Decitabine gene modulation sensitizes human non–small-cell lung cancer (NSCLC) to NY-ESO-1 TCR immunotherapy (letetresgene autoleucel; GSK3377794) in vivo

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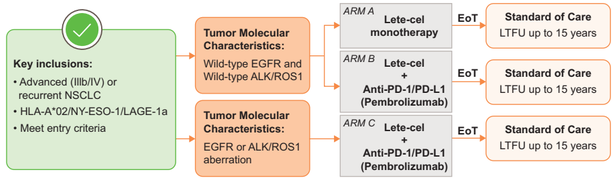
Background and previous work

- New York esophageal squamous cell carcinoma-1 (NY-ESO-1)–specific T cells (lete-cel; letetresgene autoleucel) are autologous CD4+ and CD8+ T cells transduced to express a high affinity T-cell receptor (TCR) capable of recognizing NY-ESO-1 and L antigen family member 1 A (LAGE-1a) antigens in complex with human leukocyte antigen A*02 (HLA-A*02).
- NY-ESO-1 and LAGE-1a are intracellular tumor associated antigens (TAA), that generate a shared SLLMWITQC peptide bound to HLA-A*02 that is expressed across multiple malignancies, including non–small-cell lung cancer (NSCLC).
- A separate study using engineered T cells targeting NY-ESO-1 have shown a partial response in a patient with advanced lung adenocarcinoma.[1] Decitabine (DAC) is a hypomethylating agent and potent inducer of TAA, including NY-ESO-1.[2]
- We have reported in vitro use of DAC to selectively modulate TAA expression in TAA low-expressing tumor cell lines in order to enhance lete-cel therapy.[3]
- GSK is currently running a Phase Ib/IIa, multi-arm, open-label pilot study (NCT03709706) of lete-cel as a monotherapy or in combination with pembrolizumab in HLA-A*02 positive patients whose tumors express NY-ESO-1 and/or LAGE-1a (**Figure 1**).
- Treatment of solid tumors remains challenging. Possibly due to the immunosuppressive tumor micro-environment (TME), heterogeneity of antigen expression and unfavorable metabolic conditions.

Figure 1. Study design of lete-cel in NSCLC[4]



ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; EoT, end of treatment; HLA, human leukocyte antigen; LAGE-1a, L antigen family member 1 A; lete-cel, letetresgene autoleucel; LTFU, long-term follow-up; NSCLC, non-small cell lung cancer; NY-ESO-1, New York esophageal squamous cell carcinoma 1; PD-1, programmed cell death receptor 1; PD-L1, programmed cell death ligand; ROS, ROS proto-oncogene 1.

Aims

- The aim of this study was to assess enhancement of combination therapy with lete-cel and DAC in an in vivo NSCLC model.
- Use a clinically relevant mouse model that mimics developed NSCLC.
- Evaluate the impact of combination therapy with lete-cel and DAC on tumor burden, NY-ESO-1 and LAGE-1a antigen expression on tumor cells, and activation of lete-cel by interferon gamma (IFN-γ) production.

Methods

- NOD scid gamma (NSG) mice (n=9/group) were injected subcutaneously with the human NSCLC tumor cell line 5×10^6 NCI-H1703. Upon engraftment (confirmed by size measurement), tumor-bearing mice were treated with 3 mg/kg DAC or phosphate buffered saline (PBS) control intraperitoneally every other day for a total of 3 doses (total dose 9 mg/kg).
- 72-hour post final DAC dose unmodified (NTD) or lete-cel T cells were infused intravenously at a total dose of 5x10^6 cells.
- RNA was isolated from tumor FFPE blocks and levels of NY-ESO-1 and LAGE-1a transcript were measured by RT-qPCR.
- Expression pattern of the NY-ESO-1 protein was assessed via Immunohistochemistry (IHC).
- Whole blood sampling was performed at study Day 9 (baseline), 26 (Day 7 post T-cell infusion) and 40 (Day 21 post T-cell infusion).
- Efficacy was defined by changes in tumor volume and systemic IFN-γ secretion using serum sample.
- Cell lines were supplied by Drs. Gazdar and Minna of the National Cancer Institute, under agreement with the NIH.

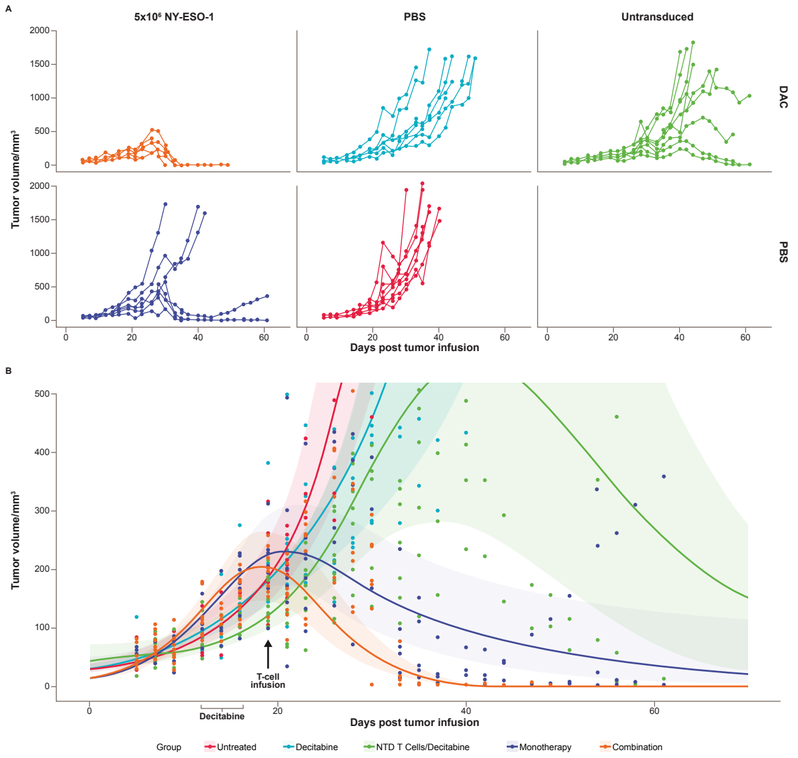
Results

- Lete-cel in combination with DAC significantly enhanced anti-tumor efficacy in a NSCLC model in vivo, compared with lete-cel monotherapy (**Figure 2**).
- Mice that received DAC treatment only did not show statistically significant tumor reduction compared to lete-cel monotherapy treated mice (**Figure 2**).

Figure 2: In vivo efficacy of NY-ESO-1 TCR (lete-cel) in combination with decitabine to target H1703 xenografts

Expression of the target antigens NY-ESO-1 and LAGE-1a can be enhanced independently of one another in antigen-low tumor cell lines following DAC treatment to combined levels to induce specific responses by lete-cel. Highlighting the additive effect of NY-ESO-1 and LAGE-1a in their contribution to the triggering of a T-cell response.

(A) Individual curves (B) Mean tumor volumes. At 12 days post T-cell infusion, significant differences in tumor volume were noted between the combination and other treatment groups with no significant difference between decitabine and untreated.



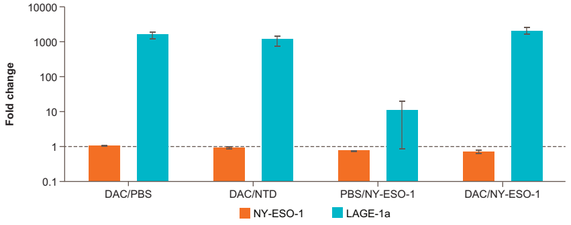
DAC, decitabine; LAGE-1a, L antigen family member 1 A; NY-ESO-1, New York esophageal squamous cell carcinoma 1.

- Consistent with our previous in vitro studies, DAC treatment in vivo resulted in induction of LAGE-1a (RT-qPCR) in the NSCLC (NCI-H1703) tumor model. Endogenous expression of NY-ESO-1 levels as assessed by RT-qPCR, was high in the NSCLC (NCI-H1703) and no further increase was observed following DAC treatment (**Figure 3**).
- Anti-tumor response in lete-cel monotherapy and combination groups was confirmed in a clinically relevant model, mimicking developed NSCLC.
- Anti-tumor efficacy was associated with increased IFN-γ secretion at early timepoints (Day 7 post T-cell infusion; **Figure 4**). Results at the later timepoints Day 21 post T-cell infusion were confounded by onset of graft versus host disease.

Figure 3: Antigen upregulation profile observed during combination efficacy study

(A) Fold change of antigen upregulation of treatment group over untreated control group (B) dCq values of treated and untreated control groups. Effects seen in PBS/PBS=9, DAC/PBS=9, DAC/NTD=7, PBS/NY-ESO-1=4, DAC/NY-ESO-1=2. The horizontal dotted line indicates a fold change of 1 (ie, no difference).

A



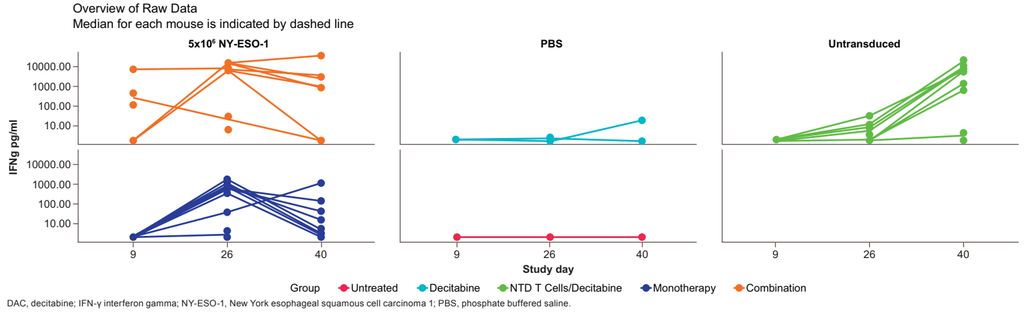
B

	Mean NY-ESO-1 (dCq)	Mean LAGE-1a (dCq)	Mean Total Target (dCq)
PBS/PBS	–1.07	15.70	–1.07
DAC/PBS	–1.14	5.25	–1.16
DAC/NTD	–0.97	6.19	–0.98
PBS/NY-ESO-1	–0.66	14.52	–0.66
DAC/NY-ESO-1	–0.64	4.71	–0.68

DAC, decitabine; LAGE-1a, L antigen family member 1 A; NY-ESO-1, New York esophageal squamous cell carcinoma 1; PBS, phosphate buffered saline.

Figure 4. Production of IFN-γ in vivo at baseline, 7 days, and 21 days post T-cell infusion

IFN-γ was shown to increase from baseline in the monotherapy and combination therapy groups. The change in cytokine production from baseline in combination therapy groups is nominally (but not significantly) greater than in monotherapy groups - 8.97x (p=0.169) at Day 7 post infusion.



DAC, decitabine; IFN-γ interferon gamma; NY-ESO-1, New York esophageal squamous cell carcinoma 1; PBS, phosphate buffered saline.

Conclusions

- **Pre-treatment of NCI-H1703 with DAC resulted in increase in expression of LAGE-1a in vivo. This was associated with a reduction in tumor burden and a nominal increase in IFN-γ in lete-cel in combination with DAC compared with treatment with lete-cel alone.**
- **GSK is currently enrolling a Phase Ib/IIa, multi-arm, open-label pilot study (NCT03709706) of lete-cel as a monotherapy or in combination with pembrolizumab in HLA-A*02 positive patients whose tumors express NY-ESO-1 and LAGE-1a. This work may support rationale for the use of DAC in combination with lete-cel to improve the efficacy of NY-ESO-1–specific T cells, by increasing levels of target antigens and anti-tumor effect in NSCLC.**

References

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Disclosures

The presenting author, **Dmitry Pankov**, declares the following real or perceived conflicts of interest during the last 1 year and 10 months in relation to this presentation: an employee and stock/shareholder in GSK. **IE**, **AD**, **SJB**, **LP**, **MAK**, **GT**, **JE**, **JK**, **KS**, **MD**, **MDWH**, **MG**, **AS** are employees of and stock/shareholders in GSK. **CMB** is a stock/shareholder and was an employee of GSK.

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