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# Treatment with obinutuzumab plus venetoclax reshapes the TRB repertoire of CLL patients



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To the Editor,

As T cell-mediated immunotherapies are being explored as a treatment strategy for relapsed and refractory chronic lymphocytic leukemia (CLL), the study of autologous T cells in patients with CLL has intensified [1, 2]. The repertoire of αβ T cell receptors (TRA/TRB repertoire) in CLL patients exhibits several abnormalities. These include skewing of TRBV gene usage, marked oligoclonal $CD8^+$ T expansions and the existence of stereotyped TRB rearrangements that are shared by unrelated patients [3–5].

Anti-leukemic treatment affects autologous T cells through on-target, off-target, and/or indirect mechanisms. As the use of T cell-mediated immunotherapy has thus far been largely confined to the relapsed and refractory setting, it is becoming crucial to understand how previous lines of therapy have affected the autologous T cell population. Previous studies have investigated the impact of chemoimmunotherapy and ibrutinib on the TRB repertoire [6–9]. However, how the TRB repertoire is affected by treatment with obinutuzumab and venetoclax, an increasingly favored option for the initial treatment of CLL, is unknown. Here, we have used next-generation sequencing to longitudinally characterize the TRB repertoire of previously untreated CLL patients who received treatment with obinutuzumab plus venetoclax in the HOVON-139/GIVE trial [10].

## RESULTS

The TRB repertoire was characterized in 137 samples, representing 59 patients from the HOVON-139/GIVE trial at trial entry, after one year of obinutuzumab plus venetoclax treatment (end of induction treatment; EOiT), and one year after randomization (R + 12), after patients had received either venetoclax maintenance or no further therapy (Supplementary Fig. 1A, Supplementary Table 1). Cell counts are provided in the Supplementary Material and Supplementary Fig. 1B–G.

A total of 287,548 productive TRB clonotypes were identified, corresponding to 502,550 cell equivalents. The mean number of clonotypes per sample was 2099, with a mean clonotype size of 1.7 cell equivalents. The number of detected TCRαβ cell equivalents per sample strongly increased following treatment with one year of obinutuzumab and venetoclax (at trial entry 840 [95%CI 594–1086] *vs* EOiT 5225 [95%CI 4136–6313], $P < 0.001$) (Fig. 1A). The number of identified T cell equivalents was rank-correlated to the fraction of $CD3^+$ cells in the leukocyte pool (Spearman's $\rho = 0.69$, $P < 0.001$) (Fig. 1B).

Following one year of treatment with obinutuzumab plus venetoclax, TRB repertoire diversity increased (Shannon's *H*; at trial entry 5.3 [95%CI 4.9–5.5] *vs* EOiT 6.1 [95%CI 5.6–6.6], $P = 0.001$) (Fig. 1C). This observation was confirmed in a complete cases analysis (mean difference in Shannon's *H* 0.9, [95%CI 0.3–1.5], $P = 0.006$) (Fig. 1D). To assess TRB clonality, we calculated the cumulative frequency of the top ten major clonotypes (CF10) per sample. At trial entry, the TRB repertoire was markedly oligoclonal, harboring multiple prominently expanded clonotypes (mean CF10 28.9%, [95%CI 24.3–33.4]) (Fig. 1E). Notably, treatment with obinutuzumab and venetoclax did not ameliorate TRB oligoclonality (EOiT, CF10 32.8% [95%CI 25.9–39.6], $P = 0.3$; R + 12 CF10 31.6% [95%CI 25.0–38.2], $P = 0.5$) (Fig. 1E). A complete cases analysis yielded comparable results (Fig. 1F), suggesting that treatment with obinutuzumab and venetoclax improves but does not completely restore TRB repertoire diversity.

To characterize the longitudinal evolution of clonotypes, the cohort of patients with complete sample availability ($n = 31$) was analyzed, and clonotypes were classified as those that were 'persisting' (detected both at trial entry and at R + 12), 'disappearing' (detected at trial entry but not at R + 12), or 'emerging' (not detected at trial entry but detected at EOiT and/or R + 12) (Fig. 2A). The majority of clonotypes were emerging (median 87.3%, range 37.6–99.8), but the size of individual emerging clonotypes was mostly very small (median 1.0 cell equivalents). Only a small proportion of clonotypes was persisting (median 1.7%, range 0.0–5.5%), which were mostly stable in size, with a minority demonstrating significant expansion or contraction (mean fold change 7.3, range 0.01–431) (Fig. 2B). Notably, the mean cumulative frequency of the original top 10 clonotypes, which were dominant at trial entry, remained persistently high over time (at trial entry; 30.3%, [95%CI 24.1–36.4%], EOiT; 20.2%, [95%CI 13.4–26.9%], $P = 0.0002$ and R + 12; 23.1% [95%CI 14.7–31.6%], $P = 0.02$) (Fig. 2C).

A total of 7277 (2.5%) TRB clonotypes were shared among patients (Fig. 2D). Whereas most of these were shared among only 2 patients, a smaller number of clonotypes could be detected in the TRB repertoire of ≥7 patients ($n = 110$), with one single clonotype detected in 22 patients (Fig. 2D). Cross-referencing these shared clonotypes with the VDJdb public clonotype compendium, 237/7277 (3.2%) were found to have known antigen specificity, predominantly against cytomegalovirus (CMV), Epstein-Barr virus (EBV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) and *Homo Sapiens*-derived autoantigens (Fig. 2E). The 39 shared clonotypes with human auto-antigenic specificity were found to target the proteins melan-A (*MLANA*, $n = 32$), tetherin (*BST2*, $n = 6$) and insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*, $n = 1$).

To assess whether changes in the TRB repertoire may be related to pre-treatment patient- or disease characteristics, we tested for associations between TRB repertoire diversity or oligoclonality, and patient sex, age, Rai stage, IGHV mutational status, genomic complexity, *TP53* status, presence of $IGLV3\text{-}21^{R110}$ and *SF3B1* mutations at all three timepoints (Supplementary Tables 2 and 3, Supplementary Fig. 2A–D). Out of these, statistically significant differences in TRB diversity were observed only in relation to

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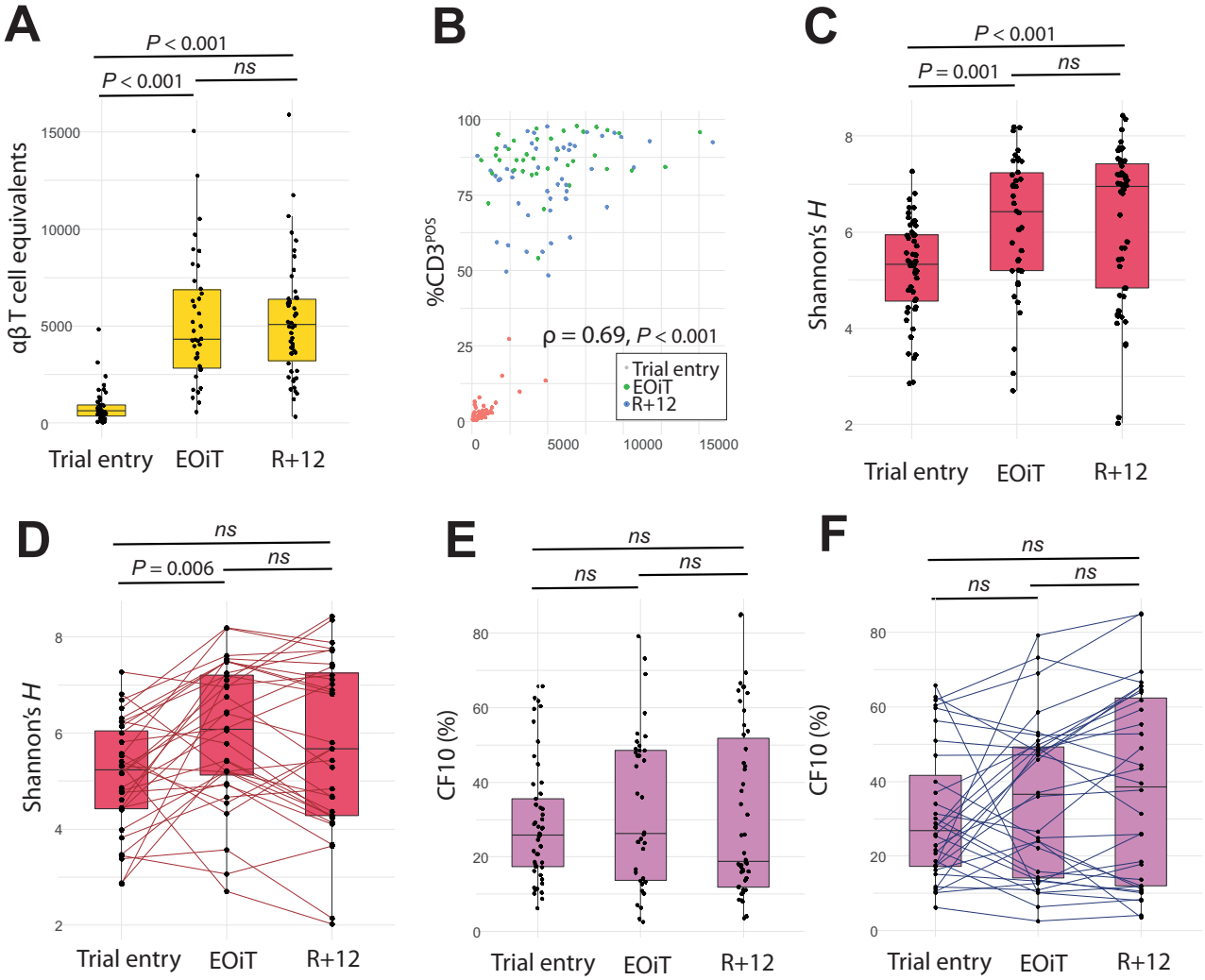



**Fig. 1 TRB diversity and clonality. A** Boxplots illustrating αβT cell equivalents, stratified per timepoint. *P*-values were calculated using a Welch's *t*-test. The black lines indicate median values, the vertical size of the boxes indicates the interquartile range. The whiskers extend 1.5x the interquartile range. Cell equivalents were calculated using the clT-QC. **B** Scatterplot illustrating the association between the $CD3^+$ leukocyte fraction and T cell equivalents. The correlation coefficient and *P* value were computed using a Spearman's correlation test. **C**, **E** Boxplots illustrating Shannon's index (**C**) or the cumulative frequency of the ten major clonotypes (**E**), stratified per timepoint. *P*-values were calculated using a Welch's *t*-test. The black lines indicate median values, the vertical size of the boxes indicates the interquartile range. The whiskers extent 1.5x the interquartile range. **D**, **F** Box- and lineplots illustrating Shannon's index (**D**) or the cumulative frequency of the ten major clonotypes (**F**), stratified per timepoint, for cases with complete sample availability. *P*-values were calculated using a paired *t*-test. The black lines indicate median values, the vertical size of the boxes indicates the interquartile range. The whiskers extend 1.5x the interquartile range. CF10 cumulative frequency of the top 10 major clonotypes, EOiT end of induction treatment, ns not significant, R + 12 twelve months after randomization.

$IGLV3\text{-}21^{R110}$, the presence of which was associated with weaker recovery of TRB repertoire diversity at R + 12 (mean Shannon's *H* 4.8 [95%CI 3.7–5.9] *vs* 6.7 [95%CI 6.2–7.1], $P = 0.003$) (Supplementary Fig. 2C). In addition, patients with $IGLV3\text{-}21^{R110}$ had significantly more pronounced TRB oligoclonality at R + 12 (mean CF10 48.4% [95%CI 30.8–66.0] *vs* 25.9% [95%CI 19.5–32.3], $P = 0.02$) (Supplementary Fig. 2D).

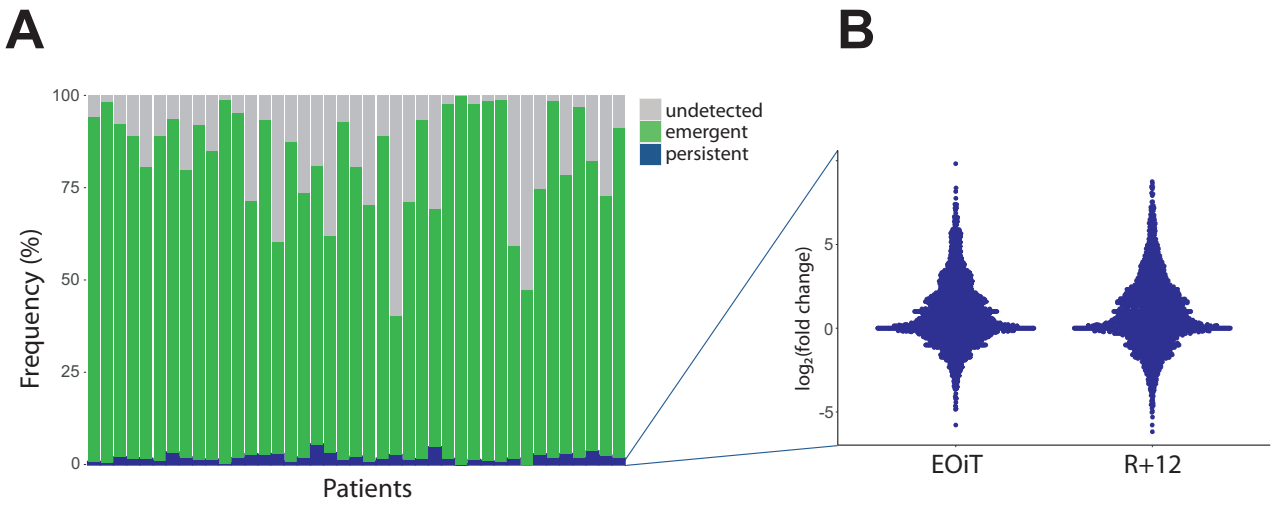
Current or future MRD status and venetoclax maintenance therapy were not associated with changes in the TRB repertoire (Supplementary Table 4). Poisson regression modeling demonstrated that TRB diversity and oligoclonality were not associated with a significantly higher risk of infection in the first year of the trial (data not shown).

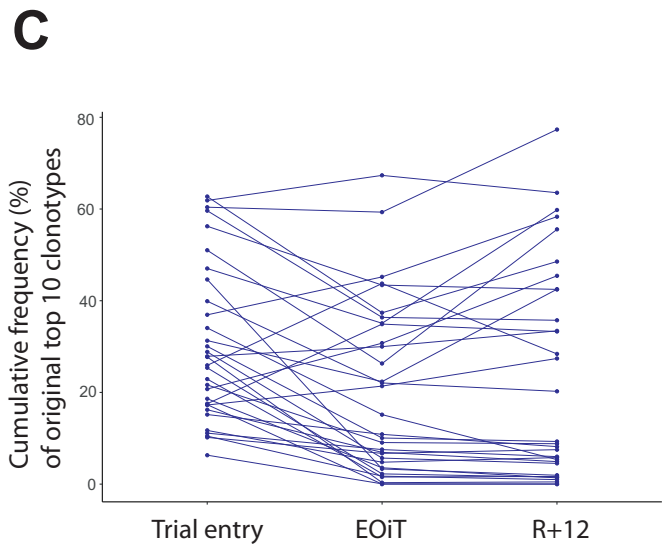
## DISCUSSION

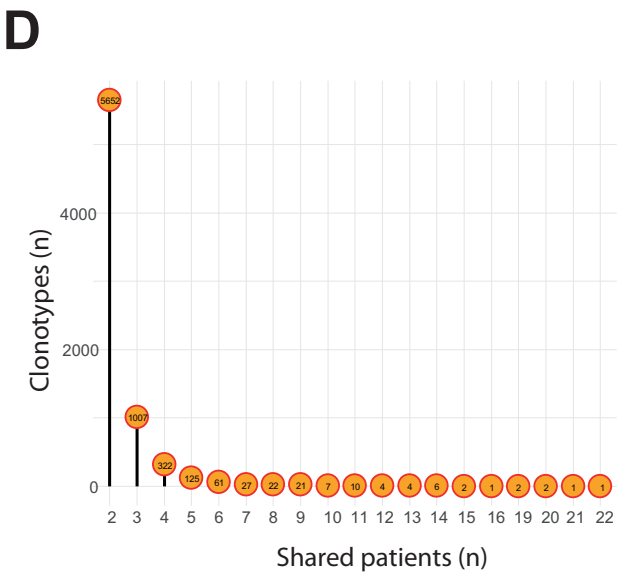
Here, we report the first characterization of the longitudinal changes in the TRB repertoire of CLL patients receiving first-line treatment with obinutuzumab plus venetoclax. Over the course of treatment, TRB repertoire diversity increased, mainly due to the emergence of a polyclonal population of small-sized, diverse clonotypes.

In line with previous work, we show that CLL patients harbor oligoclonal TRB expansions at trial entry [3–6]. Interestingly, although 1-year fixed-duration treatment with obinutuzumab plus venetoclax increased TRB diversity, it did not ameliorate TRB oligoclonality. We show that this effect is attributable to the persistence of expanded TRB clonotypes that were already dominant at trial entry. Similar dynamics have been observed after treatment with ibrutinib monotherapy and rituximab-idelalisib [6].

TRB oligoclonality is predominantly attributable to the $CD8^+$-T cell fraction [4]. It has been proposed that these $CD8^+$-T cell expansions may represent antitumor populations with antigenic specificity for CLL-derived neoantigens, such as epitopes originating from the clonotypic B cell receptor or (cyto)genetic lesions [3, 5, 11]. However, treatment with venetoclax plus obinutuzumab induces deep and



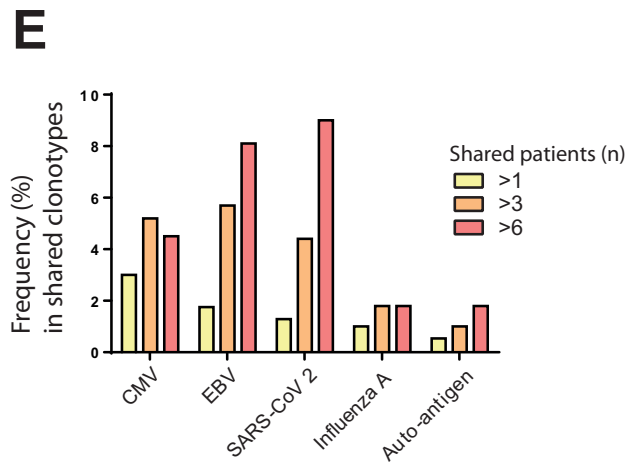




**Fig. 2 Longitudinal clonotype evolution. A** Stacked, patient-level parts-of-whole bar plot, in which TRB clonotypes are classified as 'disappearing' (detected at trial entry but undetectable at R + 12), 'emerging' (undetectable at trial entry, but detected at EOiT and/or R + 12), and 'persisting' (detectable at both trial entry and R + 12). **B** Dot plot showing the relative $log_2$-transformed fold change of persisting clonotypes, relative to trial entry. **C** Dot- and line plot, showing the longitudinal change of the cumulative frequency of the top ten major clonotypes that dominated at trial entry. Lines indicate paired samples. **D** Lollipop plot, illustrating the number of shared clonotypes and the number of patients that harbor them. **E** Bar chart showing the amount of shared clonotypes with known antigenic specificity, as a fraction of the total number of clonotypes shared among the respective number of patients. CMV cytomegalovirus, EBV Epstein-Barr virus, EOiT end of induction treatment, R + 12 twelve months after randomization, SARS-CoV2 severe acute respiratory syndrome coronavirus 2.

durable levels of MRD, with most patients rapidly reaching uMRD5 [12]. It seems less likely that, after a two-year absence of tumor-derived antigenic stimulation, antitumor clonotypes would remain oligoclonally expanded. Moreover, we could not identify any impact of the concurrent presence of MRD on TRB oligoclonality, nor did strong oligoclonality prevent future MRD resurgence.

Among TRB clonotypes shared between patients, we could identify clonotypes with reactivity against commonly encountered viruses. In addition, a subgroup of small-sized shared clonotypes were found to have auto-antigenic specificity. The most common target antigen was melan-A, which is an auto-antigen commonly targeted by cytotoxic T cells in healthy individuals with an HLA-A2 genotype [13]. As melan-A is only expressed in melanocytes, these clonotypes are unlikely to be relevant in the context of CLL. In contrast, the second most commonly target autoantigen, tetherin (CD317), is constitutively expressed by B cells and is overexpressed by CLL cells [14]. Whether these shared clonotypes can effectively target and lyse CLL cells, and may thus represent a true antitumor cytotoxic population, warrants further research. In this context, it is worth mentioning that recently developed CD317-CAR T cells have shown efficacy in targeting glioblastoma [15].

Our study has limitations. First, as we amplified TRB rearrangements from bulk PBMC DNA, our conclusions regarding TRB diversity apply to the TRB repertoire in relation to the PBMC pool, rather than specifically in relation to the total count of circulating T cells. That said, a similar study, which amplified TRB rearrangements from purified T cell DNA in the context of ibrutinib treatment, obtained comparable results [7]. Our approach also precludes the ability to draw separate conclusions on the TRB repertoires of $CD4^+$- and $CD8^+$-T cell fractions. Secondly, we have noticed that our protocol induces relative overamplification of TRBV20-1 rearrangements (data not shown). Any inferences based solely on TRBV20-1 clonotypes should therefore be interpreted with caution.

In summary, we show that treatment with obinutuzumab plus venetoclax restores TRB repertoire diversity, but does not improve TRB oligoclonality, due to the persistence of large pre-treatment clonotypes. The concurrent presence or future resurgence of MRD did not impact the TRB repertoire. These findings lay the foundation for future exploration of the viability and effectiveness of autologous T cells for T cell-mediated immunotherapy in patients that previously received obinutuzumab plus venetoclax.

## METHODS

For the "Methods" section, please see the supplementary material.

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## AUTHOR CONTRIBUTIONS

PJH, PMK, PEW, MDL, and AWL conceived of the study. PJH and JSV contributed to data collection. JMND, SK, APK, and MDL provided the patient samples. PJH, PMK, MDL, and AWL contributed to data analysis. All authors contributed to data interpretation and approved the final version of the manuscript.

## COMPETING INTERESTS

JMND has received research funding from Roche/Genentech. SK has received personal fees from Janssen, AbbVie, Novartis, Gilead, and Celgene; and research funding from AbbVie, Janssen, AstraZeneca, and Roche/Genentech. APK has received personal fees from AbbVie, LAVA, Genmab, Janssen, AstraZeneca, Roche/Genentech, and Bristol Myers Squibb; and research funding from AbbVie, Janssen, AstraZeneca, Roche/Genentech, and Bristol Myers Squibb. AWL has received research funding via an unrestricted grant from Roche-Genentech and speaker-fees from Janssen. M-DL has received personal fees from AbbVie, Janssen, and Roche; and research funding from AbbVie, Janssen, AstraZeneca, and Roche/Genentech. The remaining authors declare no competing financial interests.

## ADDITIONAL INFORMATION

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