Systemic Administration of Ladiratuzumab Vedotin Alone or in Combination with Pembrolizumab Results in Significant Immune Activation in the Tumor Microenvironment in Metastatic Breast Cancer Patients

Lajos Pusztai[1], Hailing Lu[2], Christopher Hale[2], Anne Grosse-Wilde[2], Jennifer Specht[3], Shanu Modi[4], Hyo Han[5], Javier Cortes[6], Mafalda Oliveira[7], Phillip Garfin[2], Zejing Wang[2], Matthew Onsum[2]

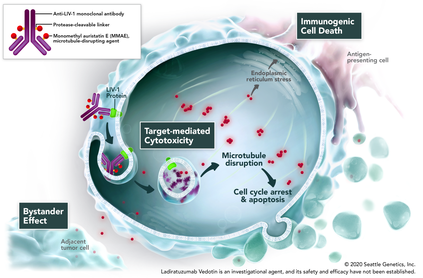
[1]Yale School of Medicine, New Haven, CT; [2]Seagen Inc., Bothell, WA; [3]Seattle Cancer Care Alliance, University of Washington, Seattle, WA; [4]Memorial Sloan Kettering Cancer Center, New York, NY; [5]H.Lee Moffitt Cancer Center and Research Institute, Tampa, FL; [6]IOB Institute of Oncology, Quiron Group, Madrid & Barcelona, Vall d´Hebron Institute of Oncology (VHIO), Barcelona, Spain; [7]Hospital Universitari, Spain

Background

- Ladiratuzumab vedotin (LV) is an investigational antibody-drug conjugate (ADC) composed of a humanized anti-LIV1 Immunoglobulin G1 (IgG1) conjugated with monomethyl auristatin E (MMAE), a microtubule-disrupting agent.
- LV targets LIV-1, a transmembrane protein expressed by various cancers.[1]Along with a cytotoxic effect, LV has been shown to induce immunogenic cell death (ICD) in preclinical studies.[2]
- LV is currently being investigated as a monotherapy (SGNLVA-001, hereafter referred to as LVA-001/monotherapy) and in combination with pembrolizumab (pembro; SGNLVA-002, hereafter referred to as LVA-002/LV+pembro) in patients with metastatic breast cancer and other solid tumors.[3,4,5]
- This correlative biomarker study aims to assess the ability of LV to modulate the tumor microenvironment (TME) in triple negative breast cancer (TNBC) patients.

Proposed Mechanism of Action

- LV
  - Humanized IgG1 ADC
  - Selectively binds to cells expressing LIV-1
  - Conjugated to MMAE
- LV-mediated delivery of MMAE drives antitumor activity through
  - Cytotoxic cell killing
  - Inducing ICD[2]



Methods

- Patients with locally advanced or metastatic TNBC were enrolled in LVA-001 (LV monotherapy, 2~2.5mg/kg, every 3 weeks [q3w], in 2~5th line of therapy) and LVA-002 (LV plus pembro 200mg, q3w, 1st line of therapy)
- Tumor biopsies were collected at baseline and after the first dose of treatment (cycle 1 day 5 [C1D5] in LVA-001; C1D5 or C1D15 in LVA-002)

- **RNAseq:** RNA extraction and RNAseq was performed at Q2 Solutions. Differential gene expression was assessed using the DESeq2 package[6]. Gene ontology (GO) analysis was carried out using the TopGO R package[7] using a Fisher test in conjunction with the "weight" scoring algorithm to assess significantly enriched biological process nodes. Scoring of potential cell type enrichment was done using the xCell method[8] implemented in R. Unless otherwise noted, false discovery rates (FDRs) for individual gene expression comparisons were calculated by the Benjamini-Hochberg method and DESeq p-values.
- **Immunohistochemistry (IHC):** Cluster of differentiation 8 and 68 (CD8 and CD68), as well as programmed cell death 1 ligand 1 (PD-L1; 22C3) IHC evaluation were performed at Phenopath (Seattle, WA). Combined Positive Score (CPS) was scored according to Agilent's PD-L1 IHC 22C3 pharmDx interpretation manual.

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Results

1. LV Monotherapy Induced Immune Activation in TME in Breast Cancer Patients

LV Monotherapy Induces Immune Activation in TME as Measured by RNA Seq

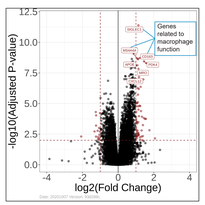


Figure 1. Volcano plot showing differentially expressed genes induced by LV monotherapy. Paired baseline and on-treatment (C1D5) biopsies were used for RNAseq (N=59). Differential expression was assessed by comparing paired baseline-C1D5 RNAseq profiles. We identified differentially expressed genes (highlighted in red) using fold-change and significance cutoffs (absolute fold-change > 2, adjusted p-value/FDR < 0.01). We identified 59 up-regulated genes at C1D5 and 15 down-regulated genes at C1D5. Some of the top differentially expressed genes (SIGLEC1, MS4A4A, CD163) relate to macrophage function. Genes differentially expressed at an FDR<1e-7 are shown with labels. Some outlier points removed for visualization purposes.

Table 1. GO Terms Highlight Activation of Innate Immune Response – Top 10 Terms

GO.ID	Term	P-value
GO:0006956	complement activation	1.80E-06
GO:0042742	defense response to bacterium	2.50E-06
GO:0042116	macrophage activation	1.50E-05
GO:0070613	regulation of protein processing	3.50E-05
GO:0006898	receptor-mediated endocytosis	5.10E-05
GO:0006910	phagocytosis, recognition	0.00012
GO:0006954	inflammatory response	0.00019
GO:0006641	triglyceride metabolic process	0.00033
GO:0006953	acute-phase response	0.00048
GO:0071223	cellular response to lipoteichoic acid	0.00061

LV Increases Macrophage Infiltration, Induces MHC, Co-stimulatory Molecules, and PD-L1 Expression

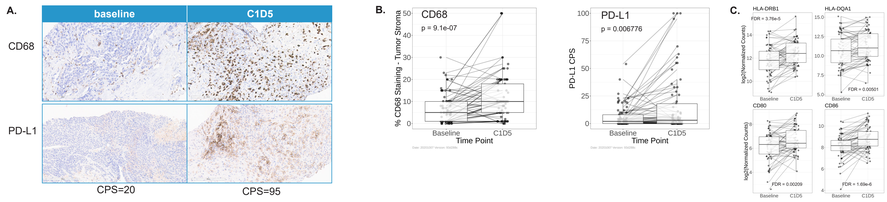


Figure 2. Increased macrophage infiltration and PD-L1 expression and induction of major histocompatibility complex (MHC) and co-stimulatory molecules by LV monotherapy. A: representative IHC pictures showing macrophage (CD68) and PD-L1 (22C3) staining in tumor biopsies collected at baseline and C1D5 of an LVA-001 patient. **B:** Summary graphs showing CD68 IHC results (% of cells positive for CD68 in tumor stroma) and PD-L1 CPS scores in all LVA-001 patients with evaluable samples (N=79 for CD68; N=73 for PD-L1). **C:** Summary graphs showing induction of MHCII genes (HLA-DRB1 and HLA-DQA1) and co-stimulatory molecules (CD80 and CD86) by RNAseq analysis (N=59). There was also significant induction of MHCI genes, inflammatory cytokines, and overall increase in tumor inflammation score (not shown).

2. LV + Pembro Induced Immune Activation in TME in Breast Cancer Patients

Initial RNAseq Data from LV+Pembro Shows Stronger Immune Activation at C1D15

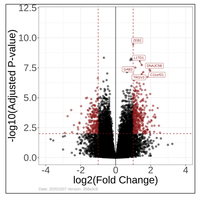


Figure 3. Volcano plot showing differentially expressed genes induced by LV plus pembro. Paired baseline and on-treatment (C1D15) from initial data set were used for RNAseq (N=16). Differential expression was assessed by comparing paired baseline-C1D15 RNAseq profiles. We identified differentially expressed genes (highlighted in red) using fold-change and significance cutoffs (absolute fold-change > 2, adjusted p-value/FDR < 0.01). We identified 342 up-regulated genes at C1D15 and 192 down-regulated genes at C1D15. ZEB2, L1TD1, DNAJC5B, GAB3, C11orf21 and TRGV3 were upregulated at an FDR<1e-7 (shown with labels). Some outlier points were removed for visualization purposes.

Table 2. GO Terms Highlight Activation of Adaptive Immune Response – Top 10 Terms

GO.ID	Term	P-value
GO:0002250	adaptive immune response	< 1e-30
GO:0006955	immune response	1.80E-24
GO:0006952	defense response	5.30E-23
GO:0050863	regulation of T cell activation	1.90E-19
GO:0030217	T cell differentiation	1.70E-16
GO:1903039	Pos reg. of leukocyte cell-cell adhesion	3.70E-16
GO:0001909	leukocyte mediated cytotoxicity	2.30E-14
GO:0032609	interferon-gamma production	2.90E-14
GO:0032655	regulation of interleukin-12 production	1.60E-13
GO:0045619	regulation of lymphocyte differentiation	1.60E-13

Increased Infiltration of T Cells and Induction of PD-L1 on C1D5 in an LVA-002 Patient

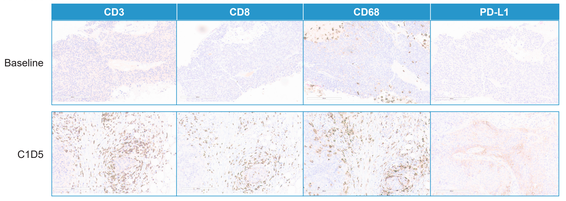


Figure 4. Representative IHC pictures showing the increased infiltration of CD3⁺ and CD8⁺ T cells, CD68⁺ macrophages, and increased PD-L1 staining in C1D5 biopsy as compared to baseline biopsy collected from an LVA-002 patient. Brown color indicates cells stained positive for each marker.

3. Comparison of LV Monotherapy and LV+Pembro Combination Biomarker Data

Stronger Induction of DC and CD8 T-Cell Signatures in LVA-002 (LV+Pembro) at C1D15 by xCell Analysis*

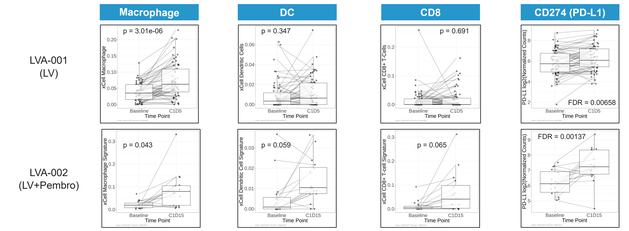


Figure 5. Comparison of xCell analysis (RNAseq) results on tumor infiltrating immune cells (macrophage, dendritic cells [DC], and CD8 T cells) and CD274 (PD-L1) gene expression in LV monotherapy (LVA-001) and LV+Pembro (LVA-002). P-values for xCell gene signatures were calculated using a paired t-test. For PD-L1, the FDR is derived from the differential expression analysis comparing C1D5/C1D15 and baseline. N=59 for LVA-001; N=16 for LVA-002 (more patient data pending).

*Data remains to be confirmed via a direct comparison with more data from LVA-002 samples collected at C1D5.

Stronger Induction of CD8 Tumor Infiltration in LVA-002 (LV+Pembro) at C1D15 by IHC Analysis

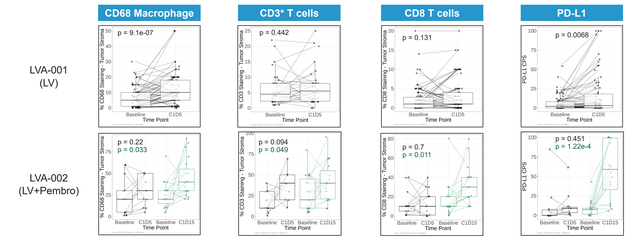


Figure 6. Comparison of IHC analysis on tumor infiltrating immune cells (CD3⁺ and CD8⁺ T cells, CD68⁺ macrophages) and CD274 (PD-L1) staining in LVA-001 and LVA-002. P-values were calculated by a Wilcoxon signed-rank sum using the "coin" permutation framework in R[9]. On-treatment biopsies were collected around C1D5 in LVA-001 and around C1D5 or C1D15 in LVA-002.

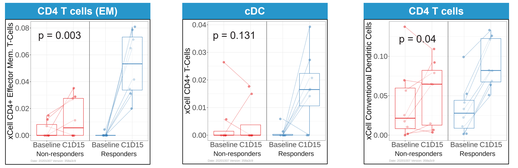
* For LVA-001, CD3 IHC was only performed on samples from one cohort of the study.

Black: C1D5 pairs

Green: C1D15 pairs

4. LV+Pembro-induced Immune Activation is Associated with Clinical Response

Treatment-induced Increase in CD4 T Cells and DC in Tumor are Associated with Clinical Response (LVA-002: LV+Pembro)

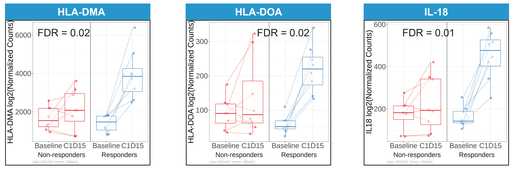


Red: Non-responders (NR): stable disease (SD) or progressive disease (PD)

Blue: Responders (R): partial response (PR) or complete response (CR)

Figure 7. xCell gene signature analysis of RNAseq data showed that induction of genes associated with CD4 T cells (especially Effector Memory [EM] CD4 T cells) and conventional DC (cDC) are associated with clinical responses. P-values were calculated by using a likelihood ratio test of two nested linear models. The full model considered the xCell score as a dependent on response, timepoint, and the interaction of those two features. The simpler, nested, model considered the xCell score as dependent on just response and timepoint. Thus, the resulting likelihood ratio test assessed the importance of the interaction between time and response and the relationship to xCell signature values. A more significant p-value indicates that xCell signatures are behaving differently over time between responders and non-responders.

Patients with Tumors that Respond to Therapy Have Greater MHC and Inflammatory Cytokine Induction (LVA-002: LV+Pembro)



Red: Non-responders (NR): stable disease (SD) or progressive disease (PD)

Blue: Responders (R): partial response (PR) or complete response (CR)

Figure 8. Induction of MHC genes (HLA-DMA and HLA-DOA) and the inflammatory cytokine interleukin-18 (IL-18) are associated with clinical responses. FDRs are calculated by a Benjamini-Hochberg correction of multiple testing for p-values generated by a DESeq-based likelihood ratio test of two nested models. The term tested between these two models is the interaction between timepoint and response. Overall we have observed 93 genes that are differentially regulated (FDR<0.05) across time between Responders and Non-responders (data not shown).

Conclusions

- LV monotherapy results in immune activation in the TME in patients with metastatic TNBC with a significant induction of macrophage infiltration, increase in MHCI/II, co-stimulatory molecules, pro-inflammatory cytokines/chemokines, and PD-L1. LV-induced TME changes in cancer patients are consistent with preclinical evidence that LV induces ICD.
- Combination of LV plus pembro results in an enhanced immune activation in the TME with activation of adaptive immune response pathways and increased infiltration of CD8 T cells in addition to macrophages.
- Preliminary analysis showed that infiltration of T cells and DC, and induction of immune activation genes (MHC and cytokines) are associated with clinical responses in the context of a LV and pembro combination.

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