IMMUNE RESPONSE RESULTS FROM VESIGENURTACEL-L (HS-410) IN COMBINATION WITH BCG FROM A RANDOMIZED PHASE 2 TRIAL IN PATIENTS WITH NON-MUSCLE INVASIVE BLADDER CANCER (NMIBC)

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Background

Bacillus Calmette-Guérin (BCG), is an intravesical immunotherapy which remains the mainstay of non-muscle invasive bladder cancer (NMIBC) treatment since 1976. With adequate surgical resection and adjuvant BCG treatment, about 53-63% of newly-diagnosed T1 and high-grade Ta patients will remain disease free at 1-year. The current model of BCG's mechanism of action (MOA) proposes direct cytotoxicity of the urothelium (and tumor) and an indirect effect by immune cell mediation. Given the MOA, combining T-cell activation strategies with BCG could potentiate the effect of BCG.[1,2]

Vesigenurtacel-L (HS-410) is a vaccine comprised of an allogeneic cell line, PC3, selected from a series of cell lines for high expression of known bladder cancer antigens. The cell line was stably transfected with a plasmid that induces the expression of a modified, secretable, heat shock protein, gp96-Ig, which is a versatile tumor antigen chaperone and adjuvant protein.

We conducted a randomized Phase 2 trial with vesigenurtacel-L in combination with BCG in NMIBC. Overall, the vaccine arms did not show a statistical improvement over the placebo arm in the primary endpoint (1-year recurrence free survival). However, vaccine patients experienced increases in IFN-γ secreting CD8+ T-cells which was not seen in patients treated with placebo, compelling further evaluation of these immune responses.[3]

Here we present immunologic correlative data from the Phase 2 trial to advise future development with vesigenurtacel-L. Trial ID: NCT02010203

Vaccine Mechanism of Action

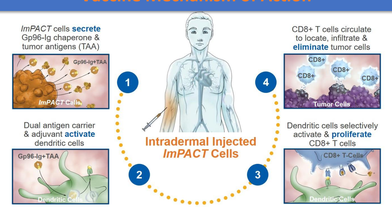

Figure 1: Vaccine Mechanism of Action

Vesigenurtacel-L (ImPACT) cells are intradermally injected into the patient. Vesigenurtacel-L cells secrete TAA-gp96-Ig protein complexes (1), which act as a dual antigen carrier and adjuvant. Dendritic cells are subsequently activated (2) leading to the selective antigen specific activation of CD8+ T-cells (3). CD8+ T-cells circulate within the patient's body and eliminate encountered tumor cells (4). Proof of Concept data have confirmed this mechanism of action in clinical trials.

Immune Correlative Analyses

The HS410-101 protocol incorporated correlative immune analyses as endpoints (other safety and efficacy endpoints were also included, not shown). Data supporting bolded endpoints are presented here:

SECONDARY ENDPOINTS (IMMUNE RELATED):

- **Immunologic response of CD8 cells via ELISPOT**

EXPLORATORY ENDPOINTS (IMMUNE RELATED):

- **Immunologic response of PBMCs by flow cytometry**
- Evaluation of tumor tissue by mRNA expression/sequencing for: antigen expression, expression of MHC1, and expression of immunosuppressive molecules
- **Evaluation of tumor tissue for presence of infiltrating T-lymphocytes (TILs)**
- T cell receptor sequencing of PBMCs and tumor tissue
- Immune cell infiltration and inflammatory cytokine levels in urine

Trial Design

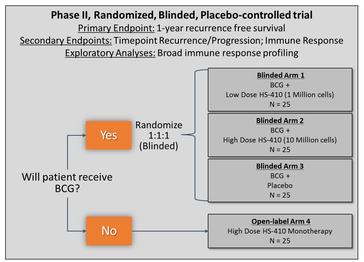

Figure 2: HS410-101 Trial Design

Patients who would receive BCG were centrally randomized into one of three blinded treatment arms. Arms were stratified at the time of randomization by baseline risk category (intermediate- or high-risk) or concomitant CIS (yes or no). Patients who would not receive BCG were assigned sequentially to the open label monotherapy arm. Vaccine was given in combination with 6 weeks of induction BCG, followed by 6 more weeks of HS-410 in the induction phase. Maintenance treatment consisted of 3-weekly treatments at the following timepoints: 3 months, 6 months, 12 months.

Interferon gamma ELISPOT Immune Response

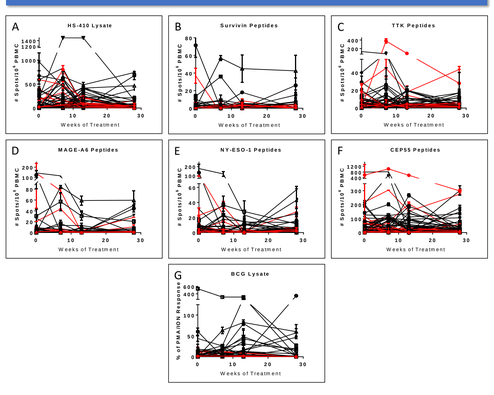

Figure 3: Vaccination induced antigen-specific cytotoxic immune responses in peripheral blood T-cells

Peripheral blood cells were re-stimulated with whole vaccine lysate (A) and overlapping peptides to tumor antigens contained within the vaccine (B-F), or BCG lysate (G). Increases in spots indicates activated T-cells recognizing antigen and producing cytotoxic interferon gamma (IFN-g). Placebo treated patients (red lines) did not consistently demonstrate increases in IFN-g secreting T cells. Increases in these cells were seen in vaccine patients (black lines) in an immune priming pattern. Most patients did not mount IFN-g response to BCG lysate.

Week 7 timepoint: after vaccine + BCG induction, Week 13: after 6 weeks of vaccine only, Week 28: after second maintenance doses.

Demographics and Patient Characteristics

	Arm 1: BCG + Low Dose Vesigenurtacel-L (N=26)	Arm 2: BCG + High Dose Vesigenurtacel-L (N=26)	Arm 3: BCG + Placebo (N=26)	Arm 4: High Dose Vesigenurtacel-L (N=16)
Age (Years)				
Median	72	69.5	70.5	73
Gender n(%)				
Male	23 (88.46%)	21 (80.77%)	20 (76.92%)	13 (81.25%)
Treatment Status				
BCG Naïve	18 (69.23%)	16 (61.54%)	16 (61.54%)	7 (43.75%)
BCG Recurrent (>12 mo)	8 (30.77%)	10 (38.46%)	10 (38.46%)	9 (56.25%)
TNM Classification				
T1	12 (46.15%)	7 (26.92%)	11 (42.31%)	4 (25.00%)
TA	7 (26.92%)	11 (42.31%)	10 (38.46%)	11 (68.75%)
TIS	7 (26.92%)	8 (30.77%)	5 (19.23%)	1 (6.25%)
Carcinoma in situ				
Yes	11 (42.31%)	11 (42.31%)	9 (34.62%)	4 (25.00%)
WHO 2004 Grade				
High	25 (96.15%)	22 (84.62%)	24 (92.31%)	15 (93.75%)

Table 1: Demographics and Baseline Disease History

Baseline demographic and disease status were well balanced across treatment arms. All patients had urothelial carcinoma except 1 patient in Arm 3 who had squamous cell carcinoma.

Clinical Efficacy (1-year Recurrence Free Survival)

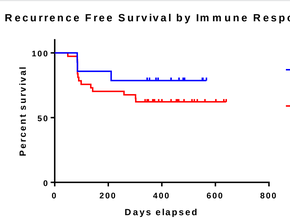

Figure 4: Clinical Efficacy Data by Immune Response

Immune Responders may have a better disease free interval when compared to Non-Immune Responders, though these are small data sets. Overall, vaccine-containing arms are not statistically different from the placebo-containing arm at 1-year (data not shown but presented previously [3]). These immune responses compelled additional immune profiling to define potential patterns of response and potential pretreatment biomarkers.

Tumor Infiltrating Lymphocyte (TIL) at Baseline

	Vaccine Arms		Placebo Arms		
Baseline TIL	Enrolled Patients	Patients Recurred	Enrolled Patients	Patients Recurred	TOTAL
High CD8 TIL	8	2 (25%)	5	1 (20%)	3/13 (23%)
Low CD8 TIL	28	8 (29%)	13	5 (38%)	13/41 (32%)

Table 2: Recurrence rate by baseline CD8 Tumor Infiltrating Lymphocyte (TIL) status and treatment arm

Previous clinical trials with the *ImPACT* platform have shown CD8+ TIL induction after vaccination. In this trial, patients with High TIL at baseline had fewer disease recurrences than patients with Low TIL at baseline – patients with inflamed tumors have better outcomes in many tumor types. However, Low TIL patients treated with vaccine appear to have a similar recurrence rate to High TIL patients. These data support the vaccine mechanism of action of stimulating a CD8+ T-cell response that may be related to clinical outcome.

Immunohistochemistry

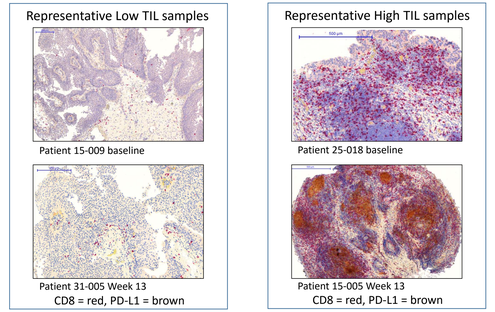

Figure 5: Representative Immunohistochemistry Images

All patients had baseline biopsies; 64 of 78 were evaluable for CD8+ tumor infiltrating lymphocytes (TIL, red) and PD-L1 expression (brown). Thirty-nine (39) patients had follow-up biopsies as clinically indicated (disease recurrence). Most patients in this trial did not have CD8+ TIL: 41 of 64 evaluable biopsies were low CD8+ TIL. PD-L1 staining was sparse in most samples, but was localized in some patients in areas that appear to be granulomas, consistent with literature reports. Further post-treatment biopsies may contribute to understanding the role of TIL (Table 2).

Flow Cytometry Profiling

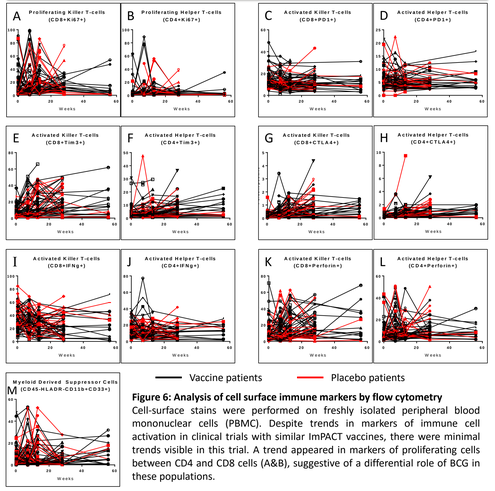

Figure 6: Analysis of cell surface immune markers by flow cytometry

Cell-surface stains were performed on freshly isolated peripheral blood mononuclear cells (PBMC). Despite trends in markers of immune cell activation in clinical trials with similar ImPACT vaccines, there were minimal trends visible in this trial. A trend appeared in markers of proliferating cells between CD4 and CD8 cells (A&B), suggestive of a differential role of BCG in these populations.

Discussion

The HS410-101 trial was designed to treat patients in a minimal residual disease setting to test the combination of HS-410 vaccine and standard of care BCG against BCG alone. The proposed mechanism of BCG, with direct cytotoxicity and recruitment of immune cells, provided a compelling rationale to use HS-410 to activate T-cells while BCG helped attract those activated T-cells to the bladder to lower the incidence of malignant recurrence.

Immune responses generated in this trial (and other ImPACT vaccine combination trials) demonstrate:

1. HS-410 activates CD8+ T-cells.
2. Those activated CD8 cells secrete cytotoxic IFN-g when re-stimulated with vaccine lysate and individual cancer antigens.
3. Patients who generate this type of immune response appear to have a lower recurrence rate.

These data support the vaccine mechanism of action and clinical proof of concept. Further, this vaccine treatment strategy could be evaluated in more advanced bladder patient populations where immunotherapy has been shown to be effective but where all patients do not respond due to insufficient T cell activation and expansion.

Conclusions/Next Steps

1. Immune response as measured by ELISPOT analysis on peripheral blood lymphocytes drawn from patients on treatment suggest a better recurrence free survival outcome than non-immune responders.
2. In the placebo arm, Low TIL at the start of treatment had a higher incidence of recurrence compared to patients with High TIL at the start of treatment. Further patient follow-up and post-treatment biopsies may advance the understanding of this trend.
3. Interestingly, in the vaccine treated group, recurrence levels were essentially the same between the High and Low TIL subgroups.
4. These robust markers of immune activation coupled with the previously-reported low incidence and severity of safety signals may warrant evaluation of HS-410 in patients with more advanced disease.
5. HS-410 in combination with BCG continues to be generally well tolerated.

References

1. C. Biot, et al, Preexisting BCG-specific T cells improve intravesical immunotherapy for bladder cancer. Sci. Transl. Med. 4, 137ra72 (2012).
2. G. Redelman-Sidi, et al. The mechanism of action of BCG therapy for bladder cancer—a current perspective. Nature Reviews Urology 11, 153–162 (2014)
3. HS410-101 Data presented at SUO Annual Meeting 2016

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