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Cancer-testis antigen detection by targeted RNA sequencing

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INTRODUCTION

Cancer-testis antigens (CTAs) have restricted expression in normal adult tissues but have been found to be overexpressed in multiple tumors. This and their ability to elicit spontaneous cellular and humoral immune responses have rendered CTAs as good candidate targets for cancer immunotherapy. CTA overexpression is variable and influenced by factors such as tumor stage, grade, treatment and detection methods. Here we report the use of a targeted RNA sequencing panel that accurately detects CTAs in solid tumors as a screening tool for cancer vaccine and cell-based immunotherapies.

METHODS

Studies were designed to characterize the analytical performance of an RNA sequencing assay targeting expression of six CTAs which have shown potential as prognostic biomarkers and immunotherapeutic targets: NY-ESO-1 (CTAG1B), LAGE-1A (CTAG2), MAGEA1, MAGEA3, MAGEA4, and SSX2. Ion AmpliSeq targeted library chemistry using the Oncomine Immune Response Research Assay (OIRRA) was utilized followed by Ion Chef system templating and Ion S5XL 540 chip sequencing (Thermo Fisher Scientific, Figure 1)[1]. FFPE specimens (n=180) included cell lines, fine-needle aspiration biopsies, punch biopsies, needle core biopsies, incisional biopsies, excisional biopsies, and resection specimens of multiple histologies. A ≥20 nRPM cutoff was adopted as the "Positive" threshold for RNA-seq CTA expression. Performance variables with respect to gene-specific amplicon specificity, linearity and limits of detection were estimated with various mixing studies and input RNA levels. The effects of the tumor micro-environment (adjacent benign tissue, necrosis) on CTA expression was evaluated by including these potential interferents in the RNA-seq assay. Analytical precision including intra-assay, inter-assay, and inter-operator reproducibility was measured by testing replicate RNA isolates. Accuracy was determined by comparing the CTA transcripts with those from established IHC, orthogonal TaqMan qPCR and TCGA whole transcriptome assays.

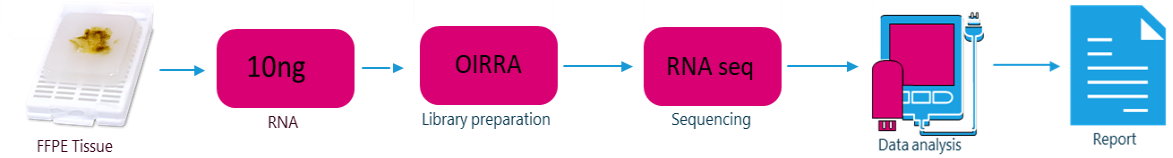

Figure1: RNA-seq workflow for detection of Cancer-testis antigen expression

Table 1: 189 clinical trials available for >25 tumor types with Cancer Testis antigen expression

Cancer Testis Antigen	NCBI_NAME	NCBI_ACCESSION	Clinical trials*
NY-ESO-1 (CTAG1B)	cancer_testis_antigen_1B	NM_001327	140
MAGEA3	MAGE_family_member_A3	NM_005362,NM_005363	29
MAGEA4	MAGE_family_member_A4	NM_001011548	8
LAGE-1A (CTAG2)	cancer_testis_antigen_2	ENST00000369585	8
SSX2	SSX_family_member_2	NM_003147	2
MAGEA1	MAGE_family_member_A1	NM_004988	2

* https://clinicaltrials.gov/ October 2020

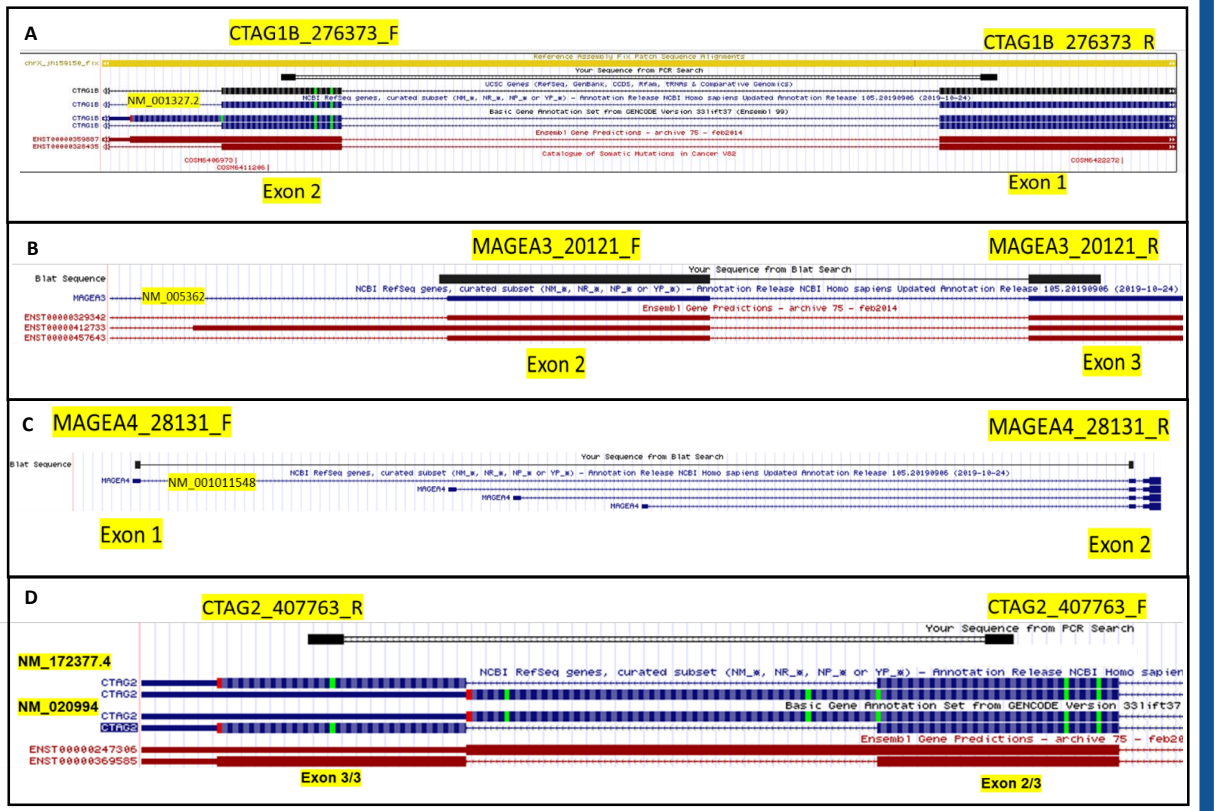

Figure 2: (A) NY-ESO-1 (CTAG1B), (B) MAGEA3, (C) MAGEA4 and (D) LAGE-1A (CTAG2) target positions in mRNA reference sequences showing specificity of RNA-seq assay.

RESULTS

Table 2. Analytical Accuracy across platforms: correlation values for comparison of OIRRA RNA-seq, TaqMan qPCR, and TCGA gene expression results for CT antigens in expressing samples. R = Pearson correlation results.

Level	R (OIRRA nRPM – qPCR)	R (OIRRA nRPM – TCGA RPM)	R (TCGA RPM – qPCR)
Average	-0.9104	0.7369	-0.8372
Median	-0.9697	0.9892	-0.9515
Maximum	-0.4815	1.0000	-0.1387
Minimum	-1.000	0.2740#	-1.0000

#Sample with lowest concordance is likely due to sample heterogeneity between matched FFPE (OIRRA) and FF (TCGA) samples.

Table 3. Analytical Accuracy across genes: correlation values, PPA and NPA of OIRRA RNA-seq and TaqMan qPCR expression results for CT antigens in expressing samples at defined cutoffs.

CTA Gene	R (OIRRA vs qPCR)[1]	PPA[2]	NPA[3]
NY-ESO-1	-0.9837	75%	100%
LAGE-1A	-0.9339	63%	100%
SSX2	-0.3572	100%	100%
MAGEA3	-0.9239	100%	94%
MAGEA1	-0.8794	100%	100%
MAGEA4	-0.8537	100%	95%
average	-0.8220	90%	98%

[1] *R = pearson correlation results*

[2] *PPA = positive percent agreement = [(# OIRRA positive calls) ÷ (# TaqMan positive calls)] / x 100*

[3] *NPA = negative percent agreement = [(# OIRRA negative calls) ÷ (# TaqMan negative calls)] x 100*

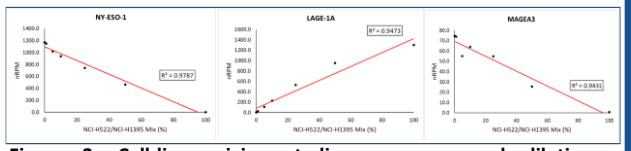

Figure 3: Cell-line mixing studies across several dilutions demonstrating linear reduction in RNA-seq CTA normalized reads per million (nRPM) to background levels (nRPM <20).

RNA stability was demonstrated by high degree of CTA expression correlation between matched frozen and FFPE samples, and samples with varying tumor purity and necrosis (data not shown). Reproducibility results show minimal gene expression nRPM variation within runs, and between runs and operators (R range 0.82 – 0.99).

CONCLUSIONS

The analytical performance of the RNA sequencing assay for reporting CTAs has been validated for clinical use using FFPE specimens from multiple tumors. With ability to process many samples within a single run and a 10ng RNA input each, the assay is a robust method for identifying tumors that overexpress tumor-specific CTAs as potential targets for immunotherapies including cancer vaccination and adoptive T-cell transfer with chimeric T-cell receptors.

REFERENCES

[1]Conroy JM, Pabla S, Nesline MK, *et al;* Next generation sequencing of PD-L1 for predicting response to immune checkpoint inhibitors. *Journal for ImmunoTherapy of Cancer 019; 7:18.*