Comprehensive immune profiling of clinical samples from subjects with advanced recurrent epithelial ovarian cancer treated with a novel T cell activating therapy, DPX-Survivac

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ABSTRACT# P586

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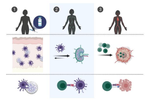
DPX-Survivac is a novel lipid-based formulation designed to elicit *de novo* cytotoxic T cell response to survivin-expressing tumors. DeCidE[1] trial is assessing the clinical utility of DPX-Survivac in combination with low dose intermittent cyclophosphamide and with or without epacadostat in advanced ovarian cancer patients. To understand the underlying mechanism of action of this immunotherapy, we performed immune-profiling of PBMC and tumor samples from this study.

Treatment with DPX-Survivac induced systemic survivin-specific T cell response in nearly all evaluable subjects as assessed by ELISPOT. On-treatment enrichment in systemic survivin-specific T cells was also detected after *in vitro* expansion and tetramer analysis, confirming activated T cells are functional and proliferative. Immunophenotyping of PBMC did not show substantial increases in the expression of immunosuppressive markers (CTLA-4, PD-1, Tim-3) within T cell population in subjects that responded to the treatment, further suggesting that induced T cells remained active over time. RNA-profiling of immune cell within the TME revealed on-treatment overexpression of genes related to T cell activation and cytolysis as well as enrichment of B cell and NK cell specific signatures, suggesting the strong potential of treatment to induce infiltration and activation of cytotoxic cells. Infiltration of T cells into tumor post treatment did not correlate with highest levels of systemic survivin-specific T cells, suggesting migration of circulating activated T cells into tumors. Analysis of TCRβ repertoire in pre- and on-treatment tumors demonstrated that DPX-Survivac therapy can promote proliferation and tumor-infiltration of new T cell clones.

In conclusion, DPX-Survivac combinational therapy induces robust and sustained survivin-specific responses and promotes T cell infiltration of tumors, without a loss in functionality. The infiltration immune cells beyond T cells has been demonstrated in tumor tissue yet was not consistently detected in PBMCs; a finding that emphasizes the need for immune-profiling of both blood and tumor to gain a full understanding of the mechanism of action of novel immunotherapies and combinations.

Materials and methods



Mechanism of action of DPX-Survivac (1) Injected DPX is actively engulfed by antigen-presenting cells (APCs). (2) APCs traffic to lymph nodes to activate survivin-specific T cells. (3) Survivin-specific T cells traffic to target survivin-expressing tumor cells

Subjects with advanced ovarian cancer (stage IIc-IV with evidence of disease progression) were treated with DPX-Survivac (2 priming doses q3w and up to 6 boosting doses), CPA (50 mg BID), and epacadostat (up to 300 mg BID).

Pre-treatment and longitudinally collected on-treatment PBMCs were used to assess survivin-specific T cell response by *ex vivo* IFN-γ ELISPOT and *in vitro* MHC multimer staining. Immune-phenotyping was performed on a subset of PBMC samples collected from eight subjects prior to treatment at study day 0 (D0) and at different time points through the treatment. Multi-parametric flow cytometry analysis was performed using FACS Celesta and FlowJo software. Among eight subjects 2 achieved PR, 2 achieved SD and 4 with PD. Representative results from 3 subjects with different clinical outcomes are shown (these are the same 3 subjects as Figures 1-5).

Pre- and on-treatment (D56-70, after priming phase) tumor biopsies were subjected to whole transcriptome RNA sequencing. Sequencing and data normalization were performed by Q^2 Solutions. In total 58 samples from 29 subjects were analysed. Representative results from three subjects are shown.

TCR repertoires were assessed in pre- and on-treatment (D56-70, after priming phase) tumor biopsies and PBMC samples from nine subjects by sequencing of the TCRβ loci using the ImmunoSEQ survey level assay by Adaptive Biotechnologies (Seattle, WA). Subject 1 is the same as Figures 1-5, subjects 4-11 are different, subjects 2 and 3 were not included in this analysis.

Further information

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De novo activation of durable and functional T cells in response to DPX-Survivac immunotherapy

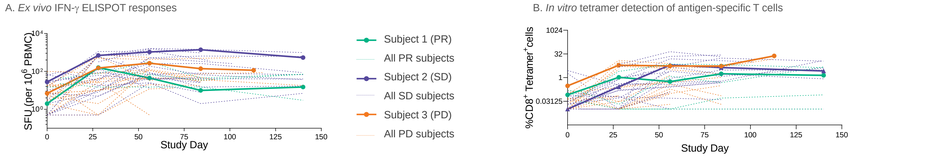


Figure 1. Increases in antigen-specific T cells were detected in PBMCs of 31/40 (77.5%) of all evaluable subjects (based on currently evaluable subjects) by *ex vivo* IFN-γ ELISPOT and *in vitro* tetramer analysis. (A) Patient PBMCs were stimulated overnight with survivin peptides in an IFN-γ ELISPOT assay. **(B)** Antigen-specific immune responses detected by tetramer staining after 10-day *in vitro* expansion. **DPX-Survivac induces systemic survivin-specific T cell responses in the majority of subjects. Survivin-specific T cells retain proliferation potential and are capable of *in vitro* expansion.**

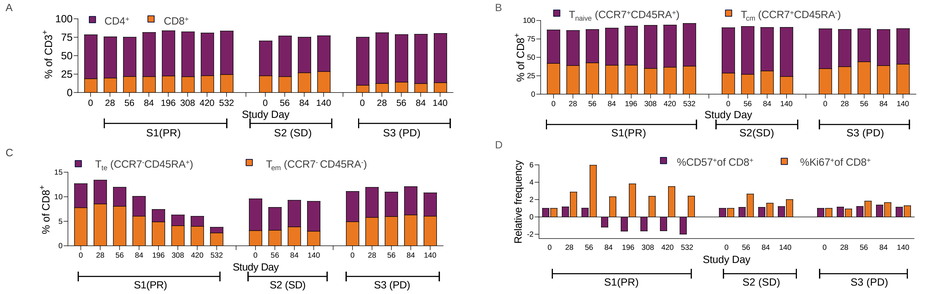


Figure 2. Phenotypic and functional characterization of T cell population in peripheral blood samples from three representative subjects. (A) Frequencies of CD4$^+$ and CD8$^+$ T cells within CD3$^+$ T cell population **(B)** Frequency of naïve CD8$^+$ T cells (T_{naive}) and central memory T cells (T_{cm}) within CD8$^+$T cell population **(C)** Frequency of effector memory (T_{em}) CD8$^+$T cells and terminal effector CD8$^+$ T cells (T_{te}). (D) Relative frequency of proliferative Ki67$^+$ and highly cytotoxic "senescent" CD57$^+$ T cells within CD8$^+$ T cell population; data are expressed as fold of change in frequency relative to the cell frequency at D0 which is arbitrarily set at 1. **Ratios of CD8/CD4, T_{naive}, T_{cm} cells and proliferation capacity of circulating T cells sustain through the treatment, however frequencies of T_{em} and T_{te} and, consistently, frequency of terminally differentiated cytotoxic CD8$^+$ T cells are reduced during treatment in PBMCs of a subject with partial response, suggesting that these cells may be recruited to tumor sites**.

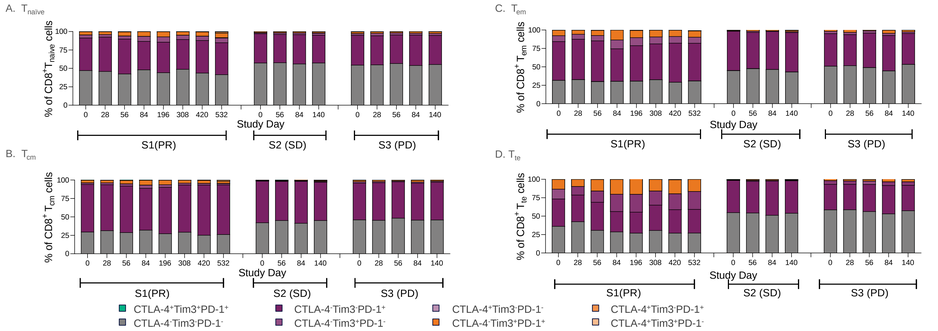


Figure 3. Expression of inhibitory checkpoint molecules (Tim-3, CTLA-4, and PD-1) on different subsets of CD8$^+$ T cells. Ratios of CD8$^+$T cells that express one, two or three checkpoint molecules or display triple negative phenotype are shown for **(A),** naïve, **(B)** central memory, **(C)** effector memory and **(D)** terminal effectors CD8$^+$ T cells. **Expression of checkpoint molecules was not enhanced in response to DPX-Survivac, suggesting that all tested subsets of T cells remained functional and active over time.**

Increase in immune infiltration in tumors correlate with clinical responses

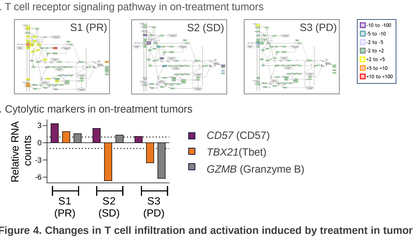
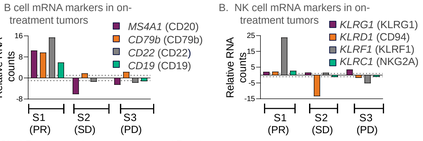
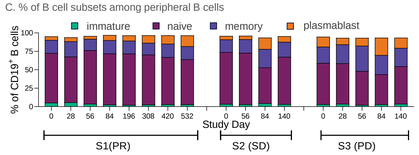


Figure 4. Changes in T cell infiltration and activation induced by treatment in tumors of 3 representative subjects. Analysis is performed at the RNA level using whole transcriptome sequencing of tumor biopsy samples. Fold-changes in the expression of each transcript are calculated relative to baseline levels **(A)** Activation of T cell receptor signaling pathway. Fold-changes in the expression of each transcript and are color-coded. **(B)** Changes in the expression of cytolytic markers. **PR-** Partial Response; **SD-** Stable Disease; **PD-** Progressive Disease. **On-treatment infiltration of T cells into tumors correlates with clinical responses.**





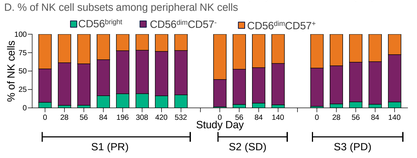


Figure 5. Analysis of B and NK cells in peripheral blood and tumor samples from three representative subjects. **(A and B)** On-treatment changes in **(A)** B and **(B)** NK signatures in tumors**.** Fold-changes in the expression of each transcript are calculated relative to baseline levels that are shown by dotted lines. **(C and D)** Characterization of peripheral B and NK cell populations by flow cytometry. Cell subsets shown in order of their maturation status. **(C)** Frequency of immature (CD10$^+$ CD27$^-$CD38^{++}), naive (CD10$^-$CD27$^-$ CD38$^{(+)}$), memory (CD10$^-$ CD27$^+$ CD38$^{(+)}$) and plasmablast (CD10$^-$CD27$^+$ CD38^{++}) subsets within B cell population (CD19$^+$). **(D)** Frequency of CD56^{bright} (CD56^{dim} precursors and main cytokine producers), CD56^{dim}CD57$^-$ (proliferative cells) and CD56^{dim}CD57$^+$ (terminally differentiated highly cytotoxic cells) within NK cell population (CD3$^-$CD56$^+$). **Strong on-treatment increase in B cells and some enrichment of NK cells in the on-treatment tumors were observed mainly in subjects demonstrating clinical response. In contrast, changes in NK and B maturation profiles detected in blood were not associated with clinical response to treatment.**

DPX-Survivac based immunotherapy impacts the T cell repertoire of treated tumors

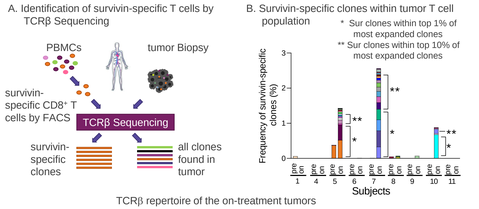


Figure 6. Analysis of TCRβ repertoire in pre- and on-treatment tumors and identification of survivin-specific T cells by TCRβ sequencing. (A) Diagrammatic representation of the detection of survivin-specific TCRβ sequences. **(B)** % of the survivin-specific T cells in the pre- and on-treatment tumors of nine subjects. Different colors represent different clonotypes. * clones found within 1% and ** clones found within 10% of most expanded clones in the tumor T cell population. **(C, D)** Analysis of the TCRβ repertoire in the on-treatment tumors of the same nine subjects. Results presented as **(C)** % of unique clonotypes found in the on-treatment tumor, but not in the pre-treatment tumor vs. % of clonotypes shared between both timepoints and **(D) Σ** frequency of all clones detected in the on-treatment tumor, but not in the pre-treatment tumor vs. **Σ** frequency of shared clones.

- **On treatment TCRβ repertoire contains 51% to 96% new clonotypes, and these novel clonotypes cumulatively comprise 29% to 90% of intratumoral T cell population.**
- **Survivin-specific clonotypes can be detected in patient tumors and are strongly expanded on-treatment**
- **Limitations of this analysis preclude correlation with clinical responses; these include that quality of biopsy samples are highly variable, identification of the survivin-specific clones are determined from limited PBMC samples, biopsies are collected at study day 56, prior the clinical responses in some patients**

CONCLUSIONS

- DPX-Survivac based immunotherapy elicits robust systemic immune responses in ovarian cancer patients.
- De novo and unique mechanism of T cell activation provided by DPX platform allows T cells to avoid replicative senescence and exhaustion and to remain active and proliferative over time.
- Induction of cytotoxic T cell infiltration of tumors may promote CTL- mediated tumor rejection, correlating with objective clinical responses to combination therapy.
- Repopulation of TME with new T cell clonotypes and the enhancement of clonal expansion and diversity of survivin-specific T cells is observed with DPX-Survivac combination therapy, indicative of its impact on the tumor T cell repertoire
- Comprehensive analysis of both blood and tumor tissues is essential to maximize our understanding of the mechanism of action of novel immunotherapies