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Rapid-CNS² Combined with MNP-Flex Enables Next-Day Comprehensive Molecular Diagnostic Profiling of CNS Tumors in a Global Cohort

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Introduction: The 2021 WHO classification of central nervous system (CNS) tumors underscores the integration of molecular data in CNS tumor diagnosis. Traditional molecular diagnostics are challenged by high costs, complex protocols, and slow turnaround times, limiting their translational application. Methylation classification by the Heidelberg methylation classifier distinguishing 184 classes of CNS tumors is currently limited to methylation array data. We introduce Rapid-CNS² and MNP-Flex, innovative tools designed to overcome these barriers, facilitating rapid, accurate CNS tumor diagnosis and treatment planning. **Methods:** Rapid-CNS², a nanopore sequencing workflow, was applied to 190 CNS tumor samples at University Hospital Heidelberg and University of Nottingham, facilitating real-time intraoperative results within 30 minutes and comprehensive genomic profiling within 24 hours. MNP-Flex, a platform-agnostic methylation classifier, was developed to encompass 184 CNS tumor classes. We validated MNP-Flex on a global cohort of more than 78,000 samples from various methylation array and sequencing-based technologies. **Results:** Rapid-CNS² demonstrated precise integrated diagnostics within a crucial 30-minute window, offering immediate broad methylation classes and copy number alterations, followed by detailed molecular insights including mutations, focal copy number alterations, O-6-methylguanine-DNA methyltransferase promoter methylation, structural variants, and fine-grained methylation classification by MNP-Flex on the next day. In a global cohort consisting of diverse technologies, MNP-Flex achieved a remarkable 92% accuracy, confirming its robust applicability in clinical and research environments. **Conclusions:** The combination of Rapid-CNS² and MNP-Flex represents a significant advancement in CNS tumor diagnostics, providing clinicians with rapid, actionable molecular insights essential for personalized treatment strategies. These technologies enable quick, comprehensive molecular analysis, essential for tailored patient care and treatment optimization. Their high accuracy and rapid turnaround time offer a promising solution to the current limitations of CNS tumor diagnostics, demonstrating the potential of next- and third-generation sequencing and methylation classification in translational medicine. Rapid-CNS² and MNP-Flex not only streamline the diagnostic process but also enhance the accessibility of precision oncology for CNS tumors.