

Dual Immunotherapy in Aggressive Lymphoma (DIAL) study, a randomized phase 2 trial of Varlilumab in combination with Nivolumab in patients with relapsed/refractory B-cell non-Hodgkin lymphoma

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Article

Keywords:

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10845316/v1>

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Additional Declarations: **Yes** there is potential conflict of interest. Stephen M Ansell receives research funding for clinical trials from Takeda, Bristol Myers Squibb, Regeneron, Pfizer, AstraZeneca, ADC Therapeutics, Affimed and StepPharma. Aleksandr Lazaryan receives research funding from the U.S. Department of Defense and Sanofi; serves on advisory boards and as a consultant for Sanofi; provides consultancy services for Incyte and Pierre Fabre; receives honoraria from Sanofi, Incyte, and Pierre Fabre; and holds stock in UnitedHealth Group and American Well Corporation. Reem Karmali serves on advisory boards for Genentech/Roche, Genmab, Bristol Myers Squibb, Kite/Gilead, AvenCell, Miltenyi, and AbbVie; serves on speakers bureaus for AstraZeneca, Bristol Myers Squibb, and BeOne; and receives institutional research funding from Merck, Bristol Myers Squibb, Kite/Gilead, and AbbVie.

Abstract

Varlilumab is a monoclonal antibody with agonistic activity on CD27. The efficacy of single-agent PD-1 inhibitors on aggressive B-cell non-Hodkin lymphoma is limited. Preclinical and phase 1 data suggest that Varlilumab is safe and may have synergistic activity with PD-1 inhibitors. In the DIAL study, we explored whether dual immunomodulatory therapy with Varlilumab and Nivolumab in patients with relapsed/refractory aggressive B-NHL was safe and effective.

We conducted a randomized phase II clinical trial on adult patients with relapsed/refractory aggressive B-NHL. Patients were randomized to receive Nivolumab monotherapy in the control group and Varlilumab combined with Nivolumab in the experimental group. Crossover to the experimental group was allowed upon progression in the control group. The primary endpoint was the overall response rate based on the Lymphoma Response to Immunomodulatory Therapy Criteria (LYRIC).

54 patients underwent randomization, and 51 received assigned intervention and were included in the intention-to-treat analysis. Objective response was observed in 6 out of 51 patients (ORR 12%). There were 4 responders (ORR 16%) in the Nivolumab group and 2 responders (ORR 7.6%) in the Nivolumab and Varlilumab combination group ($p = 0.3$). None of the 7 patients that crossed over to the experimental group responded to the addition of Varlilumab. The ORR of patients who were previously exposed to CAR-T cell therapy was 13% (5 out of 39) compared to 8.3% (1 out of 12) in the unexposed patients ($p > 0.99$). The rate of treatment emergent adverse events was similar between groups, and there was no fatal TEAE in the study.

The addition of Varlilumab to Nivolumab did not improve outcomes in patients with aggressive B-NHL. Even though this trial was not designed to evaluate immunotherapy as a rescue therapy after CAR-T failure, most patients (76%) had previously received CAR-T therapy, and their outcomes were equally poor.

BACKGROUND

Diffuse large B-cell Lymphoma (DLBCL) is an aggressive malignancy and the most common lymphoma worldwide¹. Standard frontline therapy in fit patients consists of a combination of anthracycline-based multi-agent chemotherapy with a CD20-targeting monoclonal antibody. Unfortunately, approximately one third of patients¹ will have disease that is refractory to or that relapses after an initial response. Additional treatment with high dose chemotherapy and autologous stem cell rescue or chimeric antigen receptor (CAR) T cell therapy may still offer a chance of cure for some of these patients but a considerable proportion of them will die of progressive disease. Patients who relapse after CAR T cell or autologous stem cell transplant therefore have a poor prognosis and are in need of novel therapies.

Varlilumab (CDX-1127) is a fully human monoclonal antibody with agonistic activity on the CD27 receptor (TNFRSF7), a member of the tumor necrosis factor (TNF) receptor superfamily. CD27 is constitutively expressed on most mature T-cells, memory B-cells, and a portion of natural killer (NK)

cells. Once bound to its cognate ligand (CD70), CD27 promotes a variety of immunological effects such as activation, proliferation, survival, maturation of effector capacity and memory in T-cells; differentiation of B-cells to plasma cells and the production of immunoglobulins; and enhancement of cytolytic activity in NK cells. Additionally, CD27 expression on B-cell lymphomas has been well documented²⁻⁷. In pre-clinical models, Varlilumab was shown to have direct anti-tumor activity in CD27-expressing B-cell lymphoma models⁸.

Nivolumab is a fully humanized monoclonal antibody with antagonistic activity on the human programmed death-1 (PD-1 or CD279) receptor. By blocking the interaction between PD-1, expressed on effector T-cells, and its cognate ligands (PD-L1 and PD-L2) expressed on tumor cells and antigen-presenting cells (APCs), Nivolumab promotes anti-tumor T-cell activity. PD-1 blockade is approved for clinical use in a variety of cancers and has become a cornerstone of modern immunotherapy⁹.

The safety and efficacy of PD-1 blockade in B-cell non-Hodgkin lymphomas (B-NHL) has been evaluated in clinical trials. The clinical activity of PD-1 blockade in aggressive B-NHL has been limited, potentially owing to profound immunosuppression and exhaustion mediated by the tumor microenvironment (TME) in this disease¹⁰⁻¹⁷. Preclinical data suggesting potential synergistic activity between PD-1 blockade and CD27 stimulation¹⁸ along with early clinical data demonstrating the safety of this combination in solid tumors¹⁹ provided the final pieces of the rationale for this study.

Based on this background, we hypothesized that therapy combining Nivolumab and Varlilumab in patients with relapsed/refractory aggressive B-NHL was safe and effective. We anticipated that Varlilumab would provide an agonist signal to stimulate an effective antitumor immune response and Nivolumab would prevent immune inhibition and exhaustion of the activated immune cells. To this end, we designed DIAL (Dual Immunomodulatory therapy in Aggressive B-cell Lymphoma; NCI10089, NCT03038672), a multi-center randomized phase II study.

METHODS

Patient selection

Eligible patients were adults (age ≥ 18 years) with a histopathologically confirmed diagnosis of an aggressive B-NHL classified according to the 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms²⁰ belonging to any of the 22 histological subtypes specified in supplemental table 1. Patients should have disease that had relapsed after or was refractory to at least 2 lines of standard therapy and measurable lesions, defined as at least one lesion that is > 15 mm in the longest axis on cross-sectional imaging and measurable in two perpendicular dimensions per computed tomography (spiral CT), positron emission tomography-computed tomography (PET-CT) or magnetic resonance imaging (MRI). Additionally, preserved organ and bone marrow function, and Eastern Cooperative Oncology Group (ECOG) performance status < 2 were required. Main exclusion criteria included recent treatment (chemotherapy, targeted agent, or radiotherapy within 4 weeks or at least 5

half-lives, whichever was longer, prior to registration); prior treatment with an immune checkpoint inhibitor (anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody) or any other antibody or drug specifically targeting T-cell co-stimulation; prior allogeneic stem cell or solid organ transplantation; evidence of active central nervous system (CNS) disease; recent autologous stem cell transplantation ($\leq$ 12 weeks prior to the first dose of study drug). Patients treated with CAR-T cell therapy were allowed to participate. The trial was developed through the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI) and available to sites participating in the Experimental Therapeutics Clinical Trials Network (ETCTN), Early Drug Development Opportunity Program (EDDOP), and the Create Access to Targeted Cancer Therapy for Underserved Populations (CATCH-UP) program. An independent Data and Safety Monitoring Board (DSMB) had oversight of the study. The full study protocol is available in the supplemental material.

Study design and treatment

DIAL was designed as a randomized phase II study consisting of 1 control arm (group 1, single-agent Nivolumab) and 1 experimental arm (group 2, Nivolumab combined with Varlilumab). Primary endpoint was overall response rate (ORR) based on the Lymphoma Response to Immunomodulatory Therapy Criteria or LYRIC (supplemental table 2)²¹. Secondary endpoints included safety, tolerability, duration of response (DoR), progression-free survival (PFS), and overall survival (OS). Exploratory outcomes included changes in peripheral blood immune profile measured with mass cytometry, cellular indexing of transcriptomes and epitopes (CITE-seq), and multiplex enzyme-linked immunosorbent assay (ELISA) technology.

Eligible patients were randomized (1:1) using a computer-based system to treatment in one of the 2 groups. Both groups received Nivolumab at the dose of 240 mg intravenously (IV) every 2 weeks for the first 4 months on study. After four months, Nivolumab was administered at the dose of 480 mg IV every 4 weeks. Patients allocated to group 2 additionally received Varlilumab at the dose of 3 mg/kg IV every 4 weeks. Response assessment was performed every 12 weeks using PET-CT. Patients in group 1 who met criteria for disease progression at any point were allowed to cross-over to group 2 and continue on study (a repeat biopsy was required to confirm progression). Study schema is summarized in Supplemental Fig. 1. Participants were allowed to remain on study for a maximum of 2 years, until disease progression (except for patients who crossed over from group 1 to group 2), or unacceptable toxicity. Patients who completed participation were followed for additional 100 days after end of therapy or until death, whichever occurred first. Patients removed from therapy for unacceptable adverse events were followed until resolution or stabilization of the adverse event.

Statistical analysis

Primary endpoint of the study was overall response rate. At the time of study conception, available data estimated the response rate of single agent Nivolumab in DLBCL between 20–27%²². Based on this data

we selected 25% as our best estimate of response for the control arm and calculated that a sample size of 48 patients per group would provide 80% power to detect an improvement in response rate from 25% to 45%, using a one-sided test at a significance level of 0.15. We utilized a 1:1 randomization ratio stratified by age (< 65 vs. ≥ 65 years), previous autologous stem cell transplant, and diagnosis category (A vs. B, Supplemental Table 1).

An interim futility analysis was planned after half of the patients (24 patients in each group) completed their first planned radiologic assessment (after 12 weeks of treatment or up to 20 weeks if treatment delays occurred). At this pre-specified analysis timepoint, if overall response rate in the experimental group were found to be lower than that of control arm the study would be terminated for futility.

Comparison of overall response rates between the two treatment groups was performed using a one-sided Fisher's exact test at significance level 0.15. The overall adverse event rates for Grade 3 or higher hematologic and non-hematologic adverse events at least possibly related to treatment were compared between groups using the Chi-square test (or Fisher's exact test if the data in the contingency table was found to be sparse). DoR, PFS, OS were estimated using the Kaplan-Meier method, and comparisons between arms were carried out using the log-rank test. Exploratory endpoints were reported descriptively. Patients were analyzed in the group to which they were initially randomized, regardless of cross-over (intention-to-treat).

Correlative science specimens

Peripheral blood was collected from study participants at baseline and every 2 weeks for the first 12 weeks on treatment. Plasma and peripheral blood mononuclear cells (PBMC) aliquots were generated from blood specimens and cryopreserved for analysis in batches after knowledge of clinical outcomes.

Multomics Assay

Multi-omics analysis was performed by BostonGene on formalin-fixed paraffin-embedded (FFPE) tumor tissue and paired cryopreserved peripheral blood mononuclear cells (PBMC). DNA and RNA from FFPE tumor was extracted using the Qiagen AllPrep DNA/RNA FFPE Kit, while PBMC frozen samples were first thawed to 37°C and then DNA was extracted using the Qiagen AllPrep DNA/RNA Mini Kit. RNA Libraries were constructed using the Agilent SureSelect XT HS2 RNA kits with V7 + UTR probes. All RNA libraries were sequenced on NovaSeq 6000 with target 50M reads. Whole exome sequencing (WES) was performed using the SureSelect XT HS2 DNA system (Agilent) for exome capture. SureSelect Human All Exon V7 and SureSelect Human All Exon V8 (Agilent) with a custom probe set were used for hybridization procedures. Libraries were sequenced on NovaSeq 6000 with a median exon coverage of 150x for tumor samples and 100x for normal samples. NGS data quality control was performed using FastQC v0.11.5, FastQ Screen v0.11.1, RSeQC v3.0.0 and MultiQC v1.6 on all NGS samples. Sample correspondence was checked by HLA comparison from RNA-Seq using OptiType70. NGS data analysis

was performed using the BostonGene TumorPortrait test, as previously described²³. The platform integrates multi-omic data to identify genetic alterations, cell of origin, lymphoma microenvironment subtypes, and transcriptomic signatures, enabling comprehensive tumor characterization.

RESULTS

Patient characteristics and disposition

From November 2018 to August 2022, a total of 54 patients were enrolled and randomized in the study. Three patients were excluded from the clinical trial before treatment initiation as shown in Figure 1.

Figure 1. CONSORT 2025 Flow Diagram

A total of 51 patients were evaluable for safety and efficacy analysis, 25 in the group 1 and 26 in group 2. Seven patients in the control arm (group 1) crossed over to the experimental group 2 during the clinical trial. The median follow up was 8.8 months. Patient baseline characteristics were equally distributed between the groups as demonstrated on Table 1. The median age was 67 years and 71% of the patients were male. Patients were heavily pre-treated with 88% having received at least 3 prior lines of therapy; 76% had previously received CAR-T cell therapy and 14% had previously undergone autologous stem cell transplant. Most patients had Diffuse Large B-Cell Lymphoma (88%), 43% germinal-center cell subtype, 20% activated B-cell subtype and 25% not otherwise specified. Other less frequent histologies were High-Grade B-cell Lymphoma (4%), T-Cell/Histocyte Rich Large Cell Lymphoma (4%), Plasmablastic Lymphoma (2%) and Aggressive B-cell Lymphoma NOS (2%).

Table 1. Demographics and patient characteristics at baseline

Safety

The rate of treatment-emergent adverse events (TEAE) was similar between groups. Table 2 summarizes the common (>5%) and severe (grade 3 or higher) TEAEs for study participants, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.

Table 2. Common and Severe Treatment Emergent Adverse Events

* Indicates potential immune-related adverse events (IrAEs).

All patients in the study experienced a TEAE of any grade. The rate of serious TEAE (grade 3 or higher) was 76% in the patients that received Nivolumab monotherapy and 80% in the patients that received the combination of Nivolumab and Varlilumab. Grade 3 or higher hematologic events occurred in 44% of the monotherapy group and 50% in the combination group. Potentially immune-related adverse events (IrAEs) were defined as any side effect that could be immune mediated and was not explained by other treatment or condition. IrAEs were present in the majority of patients, but grade 3 or higher happened in only 24% of patients of the monotherapy group and 23% of the combination therapy group. Supplemental table 3 summarizes all TEAEs according to study group. In the subgroup of patients that crossed-over, grade 3 or higher adverse events occurred in 71% of the patients. Grade 3 or higher hematologic toxicity was experienced in 42% of these patients, while grade 3 or higher immune-related adverse events occurred in 14%. Only one patient (from the Nivolumab monotherapy group) left the study due to a severe adverse event, related to rapidly progressing tumor gastric ulcer and gastric hemorrhage. There were no treatment related deaths.

Anti-tumor activity

Objective response was observed in 6 out of 51 patients (ORR 12%). There were 4 responders (ORR 16%) in the Nivolumab group and 2 responders (ORR 7.6%) in the Nivolumab and Varlilumab combination group ($p=0.3$). None of 7 patients that crossed over from Group 1 responded to the addition of Varlilumab. There were 3 patients (CRR 12%) in the Nivolumab group who achieved complete response and one patient (PRR 4%) who achieved a partial response. In the combination therapy group, there was one partial response (PRR 3.8%) and one complete response (CRR 3.8%). These findings are summarized in Figure 2. A sub-group analysis showed that the overall response rate in patients previously exposed to CAR-T was 13% (5 out of 39) compared to 8.3% (1 out of 12) in the unexposed patients ($p>0.99$). In a pre-planned interim analysis, the study was stopped early due to futility.

Figure 2. Swimmer's Plot

*Patient 30 left study due to a severe adverse event and patient 41 withdrew consent for personal reasons.

The median overall duration of response in the full cohort was 23.5 months, ranging from 5.8 to 35 months. The median duration of response in the Nivolumab monotherapy was 24.4 months (ranging

from 23 months to 35 months), while in the combination group was 5.88 months.

The overall conversion rate from indeterminate response (according to LYRIC criteria) to partial or complete response was 13.3%.

The median overall survival was 6.2 months in the control group and 3.86 months in the experimental group ($p=0.07$), while the median progression free survival was 2.7 months and 1.1 months respectively ($p=0.08$).

When only analyzing patients previously exposed to CAR-T, the Nivolumab single-agent group had a median overall survival of 6.2 months compared to 3.9 months in the combination therapy group ($p=0.07$). The median progression free survival of this sub-group was 2.7 months and 1.2 months, respectively ($p=0.04$). Time-dependent endpoint analysis are shown in Figure 3.

Figure 3. Overall Survival and Progression Free Survival

Exploratory Analysis

Exploratory analysis using BostonGene Tumor Portrait was attempted in 11 patients with available specimens. The tumor sample of one of these patients did not pass quality control and was excluded from analysis. The tumor sample from two other patients had a low tumor content which limited the multi-omics analysis.

Out of the 10 patients that were analyzed, 4 responded to immunotherapy and 6 did not respond. 9 of these patients had been previously exposed to CAR-T. These samples were analyzed based on the genomic, transcriptomic, and B-cell receptor (BCR) repertoire and were later classified according to the cell of origin, double-hit gene signature, lymphoma microenvironment cluster, and LymphGen classification. None of the analyzed features were associated with patient outcomes in the DIAL study. Additionally, the assay was not designed to detect CAR-T structures beyond 30 days of infusion, therefore becoming unreliable to detect CART persistence on this cohort of patients who averaged 60 to 90 days post CAR-T therapy. A summary of these results can be found in Figure 4, while the complete analysis can be found in the supplementary appendix 2.

Figure 4. Boston Gene Exploratory Analysis

DISCUSSION

The DIAL study demonstrated that the addition of Varlilumab to Nivolumab did not enhance the antitumor activity in relapsed/refractory aggressive B-NHL. The dual immunomodulatory therapy group achieved similar response rates to Nivolumab monotherapy and did not increase the rate of adverse events. Even though the ORR was poor (12%), the few patients that responded to Nivolumab had durable responses. Prior exposure to CAR-T therapy was well represented in this trial, however their outcomes were equally poor, suggesting that immune checkpoint inhibition (ICI) is not an effective salvage strategy for this population.

The result of this clinical trial corroborates the previously published data on the very modest efficacy of ICI in relapsed/refractory aggressive B-NHL. This treatment strategy has a low response rate irrespective of prior CAR-T exposure, with ORR to single agent ICI ranging from 19% to 32%^{24,25,26,27}. These results differ significantly from preliminary data from small cohorts of patients failing CAR T cell therapy and subsequently treated with PD-1 blockade, which reported response rates of more than 50%^{28,29}. The only histology that consistently demonstrates a significant response to checkpoint inhibition is Primary Mediastinal B cell Lymphoma with ORR ranging from 41% to 62%^{24,25,30}. Unfortunately, the addition of Varilimumab to Nivolumab did not improve outcomes in our clinical trial. This finding goes along with the previously published phase 1 study of Varililumab monotherapy in R/R lymphomas³¹. In that cohort of 18 patients with B-NHL, none had an objective response, and 3 patients had stable disease.

The disconnection of our results from the pre-clinical data showing synergistic effects of Varlilumab with PD-1 inhibitors highlights the complexity of the immune response in such aggressive lymphomas. The failure of a dual immunotherapy to overcome immune evasion, even among patients who had previously received CAR-T therapy, may suggest either irreversible T-cell exhaustion or a suppressive tumor microenvironment characterized by immune exclusion and T-cell independent resistance. Unfortunately, the exploratory multi-omics analysis was unable to detect biomarkers predictive of treatment response. Most patients, however, exhibited a lymphoma microenvironment consistent with mesenchymal or immune-depleted subtype, which corroborates the hypothesis that T-cell exhaustion might not be the only mechanism of resistance of these neoplasms.

The rate of grade 3 or higher adverse events in this study was similar in both treatment groups and there was no treatment related mortality. This supports the previous safety data of anti-PD1 therapy, which showed low rates of severe adverse events and rare treatment-related mortality^{24,25,26}. Importantly, the addition of Varlilumab to Nivolumab did not increase immune-related toxicity in our study. This is not a surprise as the medication was well tolerated as monotherapy during the phase 1 study where doses of up to 10 mg/kg were used in patients with B-cell lymphomas without dose limiting toxicity³¹

An important limitation to our study is the small number of responders, given that only 6 of 51 patients achieved a measurable response. This number was insufficient to identify significant associations between patient characteristics and correlative data that were predictive of treatment response. Although there was a large number of post CAR-T patients in our trial, this study was not specifically designed as a CAR-T salvage trial. In fact, enrollment required a wash out of 90 days post CAR-T infusion

to enter this study. When we evaluated tumor tissue from 9 of these patients with a whole transcriptome assay and specifically looked at CAR-T transcripts, only 5 patients had structures suspicious for CAR-T constructs. This was an expected result given that the number of CAR-T cells can become undetectable by this methodology after 40 days of treatment, and the assay was not originally designed to have high sensitivity for CAR transcript detection.

Another limitation of the study is the heterogeneity of the enrolled population, which included multiple aggressive B-cell lymphomas without enrichment for a specific biomarker. Although most of our patients had DLBCL, the difference in tumor biology and immune microenvironment could have obscured a response signal for a particular population and limits the mechanistic interpretation of this negative study. Few studies have specifically evaluated the use of immune checkpoint inhibitors as salvage therapy after CAR-T failure in aggressive B-NHL. Li et al prospectively evaluated the use of PD-1 inhibitors alone or in combination with chemotherapy in this context³². Patients received this salvage therapy with PD-1 blockade either at the time of relapse or 2 months after CAR-T infusion if they achieved suboptimal response (PR or SD). Out of the 21 patients who received salvage therapy, the ORR was 28.5%. This response rate was worse for patients receiving therapy upon relapse with an ORR of 12.5% compared to 38.4% for patients who had received therapy for a suboptimal response. A multicentric retrospective cohort study also evaluated the use of salvage checkpoint inhibition after CAR-T, showing comparable results with an ORR of 19% and a DOR of 221 days²⁴. Another small cohort had only 7 patients using ICI as salvage therapy, but none of them responded to the therapy³³. These findings are comparable to our data where the ORR was 13% for the subgroup that had previously received CAR-T.

DIAL was the largest study to prospectively evaluate the efficacy of ICI use after CART cell therapy in aggressive B-NHL. We were able to demonstrate that neither single checkpoint inhibition nor dual immunomodulation performs well after CAR-T relapse in this population. The fact that our study was randomized and allowed cross-over significantly reduced confounding factors and increased its power to detect any clinical benefit. Given the small number of responders, our exploratory analysis was unable to detect any patient characteristics that could predict a positive response to this treatment. However, the few responding patients in our study often exhibited a prolonged response to checkpoint inhibition, with a manageable side effect profile. This highlights the need for additional efforts to accurately identify which patients with aggressive B-NHL may benefit from ICI.

Collectively, our data discourages the use of ICI in an unselected population with relapsed/refractory aggressive non-Hodgkin B-cell lymphomas. The overall survival and response rates were poor with single checkpoint inhibition, and there was no additional benefit from dual immunomodulation, regardless of previous CAR-T exposure. Even though there were a few responders with excellent disease control, it is challenging to apply this data to clinical practice as our exploratory analysis was unable to find any patient characteristics that could predict response to such therapy. The safety of dual checkpoint inhibition was not a concern as there was no increase in adverse events in the experimental arm. Novel platforms and improved biomarkers should continue to drive research for these patients.

Declarations

CONFLICTS OF INTEREST

SMA receives research funding for clinical trials from Takeda, Bristol Myers Squibb, Regeneron, Pfizer, AstraZeneca, ADC Therapeutics, Affimed and StepPharma.

ETHICS APPROVAL

This study was reviewed and approved by the Central Institutional Review Board (CIRB) of the National Cancer Institute.

ROLE OF FUNDING SOURCE

This was an NCI-funded investigator-initiated study. No industry sponsor had any significant role in the design or conduct of the study.

FUNDING

This trial was funded by ETCTN UM1 grant number CA186709.

JCV (W81XWH-18-1-0650) and SMA (W81XWH-18-1-0652) received partial support from the Department of Defense (DOD) for conduct of this study.

AUTHOR CONTRIBUTIONS

RHS analyzed and interpreted the data, prepared tables and figures, and prepared the first draft of the manuscript. SG analyzed and interpreted the data through statistical analysis, created tables, and figures. JK, AL, NB, RK, PR, FA, CBR, FH, CD, FL and LS acquired data and critically analyzed the manuscript. EP, AB, NF performed multi-omics analysis. BC, JS and SMA edited and critically analyzed the manuscript. JCV and SMA conceived, designed, conducted the study, acquired, analyzed and interpreted data, and supervised the manuscript creation. All authors reviewed and approved the final manuscript.

ACKNOWLEDGEMENTS

Authors acknowledge patients and their caregivers for participating in the study. We also would like to extend our sincere acknowledgements to our study teams and administrative staff from each of the

participating sites and from CTEP, NCI, ETCTN, EDDOP, and CATCH-UP program.

Tables

Tables 1 & 2 are available in the Supplementary Files section.

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Figures

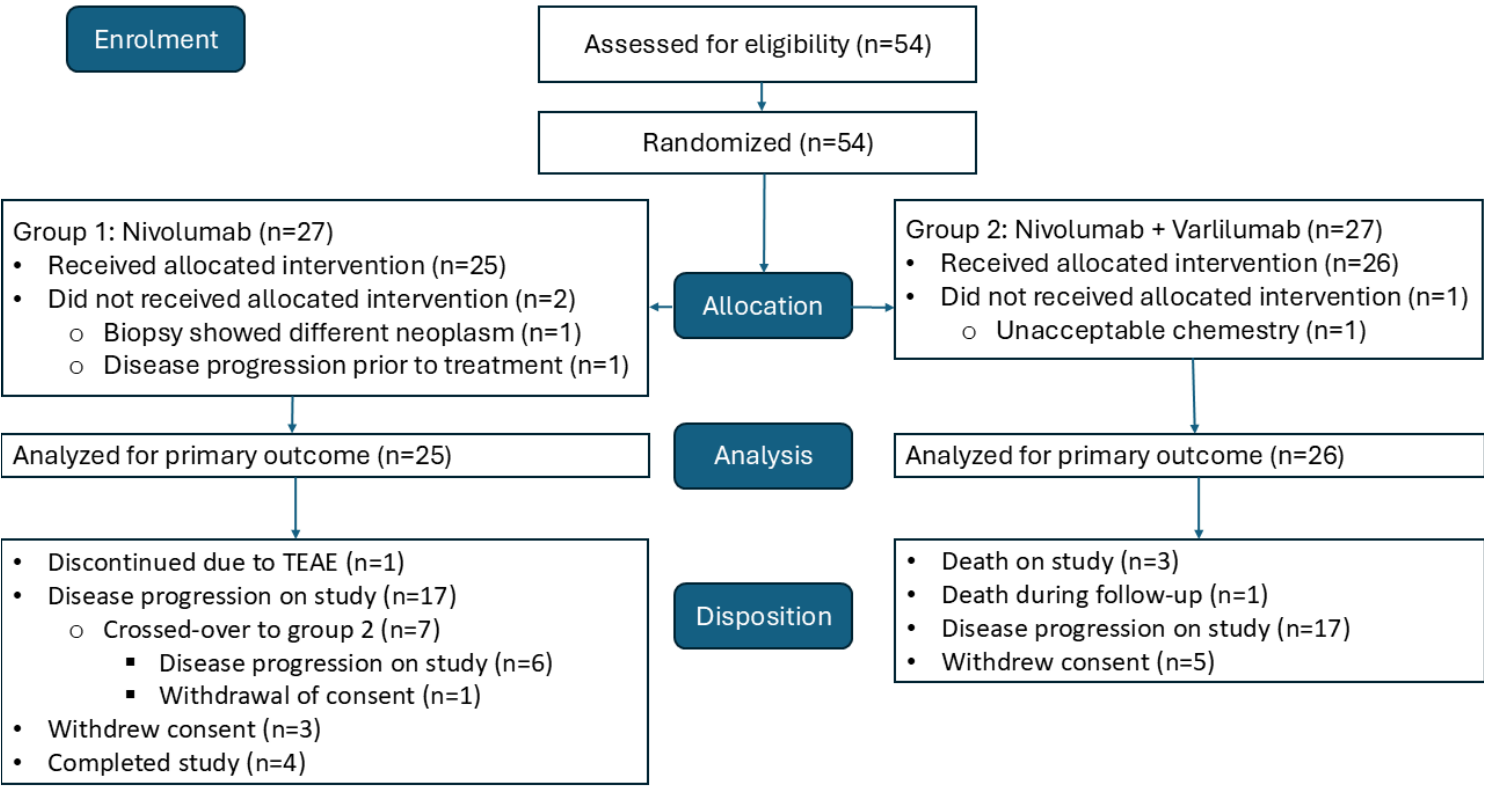


Figure 1

CONSORT 2025 Flow Diagram

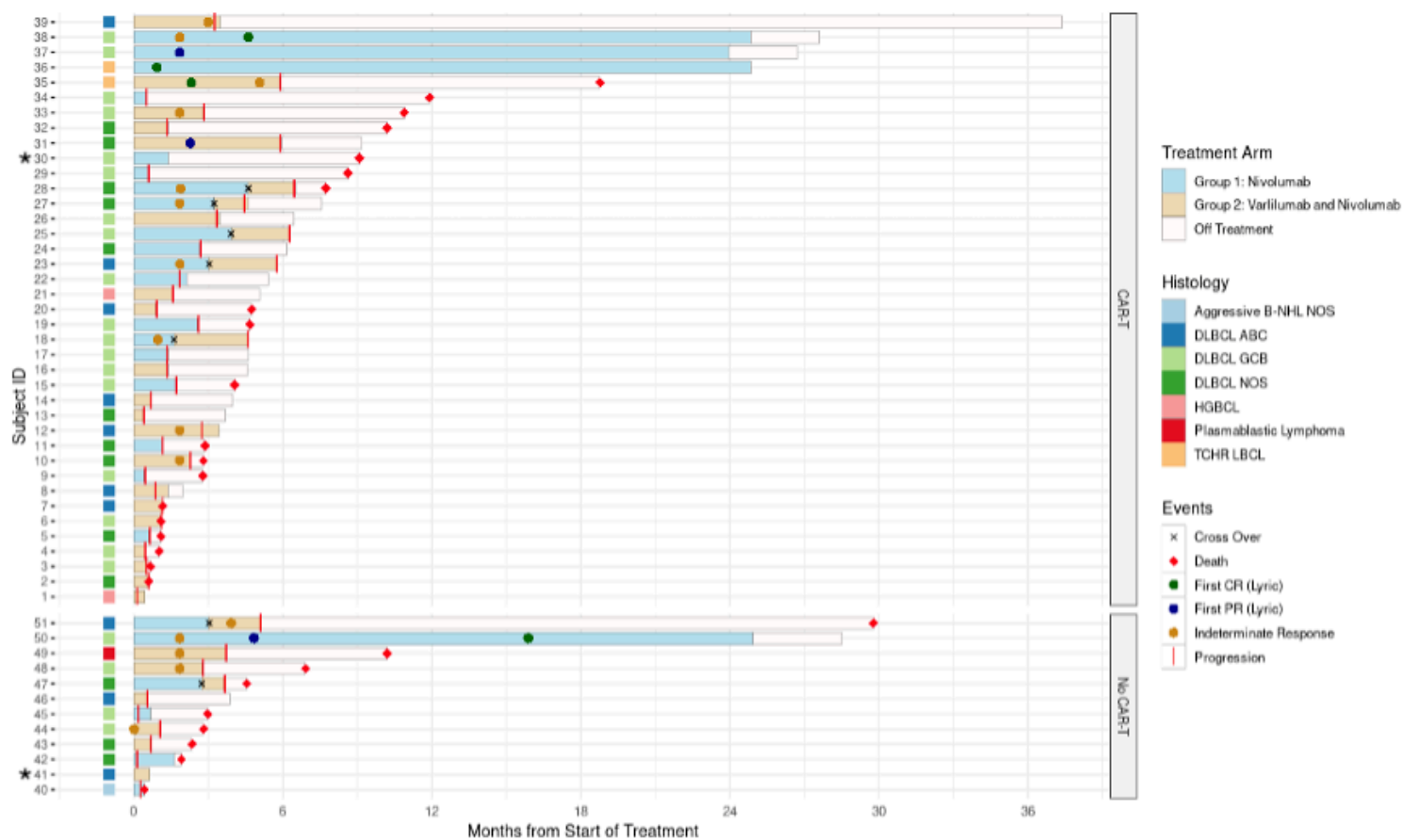


Figure 2

Swimmer's Plot

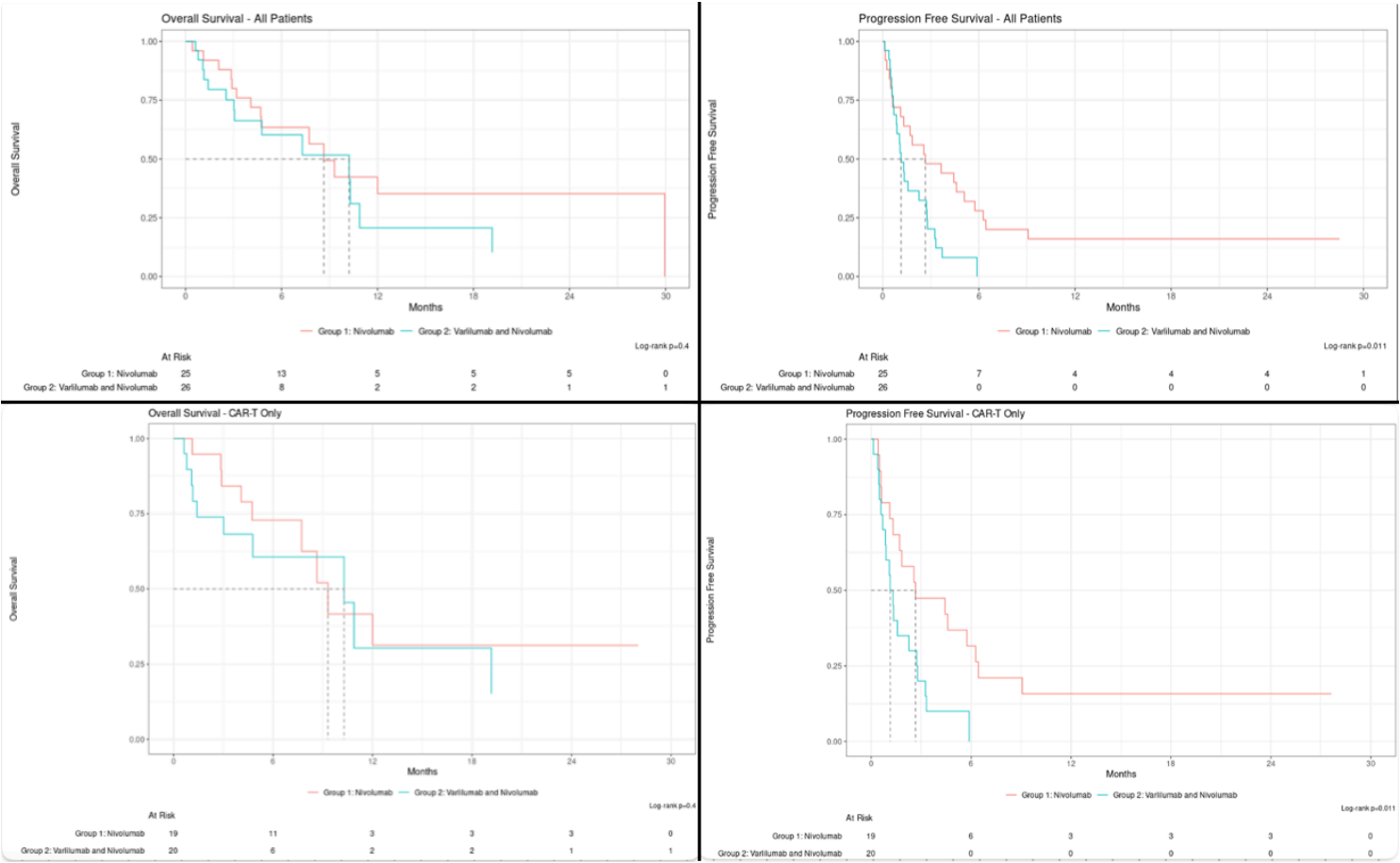


Figure 3

Overall Survival and Progression Free Survival

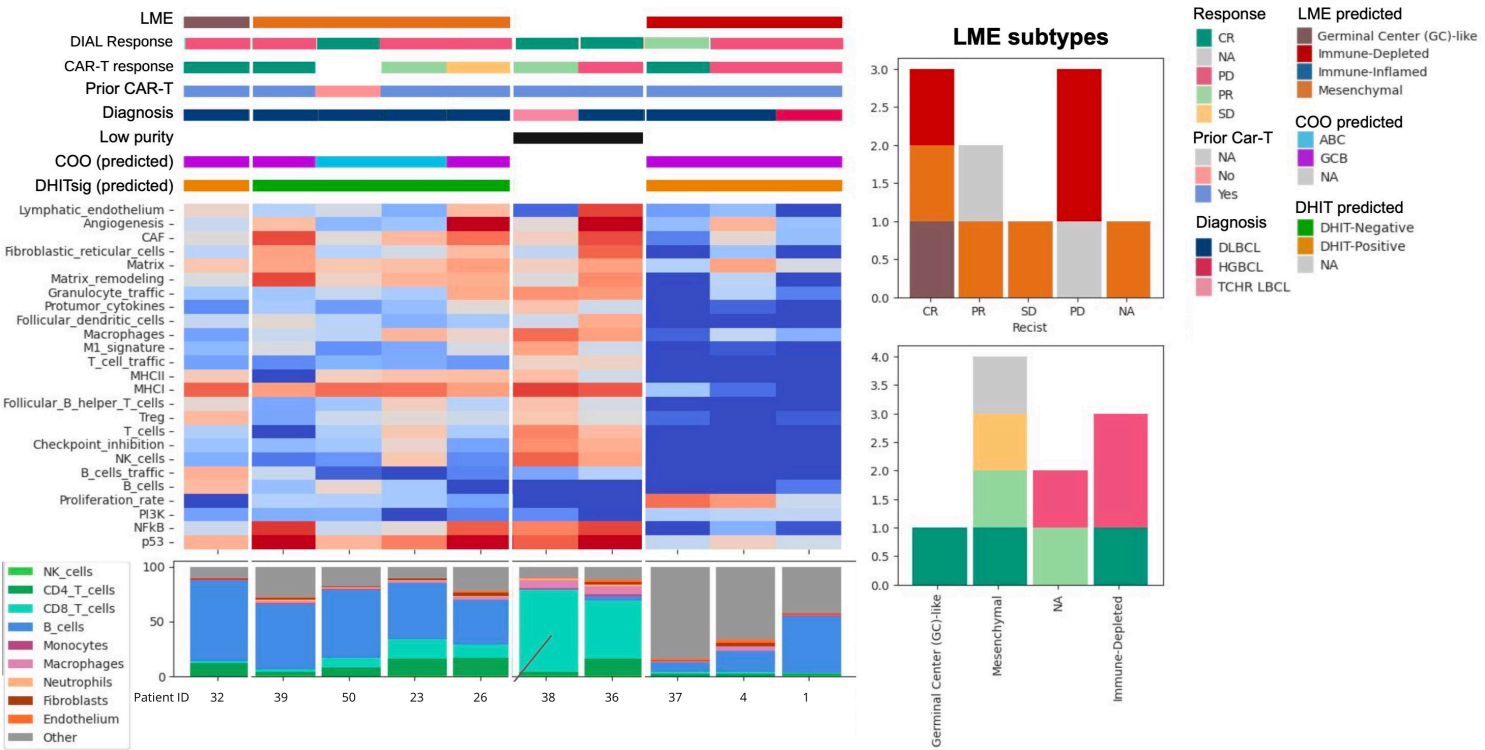


Figure 4

Boston Gene Exploratory Analysis

Supplementary Files

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- [Table1Demographicsandpatientcharacteristics.docx](#)
- [Table2Commongrade3highertoxsummaryall.xlsx](#)
- [SupplementalFigure1TrialScheme.docx](#)
- [SupplementalTable1HistologicalSubtypes.docx](#)
- [SupplementalTable2LYRICcriteria.docx](#)
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