Poster
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Favorable Tumor Immune Microenvironment and Robust CAR T-Cell Expansion May Overcome Tumor Burden and Promote Durable Efficacy With Axicabtagene Ciloleucel in Large B-Cell Lymphoma

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BACKGROUND

- Axicabtagene ciloleuel (axi-cel) is an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy approved for the treatment of adult patients with relapsed/refractory large B-cell lymphoma (LBCL) or follicular lymphoma after ≥2 prior lines of systemic therapy[1]
- In the ZUMA-1 (NCT02348216) multicenter, single-arm, registrational, Phase 1/2 study of axi-cel in patients with refractory LBCL, with a median follow-up of 27.1 months (n=101)[2]:
  - 83% Objective response rate (58% complete response rate)
  - Grade ≥3 cytokine release syndrome and neurologic events were reported in 11% and 32% of patients, respectively
- In a 4-year follow-up analysis (median, 51.1 months; n=101), median overall survival (OS) was 25.8 months, and the Kaplan-Meier estimate of the 4-year OS rate was 44%[3]
- In ZUMA-1, several factors have been shown to be associated with axi-cel response[4,5]:
  - Patients with high pretreatment tumor burden (estimated by sum of product diameters [SPD]) had lower peak CAR T-cell expansion and lower durable response rates versus patients with low pretreatment tumor burden[4]
  - The number of CD8+ and CCR7+CD45RA+ product T cells infused and favorable immune context in the pretreatment tumor microenvironment (TME), defined by the presence of activated CD8+PD-1+LAG-3+/–TIM-3– T cells, were also associated with axi-cel response[4,5]

OBJECTIVE

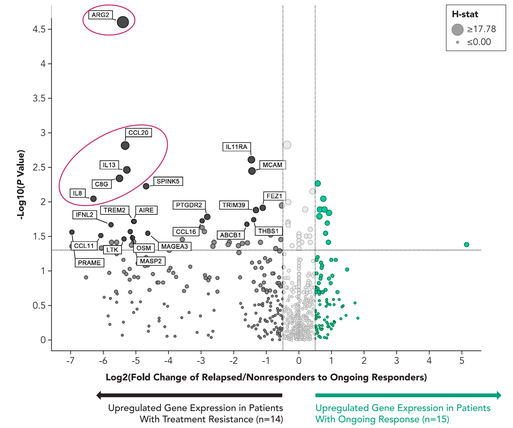
- To systematically analyze pretreatment TME characteristics that may influence CAR T-cell performance in patients with LBCL from ZUMA-1, particularly those with higher tumor burden and lower ongoing response rate

METHODS

- In this post-hoc analysis, evaluable samples from patients in ZUMA-1 Phase 1 and Phase 2 Cohorts 1–3 were analyzed; as such, n values vary by assay type
  - Cohorts 1 and 2 represent the pivotal cohorts[2,6]
  - Cohort 3, one of several exploratory safety management cohorts added to ZUMA-1, evaluated the prophylactic use of the anticonvulsant levetiracetam and the anti–interleukin-6 receptor antibody tocilizumab to minimize CAR T-cell treatment-related toxicities[7]
  - Patients in Phase 1 and Phase 2 Cohorts 1 and 2 had ≥2 years of follow-up (median, 27.1 months); patients in Cohort 3 had ≥6 months of follow-up (median, 9.8 months)
- Pretreatment immune TME was analyzed by multiplex immunohistochemistry and gene expression profiling (NanoString) as previously described[3,8]
- Baseline tumor burden (by SPD) was evaluated as previously described[4]
- Correlative analyses of the above covariates with clinical outcomes were performed by Spearman rank correlation or Wilcoxon or Kruskal-Wallis test
- Median tumor burden (by SPD) from ZUMA-1 Phase 1 and Phase 2 Cohorts 1+2 was used as a cutoff for high (>3721 mm^2) versus low (≤3721 mm^2) tumor burden
- Response definitions were defined according to response at time of data cutoff and were as follows:
  - Ongoing/durable responders: patients who achieved a complete or partial response and remained in response
  - Nonresponders: patients who experienced stable or progressive disease as best response
  - Relapsed: patients who achieved a complete or partial response and subsequently experienced disease progression

RESULTS

Figure 1. Pretreatment TME Markers Were Differentially Expressed in Patients Who Failed to Achieve Durable Response After Axi-Cel Infusion

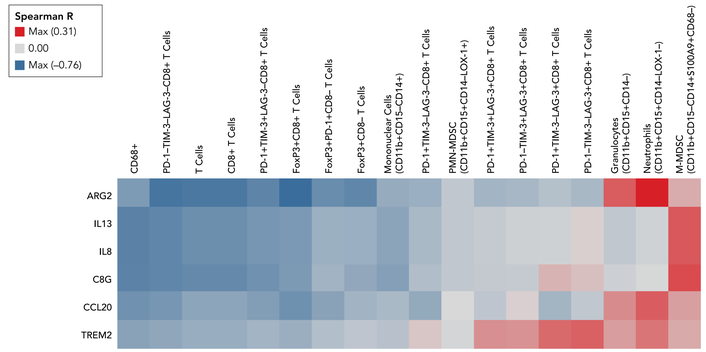


Fold change of relapsed/nonresponders to ongoing responders was determined by the ratio of "median gene count +1" from ongoing responders to relapsed/nonresponders to avoid zeros in the ratio. *P* values were obtained from Wilcoxon test. Dot size is based on the H-stat of Wilcoxon test.
ARG2, arginase 2; axi-cel, axicabtagene ciloleucel; CCL, chemokine ligand; COL3, collagen type III; EPCAM, epithelial cell adhesion molecule; IFN, interferon; IL, interleukin; ITG, integrin; MAGE, melanoma-associated antigen; MASP, mannan-binding lectin serin protease; MCM, minichromosome maintenance complex component; OSM, oncostatin M; PTGDR, prostaglandin D2 receptor; SPA, sperm surface protein; TME, tumor microenvironment; TREM2, triggering receptor expressed on myeloid cells 2; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor; XCR, X-C motif chemokine receptor.

- Pretreatment immune TME features related to suppressive myeloid-related activity, most notably *ARG2*, *TREM2*, and *IL-8* gene expression, were elevated in patients who failed to respond to axi-cel or relapsed without documented loss of CD19 expression

RESULTS *(continued)*

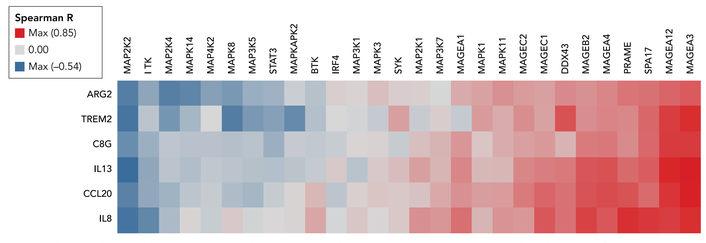
Figure 2. Genes Negatively Associated With Ongoing Response Were Positively Associated With the Myeloid Population in the TME



Data are included for 12 patients from ZUMA-1 Cohorts 1–3 with evaluable samples for both gene expression analyses and multiplex immunohistochemistry. The genes presented in the heatmap were selected based on findings from Figure 1; specifically, these genes were upregulated in patients with treatment resistance versus ongoing responders. Cell values represent the Spearman rank correlation value (R) between the covariates shown. Red and blue shading indicate positive and negative associations, respectively, between covariates.
ARG2, arginase 2; C8G, complement C8 gamma chain; CCL, chemokine ligand; FoxP3, forkhead box protein P3; IL, interleukin; LAG-3, lymphocyte-activation gene 3; LOX-1, lectin-type oxidized low-density lipoprotein receptor 1; max, maximum; min, minimum; M-MDSC, monocyte myeloid-derived suppressor cell; PD-1, programmed cell death protein 1; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; S100A9, S100 calcium-binding protein A9; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3; TME, tumor microenvironment; TREM2, triggering receptor expressed on myeloid cells 2.

- Top genes differentially expressed in nonresponders or patients who relapsed showed positive association with myeloid cells and negative association with T cells in the TME

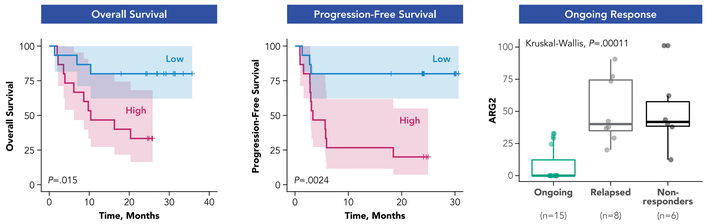
Figure 3. The Suppressive Myeloid Gene Signature Was Positively Associated With Gene Expression of Cancer Testis Antigens



Data are included for 30 patients from ZUMA-1 Cohorts 1–3 with evaluable samples for gene expression analyses. The genes presented in the heatmap were selected based on findings from Figure 1; specifically, these genes were upregulated in patients with treatment resistance versus ongoing responders. Cell values represent the Spearman rank correlation value (R) between the covariates shown. Red and blue shading indicate positive and negative associations, respectively, between covariates.
ARG2, arginase 2; BTK, Burton tyrosine kinase; C8G, complement C8 gamma chain; CCL, chemokine ligand; DDX43, DEAD-box helicase 43; IL, interleukin; IRF, interferon-regulatory factor; ITK, interleukin-2–inducible T-cell kinase; MAGE, melanoma antigen gene; MAP2K, mitogen-activated protein kinase kinase; MAP3K, mitogen-activated protein kinase kinase kinase; MAPKAPK, mitogen-activated protein kinase-activated protein kinase; max, maximum; min, minimum; PRAME, preferentially expressed antigen of melanoma; SPA17, sperm surface protein Sp17; STAT, signal transducer and activator of transcription; SYK, spleen associated tyrosine kinase; TREM2, triggering receptor expressed on myeloid cells 2.

- Cancer testis antigen genes were shown to be negatively associated with best response[8]

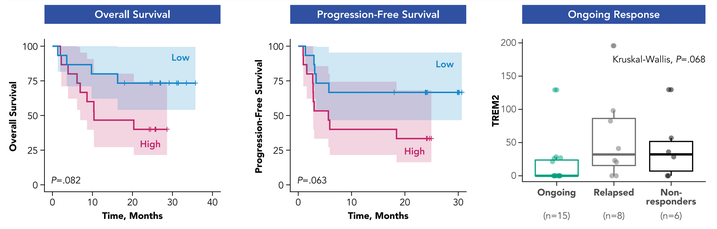
Figure 4. Expression of *ARG2* Was Negatively Associated With Durable Response



	ARG2 Low (n=15)	*ARG2* High (n=15)
ORR, n (%)	14 (93)	10 (67)
CR rate, n (%)	14 (93)	8 (53)
Ongoing response, n (%)	12 (80)	3 (20)
Grade ≥3 CRS, n (%)	1 (7)	3 (20)
Grade ≥3 NEs, n (%)	2 (13)	6 (40)
Median CAR peak, cells/μL	60	32
Median CAR peak/tumor burden (SPD), cells/mm²	0.0237	0.0068

Median expression of *ARG2* was used to determine the high versus low cutoff point in the Kaplan-Meier curves. At the time of data cutoff, responses (ie, ongoing response, relapsed, nonresponder) were known for 29 of 30 evaluable patients.
ARG2, arginase 2; CAR, chimeric antigen receptor; CR, complete response; CRS, cytokine release syndrome; ORR, objective response rate; NE, neurologic event; SPD, sum of product diameters.

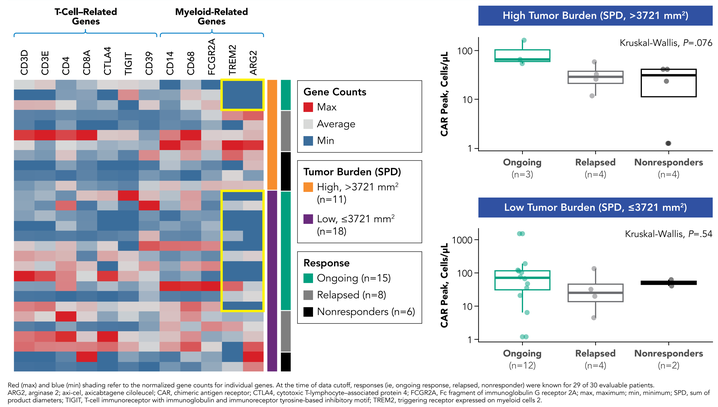
Figure 5. Expression of *TREM2* Trended Negatively With Durable Response



	TREM2 Low (n=15)	*TREM2* High (n=15)
ORR, n (%)	13 (87)	11 (73)
CR rate, n (%)	13 (87)	9 (60)
Ongoing response, n (%)	10 (67)	5 (33)
Grade ≥3 CRS, n (%)	2 (13)	2 (13)
Grade ≥3 NEs, n (%)	4 (27)	4 (27)
Median CAR peak, cells/μL	64	32
Median CAR peak/tumor burden (SPD), cells/mm²	0.017	0.008

Median expression of *TREM2* was used to determine the high versus low cutoff point in the Kaplan-Meier curves. At the time of data cutoff, responses (ie, ongoing response, relapsed, nonresponder) were known for 29 of 30 evaluable patients.
CAR, chimeric antigen receptor; CR, complete response; CRS, cytokine release syndrome; ORR, objective response rate; NE, neurologic event; SPD, sum of product diameters; TREM2, triggering receptor expressed on myeloid cells 2.

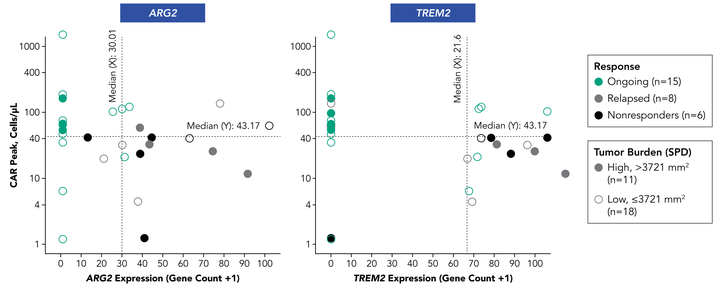
Figure 6. Association Between Pretreatment Myeloid-Related TME Gene Expression Signature and Tumor Burden, CAR T-Cell Expansion, and Efficacy



Red (max) and blue (min) shading refer to the normalized gene counts for individual genes. At the time of data cutoff, responses (ie, ongoing response, relapsed, nonresponder) were known for 29 of 30 evaluable patients.
ARG2, arginase 2; axi-cel, axicabtagene ciloleucel; CAR, chimeric antigen receptor; CTLA4, cytotoxic T-lymphocyte–associated protein 4; FCGR2A, Fc fragment of immunoglobulin G receptor 2A; max, maximum; min, minimum; SPD, sum of product diameters; TIGIT, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif; TREM2, triggering receptor expressed on myeloid cells 2.

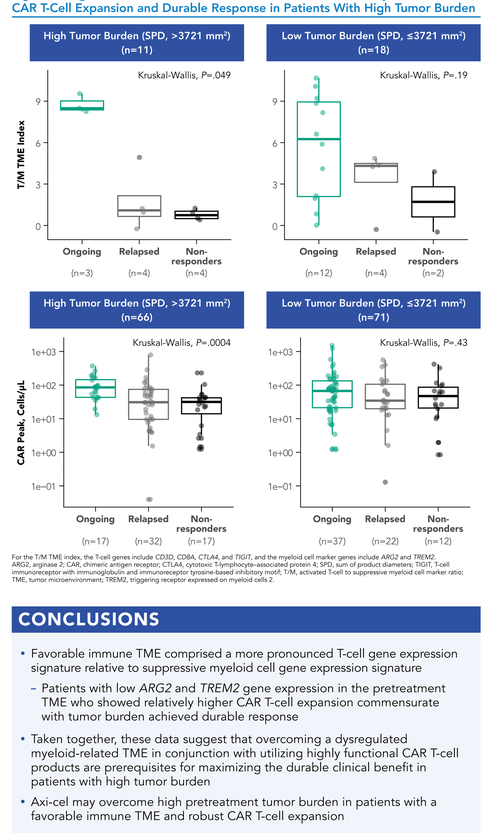
- Patients with low *ARG2* and *TREM2* levels in pretreatment TME who showed relatively higher CAR T-cell expansion commensurate with tumor burden achieved durable responses (**Figure 6** and **Figure 7**)
- Conversely, patients with high *ARG2* and *TREM2* levels in pretreatment TME were enriched for high tumor burden as were nonresponders and patients who relapsed

Figure 7. Pretreatment TME *ARG2* and *TREM2* Expression Versus CAR T-Cell Expansion and Clinical Efficacy



ARG2, arginase 2; CAR, chimeric antigen receptor; SPD, sum of product diameters; TME, tumor microenvironment; TREM2, triggering receptor expressed on myeloid cells 2.

Figure 8. High Pretreatment T/M TME Index Associated Positively With CAR T-Cell Expansion and Durable Response in Patients With High Tumor Burden



For the *T/M TME* index, the T-cell genes include *CD3D*, *CD8A*, *CTLA4*, and *TIGIT*, and the myeloid cell marker genes include *ARG2* and *TREM2*.
ARG2, arginase 2; CAR, chimeric antigen receptor; CTLA4, cytotoxic T-lymphocyte–associated protein 4; SPD, sum of product diameters; TIGIT, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif; T/M, activated T-cell to suppressive myeloid cell marker ratio; TME, tumor microenvironment; TREM2, triggering receptor expressed on myeloid cells 2.

CONCLUSIONS

- Favorable immune TME comprised a more pronounced T-cell gene expression signature relative to suppressive myeloid cell gene expression signature
  - Patients with low *ARG2* and *TREM2* gene expression in the pretreatment TME who showed relatively higher CAR T-cell expansion commensurate with tumor burden achieved durable response
- Taken together, these data suggest that overcoming a dysregulated myeloid-related TME in conjunction with utilizing highly functional CAR T-cell products are prerequisites for maximizing the durable clinical benefit in patients with high tumor burden
- Axi-cel may overcome high pretreatment tumor burden in patients with a favorable immune TME and robust CAR T-cell expansion

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