CD19 Expression by IHC Alone Is Not a Predictor of Response to Loncastuximab Tesirine: Results From the LOTIS-2 Clinical Trial and Quantitative Systems Pharmacology Modeling

Paolo F. Caimi, MD[1]*, Mehdi Hamadani, MD[2], Carmelo Carlo-Stella, MD[3], Masoud Nickaeen, PhD[4], Eric Jordie, PhD[5], Kiersten Utsey, PhD[5], Tim Knab, PhD[5], Francesca Zammarchi, PhD[6], Serafino Pantano, PhD[7], Karin Havenith, PhD[6], Ying Wang, MD, PhD[8], Joseph Boni, PhD[8]

[1]Blood and Marrow Transplant Program, Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland, OH, USA; [2]Division of Hematology & Oncology, BMT & Cellular Therapy Program, Medical College of Wisconsin, Milwaukee, WI, USA; [3]Department of Oncology and Hematology, Humanitas University and IRCCS Humanitas Research Hospital, Milan, Italy; [4]Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA; [5]Metrum Research Group, Simsbury, CT, USA; [6]ADC Therapeutics (UK) Ltd, London, UK; [7]ADC Therapeutics SA, Épalinges, Switzerland; [8]ADC Therapeutics, Murray Hill, NJ, USA

OBJECTIVE

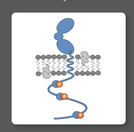
To present results from the LOTIS-2 clinical trial and quantitative systems pharmacology modeling that show that CD19 expression by IHC alone is not a predictor of response to Loncastuximab tesirine-lpyl (Lonca).

SUMMARY

Lonca may be an effective treatment option for patients with relapsed or refractory DLBCL following ≥ 2 lines of treatment, even in patients with very low CD19 tumor expression

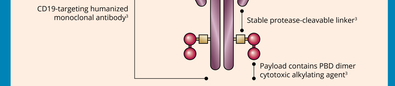
CD19 in DLBCL

CD19 is a validated target for the treatment of DLBCL, and several CD19-targeted therapies are FDA-approved. However, the optimal sequencing of these treatments has not yet been clarified.

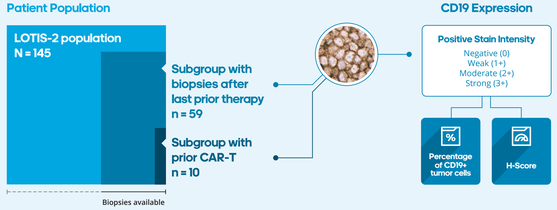


How Lonca Works

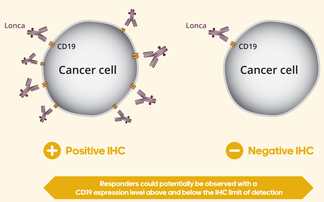
Lonca is the first CD19-directed antibody-drug conjugate that is approved by the FDA for patients with DLBCL after ≥2 systemic therapies



Methods CD19 expression by IHC was evaluated in a subset of patients enrolled in the LOTIS-2 clinical trial who had tissue samples obtained after their last anticancer therapy and prior to Lonca



Results Response to Lonca was observed in patients with very low CD19 tumor expression



Modeling CD19 expression and surface density predicts response to Lonca

- Responses to Lonca were seen in patients across all levels of CD19 expression, including patients with extremely low or no detectable CD19 expression by IHC
- Virtual patients responded to Lonca with as little as an estimated 1,000 CD19 molecules/cell, which is normally below the level of IHC detection

INTRODUCTION

- CD19 is a clinically validated target for the treatment of B-cell malignancies, and several CD19-targeted therapies have received approval, including chimeric antigen receptor T-cell (CAR-T) therapy, antibody-drug conjugates (ADCs), monoclonal antibodies, and bispecific agents.[1]
  - However, the optimal sequencing of these treatments has not yet been clarified.
- Loncastuximab tesirine (loncastuximab tesirine-lpyl; Lonca) is an ADC comprising an anti-CD19 antibody conjugated to a pyrrolobenzodiazepine (PBD) dimer cytotoxin, indicated for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) after ≥2 systemic treatments.
- Prior CD19-directed CAR-T therapy does not preclude a response to Lonca, and responses were also observed in patients who received CAR-T therapy post-Lonca.[2]
- The present analysis was performed to determine the correlation between lymphoma CD19 expression levels and disease responses in patients treated with Lonca.

METHODS

STUDY DESIGN

- CD19 expression determined by immunohistochemistry (IHC) was evaluated in a cohort of patients enrolled in the LOTIS-2 clinical trial (NCT03589469), with available tissue samples obtained after their last anticancer systemic therapy and prior to Lonca.
  - Patients who had received previous CD19-directed therapy must have had a biopsy confirming CD19 protein expression after completion of the CD19-directed therapy.
- The cohort included patients with any prior systemic therapies (n = 59).
  - Of these patients, 10 received CD19-directed CAR-T as the last therapy prior to biopsy.
- The median time from biopsy to Lonca treatment for these 59 patients was 18.0 days (1.5-185 days).

CD19 IMMUNOHISTOCHEMISTRY

- IHC was performed using the LE-CD19 antibody (Agilent Dako), and expression was analyzed using the BenchMark ULTRA platform (Ventana Medical Systems, Inc).
- CD19 expression was assessed by semiquantitative scoring of both the percentage of positive tumor cells and the HistoScore (H-score).
- The H-score is based on four IHC categories: negative (0), weakly (1+), moderately (2+), and strongly (3+) stained membranes. In each case, the H-score with a potential range of 0-300 was calculated as follows: H-score = (1 × % weakly stained cells) + (2 × % moderately stained cells) + (3 × % strongly stained cells).

PRECLINICAL ANALYSIS

- Cytotoxicity of Lonca was determined by the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS assay) (Promega). The 50% inhibitory (IC50) values were determined by using GraphPad software.
- CD19 IHC analysis was performed following the same protocol and scoring methods used for the clinical samples.
- Cell surface CD19 density was determined by flow cytometry using Bangs Laboratory Quantum MESF beads according to the manufacturer's instructions.

QUANTITATIVE SYSTEMS PHARMACOLOGY MODELING

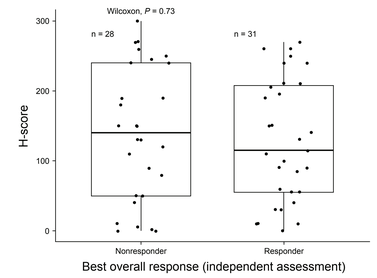
- Quantitative systems pharmacology (QSP) modeling was used to predict response to Lonca and to test hypotheses regarding patient-specific covariates, which included the following:
  - Initial tumor size and location;
  - Subject body weight;
  - Hypoalbuminemia (as neonatal Fc receptor [FcRN] expression);
  - Lonca-induced death rate of tumor cells;
  - Rate of Lonca internalization into cells;
  - Rate of payload diffusion out of cells;
  - Growth rate of tumor cells;
  - CD19 expression level from pretreatment tumor biopsies; and
  - CD19 surface density (molecules/cell) from pretreatment tumor biopsies.

RESULTS

RESPONSES TO LONCA WERE OBSERVED ACROSS ALL LEVELS OF CD19 EXPRESSION

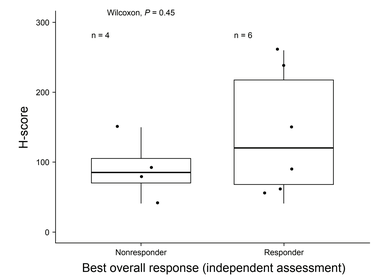
- Responses to Lonca were seen in patients across all levels of CD19 expression, including patients with extremely low or no detectable CD19 expression at baseline and extremely low H-scores and H-scores of zero (**Figure 1**).
  - Responses to Lonca were observed in patients who received CAR-T (n = 10), including those with low H-scores (**Figure 2**).

Figure 1. Baseline tumor CD19 H-score by response to Lonca (independent assessment)



Lonca, loncastuximab tesirine-lpyl.

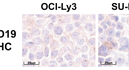
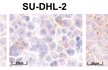
Figure 2. Baseline tumor CD19 H-score by response to Lonca (independent assessment) in post–CAR-T biopsied patients



CAR-T, chimeric antigen receptor T-cell; Lonca, loncastuximab tesirine-lpyl.

- Lonca showed potent cytotoxicity (pM range) in a panel of 6 lymphoma cell lines with different levels of CD19 expression, as measured by flow cytometry and IHC.
  - Lonca was active in cell lines with very low H-score and only 2% positive cells when expression was quantified by IHC.

Figure 3. Quantification of CD19 expression (IHC and flow cytometry) and Lonca in vitro cytotoxicity in a panel of 6 lymphoma cell lines

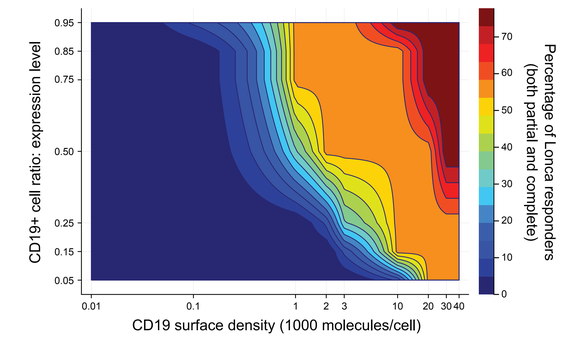
	OCI-Ly3	SU-DHL-2	TMD8	SU-DHL-16	SU-DHL-4	MEC-1
CD19 IHC						
CD19 H-score (IHC)	2	30	55	142	150	265
Percent of CD19-positive cells (IHC)	2%	30%	40%	80%	65%	90%
CD19 copy number (±SEM) (Flow cytometry)	24,420 (±24)	63,921 (±240)	61,357 (±555)	116,553 (±681)	340,761 (±2,301)	288,531 (±2,227)
Lonca in vitro cytotoxicity IC50 pM (±SEM)	216 (±15.7)	12.5 (±1.1)	47.3 (±10.7)	3.3 (±1.1)	9.6 (±3.2)	17.2 (±1.3)

IC50, half maximal inhibitory concentration; IHC, immunohistochemistry; NHL, non-Hodgkin lymphoma.

RESULTS (continued)

- Virtual patient simulations performed using the QSP model predicted disease response to Lonca with CD19 tumor cell-surface densities as low as 1,000 molecules/cell (**Figure 4**).
- QSP modeling indicated that the faster growth rate of double hit (DH) lymphomas explained the observed lack of response to Lonca, despite high CD19 positivity as measured by IHC.
- Patients with hypoalbuminemia had enhanced clearance and reduced Lonca exposure as assessed through a reduction in FcRn expression.

Figure 4. Lonca heat map profile of CD19 positive cell ratio of expression versus CD19 surface density and response



Lonca, loncastuximab tesirine-lpyl.

CONCLUSIONS

- Response to Lonca was observed in R/R DLBCL patients with very low CD19 tumor expression as measured by IHC.
- Lonca showed potent cytotoxicity (pM range) in a panel of 6 lymphoma cell lines with different levels of CD19 expression, as measured by flow cytometry and IHC. Notably, Lonca was active in cell lines with very low CD19, including one cell line where almost all cells were CD19 negative by IHC.
- QSP modeling predicts that CD19 expression level by IHC is not predictive of response to Lonca, whereas the addition of CD19 surface density improves the response prediction.
- Virtual patients responded to Lonca with estimated CD19 tumor cell surface densities as low as 1,000 molecules/cell, which is normally below the level of IHC detection.
- Our findings indicate that Lonca is an effective treatment option for patients with R/R DLBCL following ≥ 2 lines of treatment, even in patients expected to have a low level of CD19 expression.
- These results serve as a basis for future studies to address the sequencing of CD19-targeted agents.

Acknowledgments

ADC Therapeutics SA; medical writing support: CiTRUS Health Group.

References

1. Shah et al. *Front Oncol*. 2019;9:146.
2. Caimi et al. *Lancet Oncol*. 2021;22:790-800.
3. ZYNLONTA® Prescribing Information. ADC Therapeutics SA; 2022.

***Contact Information:**

Paolo F. Caimi, MD: caimip@ccf.org

Poster presented at the 64th Annual American Society of Hematology (ASH) Meeting. December 10-13, 2022, New Orleans, LA USA