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To the editor:

Corona virus disease 2019 (COVID-19) in children with cancer is usually mild and most children recover without any treatment (1,2). To et al. and others (3-6) have recently reported reinfections with severe acute respiratory syndrome corona virus -2 (SARS-CoV-2) confirmed by genome sequencing. A few recent cases of

potential reactivation/reinfection with SARS-CoV-2 have been reported in cancer patients on chemotherapy (7-9), however were not confirmed by genetic characterization of the virus. Here we report results of genome sequencing in child with neuroblastoma reported previously by us as re-infected with SARS-CoV-2 after recovering fully and developing IgG antibodies (8). Genetic characterization of the virus from both episodes confirmed reactivation.

The 3-year-old boy with stage 4 neuroblastoma was being treated as per OPEC/OJEC protocol. After 4 cycles of chemotherapy and surgery he was found to be positive on real time-PCR(RT-PCR) assay for SARS-CoV-2 on nasopharyngeal & throat swabs (Thermofisher TaqPath; Ct values N-14.59 ORF-16.84 S-17.48). As he was asymptomatic, was isolated at home and the chemotherapy was postponed. Three weeks later, the repeat RT-PCR assay was negative and was commenced on the next course of chemotherapy OJEC. Six weeks later, prior to course of next chemotherapy, the child tested positive again on routine testing for SARS-CoV-2 RT-PCR assay (Siemens Labgun ExoFast; Ct values, N-22.37 RdRp-22.15). The quantitative IgG antibody assay (by CLIA for anti S1 and anti S2 specific IgG; 306 AU/ml) was also positive. The gap between first and second episodes was 42 days. He was isolated at home for another 2 weeks, and chemotherapy was resumed after testing negative on RT-PCR (8).

The archived RNA samples were used for the library preparation using the COVID Seq assay kit which utilizes the set of 98 primers specific for the SARS-CoV-2 genome (10). The synthesized libraries were sequenced on the MiSeq sequencer (Illumina, Inc) on a 75x2 paired end sequencing recipe. Genomes were assembled using a reference-based approach following a previously published protocol (11). Briefly, quality control of the FASTQ files was performed followed by adapter/base trimming at a Phred quality score of Q30 and length cut-off 70 bps. Reads were aligned to the SARS-CoV-2 reference genome (NC_045512.2) The genomes were assembled at a depth of 12615.92X and 7834.78X, with a genome coverage of 99.84% and 99.70% for the 2 episodes E1 and E2 respectively (Fig.1). Analysis revealed 14 variants in the genome isolate from E1. 13 out of the 14 variants from E1 were also present in the isolate from E2 while 1 variant 12793G>A was present in E2 with a read count proportion of 35.8%. Of the 14 variants, 6 were non-synonymous.

Put together, the genomic analysis suggests that the virus isolated from the two episodes were not distinctly different, suggesting reactivation of the virus during the course of chemotherapy. To the best of our knowledge, this is one of the earliest cases of genetically characterized reactivation of SARS-CoV-2.

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Legend

Fig. 1. Variants in the genome isolates from the two episodes E1 and E2 with their respective read count proportions.

