Tracking and profiling of NY-ESO-1 TCR-transgenic T cells upon adoptive transfer in patients with NY-ESO-1-expressing solid tumors: association between *in vivo* differentiation and response/toxicity

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Background

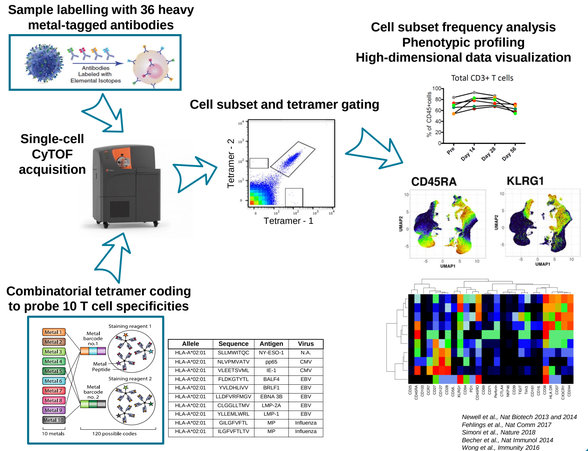
NY-ESO-1 is highly expressed in the majority of synovial sarcomas as well as other solid tumors and may be an effective target for T cell-based therapies. We conducted a clinical study of adoptive transfer of lymphocytes transduced with NY-ESO-1-specific TCR in refractory cancer patients with preconditioning (TBI-1301). High-dose of 5x10^9 autologous transduced and expanded lymphocytes, consisting of >96% T cells, was transferred into 6 patients, three of whom with synovial sarcoma. Three out of 6 patients experienced an objective clinical response (PR) and had cytokine-release syndrome (CRS) with high-levels of IL-6 and MCP-1 that could be managed with tocilizumab. Longitudinal PBMC samples were obtained for immunomonitoring. We used high-dimensional mass cytometry and combined a 36-antibody panel with a multiplexed combinatorial peptide-MHC tetramer staining approach to longitudinally track and phenotypically characterize adoptively transferred HLA-A*02:01 NY-ESO-1 transgenic TCR T cells 14, 28, and 56 days after treatment.

Summary of patient characteristics (Ishihara M, et al., ASCO 2019):

Cohort (cell dose)	Pt ID	Age	Sex	Disease	Antigen expression (positivities in tumor tissue)	Related adverse events (except hematological toxicities)	Best overall response
Cohort 2 Cy & 5x10^9 cells	TBI1301-07	46	M	synovial sarcoma	>75%	Fever	PR
	TBI1301-09	61	M	melanoma	>75%	CRS, fever, diarrhea, edema, ALT elevation	SD
	TBI1301-08	70	M	synovial sarcoma	>75%	CRS, flush, proteinuria, purpura, platelet decrease, interstitial lung injury(GI), cancer pain, fibrinogen decrease	PR
	TBI1301-14	65	F	ovarian ca	25-50%	fatigue, K elevation, uriac acid elevation, ferritin elevation, creatinine elevation	non-CR/ non-PD*
	TBI1301-16	25	M	synovial sarcoma	>75%	CRS, appetite loss, diarrhea	PR
	TBI1301-15	45	F	liposarcoma (myxoid cell)	50~75%	fever, hypoalbuminemia	SD

G, grading score by CTC-AE CRS, Cytokine release syndrome *cases without measurable lesions

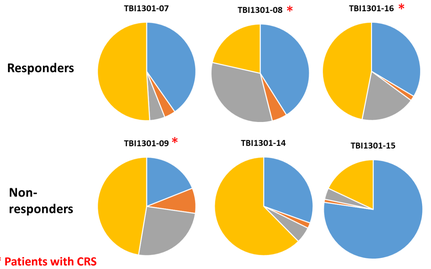
Methods: Simultaneous identification and profiling of antigen-specific T cells (targetSCAPE™)



Allele	Sequence	Antigen	Virus
HLA-A*02:01	SLLMWITQC	NY-ESO-1	N.A.
HLA-A*02:01	NLVPMVATV	pp65	CMV
HLA-A*02:01	VLEETSVML	IE-1	CMV
HLA-A*02:01	FLDKGTVTL	BALF4	EBV
HLA-A*02:01	YVLDHLIVV	BRLF1	EBV
HLA-A*02:01	LLDFVRFMGV	EBNA 3B	EBV
HLA-A*02:01	CLGGLLTMV	LMP-2A	EBV
HLA-A*02:01	YLLEMLWRL	LMP-1	EBV
HLA-A*02:01	GILGFVFTL	MP	Influenza
HLA-A*02:01	ILGFVFTLTV	MP	Influenza

Newell et al., Nat Biotech 2013 and 2014
Fehlings et al., Nat Comm 2017
Simoni et al., Nature 2018
Becher et al., Nat Immunol 2014
Wong et al., Immunity 2016

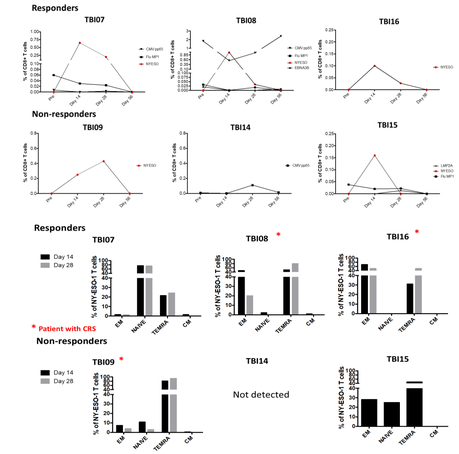
(1) Characterization of manufactured product (CD8+ T cells)



- Naïve T cell (CD3+/CD45RA+/CCR7+)
- Central Memory T cell (CD3+/CD45RA-/CCR7+)
- Effector Memory T cell (CD3+/CD45RA-/CCR7-)
- Terminal differentiated T cell (CD3+/CD45RA+/CCR7-)

Characterization of the infusion products by flow cytometry. The infusion products contained >99% CD3+ T cells, 54 to 80% of which were CD8+ T cells with variable percentages of naive, TEMRA and EM CD8+ cells; 53 to 70% of CD8+ T cells were NY-ESO-1 tetramer positive. The three patients who developed CRS had significantly higher proportion of EM T cells compared to patients who did not develop CRS (p=0.008).

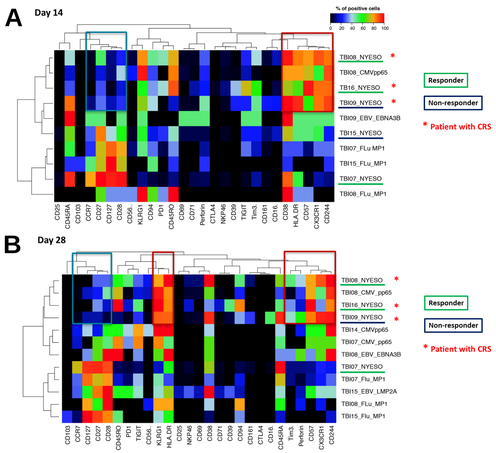
(2) Longitudinal tracking and differentiation of NY-ESO-1 -specific CD8+ T cells in patients



(A) NY-ESO-1 TCR transgenic T cells were detected at in the circulation of all responders and in 2 out of 3 non-responders. Frequencies peaked at day 14 and 28 and were undetectable in all patients by day 56. In addition, T cells specific for CMV, Flu, and EBV were detected across the total dataset.

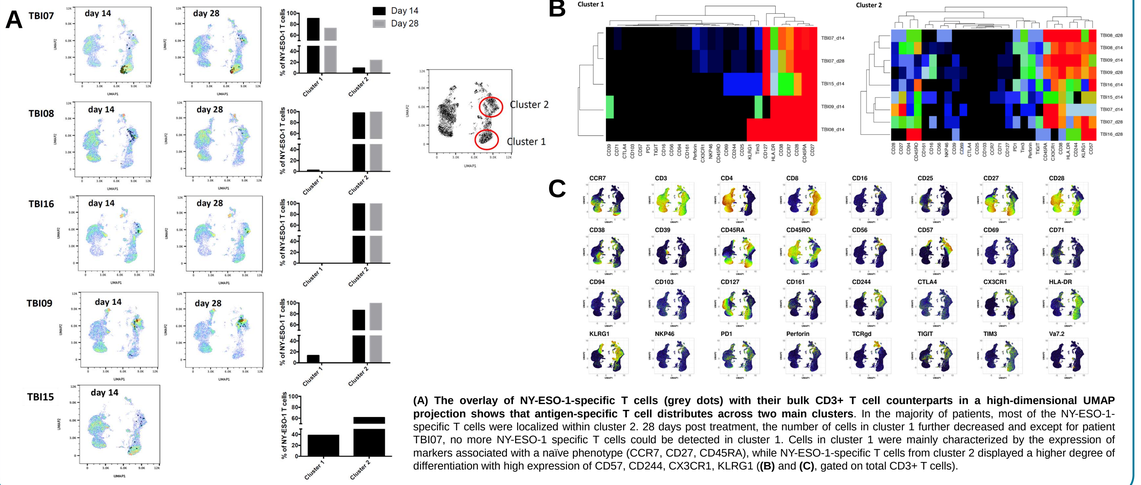
(B) Differentiation status of NY-ESO-1 specific T cells differed across patients. In 2 responders the majority of NY-ESO-1-specific T cells was differentiated into effector memory (EM, CCR7-, CD45RA-) and terminal effector memory cells (TEMRA, CCR7- CD45RA+). A trend towards increased differentiation of NY-ESO-1-specific T cells was measured in patients over time. Interestingly, NY-ESO-1-specific T cells were mostly differentiated in patients with CRS.

(3) Phenotypic characterization of NY-ESO-1 and virus-specific T cells



The phenotypes of NY-ESO-1 specific T cells from all patients were compared at day 14 (A) and day 28 (B) time points and benchmarked with the virus-specific T cells identified across the total data set. At both time points, NY-ESO-1-specific T cells from the 2 responder patients who also presented with CRS clustered together with CMV (pp65)-specific cells. These cells were characterized by an activated late-differentiated effector phenotype (CD38+ CD57+ CD45RO+, CD244+, KLRG1+, HLA-DR+). Interestingly, 28 days post-treatment NY-ESO-1-specific T cells from the non-responder patient who developed CRS also increased CD57 and CX3CR1 and clustered together with the CMV (pp65)-specific T cells. As observed above (differentiation status), NY-ESO-1-specific T cells from patient TBI07 showed high expression levels of markers associated with a naïve phenotype and clustered together with flu-specific T cells.

(4) Distribution of NY-ESO-1-specific T cells in high-dimensional space



(A) The overlay of NY-ESO-1-specific T cells (grey dots) with their bulk CD3+ T cell counterparts in a high-dimensional UMAP projection shows that antigen-specific T cell distributes across two main clusters. In the majority of patients, most of the NY-ESO-1-specific T cells were localized within cluster 2. 28 days post treatment, the number of cells in cluster 1 further decreased and except for patient TBI07, no more NY-ESO-1 specific T cells could be detected in cluster 1. Cells in cluster 1 were mainly characterized by the expression of markers associated with a naïve phenotype (CCR7, CD27, CD45RA), while NY-ESO-1-specific T cells from cluster 2 displayed a higher degree of differentiation with high expression of CD57, CD244, CX3CR1, KLRG1 (**(B)** and **(C)**, gated on total CD3+ T cells).

Summary

- **Objectives** This pilot study aimed to assess feasibility of longitudinally tracking and profiling adoptively transferred T cells in 6 cancer patients who underwent adoptive transfer of high-dose autologous T cells transduced to express HLA-A*02:01 NY-ESO-1-specific transgenic TCR.
- **Results** The infusion products had variable percentages of naive, TEMRA and EM CD8+ T cells, with the three patients developing CRS having the highest proportion of EM T cells. NY-ESO-1 TCR transgenic T cells could be detected in the circulation in 5 out of 6 treated patients, with frequencies peaking at day 14 and day 28; specific T cells were undetectable in all patients by day 56. The differentiation status of circulating NY-ESO-1-specific CD8+ T cells varied across patients and due to the small cohort size, a robust association between T cell properties and clinical response could not be drawn; however, a general tendency for increased differentiation during time was measured in all patients. In addition, in the three patients presenting with CRS, close to 100% of circulating NY-ESO-1-specific CD8+ T cells developed an activated late-differentiated phenotype (CD38+ CD57+ CD45RO+, CD244+, KLRG1+, HLA-DR+), similar to that of CMV-specific T cells and consistent with antigen experience, in vivo activation and effector function.
- **Conclusions** Adoptive transfer of NY-ESO-1 TCR-transgenic T cells has shown signs of efficacy in patients with high NY-ESO-1 tumor expression, with manageable adverse events. Our study shows feasibility of tracking evolution of phenotypic profiles of adoptively transferred tumor-antigen-specific T cells in patients and derive association between adoptive T cell status and clinical read-outs, and should be extended to a larger patient cohort.

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