Local administration of large surface area microparticle docetaxel is associated with anti-tumor immunomodulation across multiple tumor types

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INTRODUCTION

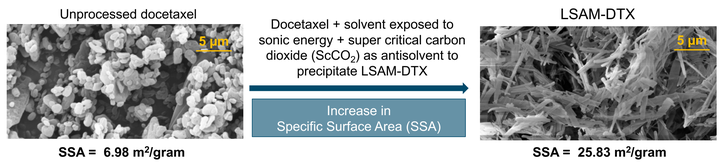
Large surface area microparticle docetaxel (LSAM-DTX) was developed for local administration to the tumor site where it acts as a depot continuously releasing drug without significant systemic toxicity or immunosuppression [1]. LSAM-DTX has been found within tumors for up to 50-days [2].

To characterize immunomodulation after local administration of LSAM-DTX, immunophenotyping was performed in 3 tumor settings:

- Before/after direct injection of LSAM-DTX at the site of transurethral resection of non-muscle invasive bladder cancer (NMIBC) + instillation in clinical trial subjects [3].
- After intratumoral (IT) LSAM-DTX in subcutaneous implanted syngeneic murine renal tumors (Renca).
- After IT LSAM-DTX ± intraperitoneal (IP) anti-CTLA-4 in syngeneic orthotopic murine metastatic luciferase-enabled breast tumors (4T-1-luc) [4].

METHODS & MATERIALS

Large Surface Area Microparticle Docetaxel (LSAM-DTX) Production Technology



Tumor Types, Treatments, and Immune Evaluations

	Non-muscle invasive bladder cancer (NCT03636256) [3]		Renal adenocarcinoma (Renca ATCC CRL-2947)	Mammary carcinoma (4T1-Luc2-1A4 luciferase-enabled) [4]
Species (N [Sex])	Human (5 [4 male/ 1 female])		Mouse (BALB/c) (20 [female]/per treatment group)	Mouse (BALB/c) (10 [female])
Tumor Site	Bladder wall		Subcutaneous implant in right flank	Subcutaneous implant in mammary fat pad
LSAM-DTX Dose	3.0-15.0mg [Direct injection[1]]	500-750mg [Intravesical instillation[2]]	0.55mg[3] [IT]	1.0mg[4] [IT]
LSAM-DTX Schedule	Once following TURBT	Post-TURBT and ≥ 4 wks later for ≤ 6 wkly cycles followed by 6 wks off and ≤ 3 wkly cycles	Qwkx3	q4dx4
Comparator	Pre-treatment biopsy		IV docetaxel [5 mg/kg; qwk x 3]	IT vehicle [q4dx4]
Tissues Analyzed (assay)	Tumor resection bed tissues (multiplex immunofluorescence)		Blood, spleen, tumor-site tissues (flow cytometry)	Blood, tumor-site tissues (flow cytometry)
Immunophenotyping Timepoints	Pre-dose and Week 18.1 (median; range = 16.4-23.1 weeks)		Pre-dose, Day 4, 11, 18, 21	Day 34
No. Immune Cell Types Analyzed	17		16	21

TURBT = transurethral resection of bladder tumor; IT = intratumoral injection; IV = intravenous

[1] Total amount of LSAM-DTX administered in ≤ 8 injections into a single tumor resection bed
[2] Total amount of LSAM-DTX administered in ≤ 9 cycles
[3] ~29mg/kg based on 0.55mg administered to ~19g animal
[4] ~52mg/kg based on 1.0mg administered to ~19g animal

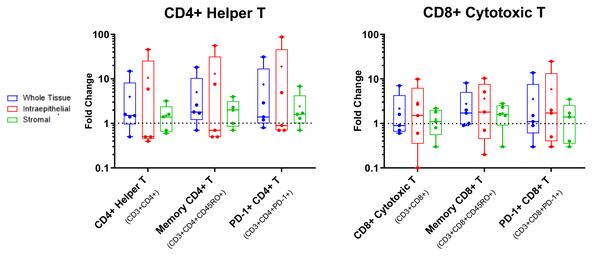
RESULTS

Non-muscle Invasive Bladder Cancer

Multiplex immunofluorescence from 5 NMIBC clinical trial subjects (NCT# 03636256) in bladder tumors pre/post LSAM-DTX therapy [3].

CD4+ and CD8+ T Cells increased in TME in subset of subjects

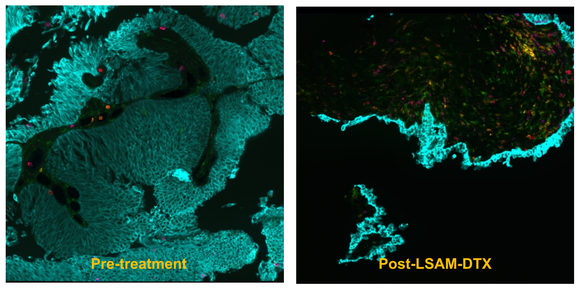
Fold-change in T Cells in TME following LSAM-DTX therapy							
	RFS (months)	CD4+ Helper T (CD3+CD4+)	Memory CD4+ T (CD3+CD4+CD45RO+)	PD-1+ CD4+ T (CD3+CD4+PD-1+)	CD8+ Cytotoxic T (CD3+CD8+)	Memory CD8+ T (CD3+CD8+CD45RO+)	PD-1+ CD8+ T (CD3+CD8+PD-1+)
Subject 3	3.8	0.5	0.7	1.2	0.6	1	0.9
Subject 2	5.3	1.5	1.8	1.4	1.6	2	1.5
Subject 1	7.0	1.6	2.6	0.8	0.9	1.7	0.3
Subject 4	11.7	14.9	18.3	31.2	7	8.1	13.8
Subject 5	13.0	1.4	1.7	2.9	0.7	0.9	1.1



Fold-change in density (cells/mm²) of CD4+ and CD8+ T cells in the TME per spatial distribution. Density is determined for the entire sample (whole tissue; blue plots), for the intraepithelial region defined by cells in contact with PanCK+ cells (intraepithelial, red plots) or for immune cells found outside of direct contact with PanCK+ cells (stromal; green plots) and only plotted if both pre-injection and resection cell densities were > 0 mm². Line in bar = median, + = mean.

Immune cell distribution in TME pre/post-LSAM-DTX in subject with complete response

Blue = PanCK+ (bladder wall and tumor); Yellow = CD3+CD4+ Helper T Cells; Magenta = CD3+CD8+ Cytotoxic T Cells



Subject 4 had no immunotherapy or TURBTs before initiation of LSAM-DTX. A representative region of interest (ROI) from a TURBT on Day 1 (left panel) found significant tumor and low infiltration of CD4+ and CD8+ T cells compared to biopsy collected 5.8 months after LSAM-DTX treatment initiation (right panel). For all ROIs evaluated, the increases in CD4+ and CD8+ density (cells/mm²) from pre- to post-LSAM-DTX, were 14.9-fold and 7-fold, respectively. Representative images (magnification = 0.20X) were selected based on similar number of cells in ROI (~7,200 for these cases).

NK cells increased in TME in subset of subjects

Whole tissue analysis found increased NK cells in TME following local LSAM-DTX in NMIBC subjects with longer recurrence free survival.

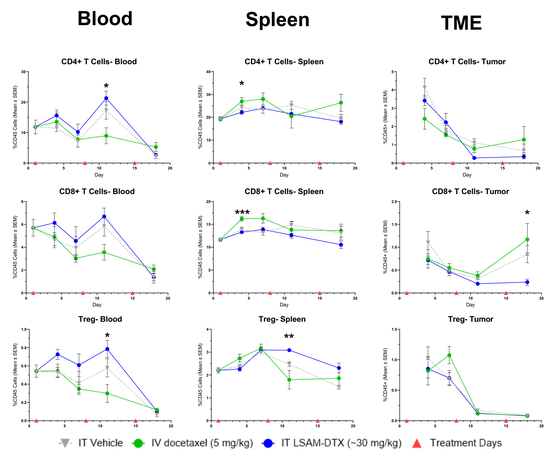
	RFS (months)	NK Cells (CD3-CD56+)
Subject 1	7.0	0.4
Subject 3	3.8	0.5
Subject 2	5.3	1.3
Subject 5	13.0	5.9
Subject 4	11.7	100.9

Renal Cancer

In the Renca tumor model [5], flow cytometry was used to evaluate immunomodulation in blood, tumor, and spleen at early timepoints. Animals (N=5/group) were treated with IT LSAM-DTX, IT vehicle, or IV docetaxel (IV doc) on Days 1, 8, & 15 and sampled for flow cytometry on Days 1, 4, 7, 11, & 18.

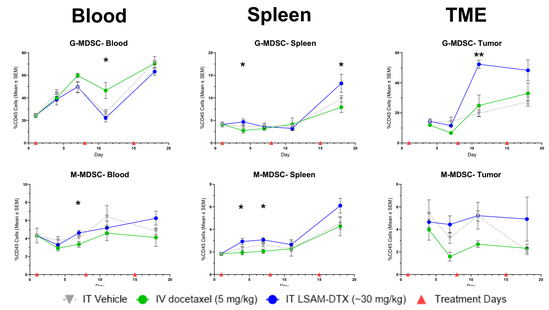
T Cells

- Circulating CD4+ and CD8+ T cells increased in IT LSAM-DTX vs. IV doc following 2 weekly treatment cycles.
- CD4+ & CD8+ T cells in the TME slightly decreased in LSAM-DTX vs. IV doc following 1 cycle and recovered to similar levels at later timepoints.
- Splenic CD4+ and CD8+ cells were similar through 2 treatment cycles.



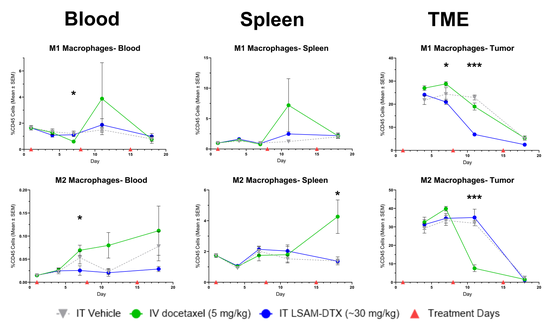
MDSC

- Reduction in peripheral G-MDSC following 2 cycles of IT LSAM-DTX vs. IV doc.
- Increased MDSC in TME during early treatment with IT LSAM-DTX vs. IV doc.



Macrophages

- Reduced circulating and splenic M2 macrophages in LSAM-DTX vs. IV doc.
- Reduced M1 and increased M2-macrophages in TME of IT LSAM-DTX vs. IV doc.



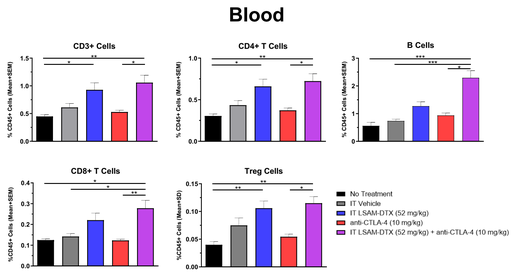
** = p < 0.05; ** = p < 0.01, *** = p < 0.001. N= 5 animals/group; statistical comparison of IT-LSAM DTX vs. IV docetaxel was made at each timepoint using ANOVA with a Tukey post-hoc test.*

Metastatic Breast Cancer

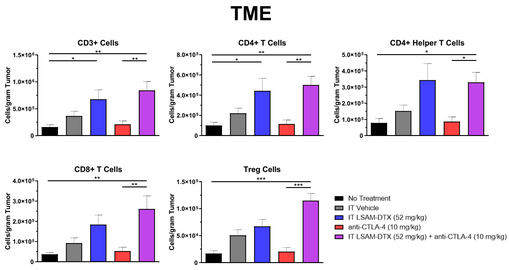
In the 4T-1-luc orthotopic model [4], flow cytometry was used to evaluate immune cells in bloods and tumors from mice administered IT LSAM-DTX (Days 10, 14, 18 & 22) and IP anti-CTLA-4 (Days 10, 13, 17, & 22). Animals (N=10/group) were sampled 34 days post-tumor implant.

T Cells

- Increased circulating CD3+, CD4+ T, CD8+ T , T reg and B cells.

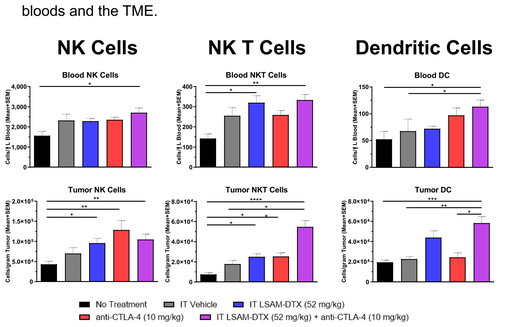


- In the TME, absolute counts of all T cells increased following addition of LSAM-DTX to anti-CTLA-4.



Natural Killer and Dendritic Cells

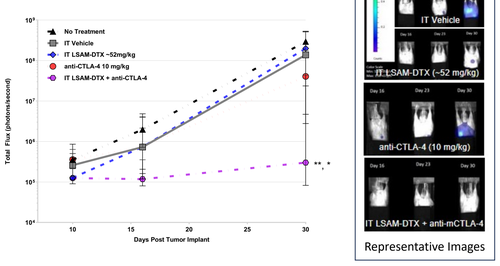
- Compared to untreated animals, NK, NK T and DC were increased in both bloods and the TME.



*Flow cytometry data analyzed with outliers removed using one-way ANOVA (Kruskal-Wallis). Significance reported as *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

Thoracic Metastatic Burden

- LSAM-DTX+anti-CTLA-4 was associated with significantly reduced thoracic metastasis; 4/10 animals in the combination group had no evidence of metastatic burden on Day 30.



*Median bioluminescence (photons/second)±IQR at days 10, 16, & 30. n = 10 mice/group. Significance determined using one-way ANOVA (Kruskal-Wallis) vs. no treatment controls, LSAM-DTX monotherapy; *p < 0.05, **p < 0.01.*

CONCLUSIONS

- Immunophenotyping in 3 diverse tumor settings found commonalities in antitumor immunomodulation following local LSAM-DTX including changes in T cells and MDSC.
- NMIBC subjects demonstrated infiltrations of CD4+T, CD8+ T, and NK cells.
- Mice administered LSAM-DTX into renal tumor xenografts had increased circulating T cells and reduced M2-macrophage levels in the blood and spleen when compared to IV docetaxel.
- Intratumoral LSAM-DTX in a metastatic breast cancer model increased T cells in the TME and reduced thoracic metastasis when combined with systemic immunotherapy.
- Preclinical/clinical antitumor immunomodulation suggests that IT LSAM-DTX may be amenable to combination with immunotherapy and warrants further clinical research to confirm.

References

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