***Comment on:* Frequent *CTLA4-CD28* gene fusion in diverse types of T-cell lymphoma, by *Yoo et al*.**

We read with great interest the recent article by Yoo *et al.* published online in the January 2016 issue of Haematologica.[1] Their study identified a fusion between the extracellular domain of CTLA4 and the intracellular domain of CD28, presumably arising from a partial tandem duplication on chromosome 2, in angioimmunoblastic T-cell lymphoma (AITL) and other peripheral T-cell lymphomas (PTCL). We believe that the investigators have identified this fusion in a few cases of T-cell lymphoma but have greatly overestimated its frequency, which they put at 58% in AITL, 23% in PTCL, not otherwise specified (NOS), and 29% in extranodal NK/T-cell lymphomas (NKTCL).

The authors cloned the breakpoint junction at the DNA level in several patients. Two cases showed the expected pattern of breakpoint regions that encompass introns of the two genes. Very surprisingly, the authors report that the breakpoint junction in 6 patients' samples and two cell lines occurred precisely at exon-intron boundaries of the two genes, resulting in an exact, direct fusion at the DNA level between *CTLA4* exon 3 and *CD28* exon 4. Although breakpoints at most positions within the 1201 bp *CTLA4* intron 3 and the 5013 bp *CD28* exon 4 would likely result in a fusion gene that would yield the fusion transcript, the authors believe that the breakpoints are usually restricted to only single specific positions in the two genes (1.7×10^{-7} of the potential breakpoint pairs), which happen to correspond to the exon-intron junctions.

Genes or pseudogenes lacking some or all introns may arise through mechanisms involving reverse transcription of processed mRNA. Such a process is extremely unlikely to explain the reported experimental result, as it would require a pre-existent fusion gene containing a fusion *CTLA4-CD28* intron as well as active reverse transcriptase activity, for which there is no evidence in most subtypes of PTCL.

Various PTCL subsets have been analyzed by RNA-seq or by whole exome sequencing (WES); but most studies failed to identify any examples of the *CTLA4-CD28* fusion. Notably, although gene fusions that occur within introns are expected, in most cases, to be missed by WES, any fusions that occur within exons or at exon-intron junctions would be expected to be captured and sequenced by WES. Recent analyses revealed rare *CTLA4-CD28* fusions in Sézary syndrome[2,3] and in adult T-cell leukemia/lymphoma (ATLL). In 2 cases of Sézary syndrome, the genomic breakpoints were cloned and were found to lie within introns, as expected, not at the exon-intron junctions.[3] Five *CTLA4-CD28* fusions were identified in 105 examined cases of ATLL, of which only 2 had the exon 3-exon 4 junction described here.[4] This study included very extensive whole genome sequencing and WES, but reported no examples of fusion transcripts with DNA breakpoints at exon-intron junctions.

We reanalyzed WES or RNA-seq data from published series of AITL (n=43), PTCL-NOS (n=16), and NKTCL (n=43)[5-9] (Table 1) and found the *CTLA4* exon 3-*CD28* exon 4 fusion only in a single sample, which was from an earlier publication by Yoo *et al.*[9] (sample PAT7), out of 9 cases with RNA-seq reported in this earlier paper. Note that this earlier RNA-seq dataset showed a significantly lower frequency (P=0.0055, two-sided binomial test) than reported in their most recent publication. Finally, no examples of *CTLA4-CD28* were identified in mate-pair genomic DNA sequencing data of 97 PTCL tissue samples and cell lines, including 37 in the AITL, PTCL-NOS, and NKTCL subtypes[10] (AL Feldman, 2016, unpublished data).

Table 1. Datasets used for re-examination.

Sample	Number	Method	Reference
AITL	11	RNA-seq	7
AITL	20	RNA-seq	8
AITL	9*	RNA-seq	9
NKTCL	15	RNA-seq	6
PTCL-NOS	10	RNA-seq	7
AITL	3	WES	7
AITL	5*	WES	9
NKTCL	25	WES	5
NKTCL	1	WES	6
NKTCL	2	WES	7
PTCL-NOS	6	WES	7

**Both RNA-seq and WES data were available for 5 cases.*

A potential explanation of the investigators' results is that after polymerase chain reaction (PCR)-cloning of the *CTLA4-CD28* fusion cDNA from their index case, there was widespread but not uniform contamination of critical reagents or samples in the laboratory by the PCR amplicon, leading to misidentification of the cDNA in many samples without the fusion transcript and in amplification of the contaminating cDNA in most of the attempts to clone the DNA breakpoint junction using PCR.

In summary, although the article by Yoo *et al.* presents a number of interesting findings in PTCL, we believe that it greatly overestimates the frequency of the *CTLA4-CD28* fusion in PTCL.

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