Phase I/II Study of Patients with Non-Muscle Invasive Bladder Cancer (NMIBC) Treated with Vesigenurtacel-L (HS-410) with or without Bacillus Calmette-Guérin (BCG)

Gary Steinberg[1] MD, Neal Shore[2] MD, Lawrence Karsh[3] MD, James Bailen[4] MD, Taylor H. Schreiber[5] MD, PhD, George Fromm[5] PhD, Melissa Price[5] PhD

[1]University of Chicago Medical Center, Chicago IL; [2]Carolina Urologic Research Center, Myrtle Beach SC; [3]The Urology Center of Colorado, Denver CO; [4]First Urology, Jeffersonville IN; [5]Heat Biologics Inc., Durham, NC.

Introduction

Vesigenurtacel-L (HS-410), consists of an allogeneic cancer cell line, selected for high expression of a series of tumor antigens that are known to be shared by a high proportion of bladder tumors. Ten patients with NMIBC who had undergone TURBT, were judged to be at an increased risk for recurrence, and were either BCG naïve or had completed previous BCG treatment >12 months prior to the most recent TURBT were treated with induction BCG and enrolled in the trial. Patients received up to 15 doses of monotherapy vesigenurtacel-L at a dose of 10^6 cells per dose, weekly for 12 weeks followed by 3 monthly doses.

Vesigenurtacel-L Mechanism

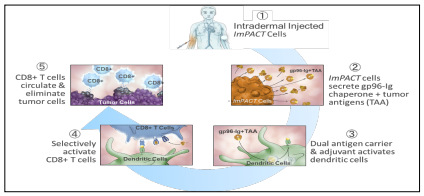

Figure 1: Vesigenurtacel-L Mechanism of Action (MOA)
Vesigenurtacel-L (*ImPACT*) cells are intradermally injected into the patient (1). Vesigenurtacel-L cells secrete TAA-gp96-Ig protein complexes (2), which act as a dual antigen carrier and adjuvant. Dendritic cells are subsequently activated (3) leading to the selective activation of CD8+ T-cells (4). CD8+ T-cells circulate within the patient's body and eliminate encountered tumor cells (5).

Phase 1 Study Design

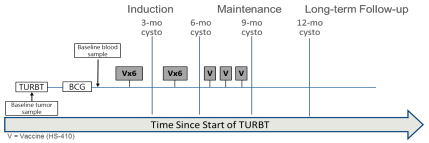

Figure 2: Phase 1 Study Design
Vesigenurtacel-L was assessed for safety in 10 patients. Tumor biopsies were collected at TURBT and at week 7 post-treatment if clinically indicated. Baseline blood was collected prior to the first vaccine dose.

Demographics and Safety

Table 1: Demographic Information

HS410-101 Phase 1 N = 10	n (%)
Male : Female	9 : 1
Race, ethnicity White, Non-Hispanic	10 (100%)
Newly diagnosed disease	9 (90%)
Prior BCG exposure	1 (10%)
Stage: Ta TIS T1	 1 (10%) 1 (10%) 8 (80%)
Grade: Low High	 0 (0%) 10 (100%)
Concomitant CIS	4 (40%)
Smoking history: Never Former Current	 3 (30%) 5 (50%) 2 (20%)

Table 2: AEs Deemed Related to Vesigenurtacel-L

Preferred Term	Grade 1	Grade 2	Total
Arthralgia		1	1
Diarrhea	3		3
Injection Site Pain	4		4
Total	**7**	**1**	**8**

Table 3: Unrelated Adverse Reactions > Grade 2

Preferred Term	Grade
Eye Hemorrhage	3
Nephropathy	3

Vesigenurtacel-L was well-tolerated and no one discontinued treatment due to adverse events (AE). There were no related SAEs reported during the study. Expected SAE rate with BCG is 25% (Colombel, M., *et. al. J. Urol.* 2006; 176: 935).

Phase I Clinical Outcomes

Table 4: Disease Characteristics and Recurrence Status

Patient	T-Class	CIS	Grade	Disease Status	Induction BCG	Vaccine doses	Maintenance BCG	3-month cysto	6-month cysto	Recurrence *
12-001	T1	NO	HIGH	NEWLY DIAGNOSED	5	15	4			No
23-001	T1	YES	HIGH	NEWLY DIAGNOSED	6	15	3			No
23-002	T1	NO	HIGH	NEWLY DIAGNOSED	6	15	6		TIS	Yes
25-001	TA	NO	HIGH	RECURRENT	3	6	0	TIS High		Yes
25-002	T1	YES	HIGH	NEWLY DIAGNOSED	3	15	3			No
25-003	T1	NO	HIGH	NEWLY DIAGNOSED	6	15	0			No
25-004	T1	YES	HIGH	NEWLY DIAGNOSED	5	12	0	Ta high	T1 high CIS	Yes
25-005	T1	NO	HIGH	NEWLY DIAGNOSED	6	15	2	Ta low		No
25-007	T1	NO	HIGH	NEWLY DIAGNOSED	6	15	0			No
25-008	TIS	YES	HIGH	NEWLY DIAGNOSED	6	15	0			No

7/10 patients were disease-free at 6 months with no additional reported recurrences for any patients >18months to-date, including 3 out of 4 carcinoma *in situ* (CIS) patients. Several patients received only 3 induction doses of BCG, instead of the standard 5-6 doses, and most patients received very little maintenance BCG. *Recurrence to same stage/grade as baseline

Tumor Antigen Expression

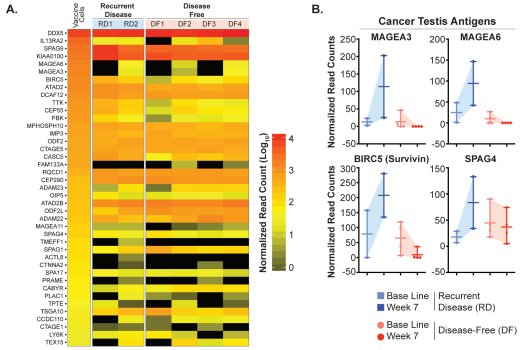

Figure 3. CTA expression overlaps with vaccine cells and patient tumors, and are differentially regulated between Recurrent-Disease and Disease-Free patients. (A) RNA-seq was performed on vaccine cells (Vesigenurtacel-L) and FFPE patient biopsies. The top 25% most highly expressed CTAs (n=41) in Vesigenurtacel-L are ranked on the left, with the corresponding expression in Recurrent-Disease (blue; RD1 and RD2) and Disease-Free (red; DF1, DF2, DF3 and DF4) patients to the right. (B) CTA expression is predictive of recurrence. Normalized read counts from RNA-seq are shown at several CTAs at Base Line and Week 7 after treatment, for Recurrent-Disease (blue) and Disease-Free (red) patients.

Intratumoral Immune Activity

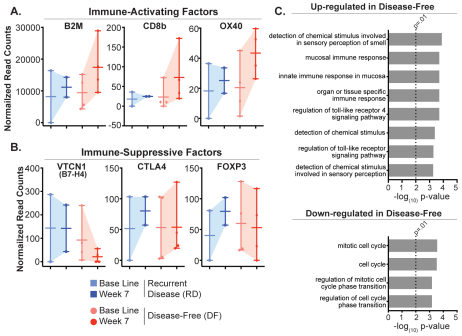

Figure 4. Expression of immune activating and suppressing genes differs based on patient response. (A) Immune-activating factors increase in expression and (B) Immune-suppressive factors decrease in expression after 7 weeks of treatment in Disease-Free (red) patients compared to Recurrent-Disease (blue) patients, despite the absence of tumor in Disease-Free patients. (C) Gene ontology analysis was performed on genes that were elevated at least 5-fold (n=1,641) or decreased at least 3-fold (n=493) in Disease-Free Patients compared to Recurrent-Disease patients using the online tool GOrilla.

Post-treatment Induction of CD8+ TIL

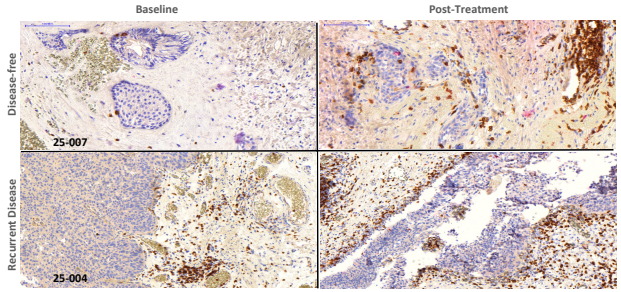

Figure 5: Representative Histology for TIL
Representative histology images of baseline and post-treatment tumors in a patient who is disease-free versus a patient with recurrent disease. Before treatment there are few CD8+ (red) TIL in the disease-free patient (25-007, upper left), whereas TIL are abundant in the recurring patient (25-004, lower left). Following treatment with vesigenurtacel-L, there is robust induction of TIL in the disease-free patient, with moderate induction in the recurring patient.

Multi-Clonal Expansion in TIL

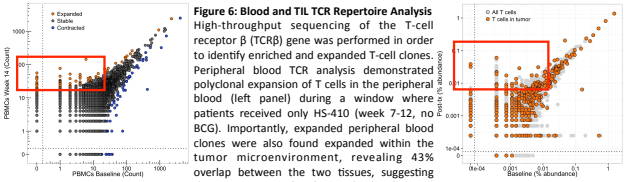

Figure 6: Blood and TIL TCR Repertoire Analysis
High-throughput sequencing of the T-cell receptor β (TCRβ) gene was performed in order to identify enriched and expanded T-cell clones. Peripheral blood TCR analysis demonstrated polyclonal expansion of T cells in the peripheral blood (left panel) during a window where patients received only HS-410 (week 7-12, no BCG). Importantly, expanded peripheral blood clones were also found expanded within the tumor microenvironment, revealing 43% overlap between the two tissues, suggesting that HS-410 stimulated a multi-clonal tumor specific T cell response in patients.

Phase 2 Study Design

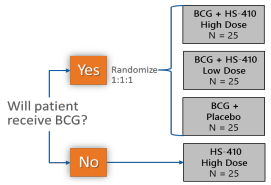

Figure 7: Phase 2 Study Design
Enrollment is complete in the three randomized arms evaluating the combination of one of two doses of vesigenurtacel-L or placebo with BCG (N=75). A fourth open-label arm enrolled 16 patients and will assess Vesigenurtacel-L as potential monotherapy replacement for BCG. The primary endpoint for the Phase 2 study is 1-year disease-free survival.

Enrollment completed Q4, 2015 for randomized arms

Enrolled 16 patients to open-label monotherapy arm

PD-L1 and TIL in NMIBC

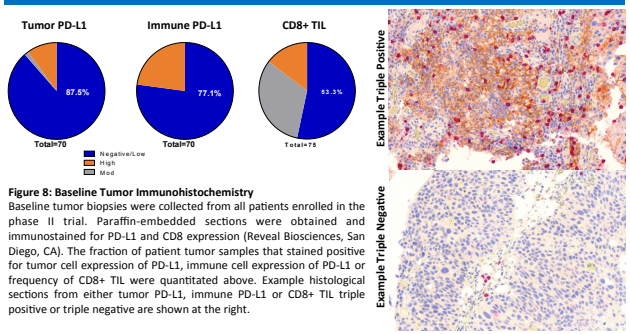

Figure 8: Baseline Tumor Immunohistochemistry
Baseline tumor biopsies were collected from all patients enrolled in the phase II trial. Paraffin-embedded sections were obtained and immunostained for PD-L1 and CD8 expression (Reveal Biosciences, San Diego, CA). The fraction of patient tumor samples that stained positive for tumor cell expression of PD-L1, immune cell expression of PD-L1 or frequency of CD8+ TIL were quantitated above. Example histological sections from either tumor PD-L1, immune PD-L1 or CD8+ TIL triple positive or triple negative are shown at the right.

Monotherapy Phase II Cystoscopy

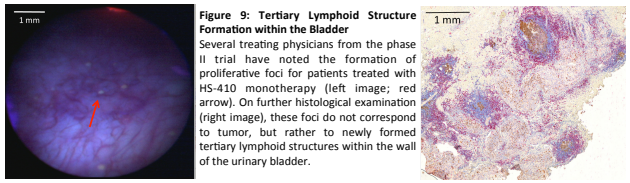

Figure 9: Tertiary Lymphoid Structure Formation within the Bladder
Several treating physicians from the phase II trial have noted the formation of proliferative foci for patients treated with HS-410 monotherapy (left image; red arrow). On further histological examination (right image), these foci do not correspond to tumor, but rather to newly formed tertiary lymphoid structures within the wall of the urinary bladder.

Conclusions

- Vesigenurtacel-L (HS-410) has been safe and well-tolerated in >100 patients to date
- Phase I data demonstrated evidence of a polyclonal T cell response following treatment with HS-410, which has now also been seen in patients treated with monotherapy in phase II
- All patients in the phase I had at least 30 antigens in common with HS-410, and several potential immune biomarkers were identified by RNA Seq
- The 12-month recurrence-free survival rate for the phase I trial was 70%, ***and no additional recurrences have been reported to date, with all patients now at least 18 months from enrollment***
- The randomized phase II trial completed enrollment in 2015 and 12-month survival data will be reported Q4 of this year