Abstract #55 | Poster

Shift towards a more naive phenotype of the T-cell compartment in patients with aggressive B-cell non-Hodgkin lymphoma undergoing high-dose methotrexate therapy

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Introduction:

Immunotherapies such as anti-CD19 chimeric antigen receptor (CAR) T-cell therapy harness patients' T cells to target malignant B cells and are approved for second- or later-line treatment of B-cell non-Hodgkin lymphoma (B-NHL). However, the effects of prior chemotherapy on T-cell function, particularly regarding CAR T-cell manufacturing, remain unclear. High-dose methotrexate (HD-MTX), frequently used as holding- and/or bridging therapy before CAR T in CNS lymphoma, has not been systematically investigated. We therefore aimed to characterize the impact of HD-MTX on the T-cell compartment.

Methods:

Peripheral blood samples were collected from patients with newly diagnosed or relapsed/refractory aggressive B-NHL receiving either MTX-based or non-MTX-based therapy. Sampling was performed at baseline and on days 10–17 after cycle 1 (c1) and cycle 2 (c2). The MTX cohort included 24 patients (MTX, median age 67; 50% male), the non-MTX cohort 32 patients (noMTX, 68; 72% male), with varying sample numbers per time point. Common T-cell maturation and memory phenotypes were analysed by flow cytometry, including CD3, CD4, CD8, CCR7, CD45RA, CD27, CD28 and others. Subset distributions were compared using the Mann-Whitney U test.

Results:

No significant baseline differences were observed. After c1, MTX-based therapy was associated with a higher proportion of $CD4^+$ and lower proportion of $CD8^+$ T cells compared to non-MTX treatment (MTX: 88.6%/7.3% vs. noMTX: 70.8%/21.6%; p=0.00057/0.0013, all for $CD4^+$/$CD8^+$ cells, respectively). Both $CD4^+$ and $CD8^+$ subsets showed a shift towards a $CCR7^+$ phenotype in c1 and 2 (c1 MTX: 74.8%/31.0% vs. noMTX: 43.5%/6.6%, p=0.0053/0.0019; c2: MTX: 72.9%/30.4% vs. noMTX: 48.6%/5.2%, p=0.021/0.0042), including increased naive&SCM ($CCR7^+CD45RA^+$) cells, alongside reduced senescent $CD27^-CD28^-$ cells in c1 after MTX. Additionally, $CD4^+$ effector memory ($CCR7^-CD45RA^-$) cells were decreased in c1 and c2 in the MTX cohort (c1: MTX: 21.4% vs. noMTX: 47.9%, p=0.0086; c2: MTX: 23.8% vs. noMTX: 47.7%, p=0.045).

Outlook:

Our data indicate a shift towards a more naive T-cell phenotype following MTX-based therapy, which may

enhance the efficacy of CAR T cells after MTX-based holding therapy. This supports the potential use of MTX as a holding therapy prior to apheresis in CNS lymphoma patients intended for CAR-T treatment. Further validation in larger cohorts, including CAR-T product and expansion data, is warranted.

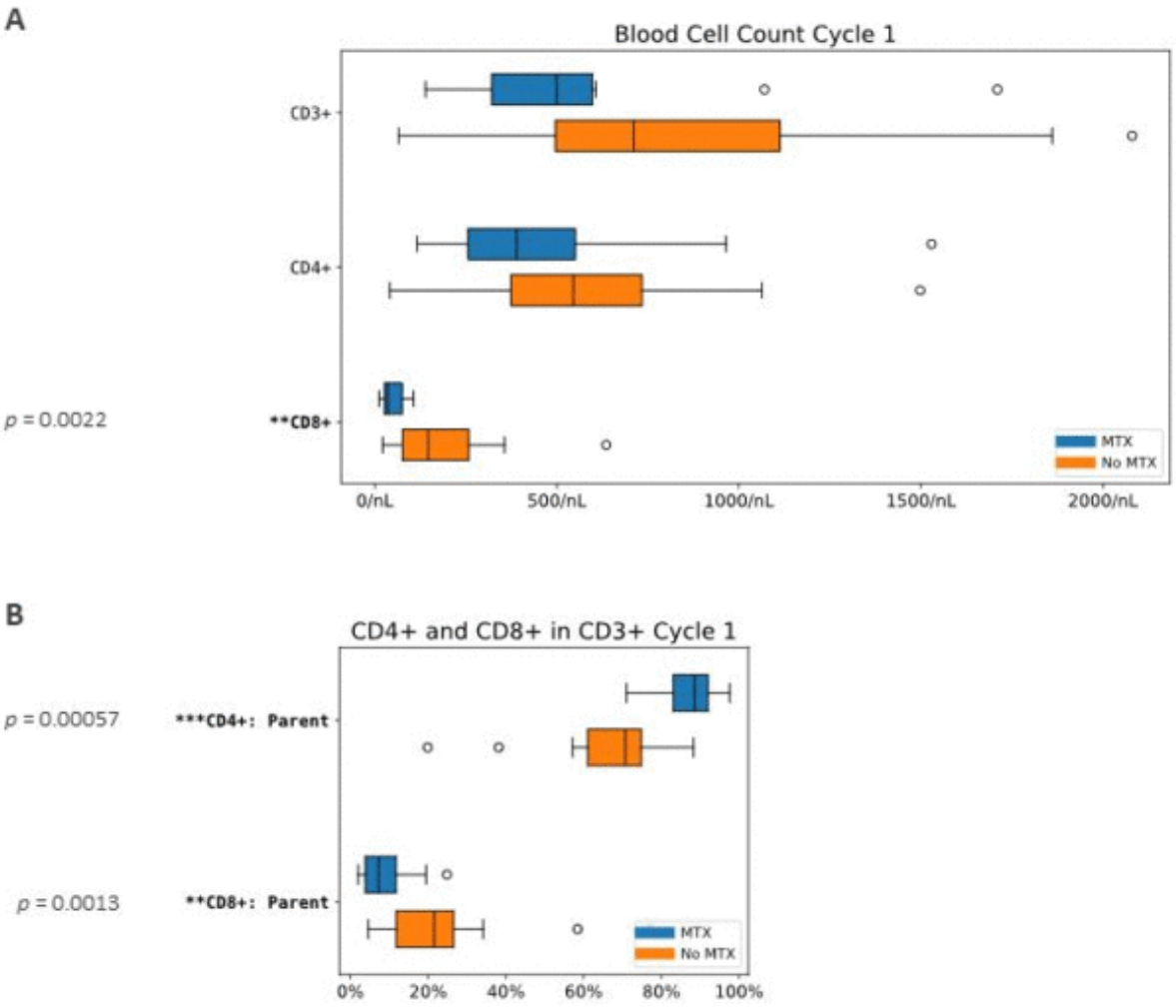

Figure 1. Absolute cell count (A) and distribution of CD4+ and CD8+ T cells within CD3+ (B) in c1

CD3+, CD4+ and CD8+ cell count per nanoliter and distribution of CD4+ and CD8+ T cells within the

CD3+ parent population in the MTX vs. noMTX cohort in cycle 1. Boxplots represent median and interquartile range; whiskers indicate the data range. Significant differences are marked by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

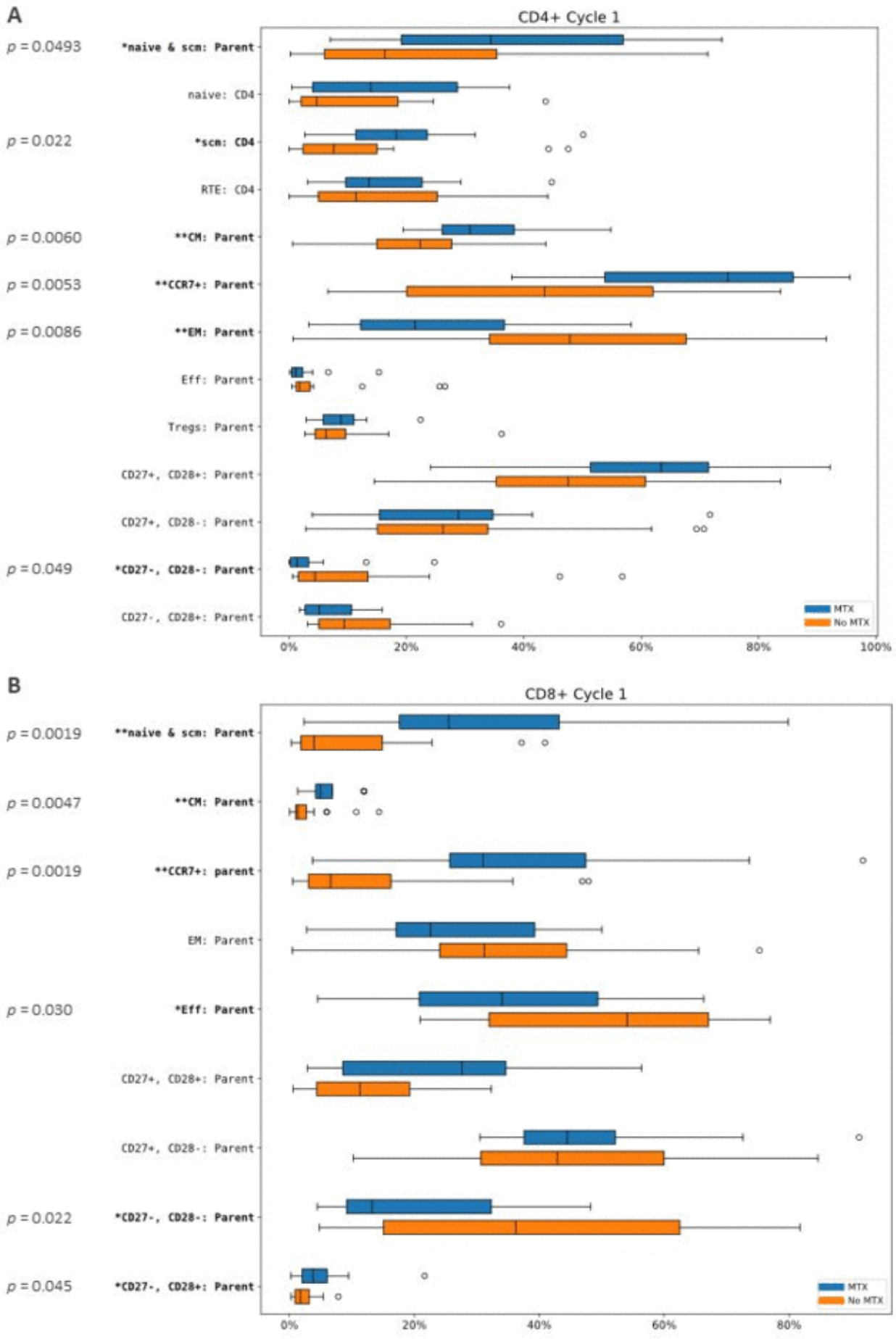

Figure 2. Distribution of CD4⁺ (A) and CD8+ (B) T-cell subsets in the MTX vs. noMTX cohort in c1
Boxplots show the relative frequencies of CD4⁺ and CD8⁺ T-cell maturation, memory, differentiation,

and regulatory subsets defined by CD45RA, CCR7, CD31, CD95, CD25, CD127, CD27, and CD28 antibody staining in cycle 1. Boxes indicate the median and interquartile range; whiskers represent the data range, and dots indicate outliers. Statistically significant differences are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

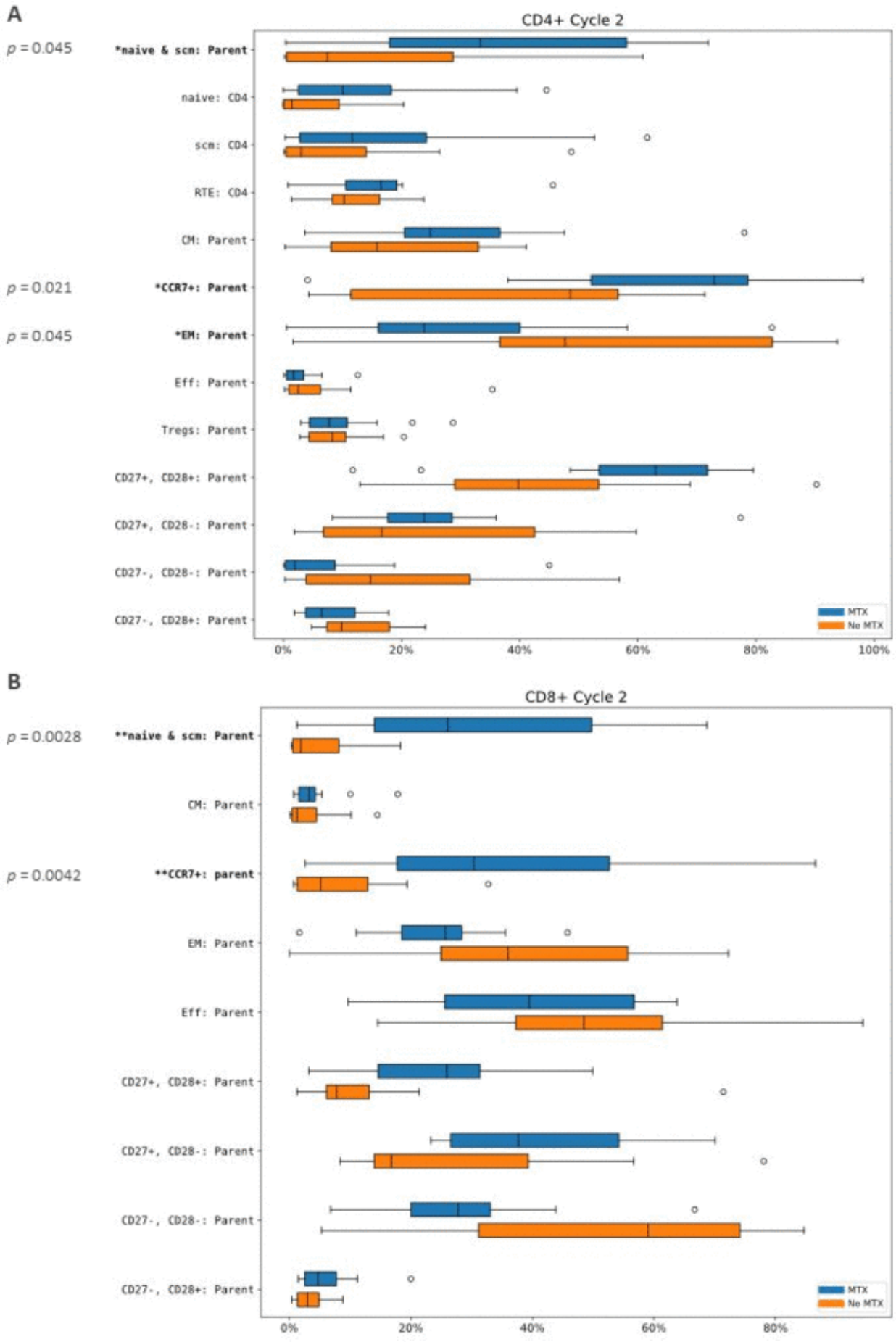

Figure 3. Distribution of CD4⁺ (A) and CD8+ (B) T-cell subsets in the MTX vs. noMTX cohort in c2
Boxplots show the relative frequencies of CD4⁺ and CD8⁺ T-cell maturation, memory, differentiation,

and regulatory subsets defined by CD45RA, CCR7, CD31, CD95, CD25, CD127, CD27, and CD28 antibody staining in cycle 2. Boxes indicate the median and interquartile range; whiskers represent the data range, and dots indicate outliers. Statistically significant differences are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).