NY-ESO-1 and LAGE-1a – an emerging target for cell therapies in solid tumours

Poster No. 1299P

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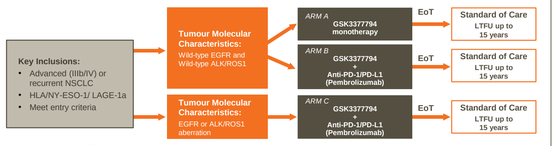
Background and previous work

- Adoptive T-cell receptor (TCR) therapy is a promising treatment for recurrent or metastatic solid and haematologic malignancies with encouraging activity demonstrated in patients with synovial sarcoma, melanoma, myxoid/round cell liposarcoma and multiple myeloma.[1-4]
- NY-ESO-1 and LAGE-1a are intracellular cancer-testis antigens that generate a shared SLLMWITQC peptide bound to HLA-A*02 that is expressed across multiple malignancies, including non-small cell lung cancer (NSCLC).
- GSK3377794 (NY-ESO-1 TCR T cells) are autologous, polyclonal, lentivirally transduced T cells engineered to express an affinity enhanced TCR recognising the SLLMWITQC/HLA-A*02:01,*02:05 and/or 02*06 peptide complex.[5]
- GSK is currently running a Phase Ib/IIa, 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**).

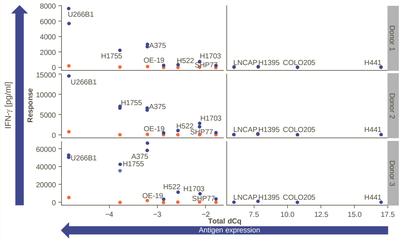
Figure 1. Study design of GSK3377794 in NSCLC[6]



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

- Treatment of solid tumours remains challenging, possibly due to the immunosuppressive tumour micro-environment, heterogeneity of antigen expression and unfavourable metabolic conditions.
- We have previously shown that GSK3377794 activation by various tumour cell lines correlates with target antigen expression levels (**Figure 2**).[7]

Figure 2. GSK3377794 activation by tumour cell lines



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

Data from three separate donor T cells are presented. Levels of NY-ESO-1 and LAGE-1a antigens were measured via quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Tumour cell lines ordered by total antigen expression levels (total antigen dCq). GSK3377794 and NTD response against tumour cell lines was measured via Meso Scale Diagnostics (MSD) for interferon (IFN)-γ (24-hour co-culture at 1:1 ratio). LSCC, lung squamous cell carcinoma.

- We have previously identified a product-specific antigen expression threshold of 1.285 dCq for GSK3377794 activation in NSCLC.[7]
- Use of epigenetic modifiers to selectively modulate tumour associated antigen (TAA) expression in tumours could potentially increase patient benefit from TAA-targeted therapies.
- Decitabine (DAC) is a demethylating agent that acts through the inhibition of DNA methyltransferase. It induces expression of TAA and modulates genes involved in antigen processing and presentation.[8] DAC induces the expression of NY-ESO-1 and LAGE-1a antigens in cancer cells enhancing antigen-specific T-cell therapy.[9-10]

Aims

- Examine the prevalence of NY-ESO-1 and LAGE-1a target antigens in various malignancies.
- Explore means to selectively modulate NY-ESO-1 and/or LAGE-1a expression in various tumours, including NSCLC, in order to further enhance the expression of the target antigens aiming to increase targeting by GSK3377794 in NSCLC and other key indications.

Methods

- Gene and transcript level RNAseq data for NY-ESO-1 and LAGE-1a were extracted from Omicsoft's OncoLand via ArrayStudio version 10 (TCGA version B38).
- Tumour cell lines were cultured in vitro, in growth media supplemented with 1 μM DAC for 72 hours that was refreshed daily. Cells were then washed and cultured in DAC-free growth media for an additional 72 hours before being harvested for antigen profiling by qRT-PCR and functional analyses. Phenotyping for viability, HLA-A*02 and PD-L1 expression was performed by flow cytometry.
- A linear mixed model was fit to IFN-γ concentration. Linear contrasts for the cytokine release following GSK3377794 versus NTD were compared under DAC treatment and untreated controls for each tumour cell line.
- 5×10^6 antigen high LSCC (H1703) or antigen low NSCLC (H1395) tumour cells were implanted in vivo in non-obese diabetic/severe combined immunodeficient/IL-2 receptor-γ null (NSG) mice by subcutaneous injection. On Day 19 post-tumour implantation, mice received intraperitoneal injections of phosphate-buffered saline or 1 mg/kg (low) or 3 mg/kg (high) total dose of DAC 3 times over 5 days. Mice were euthanised 72 hours after last injection. Tumours were collected and assessed for NY-ESO-1 expression by immunohistochemistry (IHC) and for NY-ESO-1 and LAGE-1a expression via qRT-PCR.

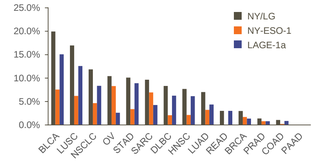
Results

- NY-ESO-1 and LAGE-1a expression varies across malignancies. Unlike synovial sarcoma and multiple myeloma where antigen prevalence is very high (60–70%) in tumour types such as lung cancer, bladder and others presented in **Figure 3**, target antigen expression is lower (4–15%).

Figure 3. NY-ESO-1 and LAGE-1a prevalence in multiple indications

Tumour Type Code	Code Description	Sample n	≥10 FPKM (Low Threshold)			≥25 FPKM (Medium Threshold)			≥50 FPKM (High Threshold)		
			NY/LG	NY	LG	NY/LG	NY	LG	NY/LG	NY	LG
BLCA	Bladder urothelial carcinoma	411	20.0%	7.5%	15.1%	12.9%	3.2%	10.9%	8.0%	0.5%	5.4%
BRCA	Breast invasive carcinoma	1107	3.0%	1.7%	1.4%	1.2%	0.7%	0.5%	0.5%	0.3%	0.1%
COAD	Colon adenocarcinoma	471	1.1%	0.2%	0.8%	0.4%	0.0%	0.4%	0.0%	0.0%	0.0%
DLBC	Diffuse large B-cell lymphoma	48	8.3%	2.1%	6.3%	6.3%	0.0%	6.3%	6.3%	0.0%	6.3%
HNSC	Head and neck squamous cell carcinoma	520	7.7%	2.1%	6.2%	4.4%	1.2%	3.3%	1.3%	0.2%	1.2%
LUAD	Lung adenocarcinoma	528	7.0%	3.2%	4.4%	3.6%	1.1%	1.7%	0.8%	0.4%	0.0%
LUSC	Lung squamous cell carcinoma	501	17.0%	6.2%	12.6%	9.4%	2.4%	6.4%	3.4%	0.8%	2.0%
NSCLC	LUAD + LUSC	1029	11.9%	4.7%	8.4%	6.4%	1.7%	4.0%	2.0%	0.6%	1.0%
OV	Ovarian serous cystadenocarcinoma	422	10.4%	8.3%	2.6%	6.9%	4.7%	2.1%	4.3%	2.8%	1.2%
PAAD	Pancreatic adenocarcinoma	178	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
PRAD	Prostate adenocarcinoma	501	1.4%	0.8%	0.8%	0.8%	0.0%	0.8%	0.2%	0.0%	0.2%
READ	Rectum adenocarcinoma	166	3.0%	0.0%	3.0%	1.8%	0.0%	1.8%	1.2%	0.0%	1.2%
SARC*	Sarcoma	259	9.7%	6.9%	4.2%	6.2%	4.2%	2.7%	3.1%	1.5%	0.8%
STAD	Stomach adenocarcinoma	416	10.1%	3.4%	8.9%	5.8%	1.2%	4.8%	2.4%	0.5%	1.4%

Percentages of patient samples expressing NY-ESO-1 (NY), LAGE-1a (LG), or sum of expression of NY-ESO-1 and LAGE-1a (NY/LG) across various tumour types at three expression thresholds (low = 10 fragments per kilobase million [FPKM]; medium = 25 FPKM; high = 50 FPKM). *Percentages shown for sarcoma are across all histologies.

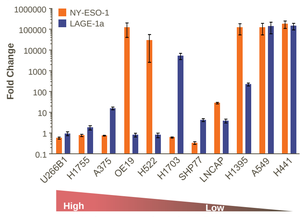


Percentages of patient samples expressing NY, LG or sum of expression (NY/LG) across various tumour types at **10 FPKM** threshold. NY/LG column shows percentages of samples where combined expression (i.e., gene/transcript sum) is above the given threshold, irrespective of gene/transcript-specific contributions.

Figure 4. In vitro upregulation of NY-ESO-1 and LAGE-1a upon decitabine treatment in tumour cell lines

- DAC efficiently upregulates the expression of NY-ESO-1 and/or LAGE-1a target antigens in **the low antigen-expressing** target tumour cell lines LNCAP, H1395, A549 and H441.
- Expression of individual antigens and total target antigen expression (total antigen dCq) is increased to levels that could induce activation of GSK3377794 (**Figure 2**).
- Treatment with DAC does not affect expression of NY-ESO-1 and/or LAGE-1a in tumour cell lines where expression of the two target antigens is high.

A.



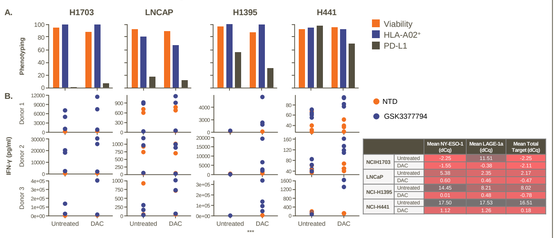
B.

		Mean NY-ESO-1 (dCq)	Mean LAGE-1a (dCq)	Mean Total Target (dCq)
U266B1	Untreated	-4.10	-3.91	-5.07
	DAC	-3.27	-3.75	-4.55
NCI-H1755	Untreated	-3.55	-2.77	-4.25
	DAC	-3.13	-3.30	-4.23
A375	Untreated	-2.84	3.54	-2.85
	DAC	-2.43	-0.36	-2.75
OE19	Untreated	13.41	-3.67	-3.67
	DAC	0.16	-3.29	-3.43
NCIH522	Untreated	13.67	-2.99	-2.99
	DAC	1.03	-2.58	-2.70
NCIH1703	Untreated	-2.25	11.51	-2.25
	DAC	-1.55	-0.38	-2.11
SHP77	Untreated	-1.10	3.63	-1.11
	DAC	-1.50	1.55	-1.66
LNCaP	Untreated	5.38	2.35	2.17
	DAC	1.60	0.46	-0.47
NCI-H1395	Untreated	14.45	8.21	8.02
	DAC	0.01	0.48	-0.78
A549	Untreated	13.40	12.60	11.88
	DAC	-0.13	-1.93	-1.85
NCI-H441	Untreated	17.50	17.53	16.51
	DAC	1.12	1.26	0.18

(A) Fold change of antigen upregulation of treatment group over untreated control. **(B)** dCq values for NY-ESO-1, LAGE-1a and total target for tumour cell lines treated with DAC and respective untreated controls. Limit of quantification was defined as Ct values of 32.6 (NY-ESO-1) and 32.0 (LAGE-1a) correlating to a dCq value of approximately 10. Post DAC treatment lung tumour cell lines express target antigen within the proposed expression threshold of 1.285 dCq. Duplicates of each treated cell line performed over two experiments is shown.

Figure 5. Decitabine further upregulates expression of target antigens in NSCLC, rendering them susceptible to specific targeting by GSK3377794

- GSK3377794 (blue dots) induce IFN-γ secretion to similar levels in response to the high antigen LSCC cell line H1703 irrespective of DAC treatment.
- In response to the low target antigen NSCLC cell line H1395, there is a 7.07x increase in IFN-γ release by GSK3377794 when combined with DAC treatment, compared with GSK3377794 monotherapy (95% CI, 3.02x-16.51x; p<0.001).
- No activation of GSK3377794 observed in response to LNCAP (p=1.00) and H441 (p=0.42) irrespective of NY-ESO-1 and/or LAGE-1a upregulation due to DAC treatment. Low levels of HLA-A*02 observed in LNCAP and high PD-L1 levels observed in H441 tumour cell lines could account for this.



		Mean NY-ESO-1 (dCq)	Mean LAGE-1a (dCq)	Mean Total Target (dCq)
NCIH1703	Untreated	-2.25	11.51	-2.25
	DAC	-1.55	-0.38	-2.11
LNCaP	Untreated	5.38	2.35	2.17
	DAC	0.68	0.46	-0.47
NCI-H1395	Untreated	14.45	8.21	8.02
	DAC	0.01	0.48	-0.78
NCI-H441	Untreated	17.50	17.53	16.51
	DAC	1.12	1.26	0.18

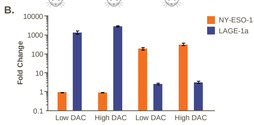
(A) Phenotype of untreated and treated target cell lines. **(B)** GSK3377794 and NTD response against tumour cell lines was measured via MSD for IFN-γ (24-hour co-culture at 1:1 ratio). Each data point represents an average of two technical duplicates of two biological duplicates performed over two experiments. Data from three separate donor T cells are presented. **(C)** dCq values of treated and untreated control.

Figure 6. Decitabine treatment of NSCLC xenograft tumour models results in upregulation of the target antigens NY-ESO-1 and LAGE-1a

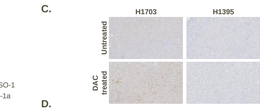
- In vivo treatment of the LSCC H1703 and NSCLC H1395 xenograft models with DAC results in successful upregulation of NY-ESO-1 and LAGE-1a target antigen expression (**Figure 6 B–D**).
- Additionally, higher DAC doses will be examined in the low-expressing H1395 NSCLC xenograft model aiming to further enhance target antigen expression before proceeding to an in vivo functional study with GSK3377794.

A.

B.



C.



D.

		Mean NY-ESO-1 (dCq)	Mean LAGE-1a (dCq)	Mean Total Target (dCq)
H1703	Untreated	-0.64	16.34	-0.64
	Low DAC	-0.50	6.06	-0.51
	High DAC	-0.51	4.88	-0.54
H1395	Untreated	13.95	6.97	6.93
	Low DAC	6.49	5.68	5.02
	High DAC	5.78	5.39	4.55

(A) Schematic of in vivo protocol. **(B)** Fold change of antigen upregulation of treatment group over untreated control group. **(C)** Representative images of IHC for NY-ESO-1 expression in DAC-treated and -untreated control groups. **(D)** dCq values of treated and untreated control groups. Effects seen in n=7–8 is shown.

Conclusions

- GSK3377794 activate and induce IFN-γ secretion in response to different tumour cell lines with variable target antigen expression.
- Expression of the target antigens NY-ESO-1 and LAGE-1a can be enhanced in the antigen low tumour cell lines following DAC treatment to levels previously identified to induce specific responses by GSK3377794.
- Combination approaches with epigenetic regulators such as DAC can further enhance target antigen expression and potentially increase targeting by GSK3377794 in NSCLC.

Ongoing work and future steps

- Ongoing GSK clinical trials in NSCLC (NCT03709706) will help us understand the relevance of these preclinical findings. Enhancing target expression in NSCLC could be evaluated with future combination work.
- Evaluate the effect of higher DAC doses in NSCLC xenograft models aiming to increase anti-tumour effect in low-antigen expressing tumours in vivo.
- Evaluate preclinically the potential for additional epigenetic modulators to upregulate NY-ESO-1/LAGE-1a expression to further enhance targeting by GSK3377794 in NSCLC and move to an exploratory medicine style combination study in key indications.

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- The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an Institutional Review Board/Ethics Committee approved protocol.
- All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Disclosures

- The presenting author, Ioanna Eleftheriadou, declares the following real or perceived conflicts of interest during the last 3 years in relation to this presentation: an employee and stockshareholder in GSK.
- SJB, AD, LP, MAK, KS, MG, JD, PF, JJ, JE, KRA, RR, SO'S, LC, MD, DP, LAJ, AS and CB are employees of and stock/shareholders in GSK.

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