Target antigen levels in NSCLC samples define a clinically relevant activation threshold for NY-ESO-1 TCR T-cell therapy

#P2.04-91

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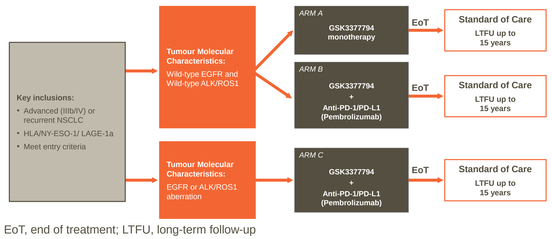
Background

- Adoptive T-cell therapy is a promising treatment for recurrent or metastatic solid and hematologic malignancies with encouraging activity demonstrated in patients with synovial sarcoma, melanoma, myxoid/round cell liposarcoma, and multiple myeloma.[1-4]
- Unlike chimeric antigen receptor T cells (CAR T) that primarily target cell surface proteins, engineered T-cell receptor T cells (TCR T) recognize intracellular antigens presented in the context of human leukocyte antigen (HLA).[5]
- NY-ESO-1 and LAGE-1a are intracellular cancer-testis antigens that generate a shared SLLMWITQC peptide bound to HLA-A*02 and are expressed across multiple malignancies, including non-small cell lung cancer (NSCLC).
- GSK3377794 (NY-ESO-1 TCR T cells) are autologous, polyclonal, lentivirally (LV) transduced T cells engineered to express an affinity enhanced TCR recognizing the SLLMWITQC/HLA-A*02:01,*02:05 and/or 02*06 peptide complex.[6]
- GSK is currently running a Phase 1b/2a, multi-arm, open-label pilot study (NCT03709706) of GSK3377794 as a monotherapy or in combination with pembrolizumab in HLA-A*02 positive patients whose tumours express NY-ESO-1 and/or LAGE-1a (**Figure 1**).

Figure 1. Affinity enhanced NY-ESO-1 TCR-T cells (GSK3377794)[7]



EoT, end of treatment; LTFU, long-term follow-up

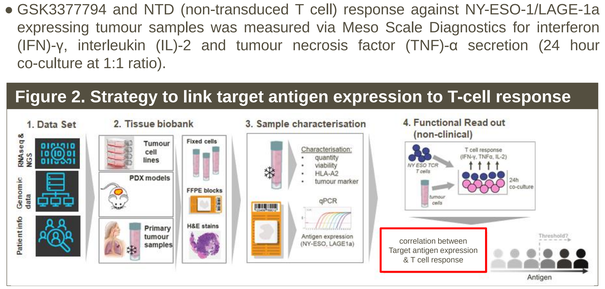
Aims

- Systematically assess the prevalence and expression levels of NY-ESO-1 and LAGE-1a target antigens in NSCLC samples.
- Determine the product-specific threshold of target antigen required to induce a specific GSK3377794 response to inform the ongoing clinical trials in NSCLC.

Methods

- A NSCLC tumour biobank consisting of (1) primary patient tumour (adenocarcinoma and squamous cell carcinoma) samples, (2) patient derived xenograft (PDX) models and (3) tumour cell lines was established.
- Expression of HLA-A*02 was characterised by flow cytometry and levels of NY-ESO-1 and LAGE-1a antigens were measured via RT-qPCR.
- Tumour samples were ordered by total antigen expression (Total antigen dCq).
- GSK3377794 and NTD (non-transduced T cell) response against NY-ESO-1/LAGE-1a expressing tumour samples was measured via Meso Scale Diagnostics for interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor (TNF)-α secretion (24 hour co-culture at 1:1 ratio).

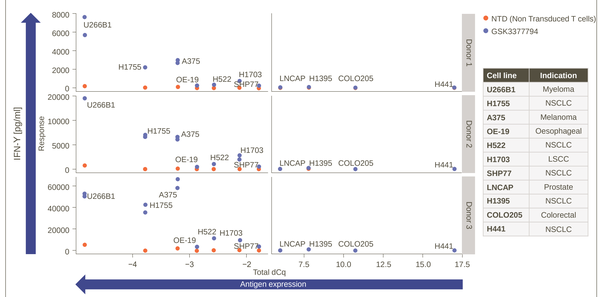
Figure 2. Strategy to link target antigen expression to T-cell response



Results

Figure 3. GSK3377794 activation by tumour cell lines correlates with target antigen expression levels

- GSK3377794 (purple dots) shows a dose dependant activation as the level of antigen expressed by individual tumour cell lines increases.
- IFN-γ is significantly elevated in positive cell lines where against H1755 it is 268x higher than NTD (95% confidence intervals (CI): 128–561, p<0.000001).
- IFN-γ release observed against antigen negative cell lines such as H1395 is not substantially elevated by the GSK3377794 where there is a 1.7x fold change compared with NTD (95% CI: 0.8–3.6, p=0.36).

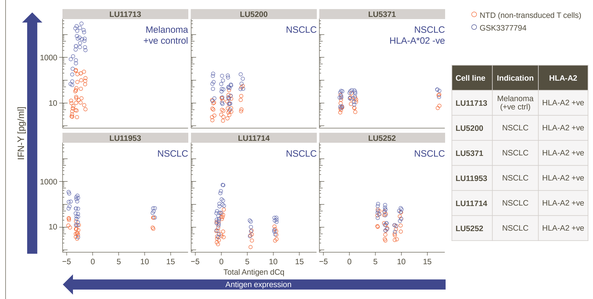


Cell line	Indication
U266B1	Myeloma
H1755	NSCLC
A375	Melanoma
OE-19	Oesophageal
H522	NSCLC
H1703	LSCC
SHP77	NSCLC
LNCAP	Prostate
H1395	NSCLC
COLO205	Colorectal
H441	NSCLC

Each data point represents a single well of duplicates performed in a single experiment. Data from three separate donor T cells are presented. LSCC, lung squamous cell carcinoma

Figure 4. IFN-γ response by GSK3377794 to NSCLC PDX tumour samples depends on level of target antigen expression

- GSK3377794 activate and induce IFN-γ secretion in response to different NSCLC PDX models with variable target antigen expression.
- IFN-γ released by GSK3377794 compared with NTD is greater in higher antigen expressing models (e.g. at dCq of -4.5, it is 7.4x higher: 95% CI: 5.7–9.6, p<0.0001).
- IFN-γ levels released by GSK3377794 in response to NSCLC models are lower than those observed in response to the positive control melanoma model.
- IFN-γ released by GSK3377794 in response to the HLA-A2 negative model is comparable to that observed against antigen negative cell lines (Figure 3).



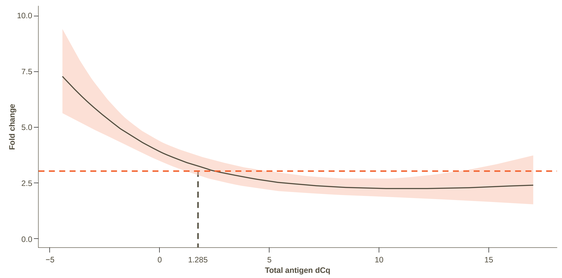
Cell line	Indication	HLA-A2
LU11713	Melanoma (+ve ctrl)	HLA-A2 +ve
LU5200	NSCLC	HLA-A2 +ve
LU5371	NSCLC	HLA-A2 +ve
LU11953	NSCLC	HLA-A2 +ve
LU11714	NSCLC	HLA-A2 +ve
LU5252	NSCLC	HLA-A2 +ve

Data from three separate donor T cells are presented.
Points are jittered in x-axis to prevent overplotting because all observations for one model have the same antigen dCq.

Figure 5. Functional readouts identify an antigen expression threshold for GSK3377794 in NSCLC

Threshold definition: Fold change (GSK3377794/NTD) for IFN-γ response with 95% two-sided CI

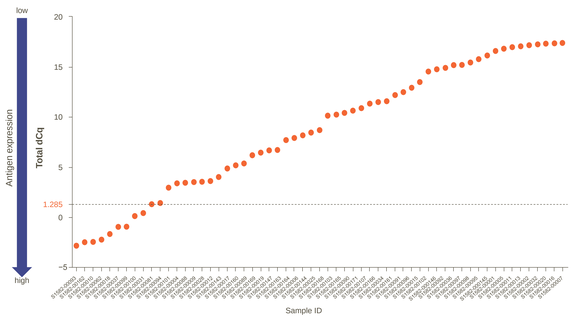
- A fold change of ~3x IFN-γ release (GSK3377794/NTD) is observed against antigen negative cell lines and is used to define background response in PDX models.
- Linear contrasts used to compare the difference in IFN-γ release between GSK3377794 vs NTD T cells in response to the 5 NSCLC PDX models.
- An antigen dCq value of 2.74 (95% CI: 1.285–5.268) is required for 3x greater IFN-γ release induced by GSK3377794 vs NTD T cells.
- A minimum total antigen dCq of 1.285 is required to reliably observe a specific GSK3377794 response.



Linear Contrast of IFN-γ release, as defined by the fold change between GSK3377794 and NTD T cells against NSCLC PDX tumours, calculated from a linear mixed model using a natural spline for the effect of antigen dCq on cytokine release. Red dotted line defines the 3-fold change.

Figure 6. Understanding of the expected GSK3377794 response towards primary patient NSCLC tumour samples based on threshold defined from PDX models

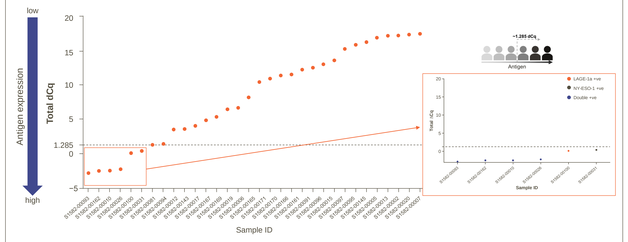
- Fifty-nine primary patient NSCLC tumour samples were recruited, processed and ordered by total levels of NY-ESO-1 & LAGE-1a antigens as defined by RT-qPCR (Total antigen dCq).
- Of this population, 9 samples (15.25%) express target antigen within the proposed expression threshold of 1.285 dCq (**Figure 5**).



Primary patient NSCLC samples ordered by total antigen expression.
Key: dotted line defines the proposed threshold value of 1.285 dCq.

Figure 7. Translating target antigen expression threshold for GSK3377794 activation to a potential patient population with NSCLC in HLA-A*02 positive samples

- Thirty-two of 59 (~54%) primary patient NSCLC samples recruited are HLA-A*02 positive.
- Of this population 6 of 32 (18.75%) samples express target antigen within the proposed expression threshold of 1.285 dCq (**Figure 7**). Such data indicates that ~18.75% of HLA-A*02 NSCLC patients tested may benefit from treatment with GSK3377794.
- Immunohistochemistry (IHC) tests, used to screen patients can identify only the NY-ESO-1 antigen. LAGE-1a cannot be detected via IHC screening.
- One of these 6 (~16.6%) samples (**Figure 7** insert) express only the LAGE-1a target antigen. Screening methods detecting both NY-ESO-1 and LAGE-1a levels can potentially increase the target population.



HLA-A*02 positive primary patient NSCLC samples ordered by total antigen expression;
Insert: target antigen prevalence in primary NSCLC tumours.

Conclusions

- We performed a systematic analysis that correlates target antigen expression in NSCLC with functional responses of GSK3377794 using a set of clinically relevant samples.
- The functional readouts identify a product-specific antigen expression threshold for GSK3377794 in NSCLC, which could translate to a potential NSCLC patient population that may benefit from cell therapy with GSK3377794.

Ongoing work and future steps

- Ongoing GSK clinical trials in NSCLC (NCT03709706) will help us understand the relevance of these preclinical findings.
- Identification of antigen expression threshold for GSK3377794 in other key indications of solid cancers.
- Evaluate preclinically the potential for epigenetic modulators to upregulate of NY-ESO-1/LAGE-1a expression to enhance targeting by GSK3377794 in NSCLC and move to an exploratory medicine style study in key indications.

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Acknowledgements

- This study was funded by GlaxoSmithKline (GSK).
- Editorial support was provided by Fiona Woodward, PhD, CMPP, at Fishawack Indicia Ltd and was funded by GSK.
- 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.
- All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

Disclosures

- The presenting author, Anna Domogala, declares the following real or perceived conflicts of interest during the last 3 years in relation to this presentation: an employee and stock/shareholder in GSK.
- IE, SJB, LP, MAK, KS, JJ, JE, KRA, RR, SO'S, LC, MD, JCK, JRS, DP, LAJ, AS and CB are employees of and stock/shareholder in GSK.

International Association for the Study of Lung Cancer World Conference on Lung Cancer, Barcelona, Spain, 7–10 September 2019

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