Analysis of NY-ESO-1 expression in specimens from a phase I/II NY-ESO-1 T-cell therapy clinical trial in non-small cell lung cancer and from exploratory studies in multiple tumor types

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Background

- New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) is a well-known cancer testis antigen. It is highly immunogenic and NY-ESO-1 expression has been reported in patients across various cancer types, including but not limited to non-small cell lung cancer (NSCLC), gastric adenocarcinoma (GAC), esophageal adenocarcinoma (EAC) and gastroesophageal junction (GEJ), urothelial carcinoma (UTC), head and neck squamous cell carcinoma (HNSCC), triple-negative breast carcinoma (TNBC), hepatocellular carcinoma (HCC), and melanoma [1–8].
- Lung cancer is one of the more common cancers, with approximately 2.1 million new cases diagnosed each year, globally [9]. NSCLC is most commonly diagnosed as Stage IV (AJCC) disease and while cancer therapies have improved in the last 5-10 years, there remains an unmet medical need for many patients [10].
- Letetresgene autoleucel (lete-cel; GSK3377794) is a potential first-in-class autologous T-cell therapy for solid tumors that expresses a genetically modified T-cell receptor (TCR) which allows a patient's T cells to recognize NY-ESO-1 complexed with human leukocyte antigen (HLA)-A*02:01, HLA-A*02:05, and/or HLA-A*02:06 [11].
- A phase Ib/IIa clinical trial (NCT03709706) aims to assess lete-cel safety and efficacy in patients with NSCLC [11]. As part of the patient screening process, NY-ESO-1 protein expression levels were determined with an investigational use only immunohistochemistry (IHC) clinical trial assay.
- The analyses presented herein evaluates NY-ESO-1 protein expression patterns and positivity rates when an IHC clinical trial assay is used to stain patient samples from a phase Ib/IIa trial. Also, an exploratory analysis of procured formalin-fixed paraffin-embedded (FFPE) specimens comprised of multiple tumor types was preliminarily assessed for staining patterns and characterization of NY-ESO-1 expression.

Aims

- To describe the protein expression patterns of NY-ESO-1 in tumor samples from patients with NSCLC using an NY-ESO-1 IHC clinical trial assay.
- To explore the expression and staining patterns of NY-ESO-1 protein expression in commercially procured GAC, EAC and GEJ, UTC, HNSCC, TNBC, HCC, and melanoma FFPE specimens.

Methods

- Patient tumor specimens from the NCT03709706 clinical trial in NSCLC and procured FFPEs from multiple tumor types were stained for NY-ESO-1 expression using an IHC clinical trial assay (**Figure 1**).
- For all clinical and exploratory testing, a run control comprised of normal kidney (negative) and normal testis (positive) were processed concurrent to each run. Stained specimens were scored by a blinded pathologist.

Figure 1. Methods and objectives

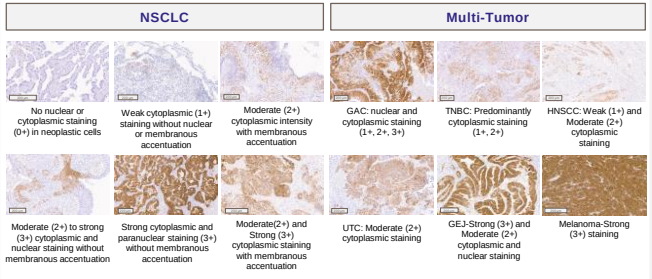
STUDY	GSK ID: 208471; NCT03709706 Phase Ib/IIa clinical trial	GSK Exploratory Study; multiple indications with the 208471 clinical trial assay
INDICATION	NSCLC	EAC, GAC & GEJ, UTC, HNSCC, TNBC, HCC & melanoma
SAMPLES	Archival FFPE tumor biopsies from screened patients	Procured FFPE tumor biopsies from donors
ASSAY	Investigational use only **IHC** clinical trial assay **Anti-NY-ESO-1** monoclonal antibody (clone E978) Cytoplasmic and nuclear staining Light microscopy A semi-quantitative **H-score** method and/or a cutoff of **≥10%Tumor and ≥1+ staining intensity**	
OBJECTIVES	❖ **Describe NY-ESO-1 expression in NSCLC and multiple tissue types using** • Distribution of H-scores and positivity when applying a cutoff of 10%Tumor • Dynamic range of staining intensity ❖ **Compare NY-ESO-1 expression patterns in the clinical NSCLC & across multiple procured tumor types (H-score & ≥10%Tumor at ≥1+ staining intensity)** ❖ **Compare NY-ESO-1 expression patterns in primary vs metastatic NSCLC**	

H-score = ∑ [(%Tumor at 1+) x 1] + [(%Tumor at 2+) x 2)] + (%Tumor at 3+) x3)].
EAC, esophageal adenocarcinoma; FFPE, formalin-fixed paraffin-embedded; GAC, gastric adenocarcinoma; GEJ, gastroesophageal carcinoma; H-score, histoscore; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; NY-ESO-1, New York Esophageal Squamous Cell Carcinoma-1; TNBC, triple-negative breast cancer; UTC, urothelial carcinoma.

Results

- NY-ESO-1 IHC (mouse monoclonal antibody, E978, Sigma) assay produced predominantly cytoplasmic and nuclear staining across the dynamic range of staining intensities (i.e., 0+, 1+, 2+, and 3+, **Figure 2**).
- In specimens that displayed NY-ESO-1 staining at lower intensities (i.e., 1+, 2+), the cytoplasmic staining was typically diffuse and generally evenly distributed within the cell, with limited to no granular presentation of the NY-ESO-1 protein. In some specimens, nuclear and/or membranous accentuation was observed (**Figure 2**).

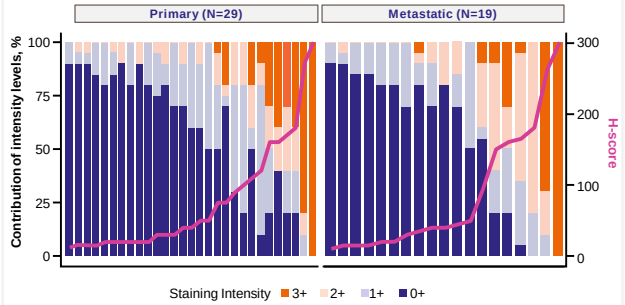
Figure 2. NY-ESO-1 cytoplasmic and nuclear expression patterns multiple tumor types



Representative images showing the dynamic range of staining intensity in NSCLC specimens and examples of NY-ESO-1 staining in GAC, TNBC, HNSCC, UTC, GEJ and melanoma.

- The observed positivity rate (using a cutoff of ≥10%Tumor and ≥1+ staining intensity) for NSCLC specimens was 15% (49/325; 95% CI [11%, 19%]) for NY-ESO-1 (**Figure 3**).
- A positivity rate for primary tumors was 15% (29/191; 95% CI [10%, 20%]) and 14% (19/132; 95% CI [8.4%, 20%]) for metastatic tumors (**Figure 3**).

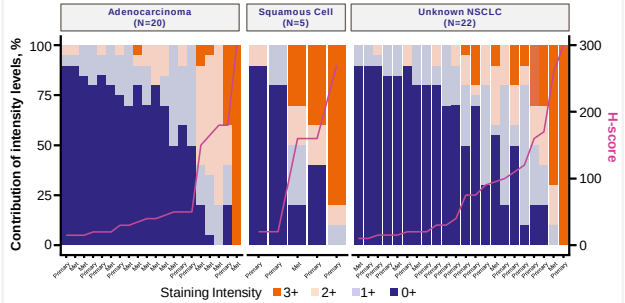
Figure 3. NY-ESO-1 scoring distribution in clinical trial NSCLC specimens, primary vs metastatic tumors (cutoff: ≥10%Tumor at ≥1+ staining intensity)



Each bar represents a single specimen. Null/weak/moderate/strong NY-ESO-1 expression was defined by IHC intensity scores 0+/1+/2+/3+, respectively. One sample had "unknown" annotation for Primary/Metastatic and was excluded from this graph.

- A positivity rate of 13% (20/159; 95% CI [7.4%, 18%]) for adenocarcinoma and 14% (5/35; 95% CI [2.7%, 26%]) for squamous cell carcinoma was observed (using a cutoff of ≥10%Tumor and ≥1+ staining intensity, **Figure 4**).

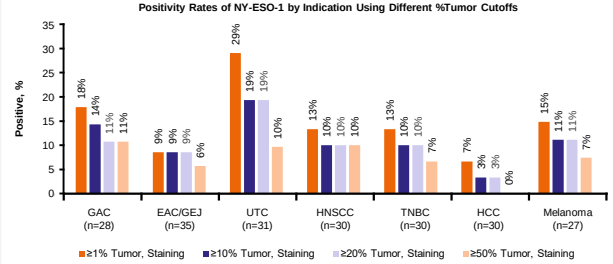
Figure 4. NY-ESO-1 scoring distribution in adenocarcinoma, squamous cell carcinoma and unknown/unclassified clinical trial NSCLC specimens (cutoff: ≥10%Tumor at ≥1+ staining intensity)



Each bar represents a single specimen. Null/weak/moderate/strong NY-ESO-1 expression was defined by IHC intensity scores 0+/1+/2+/3+, respectively. Met, metastatic.

- For exploratory purposes, we analyzed NY-ESO-1 positivity across multiple indications using %Tumor cutoffs of ≥1%, ≥10%, ≥20%, and ≥50%. In all analyses, only viable tumor cells with ≥1+ staining intensity were counted as positive (**Figure 5**).
- The following NY-ESO-1 positivity rates were observed (using a cutoff of ≥10%Tumor content and ≥1+ staining intensity): GAC, 14% (4/28); EAC and GEJ, 9% (3/35); UTC, 19% (6/31); HNSCC, 10% (3/30); TNBC, 10% (3/30); HCC, 3% (1/30); and melanoma, 11% (3/27) (**Figure 5**).

Figure 5. NY-ESO-1 positivity across multiple indications as a function of applied %Tumor cutoff (at ≥1+ staining intensity)



NY-ESO-1 protein expression was localized in the tumor cells' nuclei and surrounding cytoplasm.

Conclusions

- For NSCLC and multiple tumor types, the IHC clinical trial assay demonstrated similar NY-ESO-1 expression across the range of staining intensities and similar percentages of positivity.
- For NSCLC specimens, the staining characteristics (%Tumor, intensity) for primary vs metastatic tumor types had similar staining distribution profiles. Also, the positivity rates for NSCLC adenocarcinoma and squamous cell carcinoma subtypes were similar (note that the identified squamous cell carcinoma sample size was small, n=35).
- NY-ESO-1 expression patterns consistently displayed cytoplasmic and nuclear staining in NSCLC and other tumor types.
- The observed NY-ESO-1 positivity rate in the tested indications was consistent with the literature where the E978 clone was used.
- These findings support that an IHC assay for NY-ESO-1 detection in additional tumor types may be considered for use as a clinical trial assay.

Clinical trial advancements in T-cell therapies

- CD8α/NY-ESO-1 TCR T-cell (GSK3901961) clinical efficacy and safety are being evaluated further in patients with NSCLC with NY-ESO-1-positive tumors as part of GSK's master protocol NCT04526509.
- An NY-ESO-1 IHC assay is a robust approach to identify patients who may be eligible for CD8α/NY-ESO-1 TCR T-cell treatment.

Disclosures

BB, **MS**, **KB**, **RP**, **JK**, **NRH**, and **EC** are current employees of and own stocks/shares in GlaxoSmithKline (GSK). **MA** reports that he is on the advisory boards for GSK, Shattuck Labs, Bristol Myers Squibb, and AstraZeneca; has received speaker fees from AstraZeneca; and has received research funding (to institution) from Genentech, Nektar Therapeutics, Merck, GlaxoSmithKline, Novartis, Jounce Therapeutics, Bristol Myers Squibb, Eli Lilly, Adaptimmune, Shattuck Lab, and Gilead.

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