation in subsequent interventional precision oncology trials or treatments (eg, MEKi, RAFi, ERKi, NTRKi, FGFRi, ROSi). This is beneficial particularly for patients with supratentorial midline tumor (of which 32% were ultimately successfully analyzed) who are more likely to require treatment, whereas patients with infratentorial tumors are often cured with surgery alone.

In parallel, the expression data derived from FrFr tumor tissue can not only unfold its potential in fusion detection but also be used for other analyses such as signatures as-sociated with oncogene-induced senescence,29 and further delineate their predictive role in innovative targeted treatment approaches. For example, the expression of in- dividual genes obtained from RNA sequencing was shown to facilitate selection of tumors with potential treatment response when utilizing BCL-XL-dependent senolytics (BH3 mimetics) in senescent PA with upregulated expression of anti-apoptotic BCL-XL.30 Their predictive value might be prospectively tested in future clinical trials.30

As we did not perform a real-time analysis of the sub- mitted FrFr tumor samples but rather opted for a bulk analysis of all samples sent to the second-level diagnostic facility in Heidelberg between April 2019 and February 2021, it is not possible to derive a specific turnaround time.

Based on our experience during the diagnostic INFORM Registry31 using a similar pipeline, we estimate that we are able to perform the complete molecular workup for cases with inconclusive gene panel/methylation results within two to three weeks, providing information relevant for clinical treatment decisions in real-time. Although it is known that RNA sequencing is costly and time-intensive, the costs for panel sequencing are comparable and RNA sequencing is being more and more widely applied. By demonstrating an increased detection of clinically relevant gene fusions, hereby making targeted therapies more accessible, the im- portance of implementation of RNA sequencing becomes apparent. This is underlined when taking into consideration the possible harmful consequences of

refraining from thorough diagnostics and thus not giving children the chance to benefit from a matching targeted treatment (or even the wrong drug). RNA sequencing is necessary particularly in cases which require a precise target for eligibility in a clinical trial, as well as in trials that evaluate complex signatures reflecting MAPK activity status and senescence programs to assess and predict a patient's response to the tested drug. In these instances, a full molecular diagnostic workup including RNA sequencing is indispensable, there- fore justifying the cost of this technique. Ideally, this would translate to RNA sequencing becoming part of routine diagnostics for all pLGG patients. However, a good starting point would be to at least offer RNA sequencing in all tumors where no common MAPK alteration was identified by current routine diagnostic methods like methylation analysis and panel sequencing.

Within the first two operational years, only 178 tumor samples of 379 patients were available (Figure 1B), mostly due to lack of tissue. A few aspects attribute to this issue. Importantly, it should be taken into consideration that the establishment of the diagnostic pipeline itself was an essential goal of this project, explaining why some pediatric oncology centers had initial logistical problems and did not realize that FrFr tissue shipment was indeed a require- ment of this registry. This learning curve was expected and has been seen by comparable projects such as the diag- nostic INFORM platform as well.31 Furthermore, FrFr tissue samples were allowed to be sent batchwise in six months cycles to alleviate the shipment logistics and costs. This can have resulted in a delay in sample shipment, meaning that some more samples were theoretically available on February 17th, 2021, at the local sites than those that had made their way to the central second-level molecular diagnostic facility. Lastly, the centers did not provide detailed information on the lack of sample shipment. We were, however, able to increase the amount of arrived samples by requesting tissue shipment belatedly and reminding the centers, while undertaking ongoing efforts to improve the pipeline by clarifying repeatedly that FrFr tissue availability is mandatory for inclusion and increasing awareness during the online registration procedure.

Of all 178 submitted samples, n = 125 samples (70%) fulfilled the criteria for RNA sequencing after three-stage quality control. In order to improve this percentage, we will aim to increase the amount of available FrFr tumor tissue by refinement of sample shipment and reinforcement of size requirements at sample retrieval (eg, sufficient sample size, meaning at least one pea-sized piece of tissue; precooling the cryovials in liquid nitrogen to avoid tissue sticking to the tubes; snap freezing of tissue in liquid nitrogen as soon as possible to avoid DNA/RNA degradation, optimally within 30 minutes but no later than 3 h after re- section; correct storage at −80°C until shipment and transportation on dry ice).

When discussing the availability of FrFr tumor samples, the question arises whether FFPE tissue could be used interchangeably, thereby contradicting the rationale for restricting the analysis to FrFr tissue. Indeed, the platform would be amenable to FFPE tissue, and, in principle, identification of fusions is possible using FFPE tissue.32 While costs of both

techniques are comparable, the advantage of FrFr RNA sequencing lies in the possibilities for further exploratory research, such as the abovementioned analysis of signatures associated with oncogene-induced senescence29,30 and prediction of drug response.

Outlook

The key aim of LOGGIC Core is the establishment of a mo- lecular matching platform with integrated diagnostic, clin- ical baseline, and follow-up data to further comprehend tumor biology and behavior, predict a patient's response to therapy and determine prognostic factors as well as correl- ations between molecular LGG subgroups and clinical out- come. Over the course of the first three operational years, LOGGIC Core has been expanding across Europe and Australia in collaboration with the ZERO Childhood Cancer Program. Importantly, the eligibility criteria have recently been updated, now also including patients at progression/ relapse following a previous treatment.

The improvement of diagnostic accuracy for all pLGG patients through the addition of molecular information to reference histological evaluation, specifically the added value of RNA sequencing as part of the routine diagnostic procedures, defines the new state of the art standard molecular diagnostics for pLGG. We propose to include RNA sequencing from FrFr tumor material as part of standard diagnostics for all pLGG tumors, especially in tumors where no common MAPK alteration was identified by cur- rent routine diagnostic methods.

Keywords

actionable drivers | molecular profiling | pLGG | rare gene fusions | RNA sequencing

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