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FULL-LENGTH ARTICLE

Manufacturing and Regulatory Practices

Successful generation of fully human, second generation, anti-CD19 CAR T cells for clinical use in patients with diverse autoimmune disorders

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ABSTRACT

Background: B-cell targeting chimeric antigen receptor (CAR) T-cell therapies, which lead to profound B-cell depletion, have been well-established in hematology-oncology. This deep B-cell depletion mechanism has prompted the exploration of their use in B-cell driven autoimmune diseases. We herein report on the manufacturing of KYV-101, a fully human anti-CD19 CAR T-cell therapy, derived from patients who were treated across a spectrum of autoimmune diseases.

Methods: KYV-101 was manufactured from peripheral blood-derived mononuclear cells of 20 patients across seven autoimmune disease types (neurological autoimmune diseases, n = 13; rheumatological autoimmune diseases, n = 7). Patients ranged from 18 to 75 years of age. Duration of disease ranged from <1 to 23 years since diagnosis. Patients were heavily pretreated, and most were refractory to prior immunosuppressive treatments. Apheresis was collected across nine sites, cryopreserved, and shipped to the manufacturing facility. Healthy donor apheresis samples were collected for manufacturing comparison. Manufacturing was performed using the CliniMACS Prodigy system. Cells were enriched for $CD4^+/CD8^+$ T cells, transduced with a third generation lentiviral vector encoding the CAR, expanded in vitro, and harvested. Percent cell viability, T-cell purity, cellular expansion, and transduction efficiency were assessed. Activity was assessed using cytokine release assays for KYV-101 CAR T cells co-cultured with different $CD19^{+/-}$ target cell lines.

Results: KYV-101 was successfully manufactured for 100% of patients. Transduced cell populations were highly viable, with expansion ranging from 11 to 66 fold at Day 8, and were comparable across disease types. Healthy donor-derived controls displayed similar expansion ranges. High CAR expression and transduction rates were observed, ranging between 37 and 77% with low variation in transgene copy number (two to four

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per cell). Cell viability of the final KYV-101 drug product ranged from 87 to 97%. KYV-101 displayed robust CD19-dependent and effector dose-related release of the pro-inflammatory cytokine IFN-γ.
Conclusions: KYV-101 manufacturing yielded a CAR T-cell product with high viability and consistent composition and functionality, regardless of disease indication, pre-treatment, and heterogeneity of the incoming material. Cryopreservation of the apheresis and final drug product enabled widespread distribution. These results support the robustness of the manufacturing process for the fully human KYV-101 anti-CD19 CAR T-cell therapy drug product for patients across diverse autoimmune disease types.

Introduction

The role for B cells in driving autoimmune disease pathology has been well established [1] and is supported by the efficacy demonstrated with B-cell targeting therapies such as monoclonal antibodies [2-4]. Although existing B-cell targeting therapies show potential in treating autoimmune diseases, in most patients, multiple therapeutic approaches are required, including life-long treatments harboring risks for long-term treatment-related toxicities [5-7]. Accordingly, there is still an unmet need for novel therapies that can result in sustained treatment-free remission, reduce long-term toxicity risk, and halt progression of disability in patients with autoimmune diseases [7,8].

Chimeric antigen receptor (CAR) T-cell therapies engineered to target the B-cell protein CD19 have been approved for different B-cell malignancies [9], and demonstrate the potential for eliciting long-lasting, treatment-free remissions [10]. The clinical experience with CAR T-cell therapy in B-cell malignancies has led investigators to begin exploring CAR T-cell therapy as a means to address autoimmune diseases. Although clinical findings are still premature, initial reports leveraging CAR T-cell therapies have been encouraging, with efficacy demonstrated in different autoimmune disease types. In a case study of 5 patients with systemic lupus erythematosus (SLE) who received anti-CD19 CAR T-cell therapy, rapid and profound disease improvements were observed, and all patients remained in ongoing treatment-free remission at a median of 8 months post-infusion [11,12]. Similarly encouraging reports for CAR T-cell therapies have been reported for initial use in patients with systemic sclerosis (SSc) [13,14], myositis [15,16], and myasthenia gravis [17,18].

In the hematology-oncology setting, CAR T-cell therapies are also associated with potentially serious or life-threatening safety concerns such as Grade 3/4 cytokine release syndrome (CRS), Grade 3/4 immune effector cell-associated neurotoxicity syndrome (ICANS), and hematotoxicity [19–21]. Emerging data point to a substantially more favorable safety profile for CAR T-cell therapy use in patients with autoimmune disease [22,23]. Maximizing the efficacy while minimizing the toxicity of CAR T-cell therapies will be an important component of their establishment as autoimmune disease treatments, given that such patients often have longer life expectancies than those with malignancies, and may be less tolerant of potentially serious side effects that shift the risk/benefit ratio in an unfavorable direction. Although a variety of factors (including patient and disease-related factors) can contribute to the safety profile for a given CAR T-cell therapy, there are still areas for improvement in the CARs themselves that are currently approved for use in hematology-oncology and are being explored in autoimmune diseases.

The manufacturing process for CAR T-cell therapy involves harvesting of the patients' circulating T cells, genetic modification of cells in vitro to express the CAR transgene, and expansion of the CAR T cells to ultimately form a final functional drug product. Upon reinfusion, the CAR directs T cells, through its specificity and function, toward eliminating target cells, which include pathogenic cells (e.g., $CD19^+$ B-cells and/or B-cell-derived malignant cells) [24]. Manufacturing challenges, sometimes associated with the quality of the patient's collected cells, have been encountered in hematology-oncology. Such patients may have previously received chemotherapeutic regimens that can impact their existing T-cell populations, and certain hematological malignancies may present with abnormally elevated blasts in the peripheral blood and consequently the apheresis starting material, impacting CAR T-cell production [25]. In addition, the tumor microenvironment is known to lead to functional impairment of infiltrating T lymphocytes [26], which may lead to defects in manufacturing starting material. The bulk of CAR T-cell manufacturing knowledge has been gleaned from the hematology-oncology space thus far, and in the autoimmune disease setting, these challenges may be less of an issue. It must still be considered, however, that long-term treatment with immunosuppressants such as steroids, or the underlying disease itself (such as lymphopenia in SLE) could still represent potential obstacles to the production of functional CAR T-cell products for patients with autoimmune disease [27,28].

Since initial clinical use in autoimmune disease is still limited, reports exploring aspects of manufacturing feasibility for CAR T-cell therapies derived from patients with autoimmune diseases are also limited. One recent study published by Kretschmann et al. explored manufacturing of anti-CD19 CAR T-cell products derived from a cohort of six patients with SLE, and sufficient yields of functional CAR T-cell product were observed for all six of the patients [29]. These initial findings are encouraging, and in turn they raise questions regarding the optimization of CAR T-cell manufacturing, and how feasible it is across an even wider breadth of autoimmune diseases.

KYV-101 is a first-in-class, fully human, autologous, second generation anti-CD19 CAR T-cell therapy, which includes a CAR designed by the National Institutes of Health (Hu19-CD828z) to improve tolerability. In a phase 1 clinical trial of 20 patients with B-cell lymphomas or chronic lymphocytic leukemia, treatment with T cells expressing the fully human Hu19-CD828z construct with CD8α hinge and transmembrane domains, a CD28 co-stimulatory domain, and a CD3ζ activation domain, elicited anti-lymphoma activity and was associated with a lower incidence and severity of both CRS and ICANS compared to treatment with CAR T cells expressing an anti-CD19 CAR comprising a murine-derived antigen recognition domain, CD28 hinge, transmembrane and co-stimulatory domains, and a CD3z activation domain [30]. Hu19-CD828z CAR T-cells manufactured using cells from patients with SLE exhibited CAR-mediated and CD19-dependent activity upon co-culture with autologous primary B cells [31]. KYV-101 is currently being explored for the treatment of several types of neurological and rheumatological autoimmune diseases across multiple clinical studies and in named patient use [17,32,33].

Herein, we report the viability and potency for KYV-101, manufactured for a cohort of 20 patients with seven different types of autoimmune diseases. This analysis presents the aggregate of all individual batches and patient data from these initial 20 patients.

Methods

Patients

All patients with autoimmune disease who were included in the analysis were treated with KYV-101 through one of the following: an

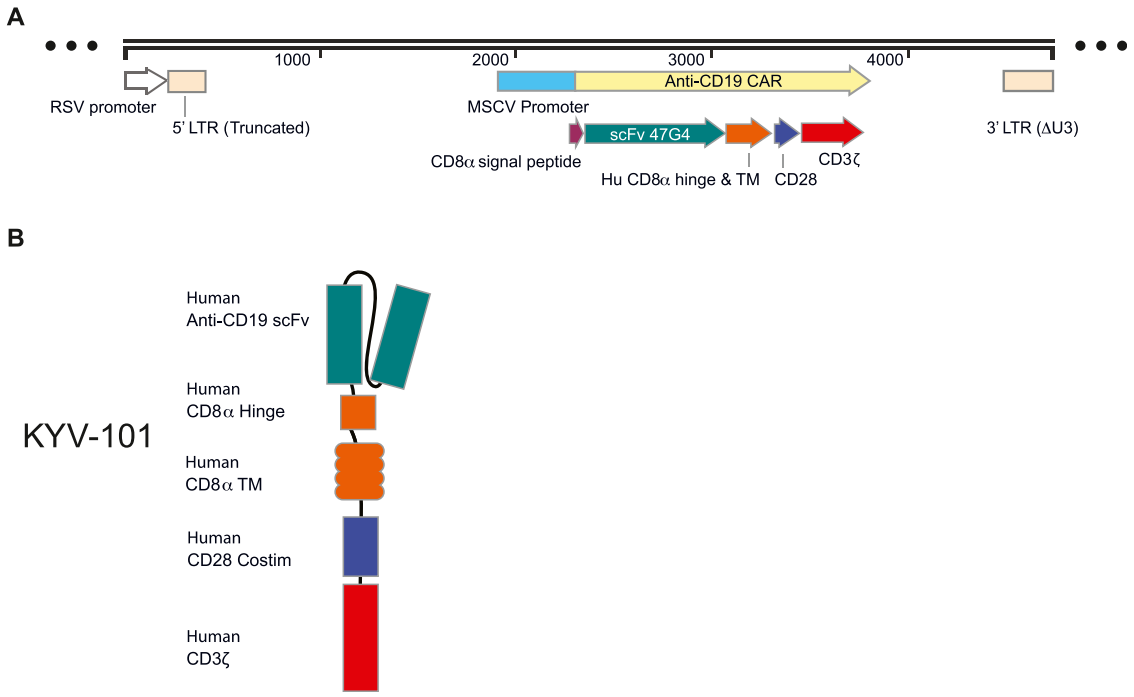

Fig. 1. Anti-CD19 CAR transgene for KYV-101. (A) Schematic depicting the anti-CD19 CAR transgene contained by the 3rd generation lentiviral vector KL-h198a28z used in KYV-101 manufacturing. (B) Schematic depicting the fully human KYV-101 construct with each individual domain of the CAR. Abbreviations: CAR, chimeric antigen receptor; Costim, costimulatory; Hu, human; LTR, long terminal repeat; MSCV, murine stem cell virus; RSV, rous sarcoma virus; scFv, single-chain variable fragment; TM, transmembrane. (Color version of figure is available online.)

individualized off-label treatment approach established as a treatment opportunity for patients with severe diseases in Germany (Individueller Heilversuch, IH), enrollment in the Phase I/II KYSA-1 trial (US-based trial in lupus nephritis [LN]; NCT05938725), or enrollment in the Phase I/II KYSA-3 trial (Germany-based trial in LN; NCT06342960). For the IH treatment pathway, treatment refractory patients with autoimmune disease were generally selected by the treating physicians after multi-line treatment and lack of effective alternatives for CAR T-cell therapy. For the KYSA-1 and KYSA-3 trials, adult patients with biopsy-proven class III or IV LN with inadequate response to ≥2 conventional therapies were eligible.

Patients provided written, informed consent to be included in the study. For treatment through the IH pathway as named patient use, under German law, such individualized treatment approaches can be made in the case of life-threatening diseases. For other patients, the Institutional Review Board of each study site approved the protocol. All procedures and aspects of the study were conducted in compliance with the principles of the Declaration of Helsinki.

Patient apheresis and chain of identity

Apheresis was performed at each clinical site. The target cell range for the apheresis collection was between $1-2 \times 10^9$ total cells distributed between two bags at $0.5-1 \times 10^9$ cells per bag. Instructions specified that two apheresis bags should be collected to ensure there was a backup source of material in case the first bag did not yield successful manufacturing.

Following cryopreservation, the apheresis products were transported to the central Kyverna manufacturing facility. The apheresis product was labeled with two unique identifiers consisting of a non-sequential pseudonymized patient number and an International Society for Blood Transfusion donor identity code or Single European Code-compliant donation identifier, which ensured chain of identity and chain of custody of the patient starting material and confirmation that each collection was suitable for further manufacturing.

Healthy donor samples

In addition to the 20 patients reported here, three healthy donor batches were also manufactured as part of the process development effort. These data served as a point of comparison for the patient derived batches. CAR T cells derived from healthy donor peripheral blood were produced using the same manufacturing process and quality control testing.

KYV-101 product manufacturing

After thawing the apheresis starting material, manufacturing was performed under strict Good Manufacturing Practice conditions using the CliniMACS Prodigy system (Miltenyi Biotec) with the T-cell transduction program and the CliniMACS TS 520 tubing set for automated closed-system processing. Cells were enriched for $CD4^+/CD8^+$ cells, activated, transduced with a third-generation lentiviral vector (KL-h198a28z) containing the Hu19-CD828Z CAR (Figure 1), and expanded. The Hu19-CD828Z CAR used in KYV-101 was developed and studied by the National Institutes of Health for the treatment of B-cell lymphoma [30]. In vitro cell expansion was assessed using the NucleoCounter NC-200 (ChemoMetec). Cells were harvested during the exponential phase of expansion and formulated in Plasmalyte, CS-10, and human serum albumin for manufacture of the KYV-101 drug product, which was cryopreserved. Manufacturing success was defined as successful generation and delivery of KYV-101 drug product to the patient without the need to re-collect apheresis starting material.

Fully human anti-CD19 CAR construct

The anti-CD19 CAR (Hu19-CD828Z) construct used for manufacturing was originally developed by the National Institutes of Health and is licensed exclusively by Kyverna for autoimmune applications. The fully human Hu19-CD828Z CAR contains a single-chain fragment variable derived from a human anti-CD19 monoclonal

Table 1
Patient demographics and characteristics.

Disease type	Patient number	Age (years)	Years since initial diagnosis	Number of prior therapies[a]
MG	1	33	11	4
MG	2	33	6	9
MG	3	45	6	5
MG	4	75	1	4
MG	5	38	5	5
MG	6	36	10	5
MG	7	24	2	5
MS	8	47	23	1
MS	9	36	4	1
MS	10	36	3	2
SPS	11	69	9	6
SPS	12	61	13	5
AIE	13	34	<1	4
LN	14	18	10	10
LN	15	28	2	8
LN	16	29	5	5
LN	17	19	7	9
LN	18	55	25	9
IgG4RD	19	59	10	3
SSc	20	53	2	2

[a]Includes immunosuppressive and immune-modulating therapies reported as treating the respective autoimmune disease.
Abbreviations: AIE, autoantibody-mediated autoimmune encephalitis; IgG4RD, immunoglobulin G4-related disease; LN, lupus nephritis; MG, myasthenia gravis; MS, multiple sclerosis; SPS, stiff person syndrome; SSc, systemic sclerosis.

antibody, CD8α hinge and transmembrane domains, a CD28 costimulatory domain, and a CD3ζ activation domain, as previously described [30].

Cell viability, T-cell, B-cell, and NK-cell (TBNK) panel, and other analyses

Viable cell counts and % viability were assessed using the Nucleo-Counter NC-200 (ChemoMetec) for the thawed apheresis product, following $CD4^+/CD8^+$ enrichment, and for the final, thawed KYV-101 drug product. Flow-cytometry assessments using a MACSQuant Analyzer 10 (Miltenyi Biotec) for the distribution of total T cells, helper T cells, cytotoxic T cells, B cells, eosinophils, monocytes, neutrophils and natural killer T (NKT) cells and $CD56^+CD16^+$ (natural killer [NK]) cells (i.e., the TBNK panel) were also performed for the thawed apheresis product, following $CD4^+/CD8^+$ enrichment, and for the final KYV-101 drug product. ANOVA was used to detect differences in distributions of TBNK subpopulations among all groups (ie, myasthenia gravis, other neurological indications, rheumatological indications, and healthy donors), followed by Tukey's honestly significant difference test (Tukey's HSD) for pairwise comparisons of TBNK subpopulations between groups. $CD4^+/CD8^+$ ratios were calculated using the results from the TBNK panel. CAR transduction efficiency was assessed using flow cytometry.

Cytokine release assays

For selected patient-derived samples, harvested anti-CD19 CAR T-cells (post-thaw), the effector cells, were co-cultured with target cells, either the well-characterized $CD19^+$ Raji cell line (as a positive control) or the $CD19^-$ CEM/C1 cell line (as a negative control). Cells were cultured at ratios of either 10:1 or 5:1 (KYV-101:target cell lines). IFN-γ cytokine release was profiled after 20–24 h of co-culture and the supernatant was removed and frozen prior to further testing. Upon thaw of the supernatants, each sample was assayed using a Quantikine IFN-γ ELISA Kit (RnDSystems). Samples were then analyzed by measuring the absorbance of the wells at 450 nm with a background subtraction at 570 nm, using a Synergy 2 Multi-Mode Microplate Reader (BioTek).

Data plotting

GraphPad Prism version 10.0.3 was used for generation of the figures.

Results

Patients

In total, anti-CD19 CAR T-cell products (KYV-101) were manufactured from 20 patients, spanning seven autoimmune disease types, who were receiving treatment from nine distinct sites. Of the 20 patients, those with neurological autoimmune diseases (n = 13) included seven patients with myasthenia gravis, three with multiple sclerosis (MS), two with stiff person syndrome (SPS), and one with autoantibody-mediated autoimmune encephalitis. Patients with rheumatological autoimmune diseases (n = 7) included five with LN, one with SSc, and one with immunoglobulin G4-related disease.

Patient ages ranged from 18 to 75 years, and duration of disease ranged from <1 to 23 years since diagnosis. Overall, patients were heavily pretreated, and most were refractory to prior treatments. Demographics and characteristics are shown in Table 1.

Characterization of apheresis and enrichment of $CD4^+/CD8^+$ T lymphocytes

For 19 (95%) patients, the minimum target apheresis yield was met at the clinical site, as described in the Methods. For one patient (Patient #1), apheresis yielded 4×10^8 total cells in a single bag. Cryopreserved apheresis products from all 20 patients were received and thawed at the manufacturing site. Cell viability of the thawed apheresis products ranged from 62 to 97% (Table 2), and the number of viable cells recovered post thaw ranged from 2.7×10^8 to 7.5×10^9 cells. Multicolor flow cytometry based TBNK analysis of the thawed apheresis products confirmed an expected distribution across multiple cell phenotypes, with the predominant population being T lymphocytes (Figure 2A). TBNK distributions were similar between patients with myasthenia gravis, patients with other

Table 2
Percent cell viability throughout the manufacturing process.

Disease type	Patient #	Cell viability, %			
		Post-thaw apheresis product	Post-enrichment	Pre-harvest	Final drug product
MG	1	95	98	97	92
MG	2	93	99	98	95
MG	3	97	98	97	95
MG	4	93	98	94	94
MG	5	91	98	98	94
MG	6	88	94	95	94
MG	7	71	93	95	95
MS	8	92	99	99	97
MS	9	93	99	93	95
MS	10	94	99	96	93
SPS	11	72	94	94	92
SPS	12	81	98	96	87
AIE	13	77	94	97	94
LN	14	62	90	98	96
LN	15	82	97	95	94
LN	16	92	97	97	96
LN	17	66	91	97	95
LN	18	64	93	96	92
IgG4RD	19	82	98	97	94
SSc	20	76	97	96	95

Abbreviations: AIE, autoimmune encephalitis; IgG4RD, immunoglobulin G4-related disease; LN, lupus nephritis; MG, myasthenia gravis; MS, multiple sclerosis; SPS, stiff person syndrome; SSc, systemic sclerosis.

neurological autoimmune diseases, and patients with rheumatological autoimmune diseases (Figure 2B–2D). All 20 patient-derived apheresis samples were deemed viable to proceed to enrichment.

Successful enrichment of $CD4^+/CD8^+$ T cells was achieved (Supplemental Figure 1A), with similar levels of enrichment observed across autoimmune disease types (Supplemental Figure 1B-1D). $CD4^+/CD8^+$

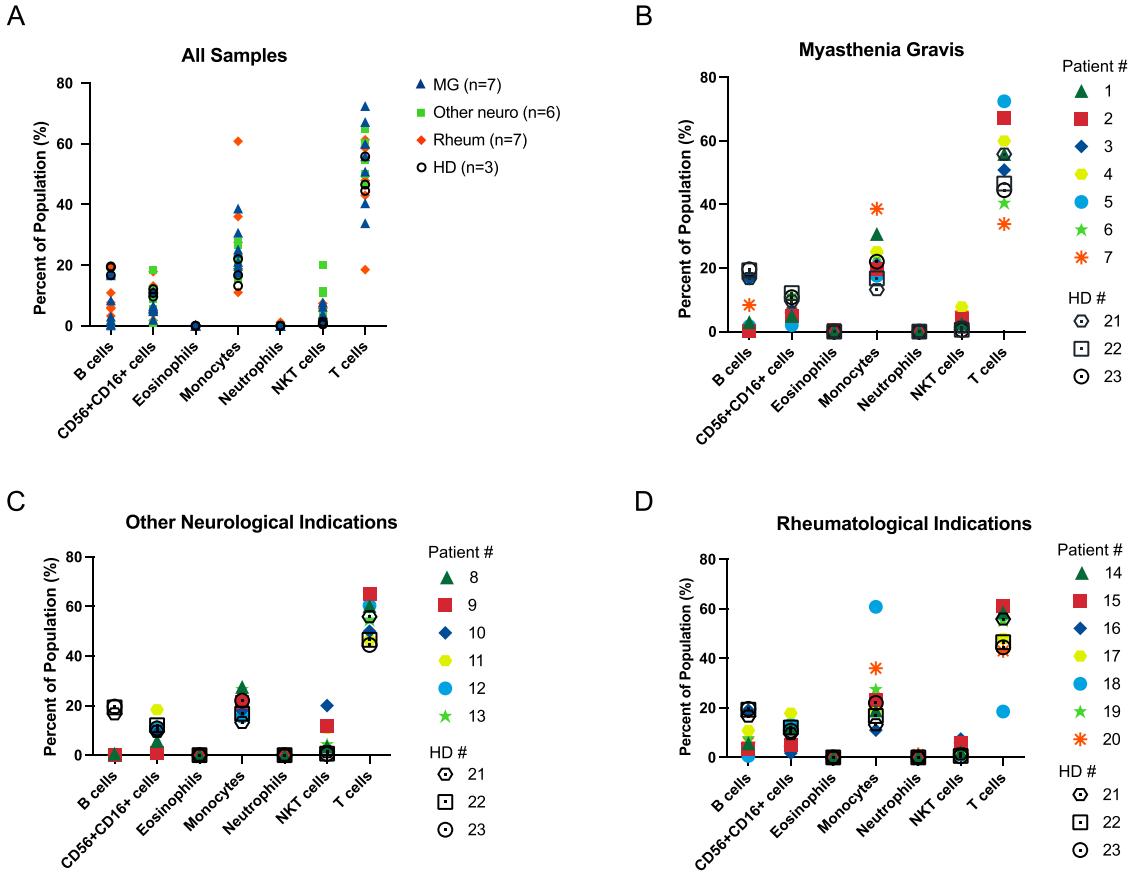

Fig. 2. Cellular composition of apheresis products. TBNK panel and percent T-cell purity analysis of the apheresis starting material for (A) all representative patient samples, (B) samples from patients with myasthenia gravis, (C) samples from patients with other neurological indications, or (D) samples from patients with rheumatological indications, each including healthy donor samples for comparison. Each symbol in the legend for panels B-D represents one patient or healthy donor, denoted by number. Data are shown as percentage of the total cell population. Abbreviations: HD, healthy donor; MG, myasthenia gravis; neuro, neurological indications; NK, natural killer; NKT cells, natural killer T cells; rheum, rheumatological indications; TBNK, distribution of total T-cells, helper T-cells, cytotoxic T-cells, B-cells, eosinophils, monocytes, neutrophils, NKT cells, and $CD56^+CD16^+$ (NK) cells. (Color version of figure is available online.)

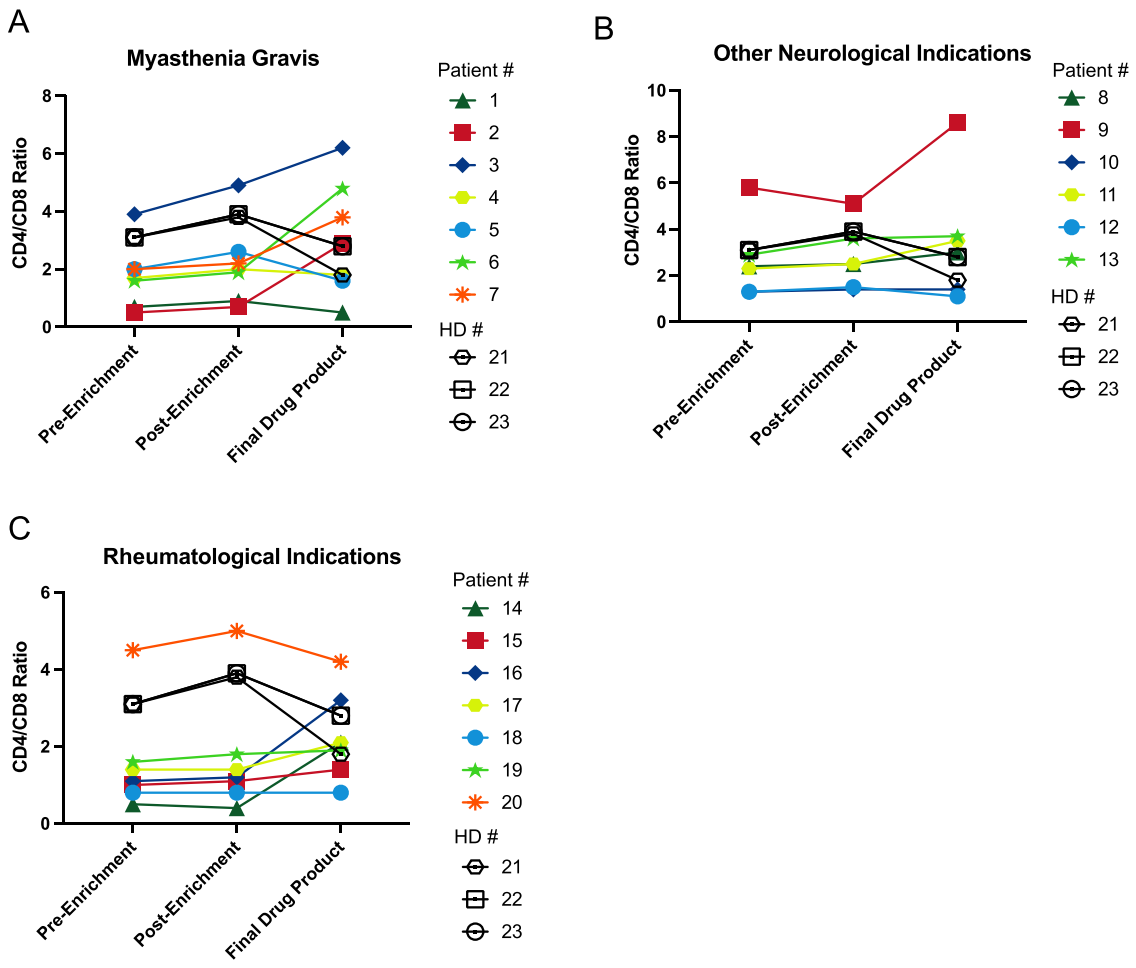

Fig. 3. CD4/CD8 ratios throughout the manufacturing process. CD4/CD8 ratios for (A) samples from patients with myasthenia gravis, (B) samples from patients with other neurological indications, or (C) samples from patients with rheumatological indications, each including healthy donor samples for comparison. Each symbol in the legend for panels A-C represents one patient or healthy donor, denoted by number. Abbreviations: HD, healthy donor. (Color version of figure is available online.)

ratios before and after enrichment are shown in Figure 3. Percent cell viability ranged from 90 to 99% across all enriched samples. ANOVA testing revealed statistically significant differences ($P < 0.05$) in the B cell and NKT cell subpopulations in the thawed apheresis starting material. In pairwise comparisons, the distribution of B cell subpopulations was different for healthy donors when compared to patients with myasthenia gravis, other neurological indications, and rheumatological indications. The distribution of NKT cell subpopulations was different for patients with neurological indications (other than myasthenia gravis) when compared to healthy donors and patients with myasthenia gravis or rheumatological indications. Regardless of the aforementioned variabilities in the apheresis starting material, $CD4^+$/$CD8^+$ T cells were successfully enriched to counts exceeding the minimum number required for subsequent steps, and all patient-derived samples were deemed suitable to proceed with anti-CD19 CAR T-cell manufacturing.

Generation of anti-CD19 CAR T-cell products

$CD4^+$/$CD8^+$ enriched T-cell samples were activated, and subsequently transduced with a third generation lentiviral vector encoding the fully human anti-CD19 CAR transgene [30]. Transduced cell populations displayed high viability, with 11-fold to 66-fold expansion at Day +8 across all 20 patient-derived clinical samples. Comparable ranges in fold expansion were observed regardless of whether patients had myasthenia gravis (Figure 4A), other neurological autoimmune diseases (Figure 4B), or rheumatological autoimmune diseases (Figure 4C). Control samples from healthy donors that were enriched and transduced equivalently displayed similar fold expansion ranges (Figure 4D), further supporting the viability of the transduced cell populations derived from patients with autoimmune diseases.

Transduction efficiency analysis (Supplemental Figure 2) revealed strong CAR expression and transduction rates ranging from 47 to 77% for neurological autoimmune diseases, 37 to 73% for rheumatological autoimmune diseases, and 50 to 75% for healthy donors (Figure 5A–5D). Low variations in CAR transgene copy number per transduced cell were also observed across samples (range, 2 to 4 per cell). In summary, these results indicated that the transduction process was both efficient and titrated appropriately, without notable distinction between rheumatological versus neurological autoimmune diseases.

Following transduction and expansion, cell viability of the harvested KYV-101 CAR T cells ranged from 93 to 98%. The final drug product displayed strong enrichment for T cells (Figure 6) that was consistent across autoimmune disease types (Supplemental Figures 3 and 4), with purity ranging from 85 to 100% across patient-derived samples. The final drug product cell viability ranged from 87 to 97% across patients, and final drug product $CD4^+$/$CD8^+$ ratios ranged from 0.5 to 8.6. CAR T-cell yield was high across the varying autoimmune disease types, and ANOVA testing revealed no significant differences among the groups (i.e., myasthenia gravis, other neurological

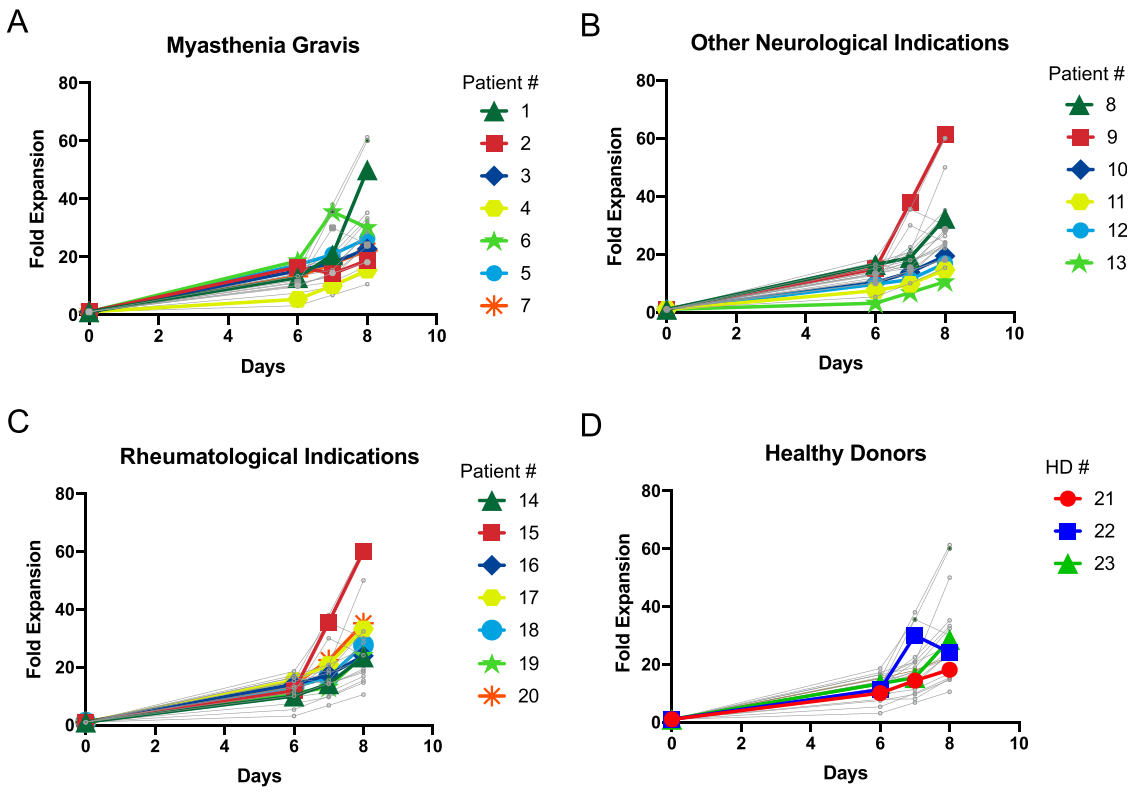

Fig. 4. Expansion of CAR T cells manufactured from patients with autoimmune diseases. Panels show in vitro fold expansion of CAR T cells derived from patients with (A) myasthenia gravis, (B) other neurological indications, (C) rheumatological indications, and (D) healthy donors. Each symbol represents one patient or healthy donor, denoted by number. Colored lines depict expansion for the disease type(s) indicated per panel, with superposition of other patients or healthy donors shown in grey for reference. Abbreviations: CAR, chimeric antigen receptor; HD, healthy donor. (Color version of figure is available online.)

indications, rheumatological indications, and healthy donors) in distribution of TBNK subpopulations in the final drug product. The overall median time from manufacturing start to release of the finished product was 29 days, with a minimum of 20 days and a maximum of 44 days. Despite the comparatively lower number of cells collected for Patient #1 during apheresis, the final drug product was successfully manufactured for this patient. Overall, the process demonstrated a 100% manufacturing success rate for all 20 patients.

In vitro activity of anti-CD19 CAR T-cell products

An in vitro cytokine release assay was used to assess the functional activity of the patient derived anti-CD19 CAR T-cell products post-thaw. When KYV-101 effector cells were co-cultured with the $CD19^+$ Raji target cell line, patient-derived anti-CD19 CAR T cells displayed robust release of the pro-inflammatory cytokine IFN-γ. Conversely, co-culture with the $CD19^-$ CEM/C1 target cell line did not elicit notable IFN-γ release, indicating that anti-CD19 CAR T-cells displayed clear target specificity. Patient-derived anti-CD19 CAR T cells also released higher levels of IFN-γ when co-cultured with target $CD19^+$ cells at a ratio of 10:1 versus 5:1, demonstrating effector cell dose-dependent activity. These observations were consistent across anti-CD19 CAR T cells derived from patients with neurological and rheumatological autoimmune diseases (Figure 7A–7C).

Preliminary clinical findings

Clinical evaluation of the KYV-101-treated patients included in this analysis is ongoing, with promising initial results. Despite the initially low apheresis yield for Patient #1 (a 33-year-old with severe, treatment-refractory, anti-acetylcholine receptor [AChR]-positive generalized myasthenia gravis), treatment with KYV-101 resulted in rapid, profound, and sustained improvements of disease severity [17]. A 70% reduction in pathogenic anti-AChR antibodies was paralleled by improvements in Besinger disease activity and Quantitative Myasthenia Gravis scores, with improved muscle strength, fatigue, arm holding time, and drastically improved walking ability without the use of supportive devices. Patient #1 also experienced no adverse events related to CAR T-cell therapy, such as CRS or ICANS [17]. Patients #2 and #3 (myasthenic syndromes) also displayed encouraging results following KYV-101 infusion, with reductions in pathogenic autoantibodies paralleled by rapid, sustained clinical recovery and full recovery of mobility [32]. Both patients experienced mild CRS (Grades 1-2), and Patient #2 experienced mild ICANS (Grade 1), all of which were successfully managed [32]. For Patients #8 and #9 (MS), KYV-101 demonstrated an acceptable safety profile with no observed neurological toxicity such as ICANS [33]. In Patient #8, a sustained, notable decrease in intrathecal antibody production was also observed following KYV-101 infusion [33]. Early analyses from the KYSA-1 trial in SLE (Patients #14 and #15 in this report) showed KYV-101 was well tolerated, with both patients experiencing only mild CRS (Grade 1), no ICANS, and showing evidence of clinical improvement [34]. Complete clinical findings from the patients included in this analysis will be published when available.

Discussion

The findings described herein demonstrate successful manufacturing of viable and functional anti-CD19 CAR T cells derived from patients with diverse B-cell-driven autoimmune diseases. Notably, manufacturing success was consistent despite several patient-related and logistical variables, including the use of samples derived from patients with neurological or rheumatological autoimmune diseases. CAR T-cell production was also successful despite a wide age

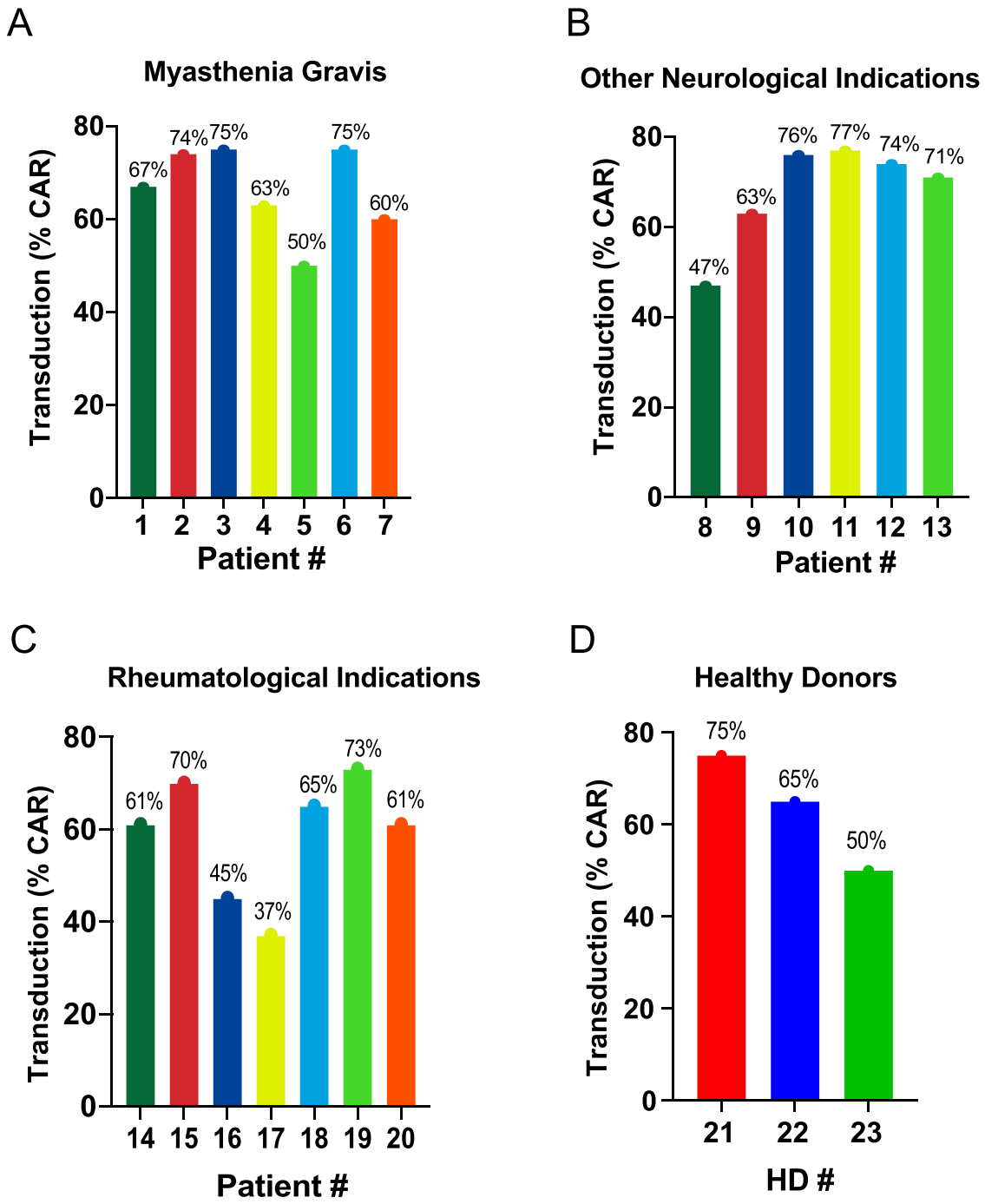

Fig. 5. Transduction efficiency for the anti-CD19 CAR construct. CAR transduction efficiency for patients with (A) myasthenia gravis, (B) other neurological autoimmune diseases, (C) rheumatological diseases, or (D) healthy donors. Each bar represents one patient or healthy donor. Abbreviations: CAR, chimeric antigen receptor; HD, healthy donor. (Color version of figure is available online.)

range (18–75 years), despite multiple prior therapies, many years since disease diagnosis in most patients, and despite apheresis harvest and cryopreservation occurring across 9 distinct treatment sites. Despite Patient #1 yielding a lower apheresis cell count initially, the starting material proved to be sufficient, leading to successful manufacture of a highly viable final drug product with expected characteristics and robust functional activity. Finally, differences in TBNK subpopulations observed between the different patient groups and healthy donors in the starting material were not observed in the final drug product.

The final anti-CD19 CAR T-cell products also demonstrated activity that was consistent across autoimmune disease sources. The 100% manufacturing success rate observed for KYV-101 (in a median of 29 days) was also comparable to or better than the 88–100% rates that have been reported for CAR T-cell therapies in the oncology setting [35–37]. Therefore, for the patients with autoimmune diseases reported herein who were generally heavily pre-treated, the high levels of disease severities did not interfere with successful CAR T-cell production. These results collectively support the robustness of the manufacturing process for the fully human KYV-101 anti-CD19 CAR T-cell product.

Herein, we report a manufacturing success rate and product purity akin to the anti-CD19 CAR T-cell manufacturing result from the cohort of 6 patients with SLE reported by Kretschmann et al. [29]. However, compared to their reported mean CAR transduction efficiency of 25.8% (standard deviation, ±5.9; [29]), we observed

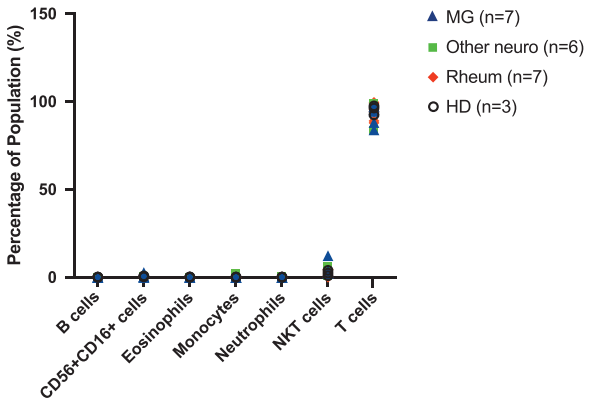

Fig. 6. Composition of final CAR T-cell product. TBNK panel and percent T-cell purity analysis of the final drug product for all representative patient samples and healthy donors. Abbreviations: HD, healthy donor; MG, myasthenia gravis; neuro, neurological indications; NK, natural killer; NKT cells, natural killer T cells; Rheum, rheumatological indications; TBNK panel, distribution of total T cells, helper T cells, cytotoxic T cells, B cells, eosinophils, monocytes, neutrophils, NKT cells and $CD56^{+}CD16^{+}$ (NK) cells. (Color version of figure is available online.)

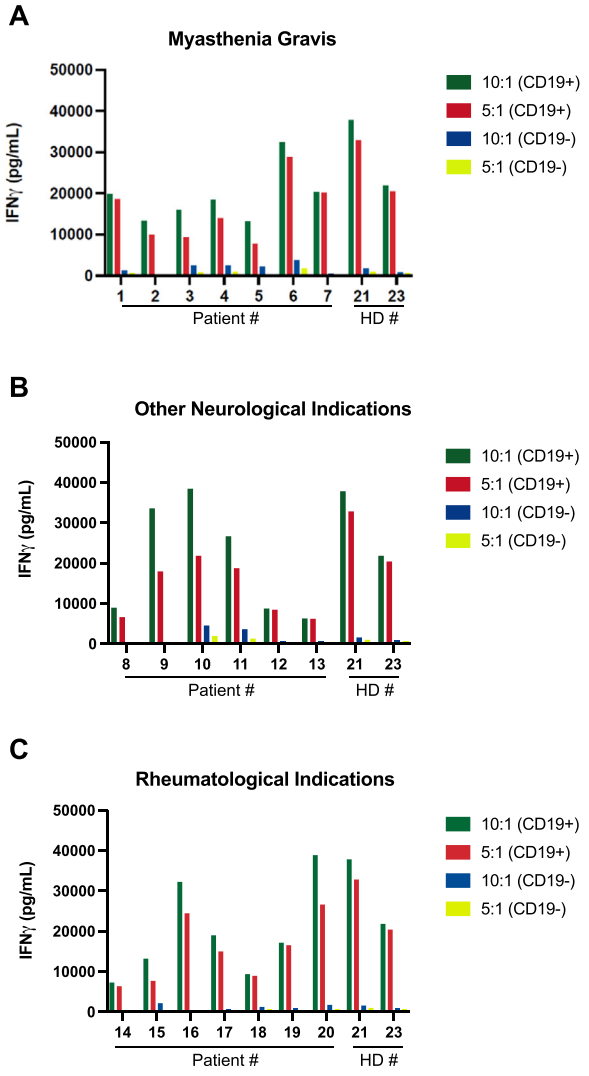

Fig. 7. In-vitro activity assays. Activity assays measuring IFN-γ secretion from thawed CAR T cells derived from (A) patients with myasthenia gravis (n = 7), (B) patients with other neurological indications (n = 6), or (C) patients with rheumatological indications (n = 7), compared with healthy donors (n = 2). CAR T cells were co-cultured at ratios of 10:1 and 5:1 with target $CD19^{+}$ controls (Raji cells) or $CD19^{-}$ controls (CEM/C1 cells). Abbreviations: CAR, chimeric antigen receptor; HD, healthy donor; IFN, interferon. (Color version of figure is available online.)

lentiviral transduction rates ranging from 37% to 70% for the five patients with LN, with similar transduction rate ranges for the other indications as well. Differences in transduction efficiency may be explained by several distinctive factors between the two manufacturing processes, including differences in lentivirus titer or multiplicity of infection values. It is worth noting that our manufacturing protocol leverages a third generation lentiviral vector to deliver the CAR transgene to target T cells, whereas Kretschmann et al. [29] use a second generation lentiviral vector (Milteny Biotec). Although it is not entirely clear whether the use of a third generation lentiviral vector is responsible for this phenomenon, differences in transduction efficiency have been reported favoring third generation over second generation lentiviral vector delivery of CARs in acute lymphoblastic leukemia models [38].

Diminished IFN-γ secretion *in vitro* for Patients 12, 13, 14, and 18 despite similar transduction efficiency to other patients may be due to differences in CAR T-cell persistence, trafficking, the tissue microenvironment, and the patients' immune systems. IFN-γ production alone does not reflect all these elements. Furthermore, variability in patient-specific factors, such as antigen expression, immune landscape, and prior treatments, can affect the relationship between *in vitro* IFN-γ production and *in vivo* efficacy. Thus, *in vitro* activity assessed by IFN-γ production should not be considered a proxy for clinical efficacy. These relationships will be explored as more clinical data become available.

Aside from transduction efficiency, there are differences among the two lentiviral vector designs that have important implications for clinical safety. Of note, third generation lentiviral vectors are designed such that the genes eliciting lentivirus production are split between four plasmids to prevent recombination, and the *tat* gene required for wild-type human immunodeficiency virus replication has been removed [39].

An important logistical difference between the manufacturing process described herein and the one reported by Kretschmann et al [29], is that KYV-101 manufacturing includes cryopreservation steps following leukapheresis and after completion of the CAR T-cell product. In contrast, cryopreservation was not applied at any time during the CAR T-cell generation in the protocol by Kretschmann et al. [29], including during apheresis, manufacturing, and before administration of the final drug product. Although the use of a fully fresh product allowed for a 2-week time span from apheresis collection to final drug product for infusion, a notable limitation for using a fresh product throughout the entire process is that collection, manufacturing, and treatment are required to be performed proximally to one another. As a result, this may limit the patients who can receive therapy to those patients who are already local or able to travel to the sites where collection and subsequent infusion take place. The cryopreservation steps leveraged in the KYV-101 protocol improve supply chain logistics, allow for reliable transfer of the apheresis product to the manufacturing facility, and allow reliable redistribution of the final product for infusion regardless of where the patient is being treated. Furthermore, the manufactured KYV-101 products were highly viable with robust target-mediated activity, demonstrating the cryopreservation approach did not compromise product activity. Overall, the manufacturing approach described herein allows for global reach and expanded access to patients while maintaining a centralized manufacturing process that can help ensure product consistency.

Although challenges for availability and viability of starting material for CAR T-cell manufacturing can be concerns for patients with malignant disorders, we report that sufficient KYV-101 products were manufactured for all 20 patients at a cell yield well above the standards typically required for anti-CD19 CAR T-cell infusion in oncology ($0.5–1 \times 10^{8}$ cells) [40]. The variability observed in CD4/CD8 ratio of the final drug product is likely due to differences in starting material across disease entities and prior therapies. Few patients

have received CAR T-cell therapy for autoimmune diseases, and not enough evidence exists to establish an ideal CD4/CD8 ratio in terms of efficacy and/or tolerability.

In summary, the robust KYV-101 manufacturing process resulted in a potent, consistent, and effective therapeutic product across multiple neurological and rheumatological autoimmune diseases. The clinical results are highly encouraging thus far and suggest that KYV-101 may change the treatment paradigm in B-cell-driven autoimmune diseases through deep B-cell depletion and immune reset with a single infusion.

Declaration of competing interest

D.M. has received speaker honoraria and consulting fees from Abbvie, BMS, Beigene, Celgene, Galapagos, Gilead, Janssen, Miltenyi, and Novartis. R.G. holds stock and received honorarium from Kyverna Therapeutics. C.H. has received speaker honoraria from Roche, Merck, Novartis. G.S. has received honoraria from Novartis, Janssen, Kyverna, BMS, and Cabaletta. A.M. has received speaker honoraria and consulting fees from BMS, KITE/Gilead, Novartis, Miltenyi Biomedicine, Century Therapeutics, and Caribou. A.P. receives research support from Novartis, Sana, Genentech-Roche, Mallinckrodt, Immune Tolerance Network, Alexion, Chinook. R.F. is a consultant and investigator for Kyverna Therapeutics. J.H.W.D. has consultancy relationships with / is part of the speaker or advisory board of AbbVie, Active Biotech, Anamar, ARXX, AstraZeneca, Bayer Pharma, Boehringer Ingelheim, Calliditas Therapeutics, Celgene, Galapagos, Genentech, GSK, Inventiva, Janssen, Novartis, Pfizer, Roche and UCB; has received research funding from Anamar, Argenx, ARXX, BMS, Bayer Pharma, Boehringer Ingelheim, Cantargia, Celgene, CSL Behring, Galapagos, GSK, Inventiva, Kiniksa, Lassen, Sanofi-Aventis, RedX, UCB; and is CEO of 4D Science and Scientific Lead of FibroCure. G.K. received speaker honoraria and consulting fees from Abbvie, BMS, Lilly, GSK, Janssen, Novartis, Takeda, UCB, Kyverna. L.B. has received speaker honoraria and consulting fees from Abbvie, Amgen, Astellas, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Gilead, Hexal, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Pfizer, Sanofi, Seattle Genetics, as well as research funding from Bayer, and Jazz Pharmaceuticals. R.Sen., M.L., M.P., A.R., and K.W. are employees of Kyverna Therapeutics, Inc and may have stock/stock options. R.Sch., A.H., J.G., N.K., R.B., and S.D. have nothing to disclose.

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Author contributions

Participation in research and design: D.M., R.G., R.Sch, A.H., C.H., N. K., G.S., A.M., A.P., J.G., R.F., R.B., J.H.W.D., S.D., G.K., L.B. All authors participated in article preparation. All authors have reviewed and approved the final version of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2024.09.008.

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