Expression Profiling of mRNA By Next Generation Sequencing and the Development of Algorithm for Predicting Response in Acute Myeloid Leukemia

#1499

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Introduction

Cellular RNA levels are tightly regulated by very complex nuclear and cytoplasmic processes. The regulation of mutant mRNA in cancer cells is rarely studied. Some studies demonstrated that in vitro studies demonstrated that synonymous mutations had a significant effect on KRAS expression with c.36 T > C (G12G) most strongly inducing KRAS mRNA and protein. In contrast C.36 T > G (G12G) had the opposite effect and significantly decreased KRAS protein expression. Data demonstrated that increased KRAS activity or the loss of wildtype KRAS as dimerization partner for mutant KRAS proteins could impact oncogenicity (Sharma et al. Nature Communications, doi.org/10.1038/s41467-019-10489-2). In addition, reported on the average five-fold higher protein and two-fold higher mRNA of KRAS: G13D mutation levels as compared with wild type in transfection experiments in HCT116 human cell lines (Lampson et al, Curr Biol. 2013 January 7; 23(1): 70–75. doi:10.1016/j.cub.2012.11.031). These data suggest that missense mutations have effects beyond the change in mino acid.

We explored the effects of mutations on mRNA levels in patients with diffuse large B-cell lymphoma (DLBCL). Using next generation sequencing (NGS) and variant allele frequency (VAF) of mutant RNA, we compared relative mutant mRNA or variant allele frequency (RNA-VAF) with variant allele frequency of mutant DNA (DNA-VAF) in the same samples from patients with DLBCL.

Methods

Samples and patients:
FFPE tissue of 441 patients diagnosed and confirmed with DLBCL. All patients were treated with Rituximab-CHOP.

Age	<60	190 (43%)	43
	>60	251 (57%)	
Gender	Male	242 (55%)	55
Cell of Origin Classification based on GEP	ABC	212 (48%)	48
	GCB	229 (52%)	52

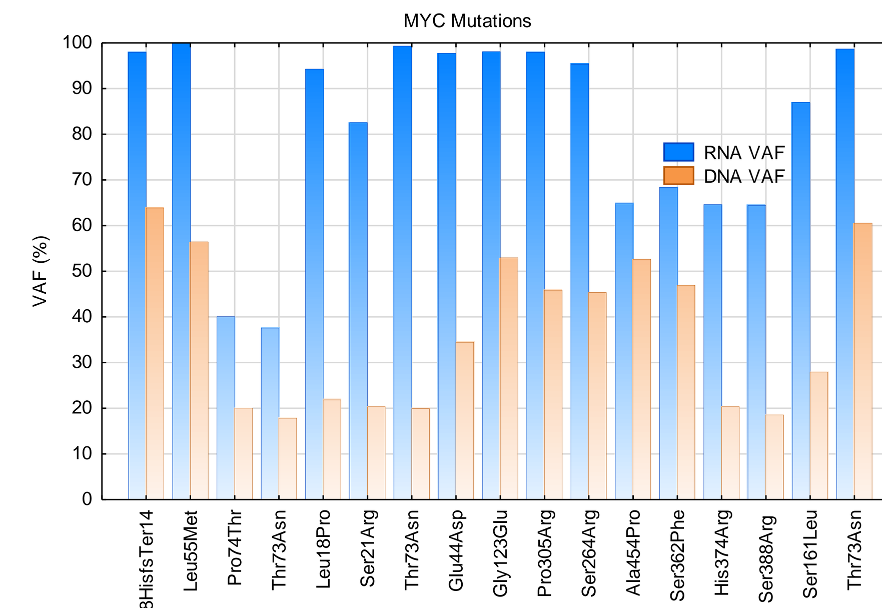
DNA and RNA Extraction:
The Agencourt FormaPure Total 96-Prep Kit is used for extracted both DNA and RNA from formalin fixed paraffin embedded human tissue. The Agencourt FormaPure Kits allows us to use a split protocol for extracting both RNA and DNA from the same FFPE lysate.

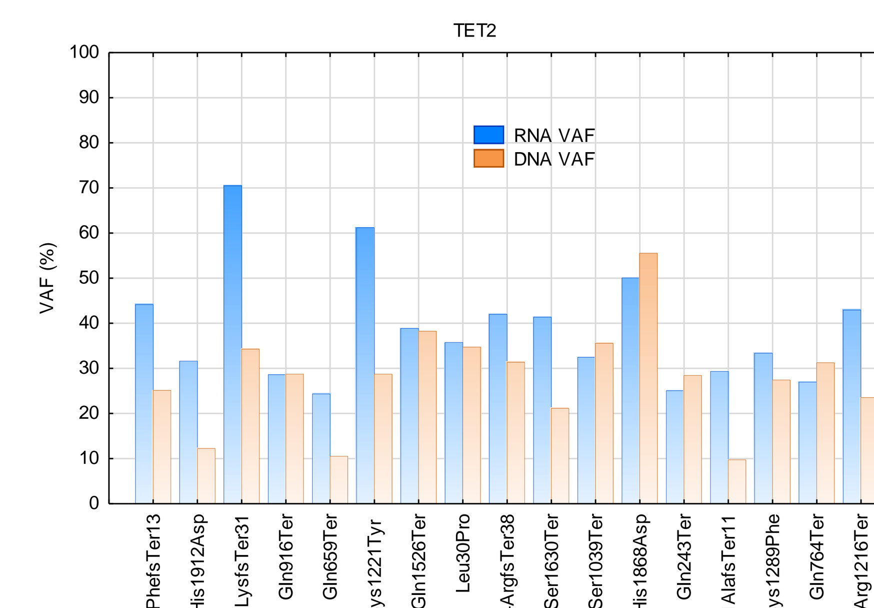
DNA Library Construction and sequencing
Target enrichment is performed post-UMI assignment using Single primer extension (SPE). The sequencing is conducted using the Illumina NextSeq 550 instrument

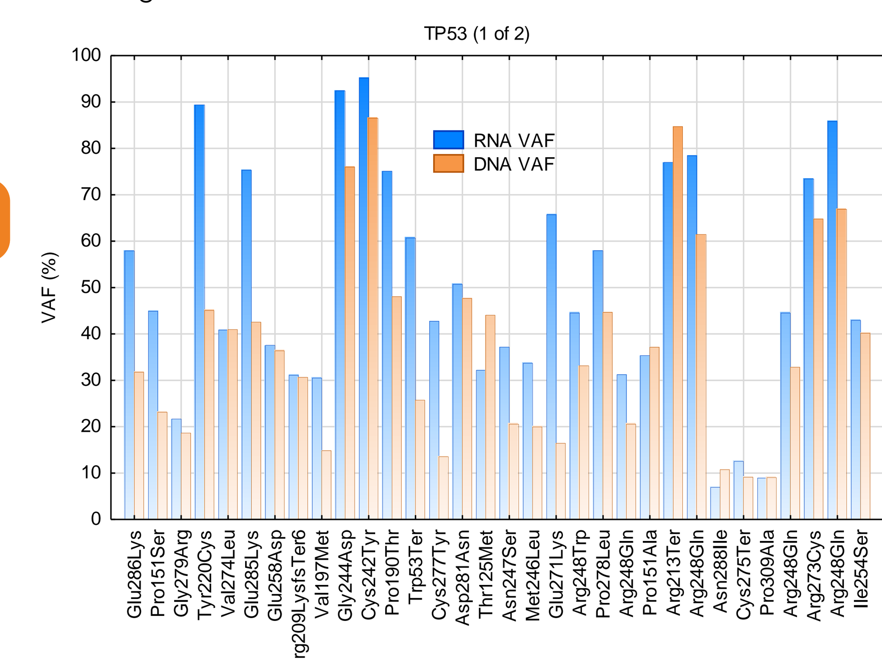
RNA Library Construction and Sequencing
Sample are selectively enriched for 1408 cancer-associated genes using reagents provided in an Illumina® TruSight® RNA Pan-Cancer Panel. Sequencing is performed on Illumina NextSeq 550. Expression levels are measured using FPKM.

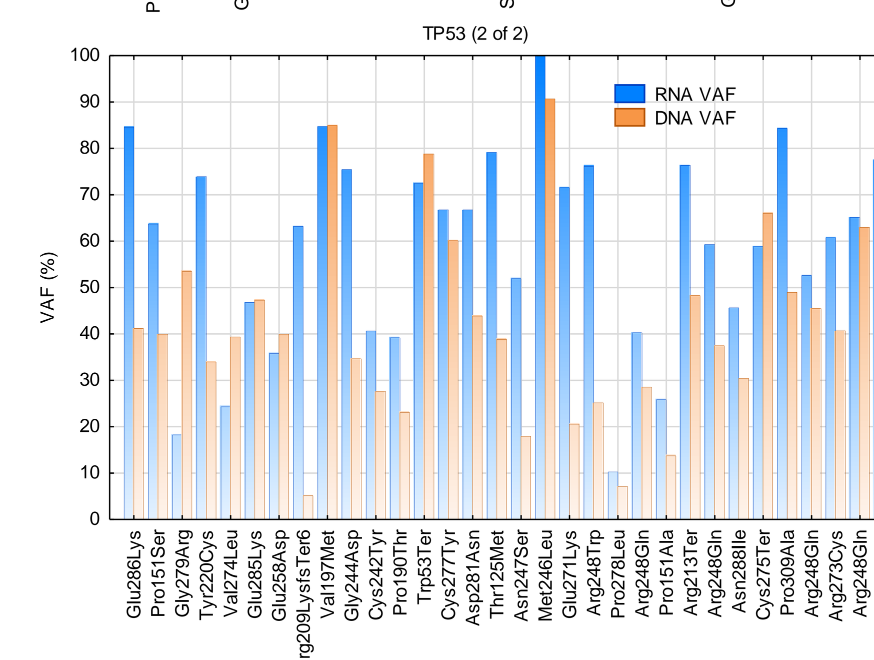
Results

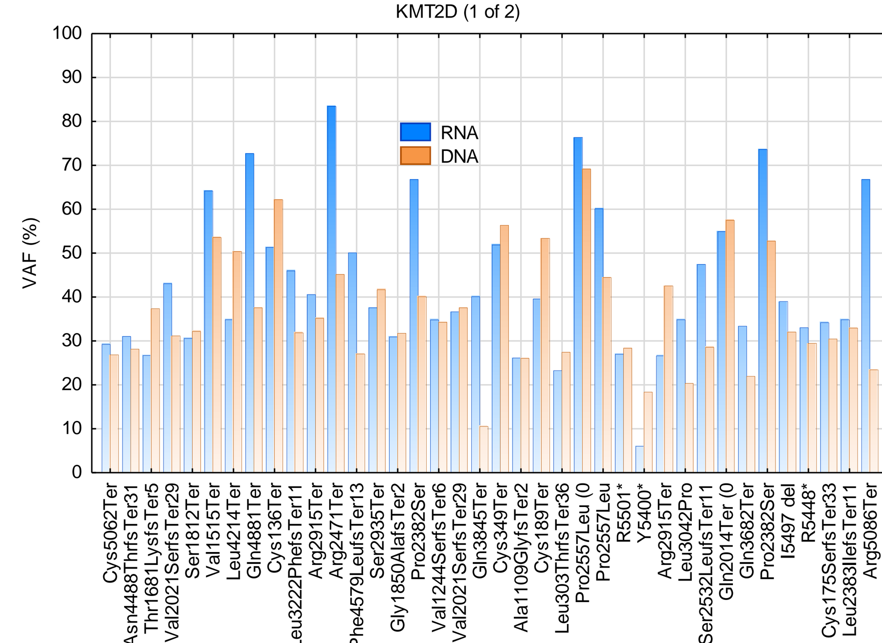
A total of 1770 mutations were detected using the-DNA panel and 2207 mutations were detected using the larger RNA sequencing panel. We focused on the most commonly mutated genes that included in both DNA and RNA panels and compared the VAF of the same mutations between DNA and RNA. The selected genes are: KMT2D, NOTCH2, CARD11, MYC, MYD88, EZH2, TP53, CD79B, BCL2, and TET2.

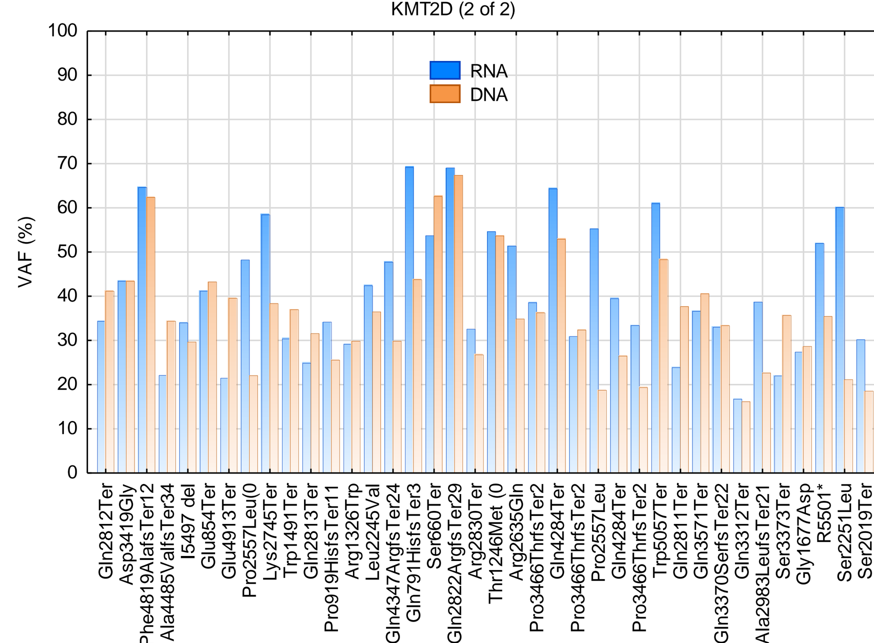


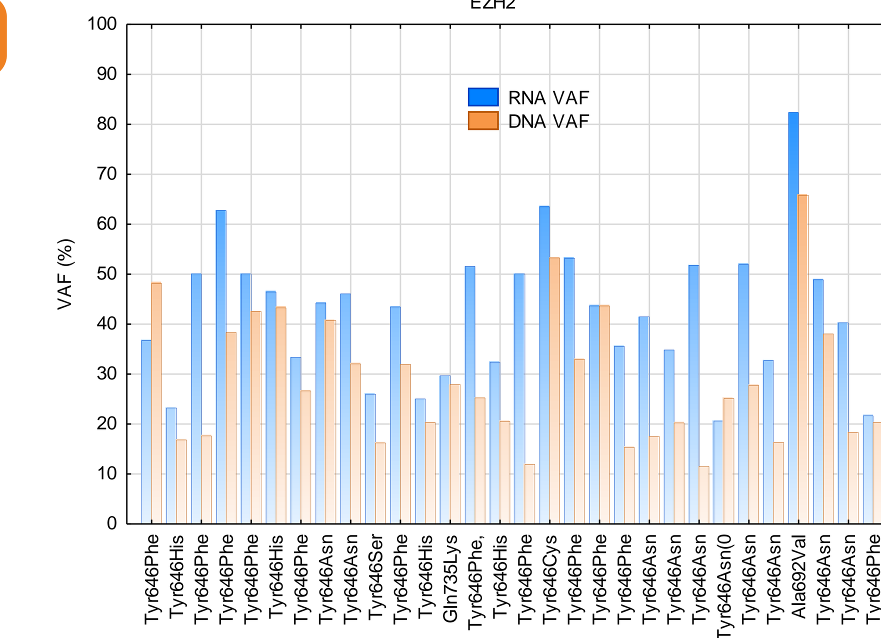


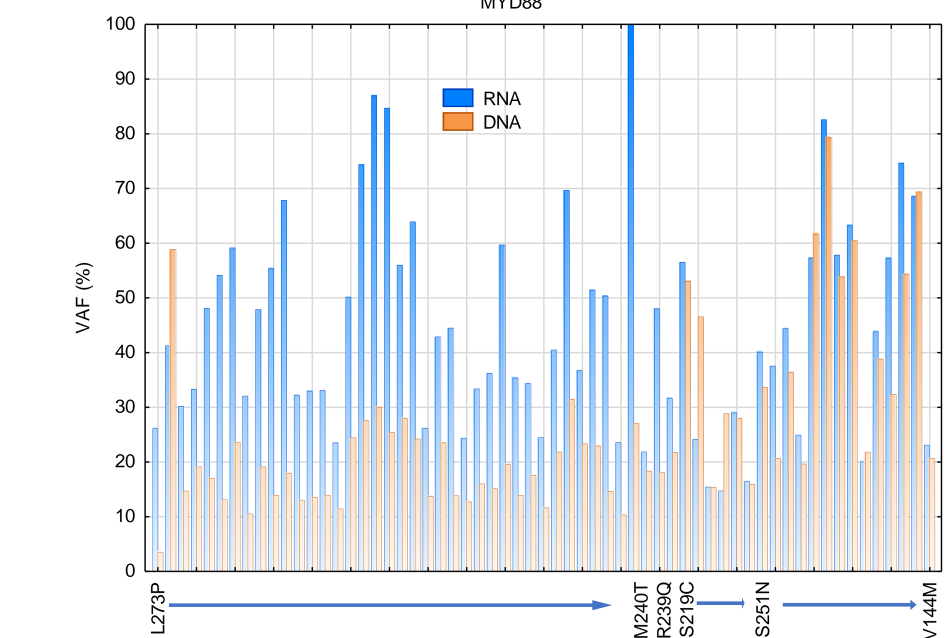




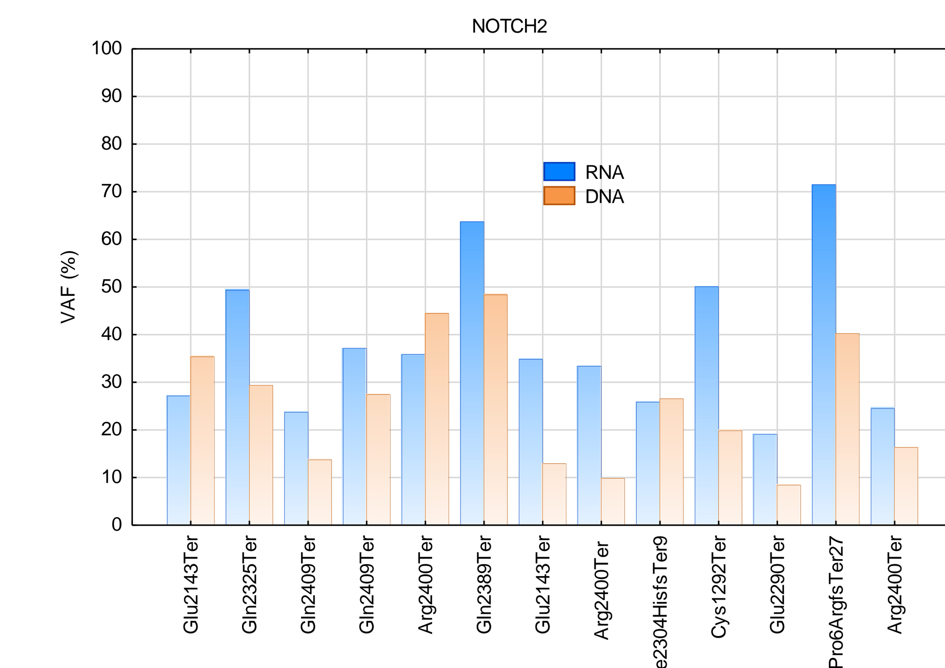








54 samples showed NOTCH2 Pro6ArgfsTer27 mutation detected by DNA, but not by RNA.



Conclusion

-The overall VAF in the RNA was significantly higher (P<0.00001) (median: 43.9%, minimum: 6%, maximum: 100%) as compared with that of the DNA (median: 28.8%, minimum: 3.5%, maximum: 95%).

-Some mutations were detected in DNA, but not in in RNA and vice versa.

-The number of mutations detected in these 10 genes using DNA sequencing was significantly (P= 0.0001) higher (#658) as compared with mutations detected in RNA (#471).

-Most of the missed mutations by RNA were termination mutations.

-The DNA testing showed 81 mutations, while the RNA testing listed only 19 mutations. Almost all NOTCH2 mutations missed by RNA sequencing were Pro6ArgfsTer27. Despite missing mutations, the VAF of NOTCH2 mRNA mutations were significantly higher (P=0.002, Kruskal-Wallis ANOVA) than that of DNA.

-This data does not rule out the possibility that wild-type mRNA may become unstable in the presence of mutant RNA.