

## I. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy affecting the development of T-lymphocytes. Approximately 8% of cases with childhood T-ALL have a rearrangement that involves the ABL1 tyrosine kinase gene at 9q34, and although BCR-ABL1 is a common fusion in B-ALL, it is not a typical finding in T-ALL. We present 8 of 35 (22.9%) documented cases of T-ALL involving chromosome rearrangements of 9q34. The goal of this study was to, 1) better characterize the gene involvement of these 9q34 gene rearrangements in T-ALL, 2) determine whether FISH can help identify most of these rearrangements, and 3) whether anchored multiplex PCR (AMP) can identify the novel gene fusion partner of ABL1.

## II. Methods

CytoScan HD<sup>®</sup> chromosome microarray analysis (CMA), AMP analysis and FISH, using two different commercial chromosome 9q34/ABL1 chromosome 22 BCR probe sets. The first is a Vysis (Abbott Molecular Company) three gene probe set that includes ASS1, ABL1 and BCR (Fig. a) and the second Kreatech (Leica BioSystems) four gene probe set comprised of ASS1, ABL1, NUP214 and BCR (Fig. b). These probes were used to evaluate T-ALL cases for DNA copy number changes, DNA linear position, gene fusion partner and FISH signal patterns. We applied a NGS-based (RNA-seq) platform combined with Archer Anchored Multiplex PCR (AMP<sup>™</sup>) technology adding molecular barcodes for error correction and read deduplication. The 87-gene panel is suitable for detecting both known and unknown fusions, based on an extensive fusion database.

## III. Results

Results: Of the eight cases analyzed, five SET/NUP214 fusions were detected by CMA through deletion of the region between the genes. FISH and AMP sequencing confirmed the deletion/fusion (Fig. 2). Patient 6 was found to have a ABL1/NUP214 gene fusion that was observed secondary to a complex duplication with two small flanking deletions that truncated the two genes centromeric to ABL1 and telomeric to NUP214 (Fig. 2). Two novel gene fusions were identified by the array and confirmed by AMP. The rearrangement in patient 7 produced an intrachromosomal SPTAN1-ABL1 gene fusion with an intervening ASS1 deletion (Fig. 3). The rearrangement in patient 8 resulted in a deletion of ASS1 and transfer of residual ABL1 to an unknown fusion partner distal to BCR on chromosome 22. AMP subsequently identified a unique fusion of TNRC6B on chromosome 22 and ABL1 (Fig. 4). Both gene fusions, to our knowledge, have not been previously reported, but like other genes that fuse with ABL1, it is reasonable to expect that it leads to increased tyrosine kinase activity. Four of the eight cases identified by microarray analysis also had FISH studies. In all cases, ASS1 was deleted.

## IV. Conclusion

1. FISH deletion of ASS1 appears to be a reliable indicator of T-ALL 9q34 gene fusions and if deleted should be re-flexed to CMA to clarify specific gene fusions associated with T-ALL. The four gene FISH probe set containing ASS1-ABL1-NUP214 at 9q34 and BCR at 22q11.
2. is a important marker to differentiate NUP214 fusions from the ABL1 fusions while both show the key deletion of the ASS1 gene probe. Furthermore, the small size residual ABL1/NUP214 contiguous red signal offers a clue to SET/NUP24 fusions. CMA is a powerful tool to identify recurrent and novel 9q34 interstitial deletions resulting in gene fusions associated with T-ALL. The CMA analysis showed a very similar intragenic splice sites for SET-NUP214 gene fusions estimated at chr9: hg19:131,459,252-134,025,284. The cytogenetically cryptic intervening ~2.6 Mb deletion that results in the SET/NUP214 fusion has previously been described in T-ALL and was the most common (5/8) in our cohort.
3. CMA in conjunction with RNA sequencing characterized and identified two novel ABL1/TRNRC6B and SPTAN1/ABL1 gene fusions. Both gene fusions, to our knowledge, have not been previously reported, but like other genes that fuse with ABL1, it is reasonable to expect that it leads to increased tyrosine kinase activity.
4. Anchored Multiplex PCR (AMP) analysis is a highly sensitive
5. Our 3-4 year cohort of 8 cases with ASS deletion and either ABL1 or NUP214 rearrangements represent about 23% (8/35) of the T-ALL patients analyzed.

## V. References

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3. Quentmeier, H. et al. SET-NUP214 fusion in acute myeloid leukemia and T-Cell acute lymphoblastic leukemia-derived cell lines. J Hem Onc 2:3 2009.
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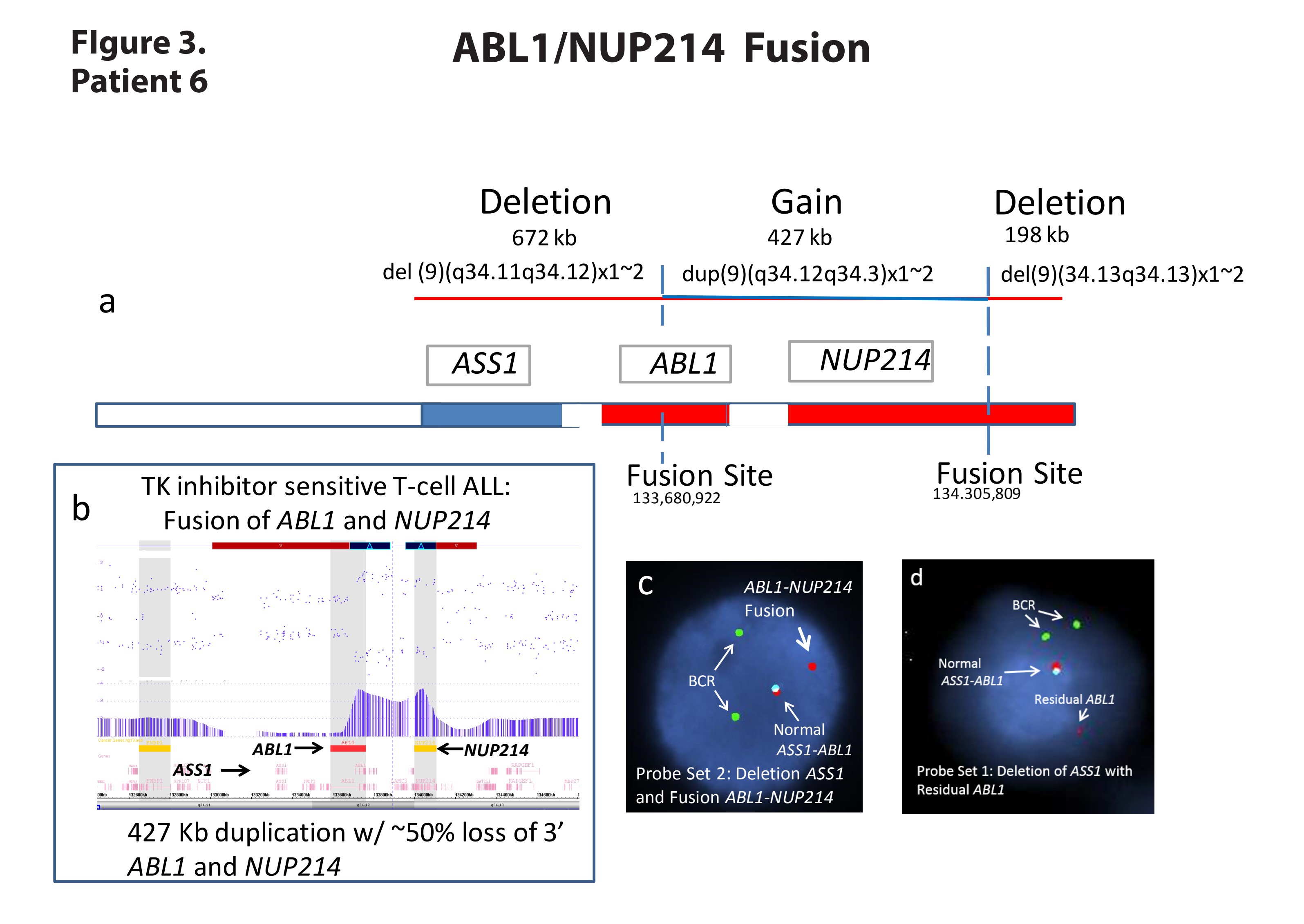
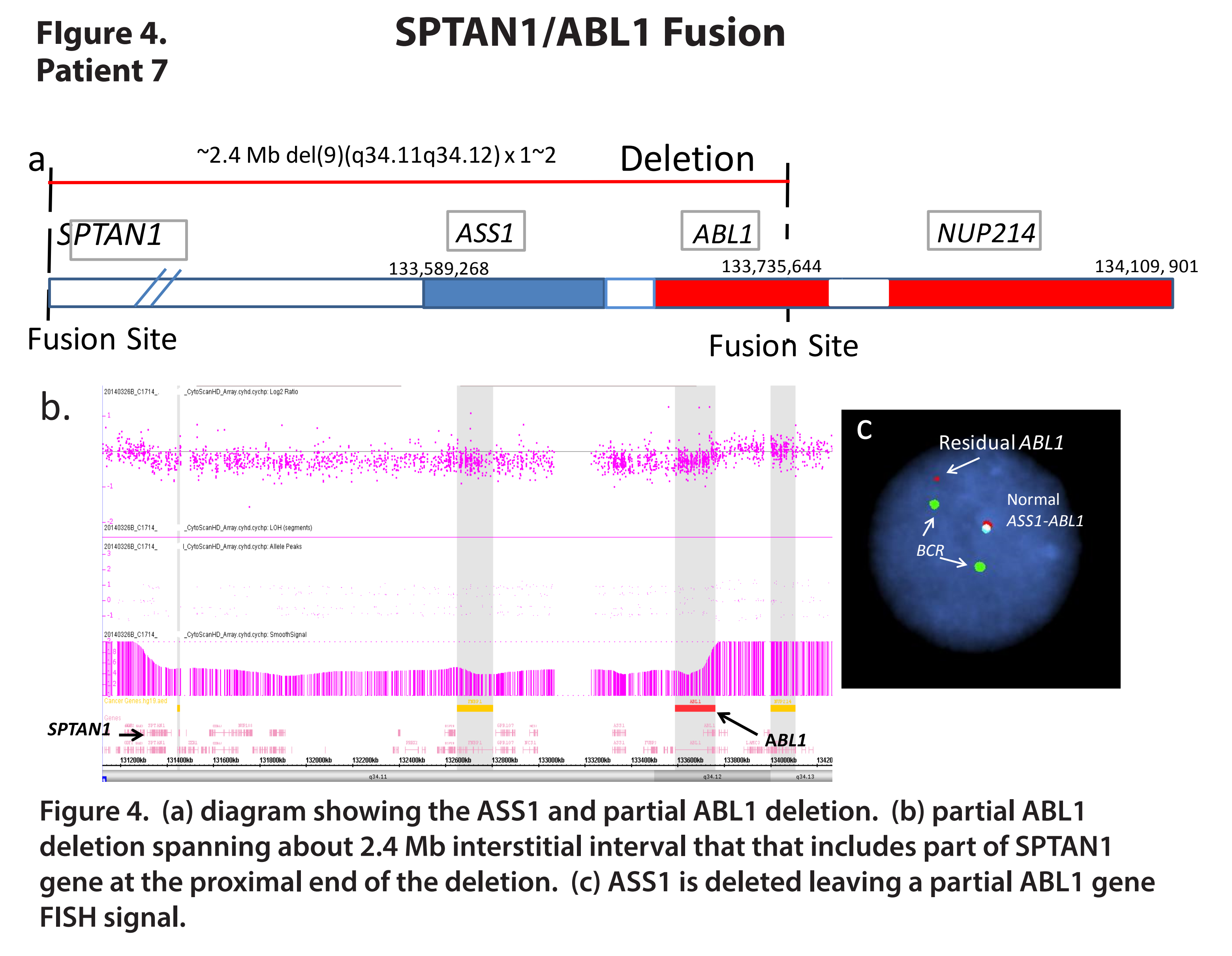
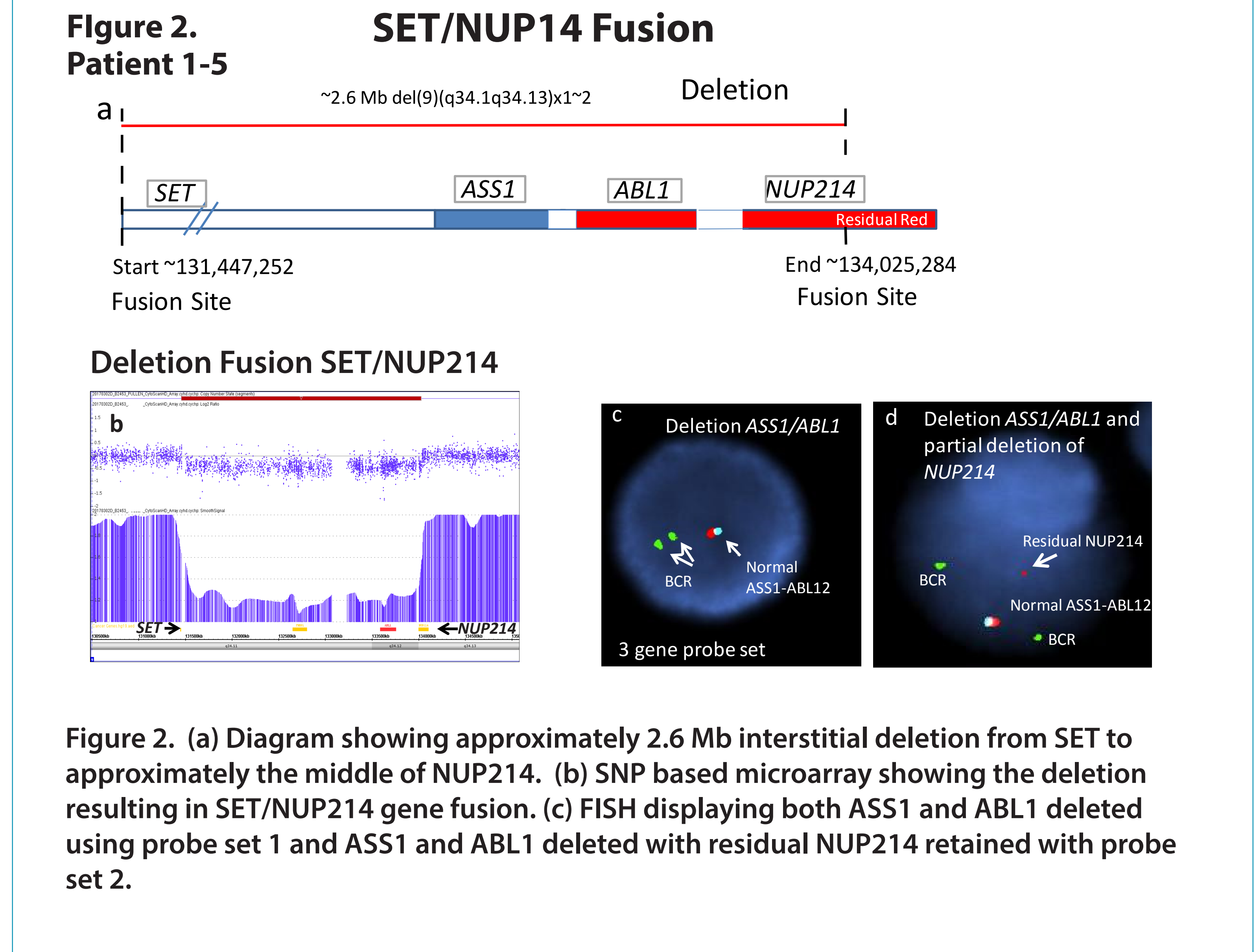


Figure 3. (a) Diagram showing unique contiguous deletion/duplication/deletion resulting in a ABL1- NUP214 gene fusion. (b) CMA showed both deletion genes and loss of the ASS1 FISH control site gene. (c) FISH showing ASS1 deletion but ABL1/NUP214 fusion appears normal with about 20% of the signal lost using probe set 2. (d) ASS1 and part of residual ABL1 retained with probe set 1.

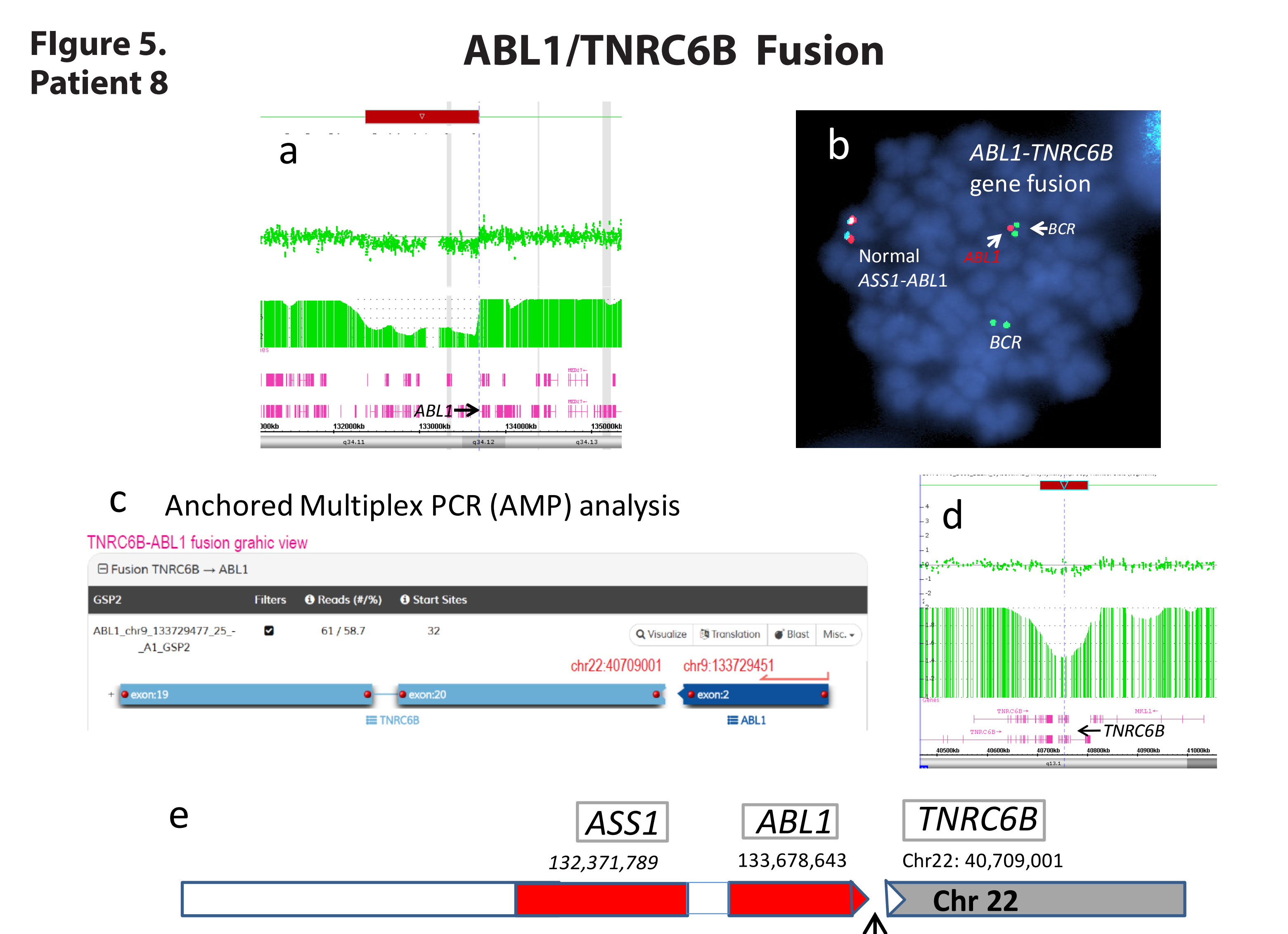


Figure 5 (a) CMA shows a deletion of ASS1. (b) FISH, using an ASS1/ABL1/NUP214/BCR showed a small ABL1 signal attached to chromosome 22 distal to BCR. (c) Follow-up AMP testing identified TNRC6B as the fusion partner. (d) A review of the CMA showed a deletion of TNRC6B on chromosome 22. (e) schematic of the t(9;22) gene fusion.