Exploratory Analysis of a Phase 2 Multicenter Study Evaluating Local Immune Activation in the Tumor Microenvironment 12 Weeks Post VP-315, an Investigational Therapy for Basal Cell Carcinoma (BCC)

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INTRODUCTION

VP-315 is an intratumorally injected oncolytic peptide in development as a non-surgical immunotherapeutic agent as a potential first line therapy in a primary or neoadjuvant setting for patients with BCC [1]. After intratumoral injection, VP-315 enters BCC cells by perturbing plasma membranes and targets mitochondria, and other organelles, causing cell death and release of danger signals (DAMPs) and a broad repertoire of tumor specific antigens [2,3].

The primary objective of this exploratory analysis was to evaluate the immune response to VP-315 treatment in the tumor microenvironment (TME) in a subset of subjects / BCC tumors (N=22/24) that were enrolled in Part 2 of a Phase 2 multi-center, proof-of-concept study designed to assess the safety, tolerability, maximum tolerated dose, and objective antitumor efficacy of once daily dosing (2 or 3 days) of 8 mg of VP-315 in up to 2 target lesions per subject [4,5,6] (NCT05188729).

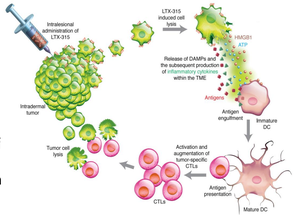

Image Key:
ATP=adenosine triphosphate; **DAMPs**=danger-associated molecular pattern molecules; **DC**=dendritic cell; **CTLs**=cytotoxic CD8+T lymphocytes; **HMGB1**=high mobility group box protein 1; **TME**=tumor microenvironment
LTX-315 is being studied as VP-315 in BCC.
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In Part 2, 82 subjects (92 tumors) completed treatment with VP-315, demonstrating safety, tolerability, and clinical efficacy:

- No Treatment-related SAEs were reported
- Treatment-related AEs were mostly mild to moderate
- All patients treated with VP-315 experienced a reduction in tumor size:
  - 51% Complete Histologic Clearance Rate
  - 71% Reduction in Tumor Size of Patients with Residual Carcinomas
  - 86% Overall Reduction of Tumor Size
  - 97% Post-Hoc Analysis of Calculated Objective Response Rate (ORR)
- A potential abscopal-like immune effect was observed with histologic reduction in size of all non-treated BCC lesions studied

METHODS

- Multiplex immunofluorescence immunohistochemistry was performed on slices from tumor tissue biopsies collected pre-treatment and at 12-14-weeks post-treatment (22 patients/24 Tumors).
- Slices were stained for T-cell subsets (CD3+, CD4+, CD8+), macrophage (CD68+), B-cells (CD20+), as well as for immunosuppressive cells expressing the FoxP3+ and PD-L1+ markers.
- Calculation of densities (cells/ mm^2) of cells expressing the individual markers, as well as specific phenotypes from combinations of the markers, was performed by quantification of fluorescent microscopic images.

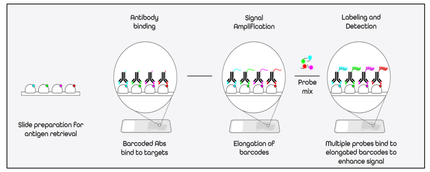
Ultivue® Markers and Phenotypes

Marker	Main Class	Phenotype	Description
CD3	T-cell	CD3+	T-cell
CD4	T-cell	CD3+/CD4+	Helper T-cell
CD8	T-cell	CD3+/CD8+	Cytotoxic T-cell
CD20	B-cell	CD20+	B-cell
GzmB	Granzyme B	CD3+/CD8+/GzmB+	Primed cytotoxic T-cell
CD68	Macrophage	CD68+/PD-L1+	Immunosuppressive M2-like macrophage
FOXP3	Transcription factor	CD3+/CD4+/FOXP3+	Regulatory T-cell (T-reg)
PD-L1	Checkpoint Protein	CD3+/CD4+/PD-L1	Immunosuppressive T helper cell
		CD3+/CD8+/PD-L1	Immunosuppressive cytotoxic T-cell
		CD3+/CD4+/FoxP3+/PD-L1	PD-L1 positive T-reg

Ultivue® Multiplex Immunofluorescence (mIF)* Technology

Step 1: Slide Preparation	The first step involves slide preparation (dewaxing) and single antigen retrieval from FFPE samples. This simplified process requires only one retrieval step, preserving the tissue
Step 2: Antibody Binding	The antibody barcode conjugates are incubated on the slide to stain the biomarkers. Each antibody is labelled with a DNA barcode that uniquely identifies it for detection. Up to 12 different antibodies can be added simultaneously. Only one single staining step is required, eliminating the need for secondary antibodies.
Step 3: Barcode Amplification	During the next step the barcodes linked to the antibodies are amplified simultaneously and at the same rate. The extended barcodes do not interfere with the immediate surroundings so there is no steric hindrance or epitope masking.
Step 4: Detection	The complementary fluorescent DNA probes are added and hybridize to the barcode targets. The samples are then ready for imaging

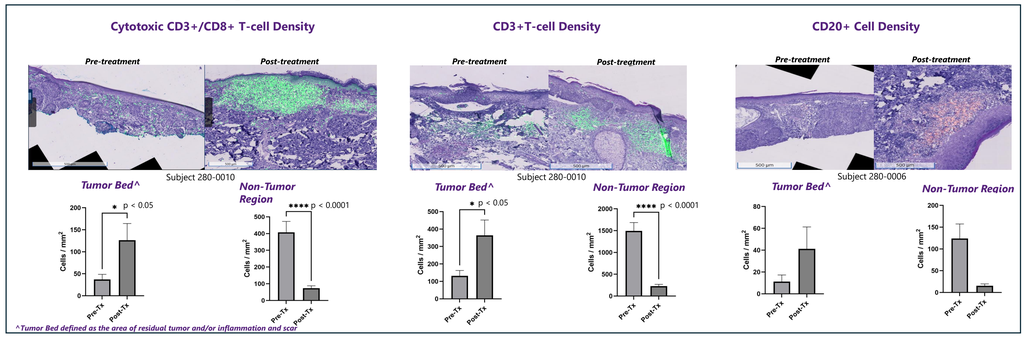
Ultivue® Multiplex Immunofluorescence Process

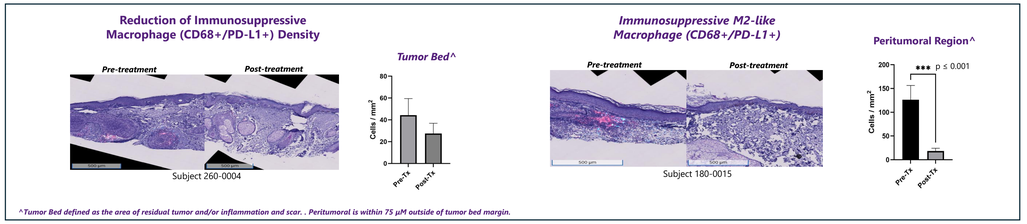


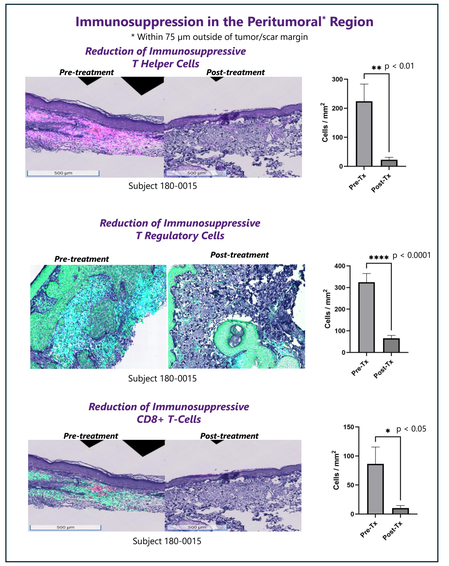
Ultivue® Multiplex Immunofluorescence (mIF)* is a proprietary technology by Vizgen, Inc.

RESULTS

- VP-315 treatment reprograms the TME as evidenced by clinically meaningful and statistically significant increases in the densities of CD3+, CD3+/CD4+ helper cells and CD3+/CD8 cytotoxic T-cells ($p<0.05$; pre- vs post-treatment) and increased B-cell infiltration (CD20+) in the tumor region.
- Furthermore, there was a reduction of immunosuppression in the TME after treatment with a decrease in the CD68+/PD-L1+ macrophage population in the overall tumor region and significant decreases in immunosuppressive CD68+/PD-L1 M2-like Macrophage ($p\leq0.001$), CD3+/CD4+/PD-L1 T-Helper Cells ($p<0.01$), CD3+/CD4+/FoxP3+ T-Regulatory Cells ($p<0.0001$), and CD3+/CD8+/PD-L1 cytotoxic T-Cells ($p<0.05$), in the peritumoral region within 75 µm of the pre-treatment tumor margin.







CONCLUSIONS

- VP-315 treatment induced a robust immune response with both cell-mediated and humoral components in the TME suggesting a reset of the immune switch from immunosuppression toward anti-tumor immunity.
- The observed increase in cytotoxic T-cell infiltration and reduction in immunosuppressive T-regs within and bordering VP-315–treated tumors support the potential for abscopal-like immune effects in non-treated lesions.
- VP-315 is a promising treatment for non-surgical first line BCC therapy in a primary or neoadjuvant setting.

References

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Disclosures

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