A Novel, Comprehensive Glimpse at NY-ESO-1 Expression, mRNA to Protein Translation, & Potential Impact on Clinical Studies

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Introduction

- As the area of precision medicine in oncology advances to a paradigm in which more than one biomarker may be used to determine treatment options for patients, there is an emergent need to leverage advanced technologies, such as whole exome and whole transcriptome sequencing, with capabilities of multi-plexing to facilitate a single test with a multiple targets model compared to traditional single-marker assays.
- An in-depth understanding of the tumor microenvironment and 'targets' of solid tumors is fundamental across all therapy modalities; this is especially useful when the expression of multiple biomarkers is needed, such as for engineered immune cells, more specifically T-cell receptor (TCR) T cells.
- Letetresgene autoleucel (lete-cel; GSK3377794) comprises autologous T cells engineered to express a high-affinity TCR capable of recognizing the human leukocyte antigen (HLA)-A*02:01, *02:05, *02:06 and SLLMWITQC antigen epitope complex.
- New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) expression has traditionally been assessed by immunohistochemistry (IHC) in certain indications and has been studied in several clinical trials investigating lete-cel and other therapies.

Objectives

In this multi-platform study, we evaluated NY-ESO-1 mRNA expression alone, L antigen family member 1 isoform A (LAGE-1a) mRNA expression alone, and NY-ESO-1 and LAGE-1a mRNA co-expression in indications that have not been well defined for these biomarkers.

Correlative analysis was performed on HLA typing between Sanger sequencing and next-generation sequencing (NGS).

Methods

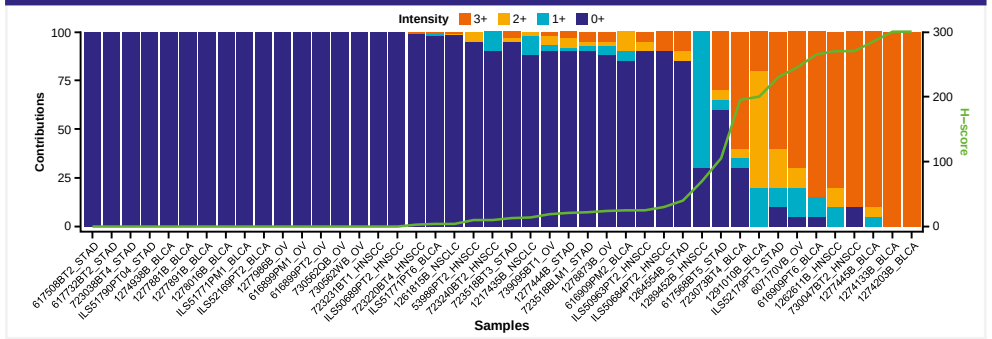
- NY-ESO-1 IHC was performed on procured stomach adenocarcinoma (STAD), bladder cancer, head and neck squamous cell carcinoma (HNSCC), ovarian cancer, and non-small cell lung cancer (NSCLC) tumor samples (cut-off: ≥10% proportion of cells at ≥1+ staining intensity; n=152).
- A subset of samples (n=42) had comparative IHC and NGS.
- An optimal cut-off for NY-ESO-1 mRNA expression to give the best concordance to the IHC results was determined by receiver-operating characteristic (ROC) analysis.
- LAGE-1a mRNA expression was determined by whole transcriptome sequencing (mRNA CTAG2 >0).
- HLA typing was determined by whole exome sequencing. For tumors with matched blood, concordance of HLA typing between Sanger sequencing and NGS was determined.

Results

NY-ESO-1 protein expression across multiple indications

- NY-ESO-1 protein expression observed across indications, at various staining intensities (**Figure 1**).
- The prevalence of NY-ESO-1 expression (IHC) was 17% in STAD, 7% in ovarian, 20% in HNSCC, and 21% in bladder based on cut-off: ≥10% proportion of cells at ≥1+ staining intensity (**Table 1**).
- Representative images show NY-ESO-1 staining pattern across the different tumor types (**Figure 2**).

Figure 1. NY-ESO-1 IHC staining intensities across multiple indications



Bladder cancer (BLCA), head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), ovarian cancer (OV), stomach adenocarcinoma (STAD).

Table 1. NY-ESO-1 protein expression across multiple indications

Indication	n	Number Positive at 10% 1+	Proportion Positive at 10% 1+
STAD	30	5	0.17
Ovarian Cancer	43	3	0.07
HNSCC	40	8	0.20
Bladder Cancer	39	8	0.21

Note: n = the number of subjects with measurements for the indication.
Note: Samples are declared positive if at least 10% of cells have at least 1+ staining intensity.
Head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma (STAD).

Comparison of NY-ESO-1 protein expression to mRNA expression

- A subset of samples had comparative IHC and NGS performed (**Figure 3**).
- Comparison of the NY-ESO-1 mRNA and protein data yielded a positive percent agreement (PPA; sensitivity) = 1 and a negative percent agreement (NPA; specificity) = 0.97 (**Figure 4**).

NY-ESO-1 and LAGE-1a mRNA expression across multiple indications

- Within subsets, the prevalence of NY-ESO-1 mRNA expression was 30% in STAD, 12% in ovarian, 30% in HNSCC, and 36% in bladder; LAGE-1a expression was found in 50% STAD, 50% ovarian, 30% HNSCC, and 64% bladder (**Table 2**).
- Further stratification of samples based on NY-ESO-1 and/or LAGE-1a mRNA expression shows differences in NY-ESO-1 or LAGE-1a mRNA expression status alone, as well as co-expression of both biomarkers, across indications (**Table 3**).

Concordance between Sanger sequencing and NGS for HLA-A locus genotyping

- A separate set of samples (n=41) had buffy coat analyzed by both NGS and Sanger sequencing for HLA-A locus typing, with valid results.
- Sanger sequencing and NGS showed a 97.6% concordance for HLA (**Figure 5**).

Figure 2. Representative images from the NY-ESO-1 IHC assessment of (A) stomach adenocarcinoma, (B) head and neck squamous cell carcinoma, (C) ovarian, and (D) bladder cancer tumor samples

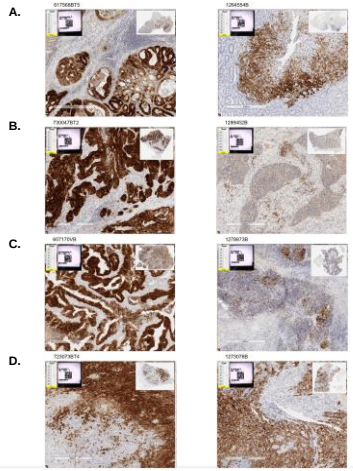
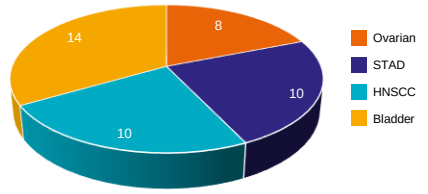
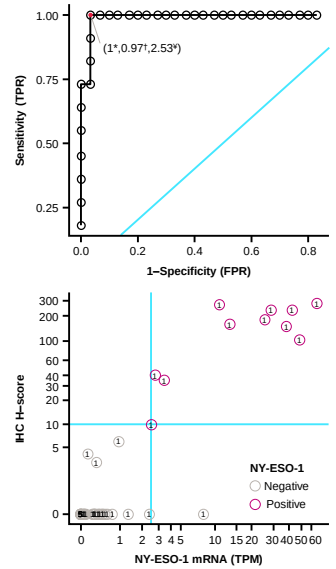


Figure 3. Subset of samples with comparative IHC and NGS analysis



Head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma (STAD).

Figure 4. ROC analysis of NY-ESO-1 protein expression by IHC to mRNA expression



(A) TPR = true positive rate, and FPR = false positive rate, (B) TPM = transcripts per million.
*Sensitivity; †Specificity; ¥mRNA cut-off.

Table 2. NY-ESO-1 and LAGE-1a mRNA expression across multiple indications

Indication	# of samples ≥2.53 TPM NY-ESO-1 / Total	# of samples >0 TPM LAGE-1a / Total
STAD	3/10 (30%)	5/10 (50%)
Ovarian Cancer	1/8 (12%)	4/8 (50%)
HNSCC	3/10 (30%)	3/10 (30%)
Bladder Cancer	5/14 (36%)	9/14 (64%)

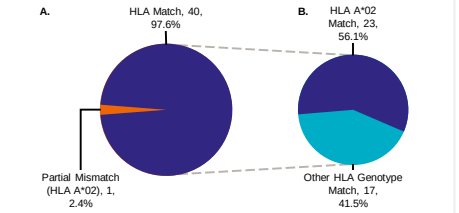
Head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma (STAD), transcripts per million (TPM).

Table 3. Single expression and co-expression of NY-ESO-1 and LAGE-1a across multiple indications by NGS mRNA

Indication	NY-ESO-1 + / LAGE-1a +	NY-ESO-1 + / LAGE-1a −	NY-ESO-1 − / LAGE-1a +	NY-ESO-1 − / LAGE-1a −
STAD	2/10 (20%)	1/10 (10%)	3/10 (30%)	4/10 (40%)
Ovarian Cancer	0/8 (0%)	1/8 (12%)	4/8 (50%)	3/8 (38%)
HNSCC	1/10 (10%)	2/10 (20%)	2/10 (20%)	5/10 (50%)
Bladder Cancer	5/14 (36%)	0/14 (0%)	4/14 (29%)	5/14 (36%)

'NY-ESO-1 +' is based on mRNA cut-off of >2.53 TPM. 'LAGE-1a +' is based on CTAG2 >0 TPM.
Head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma (STAD).

Figure 5. Distribution of HLA concordance between NGS and Sanger sequencing



(A) concordance results irrespective of genotype. The partial mismatch was due to annotation differences, versus detection differences. (B) A sample was counted under the HLA A*02 category if either one of its parental HLA alleles was A*02.

Conclusions

- NY-ESO-1 and LAGE-1a were expressed in a proportion of all samples tested in all indications investigated.
- NGS was highly correlated to IHC and Sanger sequencing and may be an alternative method for identifying a higher number of tumors that express biomarkers of interest.
- HLA sequencing in a variety of tumor types may help understand how HLA down regulation in tumor cells can act as a potential escape mechanism to TCR T-cell therapies.
- Our data show a unique platform for comprehensive assessment of disease which may improve patient stratification and management strategies.
- These findings may reduce the need for multiple assays and increase the opportunity to expand into new tumor types.

Disclosures

KB, JK, ND, GK, SB, IE employee and stockholder in GSK. **AH** was previously employed by GSK.

Ethics and Approval Statement

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

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