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Original Research Article

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Ribociclib plus Letrozole Alters the Blood Immune Profile in Older Patients with HR+/HER2- Metastatic Breast Cancer

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Abstract: Background – The combination of CDK4/6 inhibitors and endocrine therapy (ET) is a standard first-line therapy for hormone receptor positive (HR+)/HER2- metastatic breast cancer (mBC). Preliminary data suggest that CDK4/6 inhibitors can alter the host immune function and stimulate tumor cell-directed immunity. However, clinical data are scarce, and no data exist about the impact of age and frailty on this phenomenon. **Material and methods –** This biomarker substudy of the RibOB trial (NCT03956654) evaluated the impact of ribociclib and letrozole on circulating immune cell subsets and protein markers in older ($\geqslant$ 70 years) patients with HR+/HER2- mBC. Peripheral blood mononuclear cell subtyping and analysis of plasma immune response and checkpoint markers were performed using flow cytometry at baseline and after three months of ribociclib + ET. Frailty status was assessed at baseline using G8 score. **Results –** 20 patients (median age: 76 years, range: 70–87 years), 8 considered fit (G8>14), and 12 frail (G8 $\leqslant$ 14), were included. After three months of treatment, the immune subset composition showed significant increases in naïve B-, T-regulatory (Tregs), and CD4+ T-cells, while memory B-cells and Tregs were significantly decreased. In addition, consistent upregulation was seen in costimulatory receptors CD27 and CD28. Plasma immune checkpoint markers B7.2 (CD86) and PD-1 were significantly decreased. The immune subset profiles of fit *versus* frail persons showed no statistically significant difference. **Conclusions –** The study shows that the combination of ribociclib and ET modulates the immune system in older patients, potentially reversing the age-related immunosenescence process by increasing naïve T-cell and B-cell populations and decreasing memory populations.

Keywords: Breast cancer; Biomarkers; Immunity; Translational research; Geriatric oncology

1. Introduction

Breast cancer (BC) is considered an age-related disease. Due to the consistent increase in the proportion of older people in the last decades, aging biology and its interaction with cancer biology have emerged as novel areas of scientific interest [1, 2].

Aging can be appraised in terms of biological changes in certain tissues, most particularly the blood compartment and immune system. Immunosenescence is the term used to describe the age-related decline in immune function that makes older people more vulnerable to infectious disease; it is thus associated with increased morbidity and mortality [3]. Although aging and cancer are closely intertwined and share many underlying mechanisms [3], no in-depth research has been performed on the aging-related immune modifications in the setting of (older) cancer patients. Declining immunological capacity and reduced immune surveillance are two common hypotheses to explain why cancer rates rise with age [2-6]. In addition, the aging process also seems to affect the biology of breast and other tumors. While triple-negative BC (TNBC) and human epidermal growth factor 2 (HER2)-positive BC appear to be more common in younger BC patients, hormone receptor-positive (HR+)/HER2- BC, also called luminal-like BC, is the most frequent BC subtype in older women [7-11].

When patients with luminal-like BC develop distant metastases, the disease becomes incurable. Endocrine therapy (ET) is the cornerstone of treating metastatic HR+/HER2- BC, but recent publications have shown that ET in combination with CDK4/6 inhibitors leads to major improvement of disease control and prolonged survival while generally maintaining quality of life [12-17]. Progression-free survival (PFS) was significantly extended when both an endocrine inhibitor and CDK4/6 inhibitor were administered concomitantly [12, 13, 18-20]. For instance, in the MONALEESA-2 study, which included only post-menopausal women with HR+/HER2- metastatic BC (mBC), first-line ribociclib together with letrozole resulted in a median PFS of 25.3 months, as opposed to 16.0 months for the control group receiving letrozole plus placebo, with a hazard ratio of 0.57 (95% CI: 0.46-0.70) [13]. This study convincingly demonstrated that CDK4/6 inhibitors confer therapeutic benefit to older persons as well. It should be noted, however, that pivotal trials in oncology do not include many frail older persons, even though these represent a major group among the older population [18, 19].

CDK4/6 inhibitors induce cell cycle arrest in (breast) tumors through dephosphorylation of retinoblastoma (Rb), preventing cells to progress from G1 to S phase. CDK4/6 inhibitors also exert this cell cycle inhibitory effect in dividing non-cancer cells, such as white blood/immune cells, and can thus potentially affect anti-tumor immunity. Moreover, CDK4/6 inhibitors also show immunomodulatory effects, causing higher T-cell activation and depletion of T-regulatory cells after administration [16, 21]. However, the effect of CDK4/6 inhibitors on the host immune system of the older patient, which was previously already shown to be remodeled due to immunosenescence [22], has not been studied yet.

The RibOB trial (NCT03956654) is a phase IV observational study evaluating the efficacy and tolerability of CDK4/6 inhibitor ribociclib in combination with letrozole, an aromatase inhibitor, in older (⩾ 70 years) patients with HR+/HER2- mBC. In a preplanned biomarker substudy, we aimed to investigate the impact of this treatment on the immune system, by assessing treatment-induced alterations of peripheral blood mononuclear cell (PBMC) subsets and plasma immune response and immune checkpoint markers.

2. Material and Methods

2.1 Study design and patient inclusion

The RibOB study (NCT03956654) is an observational prospective, open-label, single-arm, phase IV trial that evaluates the clinical efficacy, overall safety, and tolerability of ribociclib in combination with letrozole in older women (⩾ 70 years) with HR+/HER2- mBC and no prior hormonal treatment for metastatic disease as per the indication approved by the European Medicines Authority (EMA) and as made available by Belgian national authorities in the national health care system. HER2-negative BC was defined by an immunohistochemistry (IHC) score of 0 or 1+ or a negative *in situ* hybridization with a score of 2+ or 3+ by IHC. IHC and *in situ* hybridization tests were performed by local laboratory testing according to the ASCO-CAP guidelines [23]. HR+ status was defined as histologically and/or cytologically confirmed diagnosis of estrogen receptor (ER) positive and/or progesterone receptor (PgR) positive BC (defined as ER and/or PgR ⩾ 1% or Allred score >2) by the local clinical laboratory. Patients had to have a baseline 12-lead ECG with QTcF at screening < 450 msec, adequate bone marrow and organ function, and had to be able to swallow ribociclib and letrozole tablets. Patients were excluded if they had previously received any CDK4/6 inhibitor, or systemic antihormonal therapy or chemotherapy for mBC. Patients with central nervous system (CNS) metastases were permitted if the CNS lesions were previously treated and stable. Patients with gastrointestinal impairment, high risk of developing QTc prolongation, or any other concurrent severe and/or uncontrolled medical condition that would impair the study, and patients with a known history of HIV infection were not eligible for the study.

From December 2018 until December 2022, 75 patients were enrolled in 16 Belgian sites for treatment with letrozole (2.5 mg once daily) + ribociclib 600 mg (day 1 to 21 in a 28-day cycle), which continued until disease progression, intolerable toxicity, or patient/physician decision to withdraw. During the study, patients were regularly evaluated for disease progression. Radiological examinations for response assessment were performed at 12w (+/- 2w) and 24w (+/-2w). Afterwards radiological tests were further performed every 12 to 24 weeks depending on the clinical need as long as the patient was receiving treatment or during follow-up (until progression occurred). Patients were also monitored for treatment safety, for quality of life, and geriatric assessment components including functional status at 12 weeks (+/- 2 weeks) and at 48 weeks (+/-2 weeks). This study was conducted in compliance with the principles of the Declaration of Helsinki, the Good Clinical Practice, and in accordance with all applicable regulatory requirements. All patients included in this study gave written informed consent before inclusion. The study protocol was approved by the ethics committee of our institution (Ethics Committee Research University Hospitals/Catholic University Leuven; study number: S61033; Belgian registration number: B322201838186; approval date: 29 November 2018).

Within the leading participating center (UH Leuven), a preplanned prospective blood biomarker study was conducted, which aimed at evaluating the impact of ribociclib and letrozole on the circulating immune profile. For this substudy, additional collection of PBMCs was implemented. The current manuscript specifically describes this biomarker study.

2.2 G8 assessment

The G8 questionnaire is a simple, widely-used geriatric screening instrument, that can be used as an estimate for frailty [24]. Eight items make up the G8: seven are taken from the mini nutritional assessment (MNA) questionnaire (nutritional status, weight loss, body mass index, motor skills, psychological status, number of medications, and self-perception of health), and one inquires the patient's age (<80, 80-85, >85). The G8-score ranges from 17 (not at all impaired) to 0 (heavily impaired). Patients with a score ⩽ 14 are considered as potentially frail (increased risk of underlying geriatric problems), while those with a score of >14 are regarded as fit [25, 26].

2.3 Peripheral blood mononuclear cells (PBMCs) isolation

Peripheral blood for PBMC isolation was collected in two 10 mL EDTA tubes (BD Vacutainer®) at baseline (before ribociclib initiation) and after three months of treatment (three cycles of ribociclib + letrozole treatment; sample in principle taken after one week of interruption, just before initiation of next cycle). PBMCs were isolated within one hour after blood collection via density gradient centrifugation. In brief, an equal volume of Dulbecco's phosphate buffered saline (Invitrogen) containing 2% fetal bovine serum (Invitrogen) (DPBS/2%FBS) was added to the blood. Thereafter, two SepMate-50 tubes (StemCell Technologies) were each filled with 15 mL Histopaque®-1077 (Sigma-Aldrich). The diluted blood sample was divided over the two SepMate-50 tubes and centrifuged at 1200xg for 10 minutes at room temperature (RT). After centrifugation, the top layer (containing the PBMCs) was poured into a 50 mL Cellstar tube (Greiner bio-one) and centrifuged again (400xg, 10 mins, RT). After resuspension of the cell pellet, the cells were microscopically counted and assessed for viability using trypan blue (Invitrogen). Then, the cells were washed twice with DPBS/2%FBS, resuspended in 3 mL of cold freezing medium, containing 90% FBS and 10% dimethylsulfoxide (DMSO) (Sigma-Aldrich), and equally divided over three 1.8 mL cryovials (Thermo Scientific). These were frozen using a Mr. Frosty container (Thermo Scientific) filled with isopropanol (2-propanol, Merck Millipore) in a -80°C freezer. After a minimum of four hours, PBMCs were transferred to liquid nitrogen until later use for PBMC staining.

2.4 Antibody staining and flow cytometric analysis of PBMCs

After quick thawing of the PBMCs in warm RPMI1640 medium (Invitrogen) with 10% FBS (Invitrogen), the immune subset composition profiles were analyzed using 8-color flow cytometry on a 3-laser platform (FACSVerse, BD Biosciences). The use of fluorescent antibody staining panels targeted at specific combinations of cell surface markers allowed identification and analysis of the different immune cell subsets (**Supplementary Figure S1 and S2**). In total, we used five multicolor flow cytometry staining panels to investigate changes in the immune cell subsets. Details on the specific antibodies used can be found in **Supplementary Table S3**. Briefly, the major PBMC subsets (i.e., monocytes, NK-cells, naïve and memory B-cells, NK/T-cells, and total T-lymphocytes) were identified using the markers CD3, CD14, CD16, CD19, CD27, CD56, and HLA-DR. The CD4+ and CD8+ T-cell populations were further subtyped using the markers CD3, CD4, CD8, CD27, CD28, CD45RA, CD57, and CD197/CCR7. The costimulatory receptors CD27 and CD28, as well as the senescence marker CD57, were used as additional markers to assess age-related changes in the T-cell population. T-cell activation status was assessed with a combination of CD3, CD4, CD8, CD38, CD69, CD279 (PD-1), and HLA-DR targeting antibodies. All panels also included 7-AAD as a viability stain to allow dead cell exclusion. In addition, BD Horizon™ Brilliant Stain Buffer is added to the reagent mixtures to prevent staining artifacts and ensure optimal results with BD Biosciences dyes. The staining procedure was performed as follows: after washing the thawed PBMCs with DPBS/2%FBS, 400 µL of cells were preincubated with 20 µL FC block (Human TruStain FcX, BioLegend) for 10 minutes. Thereafter, 100 µL of the cells were added to each antibody mixture of the staining panels in FACS tubes separately and incubated for 15 minutes at RT in the dark. Then, the cells from each panel were washed twice with 2 mL of cold (4°C) DPBS+2%FBS and centrifuged at 500xg, 10 mins, 4°C. The pellet was resuspended in 400 µL cold (4°C) DPBS+2%FBS and kept on ice in the dark until FACS analysis. Lastly, T-regulatory cells were examined via intracellular staining using the markers CD3, CD4, CD25, CD45RA, CD127, CD152 (CTLA-4), and FoxP3. The Fixable Viability Stain (FVS) 520 was used for discrimination between viable and non-viable cells. First, 100 µL of cells were stained with 1 µL of diluted FVS520 (1/100 with staining buffer, BD Pharmingen). Then, cells were washed twice with 2 mL staining buffer (BD Pharmingen), centrifuged at 400xg for 10 minutes at RT, and resuspended. The antibody mixture was added, vortexed, and incubated for 20 minutes at RT in the dark. Cells were rewashed in 2 mL staining buffer, centrifuged at 400xg for 10 minutes at RT, and resuspended. Thereafter, fixation and permeabilization were performed with 1x Human FoxP3 buffer (BD Pharmingen) according to the manufacturer's protocol. Then, 5 µL of conjugated FoxP3 antibody was added to resuspend and stain the cell pellet and incubated for 30 minutes in the dark at RT. Cells were washed twice with 2 mL of staining buffer and centrifuged at 500xg for 5 minutes at RT. The cell pellet was resuspended in 300 µL staining buffer and analyzed immediately on the FACSVerse. All the staining panels were optimized using fluorescence minus one (FMO) as negative controls to delineate unstained populations. Data acquisition and analysis were performed with the FACSuite software (version 1.0.6, BD Biosciences).

2.5 Plasma collection

Plasma was isolated from blood collected in a 4 mL BD Vacutainer® EDTA K2E tube at baseline (before ribociclib initiation) and after three months of treatment (three cycles of ribociclib + letrozole treatment). Within 60 minutes after blood drawing, the blood samples were centrifuged at 1600xg for 15 minutes at 4°C. Then, plasma was isolated and stored in aliquots at -80°C until future use.

2.6 Analysis of plasma immune response and checkpoint markers

Plasma samples were analyzed by multiplex cytometric bead array immunoassays, using commercially available pre-mixed kits: a 13-plex, containing the analytes IL-4, IL-2, CXCL10 (IP-10), IL-1β, TNF-α, CCL2 (MCP-1), IL-17A, IL-6, IL-10, IFN-ɣ, IL-12p70, CXCL8 (IL-8), and free active TGF-β1 (LEGENDplex™ HU Essential Immune Response Panel, BioLegend®) and a 12-plex, containing sCD25 (IL-2RA), 4-1BB, sCD27, B7.2 (CD86), free active TGF-β1, CTLA-4, PD-L1, PD-L2, PD-1, TIM-3, LAG-3, and galactin-9 (LEGENDplex™ HU Immune Checkpoint Panel 1, BioLegend®). These immunoassays were performed according to the manufacturer's protocol on 1:2 diluted plasma samples and every sample was analyzed in duplicate. After the staining procedure, standard dilutions of known concentrations and test samples were run on a FACSVerse flow cytometer equipped with FACSuite software (version 1.0.6, BD Biosciences). After flow cytometric analysis of the standard dilutions and samples, data were analyzed with the online BioLegend LEGENDplex™ software tool (https://www.biolegend.com/en-us/legendplex).

2.7 Statistical analysis

First, the impact of the combination treatment on the immune system was investigated on the whole patient cohort, as well as within the fit (G8>14) and frail (G8 ⩽ 14) populations separately. Paired analyses were performed using the Wilcoxon signed rank test to evaluate changes in PBMC subsets and plasma markers between baseline and after three months of combination therapy. Then, unpaired analyses were performed using the Mann-Whitney U test to evaluate differences in PBMC subsets and plasma markers between the fit (G8>14) and frail (G8 ⩽ 14) subpopulations, both at baseline and after three months of combination treatment. Lastly, the interaction between time and frailty was analyzed as follows: first, a change score was calculated between patients' values at three months and baseline (positive values indicating increase), and second, the difference in change score was tested by the Mann-Whitney U test. This interaction test indicates whether there is a difference between fit and frail patients with respect to their evolutions over time. All tests are two-sided and evaluated at a 5% significance level. Multiple testing correction was done by applying a false discovery rate (FDR) of 0.05. Analyses have been performed using SAS software (version 9.4 of the SAS System for Windows).

3. Results

3.1 Patient characteristics

Twenty older patients with HR+/HER2- mBC were enrolled in this biomarker study at UH Leuven. All clinical and pathological characteristics can be found in **Table 1**. Median age was 76 years (range: 70 – 87 years). Based on the geriatric assessment, 8 patients were considered fit (G8>14) and 12 patients (60%) were considered frail (G8 ≤ 14). Mean age in the fit and frail subgroups was 73.5 years and 79.1 years, respectively. Invasive Breast Carcinoma of No Special Type (IBC-NST) was the most prevalent histology (70% of participants) in our study population. Bone was the most frequent site of metastasis (70%), followed by lymph nodes (55%). Primary tumor characteristics and metastatic pattern were comparable between fit and frail subgroups. Treatment dose reductions between baseline and three months were necessary in 65% of the patients (i.e. 4 out of 8 fit patients and 9 out of 12 frail patients). For these patients, doses of ribociclib were reduced to 400 mg (30%) or 200 mg (35%). Treatment had to be discontinued in 3 patients (i.e. 1 fit and 2 frail patients). Adverse events between baseline and three months were common with this treatment regimen in the older population. The most frequent grade 3 or grade 4 adverse events were neutropenia (grade 4: 10%; grade 3: 35%), hypertension (grade 3: 60%), pain (grade 3: 20%), fatigue (grade 3: 5%), and infections (grade 3: 5%) (**Supplementary Table S4**).

Table 1: Summary of clinical and pathological characteristics (n=20 patients).

Characteristics	Result	%
Age(years)		
Median	76	
Range	[70; 87]	
Frailty status		
Fit (G8>14)	8	40%
Frail (G8 ≤ 14)	12	60%
ECOG-PS at baseline		
0	11	55%
1	7	35%
2	2	10%
Histology		
IBC-NST	14	70%
ILC	5	25%
Mixed IBC-NST/ILC	1	5%
Tumor grade		
2	13	65%
3	4	20%
Unknown	3	15%
Disease setting		
Primary metastatic (*de novo*)	8	40%
Secondary metastatic	12	60%
Tumor biopsy for HR/HER2 determination		
Primary tumor	15	75%
Locoregional lymph node	3	15%
Metastasis	2	10%
Sites of metastases		
Bone	14	70%
Liver	3	15%
Lungs	4	20%
Pleura	1	5%
Lymph node	11	55%
Locoregional	5	25%
Other	3	15%
Number of sites with metastatic disease		
1	6	30%
2	7	35%
3	7	35%
Dose reduction during ribociclib treatment		
Yes	13	65%
Dose reduction to 400 mg	6	30%
Dose reduction to 200 mg	7	35%
No	7	35%
Premature ribociclib discontinuation before three months		
Yes	3	15%
No	17	85%

G8: Geriatric 8 health status screenings score; ECOG-PS: Eastern Cooperative Oncology Group - Performance Status; IBC-NST: Invasive Breast Carcinoma of No Special Type; ILC: Invasive Lobular Carcinoma

3.2 Immune composition is altered upon ribociclib/letrozole treatment in the entire study cohort

To assess the impact of the combination treatment on the immune composition, we compared PBMC subset distribution profiles between baseline and three months in the whole patient population. Detailed results of the flow cytometry analyses can be found in **Supplementary Table S5**. First, we investigated the effect of the treatment on major PBMC subsets in the entire patient cohort, including monocytes, NK-cells, B-cells, NK/T-cells, and total T-cell population. Within the B cell compartment, memory B-cells were significantly decreased (p=0.012), whereas naïve B-cells (p=0.012) were significantly increased after three months of letrozole + ribociclib treatment (**Figure 1A**). Also, a higher proportion of the monocytes exhibited the 'intermediate (CD14^{high}CD16+)' phenotype (**Figure 1B**). Next, we looked deeper into specific T-cell subsets. Within the T-regulatory cells (Tregs), the naïve Treg subset had significantly expanded (p=0.001), while the memory Treg subset had shrunken (p=0.001) after three months of combination therapy (**Figure 1C**). Interestingly, the expression of CD25 on the naïve Tregs was also significantly higher after three months of treatment (p=0.039).

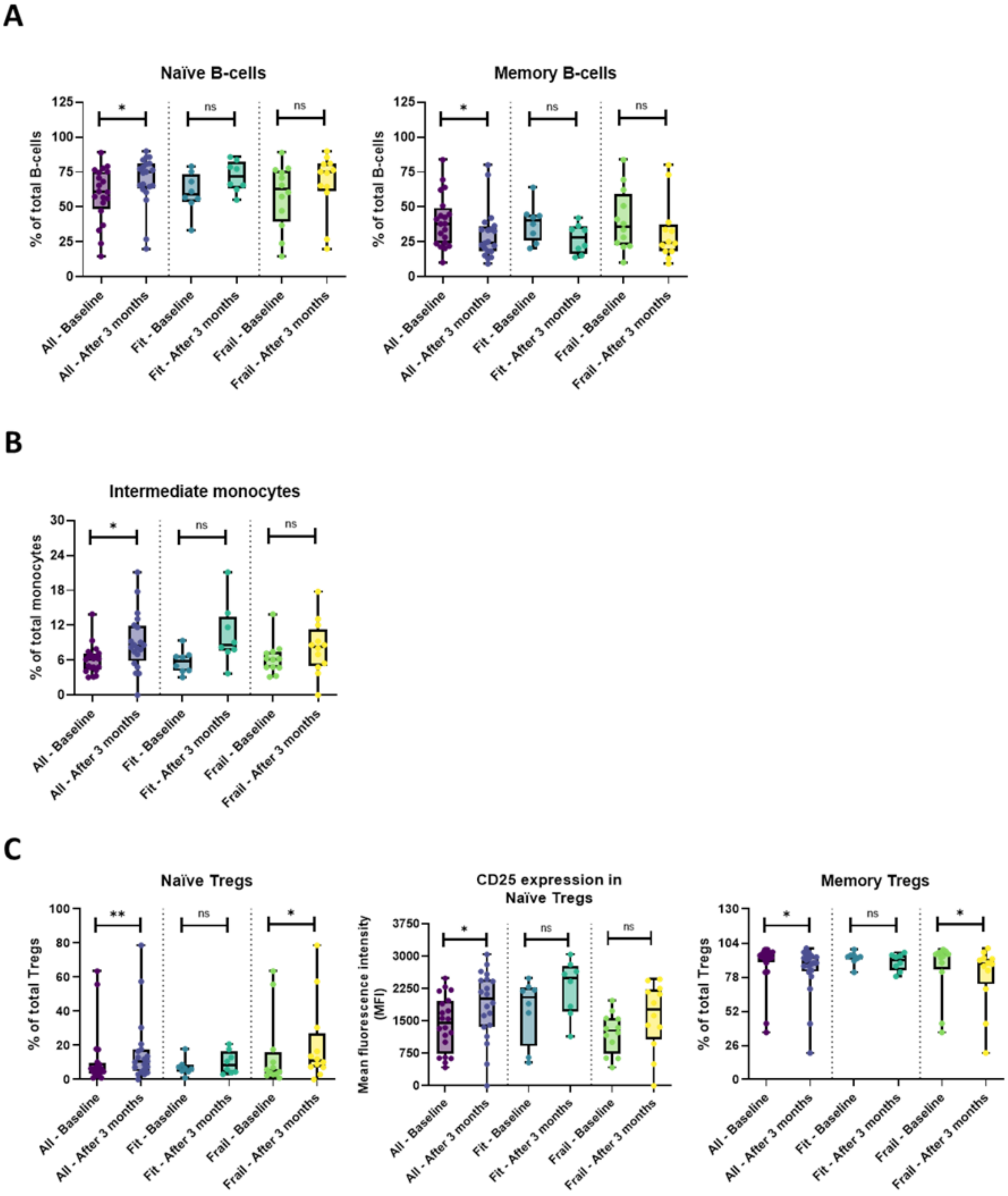
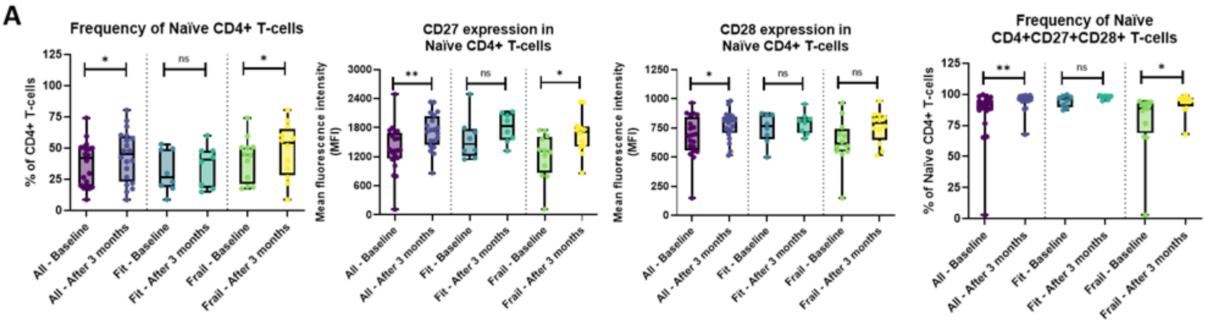


Figure 1: Overview of the B-cell, monocyte, and T-regulatory subsets in the whole patient cohort and the fit and frail

patient cohort separately. (A) After three months of combination treatment, the naïve B-cells were significantly increased, while the memory B-cells were significantly decreased in the entire patient cohort. No significant results were demonstrated in the fit and frail population. **(B)** The proportion of intermediate monocytes was significantly higher after three months of treatment in the whole patient cohort, while within the fit and frail patient cohort, no significance was found. **(C)** Within the Treg population, the percentage of naïve Tregs was significantly higher, along with an increased expression of CD25. In contrast, the memory Tregs were significantly decreased after three months of combination treatment. In the frail population, the naïve Tregs were increased as well, while the memory Tregs were significantly decreased. In the fit population, no significant results were demonstrated. The boxplots represent the IQR of the percentage of the specific immune cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leqslant 0.05$, with ** for $P \leqslant 0.01$, and with ns: no statistical significance. CD: cluster of differentiation; FDR: false discovery rate; IQR: interquartile range; Tregs: T-regulatory cells.

Within the CD4+ T-cell subset, numerous parameters significantly changed (also after multiple testing correction) during the treatment in the entire patient cohort. Naïve CD4+ T-cells increased (p=0.022) together with an upregulated expression of the costimulatory receptors CD27 (p=0.005) and CD28 (p=0.024). In addition, the double positive naïve CD4+CD27+CD28+ T-cells were also significantly more abundant (p=0.005) and exhibited upregulated expression of their costimulatory receptors CD27 (p=0.010) and CD28 (p=0.032) at three months, as compared to baseline (**Figure 2A and Supplementary Figure S6A**). Concomitant with the expansion of the naïve subset, the frequency of central memory (CM) CD4+ T-cells was decreased (p=0.026). Yet, similar to the naïve subset, the expression of the costimulatory receptors CD27 and CD28 was also significantly elevated (p=0.005 and p=0.028, respectively) on CM CD4+ T cells. Also here, the treatment appeared to promote the double positive CM CD4+CD27+CD28+ phenotype (p=0.004), along with upregulated expression of CD27 (p=0.005) and CD28 (p=0.024) on these CM CD4+CD27+CD28+ T-cells (**Figure 2B and Supplementary Figure S6B**). Although the frequency of effector memory (EM) CD4+ T-cells was not significantly altered, their expression of CD27 and CD28 did show a significant increase after three months of combination treatment (p=0.005 and p=0.022, respectively). Again, a significant expansion of the fully functional double positive EM CD4+CD27+CD28+ T-cell subset (p=0.005), which moreover showed elevated expression levels of CD27 (p=0.005) and CD28 (p=0.007), was noticed at three months (**Figure 2C and Supplementary Figure S6C**). Similar observations were made for the terminally differentiated effector memory re-expressing CD45RA (TEMRA) CD4+ T-cells: the treatment induced significant upregulation of the costimulatory receptors CD27 and CD28 (p=0.008 and p=0.022, respectively) (**Figure 2D**) and a higher fraction of the double positive TEMRA CD4+CD27+CD28+ T-cells, (p=0.005) which additionally showed increased expression of CD27 (p=0.005) and CD28 (p=0.005) (**Figure 2D and Supplementary Figure S6D**).



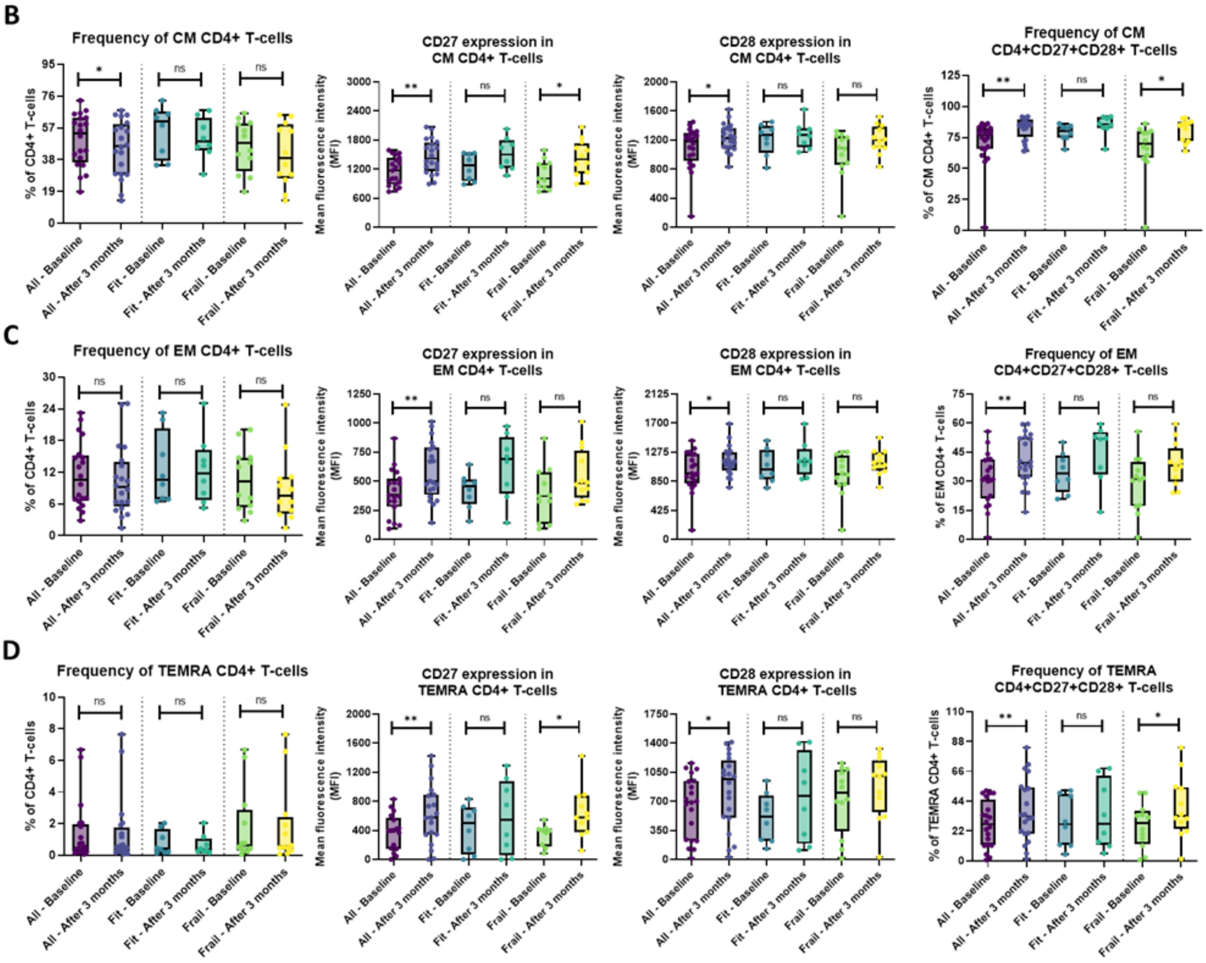


Figure 2: Overview of the CD4+ T-cell subsets in the whole patient cohort and the fit and frail patient cohort separately. (A) In the entire patient cohort, the percentage of naïve CD4+ (CD27+CD28+) T-cells was significantly higher after three months of treatment, along with a significantly increased expression of the costimulatory receptors CD27 and CD28. Within the frail patient cohort, the naïve CD4+ (CD27+CD28+) T-cells were significantly increased, along with an increased CD27 expression. No significant differences were found after three months of combination treatment in the fit patient population. **(B)** The percentage of CM CD4+ T-cells significantly decreased, while the CM CD4+CD27+CD28+ T-cell population and the CD27 and CD28 receptors' expression significantly increased in the whole patient cohort. In the frail cohort, only the increased expression of CD27 and CM CD4+CD27+CD28+ T-cell population after three months of treatment was seen. No significant results were demonstrated in the fit patient population. **(C)** In the entire patient population, after three months of treatment, the expression of CD27 and CD28 of the EM CD4+ T-cells and EM CD4+CD27+CD28+ T-cells were significantly elevated, while in the fit and frail patient population, no significance was shown. **(D)** The expression of CD27 and CD28 of the TEMRA CD4+ T-cells and frequency of TEMRA CD4+CD27+CD28+ T-cells was significantly increased in the whole patient cohort. Within the frail population, a significantly elevated CD27 expression and an increased frequency of TEMRA CD4+CD27+CD28+ T-cells were seen. Within the fit population, no significant results were demonstrated. The boxplots represent the IQR of the percentage of the specific CD4+ T-cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leq 0.05$, with ** for $P \leq 0.01$, and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

Within the CD8+ T-cell population, the treatment-induced changes basically paralleled those observed in the CD4+ T-cells, although upregulation of costimulatory receptors was rather confined to

CD27, while the intensity of CD28 staining was not significantly altered upon treatment (**Supplementary Table S5**). For example, the CD27 expression of the CD8+ naïve T-cells was significantly increased at three months (p=0.023). In addition, the frequency of naïve CD8+CD27+CD28+ T-cells (p=0.023), as well as their CD27 expression level (p=0.019), were significantly elevated (**Figure 3A and Supplementary Figure S7A**). Similar observations were seen for the CM and EM CD8 T-cell subsets (**Figure 3B,C and Supplementary Figure S7B,C**). No significant differences were found within the TEMRA CD8+ T-cell population (**Figure 3D and Supplementary Figure S7D**).

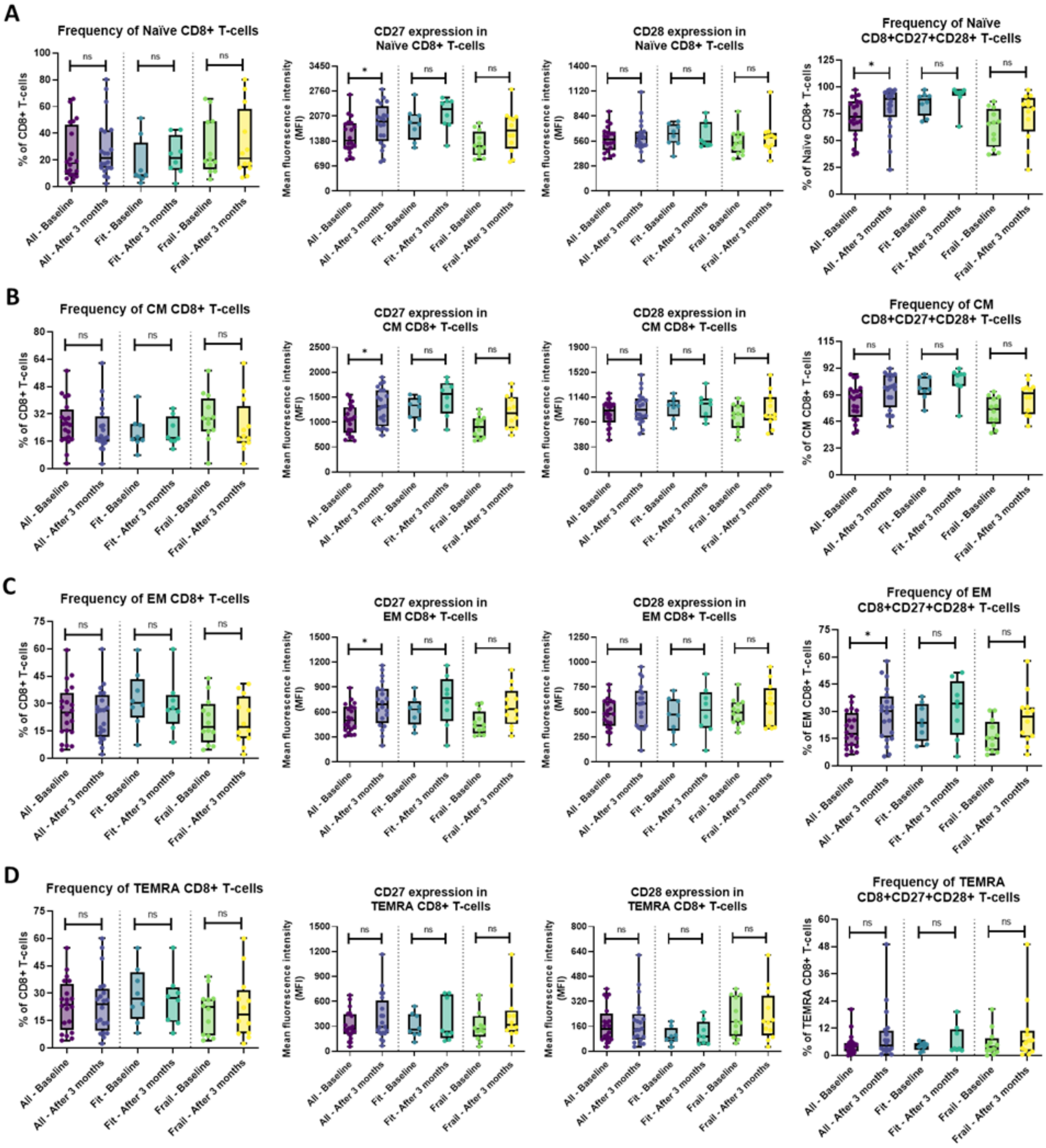


Figure 3: Overview of the CD8+ T-cell subset in the whole patient cohort and the fit and frail patient cohort separately. (A) In the entire cohort, the CD27 expression of the naïve CD8+ T-cells together with the double positive naïve CD8+CD27+CD28+ T-cells was significantly increased after three months of combination treatment, while in the fit and frail cohort, no significance was found. **(B)** In the CM CD8+ T-cell population, the expression of the costimulatory receptor CD27 was significantly increased

in the whole patient population. After three months of treatment, no significant differences were noticed within the fit and frail population. **(C)** In the entire cohort, the CD27 expression of the EM CD8+ T-cells was significantly elevated together with an increase of the EM CD8+CD27+CD28+ T-cells after three months of treatment, while in the fit and frail cohort, no significant differences were found. **(D)** No statistically significant difference was found after three months of combination therapy in the TEMRA subpopulation within the entire cohort nor in the fit and frail cohort separately. The boxplots represent the IQR of the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leqslant 0.05$ and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

Unlike the costimulatory receptors CD27 and CD28, the expression of the activation markers CD38, CD69, HLA-DR, and CD279 (PD-1) on CD4+ and CD8+ T-cells showed no marked differences between baseline and after three months of combination treatment in the whole patient cohort after correction for multiple testing (**Supplementary Table S5**).

Regarding the plasma immune response and checkpoint markers, only circulating B7.2 (CD86) and PD-1 were significantly reduced after three months of combination therapy (p=0.017 and p=0.029, respectively), when evaluated in the entire patient cohort (**Figure 4A,B**).

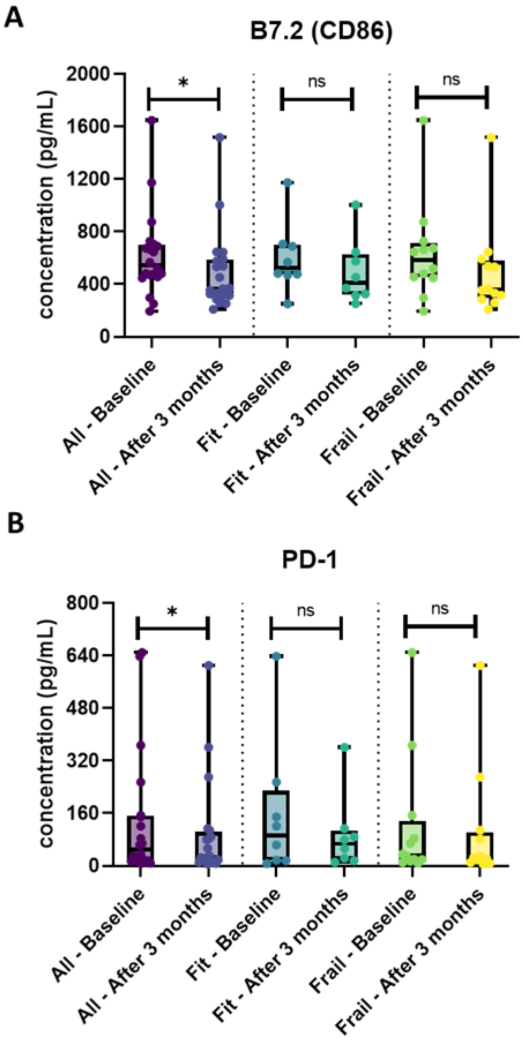


Figure 4: Overview of the plasma immune response and checkpoint markers in the whole patient cohort and the fit and frail patients cohort separately. (A) The plasma concentration of B7.2 (CD86) was significantly decreased after three

months of combination treatment in the entire patient population. No significant results within the frail and fit population were demonstrated. **(B)** In the whole patient cohort, the concentration of plasma PD-1 was significantly lower after three months of treatment, while in the fit and frail patient cohort, no significance was found. The boxplots represent the IQR of plasma immune response and checkpoint markers' concentration (pg/mL). The level of significance is indicated with * for FDR-corrected P $\leqslant$ 0.05 and with ns: no statistical significance. FDR: false discovery rate; IQR: interquartile range; PD-1: programmed cell death protein 1.

3.3 Impact of ribociclib/letrozole treatment on the immune profile in fit and frail patients

If we consider treatment-induced changes in the fit (**Supplementary Table S8**) and frail (**Supplementary Table S9**) populations separately, the naïve Tregs (p=0.019) were significantly increased and memory Tregs (p=0.019) were significantly reduced upon treatment solely in the frail, but not in the fit subpopulation (**Figure 1C**).

Also, within the frail population, significant increases of naïve CD4+ T-cells (p=0.045), with upregulated CD27 expression (p=0.038) were noted after three months of combination treatment (**Figure 2A**). Upregulation of CD27 was also found in the CM and TEMRA CD4+ T-cell populations (p=0.032 for both) after three months of treatment (**Figure 2B and 2D**). Moreover, the frequency of CD27+CD28+ double positive cells was significantly increased in the naïve, CM, and TEMRA CD4+ T-cell subsets of frail patients at three months (p-values were 0.032, 0.016, and 0.032, respectively) (**Figure 2A,B,D**). Moreover, the expression of the costimulatory receptors seemed to be increased on these CD27+CD28+ double positive subsets upon treatment in the frail population (**Supplementary Figure S6**). Interestingly, the fraction of activated CD8+ T-cells, expressing CD38, was also significantly expanded at three months compared to baseline in frail patients (p=0.047) (**Supplementary Table S9**). Conversely, in the fit population, none of the above-described changes could be demonstrated with statistical significance when applying multiple testing correction (**Supplementary Table S8**).

To further investigate whether the evolution over time of the various immune markers was different in fit *versus* frail patients, we also performed an interaction analysis between time and frailty. To this end, we calculated a change score between each patient's values at three months and baseline (positive values indicating increase), and these change scores were compared between fit and frail patients. After correction for multiple testing, no significant differences were found (**Supplementary Table S10**), indicating that in this small cohort studied here, fit and frail patients essentially did not differ with regard to their evolutions over time for any of the immune markers (PBMC subsets as well as plasma biomarkers) tested.

Lastly, when applying multiple testing correction, no significant changes in the plasma immune response and checkpoint markers could be retained, neither in fit nor in frail patients (**Supplementary Tables S8 and S9**).

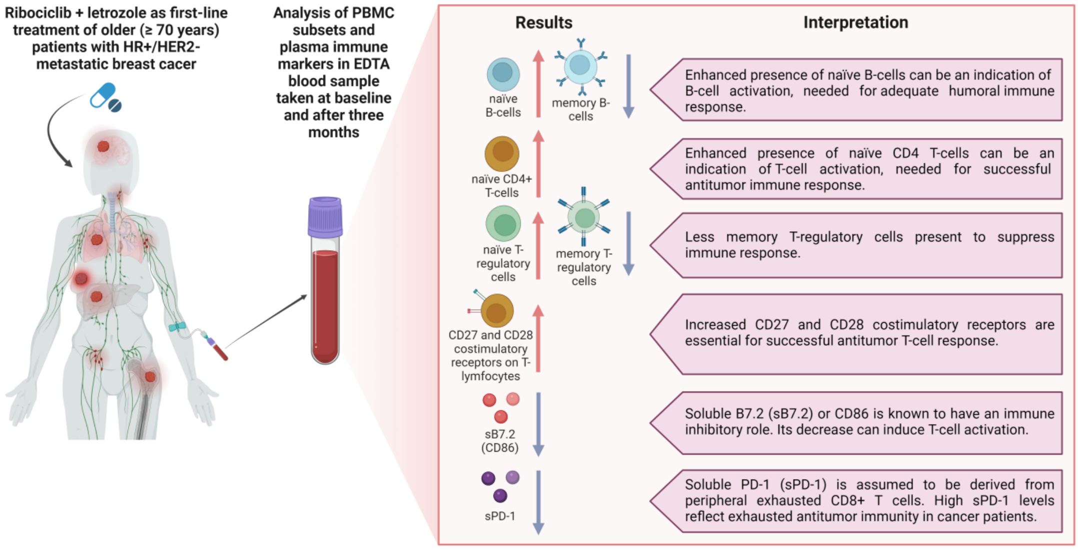
3.4 Comparison of the immune biomarkers in fit *versus* frail patients at baseline or after three months of combination treatment

Next, we evaluated if there were significant differences in the immune subset composition and/or in plasma circulating immune biomarkers between fit and frail patients, either at baseline (**Supplementary Table S11**) or after three months of ribociclib/letrozole combination treatment (**Supplementary Table S12**). No significant alterations were found after correction for multiple testing.

4. Discussion

Endocrine therapy has been used for several decades as first-line treatment option in (older) patients with HR+/HER2- mBC. However, the efficiency of treating HR+/HER2- mBC is seriously hampered by endocrine treatment resistance [27, 28]. CDK4/6 inhibitors have entered the treatment landscape, as these drugs appeared to delay endocrine resistance and to improve disease control, prolong survival, and quality of life when given in combination with ET [12-17]. Even in the understudied older population, substantial benefit is noticed when administering CDK4/6 inhibitors together with ET, as shown in the MONALEESA-2 study [13, 18, 19]. Apart from blocking the cell cycle, CDK4/6 inhibitors seem to exert immunomodulatory effects as well. A preclinical study by Deng *et al.* [21] described that CDK4/6 inhibitors promote T-cell activation, mainly due to increased T-cell recruitment and enhanced effector cell function in tumors. Furthermore, they reported that Tregs are more susceptible to CDK4/6 inhibition [21]. These findings are also confirmed by Goel *et al.* [16], who found that CDK4/6 inhibitors reduce the Treg population. The authors suggested that the selective Treg depletion might be linked to the fact that these cells express higher levels of Retinoblastoma 1 (Rb1), which is a key mediator of the CDK4/6 signaling pathway. Furthermore, they also point out that high abundance of regulatory T-cells in estrogen-positive BC tumors is associated with a poor clinical outcome [16]. However, little research has been done on how these CDK4/6 inhibitors would alter the immune profile of older HR+/HER2- mBC patients, where the immune microenvironment is already affected by the advancing aging process.

Our study explored the influence of the CDK4/6 inhibitor ribociclib in combination with letrozole, an aromatase inhibitor, on PBMC subset distribution and on a wide panel of plasma immune response and checkpoint markers in older (⩾ 70 years) patients with HR+/HER2- mBC (**Figure 5**). The main findings revealed that after three months of ribociclib/letrozole combination treatment naïve CD4+ T-cells, naïve B-cells, and naïve Tregs were significantly upregulated, whereas memory B-cells and memory Tregs were significantly downregulated. In addition, within the CD4+ and CD8+ T-cell subsets, the expression of the costimulatory receptors CD27 and CD28 was significantly upregulated after three months of this treatment. Lastly, we observed that the soluble plasma immune checkpoint markers B7.2 (CD86) and PD-1 were significantly downregulated upon treatment.



Enhanced presence of naïve B-cells can be an indication of B-cell activation, needed for adequate humoral immune response.

Enhanced presence of naïve CD4 T-cells can be an indication of T-cell activation, needed for successful antitumor immune response.

Less memory T-regulatory cells present to suppress immune response.

Increased CD27 and CD28 costimulatory receptors are essential for successful antitumor T-cell response.

Soluble B7.2 (sB7.2) or CD86 is known to have an immune inhibitory role. Its decrease can induce T-cell activation.

Soluble PD-1 (sPD-1) is assumed to be derived from peripheral exhausted CD8+ T cells. High sPD-1 levels reflect exhausted antitumor immunity in cancer patients.

Figure 5: Immunomodulatory effect of ribociclib and letrozole in older (≥ 70 years) patients with metastatic HR+/HER2- breast cancer. Blood samples were collected at baseline and after three months of therapy and changes in PBMC subsets and plasma immune markers were assessed. CD: cluster of differentiation; HER2: human epidermal growth factor 2; HR: hormone receptor; PBMC: peripheral blood mononuclear cell; PD-1: programmed cell death protein 1; s: soluble. Figure created with BioRender.

As mentioned before, within the older individuals, the immune function is already altered by the age-related process of immunosenescence [3]. This mainly results in a decreased adaptive immunity, which can impact the ability of immune cells to recognize and respond adequately to a developing tumor and thus can affect the tumor's immune response. In particular, immunosenescence is characterized by a decrease of naïve cells concurrent with an accumulation of memory cells, most notably in the T-cell population[3]. Furthermore, Koch *et al*. described that immunosenescence results in a reduced expression of the costimulatory receptors CD27 and CD28 [29]. Elyahu and colleagues reported that at old age, T-cells are more exhausted, and Treg cell frequency and functionality are elevated [30]. Also, Fulop and colleagues found that old(er) age is associated with an elevated expression of immune checkpoint markers on T-cells such as CTLA-4 and PD-1 [31]. These findings all illustrate the profound remodeling that is happening in the aging immune system. However, when we reflect on our own results, we see that the treatment with CDK4/6 inhibitors and ET actually reverses the age-related immunosenescence process, with increasing naïve T-cell and B-cell populations and decreasing memory populations. In addition, the costimulatory receptors CD27 and CD28 are increased. CD27 and CD28 costimulatory receptors are essential for T-cell survival and activation by (tumor) antigens and, consequently, for successful (anti-tumor) T-cell responses [29]. Interestingly, circulating plasma B7.2 (CD86) was significantly decreased in our study cohort after three months of combination treatment. B7.2 (CD86) is a cell surface protein-ligand predominantly expressed on antigen-presenting cells (and some tumor cells) and can bind both CD28 and CTLA-4, two receptors on T-lymphocytes that regulate the immune response. The *B7.2* (CD86)-CD28 ligand-receptor interaction evokes a costimulatory signal resulting in IL-2 production, which is essential for T-lymphocyte proliferation [32]. A soluble form sB7.2 (CD86), lacking the transmembrane domain, is generated by alternative splicing of the full-length B7.2 mRNA transcript [33]. Although the biological role of sB7.2 (CD86) is not yet fully understood, it was suggested that the release of sB7.2 (CD86) affords a powerful mechanism by which cells may modulate the costimulatory signals delivered through membrane-bound B7.2 (CD86) [33]. Several studies have reported increased levels of sB7.2 (CD86) in cancer. An immune inhibitory role of sB7.2 (CD86) was suggested via binding to CTLA-4 and inducing negative signaling to T-lymphocytes. On the other hand, an inhibitory feedback role of sB7.2 (CD86) on the costimulatory CD28 signaling induced by membrane-bound B7.2 (CD86) has also been proposed [33].This hypothesis is in agreement with our findings that circulating plasma B7.2 (CD86) is diminished concurrent with an expansion of the naïve CD4+ T-cell population after three months of ribociclib/letrozole combination therapy. This points to higher proliferation within the CD4+ T-cell pool, which could be ascribed to reinforcement of costimulatory CD28 signaling due to lower sB7.2 (CD86) levels and thus waning of the negative feedback loop. Our data however did not show an increase of circulating plasma IL-2 levels.

Besides sB7.2 (CD86), plasma circulating soluble PD-1 was also significantly decreased after three months of combination treatment. PD-1 is part of the immune checkpoint pathway PD-1/PD-L1 which dampens T-cell activity after an antigen-induced immune response, in order to avoid sustained immune activation [32, 34]. In a cancer setting, PD-1 ligation to its ligands PD-L1 and PD-L2 expressed on tumor cells or antigen-presenting cells, results in T-cell inhibition and immune escape. Blockade of the PD-1/PD-L1 axis with monoclonal antibodies (immune checkpoint inhibitors such as pembrolizumab or atezolizumab) have revolutionized the therapy of several cancer types [33, 35, 36]. Similar to sB7.2 (CD86), soluble variants of both PD-1 (sPD-1) and its ligands (sPD-L1, sPD-L2) are produced by alternative splicing. These can be detected in the plasma of healthy individuals, and elevated levels are found in autoimmune diseases, chronic infections, and various cancers, with increased levels being associated with worse outcomes in cancer patients [33, 36]. Furthermore, ribociclib plus letrozole treatment for three months resulted in an upregulation of the Tregs naïve subset. This could possibly indicate that naïve Tregs are less activated and thus exert less of their immunosuppressive effect (which is compatible with immune stimulation). This could explain the observed accumulation of naïve Tregs and concomitant reduction of the memory Treg subset in our older patient cohort after three months of treatment. Notably, these observations confirm recent findings reported by Scirocchi *et al.* [37], that the total Tregs and specifically the effector Treg subset was significantly reduced during treatment with CDK4/6 inhibitors. Nonetheless, our results could not demonstrate a statistically significant decrease in the total Treg population. Taken together, these findings strengthen the evidence that CDK4/6 inhibitors provoke an immunomodulatory effect and stimulate immune function as an additional mode of action, even in the aged BC population, where immunosenescence becomes an important determinant in the patient's immune landscape.

As already mentioned, older patients (both fit and frail) represent a major part of the HR+/HER2- BC population. CDK4/6 inhibitors appear to be beneficial in older people in general, but it is a well-known phenomenon that pivotal studies in cancer do not include frail older people, although those make up a major part of the older population [19]. As a result, safety profiles, and optimal dosing of anticancer drugs in the frail subgroup is not well documented [9, 19, 38]. It is thus critical to assess the effectiveness and tolerability of CDK4/6 inhibitors in older BC cohorts, including both fit and frail individuals. We found no statistically significant difference in any of the immune parameters tested when comparing fit and frail patients within our study cohort, neither at baseline nor at three months. In addition, the evolution in PBMC subset composition and plasma biomarkers between the two time points did not differ between fit and frail patients, as assessed by frailty*time interaction analysis with correction for multiple testing. These findings indicate that the frailty status of an older BC patient has no impact on the immune-related effects of this treatment.

This study has some limitations, most importantly the small sample size (n=20) with a relative dominance of frail patients in the cohort (12 frail compared to 8 fit patients). This could explain why the changes in immune markers observed in the frail subgroup were not significant in the fit subgroup. The subanalyses comparing fit and frail patients should thus be interpreted very cautiously and larger studies are needed to clearly dissect differential effects according to fitness/frailty status. In addition, this translational study was conducted in one single center; for feasibility reasons, PBMCs for translational endpoints of the RibOB trial were only collected from patients enrolled at the University Hospital in Leuven, Belgium. This may lead to patient selection bias, necessitating further research and validation in larger independent cohorts in multicentric prospective trials with an even distribution of fit and frail older patients. Another limitation is that we did not have access to a healthy control group without cancer and not treated with letrozole and CDK4/6 inhibitor; we could thus not monitor spontaneous fluctuations of the different PBMC subsets over time and/or with seasonal changes. Furthermore, the impact of the CDK4/6 inhibitor ribociclib in combination with ET on the immune system was only examined at two time points, i.e., baseline and after three months of treatment. In this way, it was not possible to dissect the exact dynamics of the immunomodulatory effects during treatment. Collecting PBMC and plasma samples at multiple time points in large prospective studies could give us a better insight into the immune-related effects of the combination treatment, both in the short and the long term. Furthermore, only a selection of specific PBMC subsets and plasma immune markers were evaluated to create a global picture of the immunomodulatory changes of the combination treatment. For instance, we did not include a PBMC staining panel for myeloid derived suppressor cells (MDSCs), because the currently used blood collection and processing procedure, including PBMC freezing, did not allow subsequent staining of MDSCs, which is only possible when working with freshly isolated PBMC. Within this study, we still need to evaluate a possible association between the change in the immune composition and therapy response in terms of progression-free survival (PFS) because follow-up is too short. With an expected median PFS of 24 months on first-line aromatase inhibitor plus CDK4/6 inhibitor, we plan to do this analysis in the upcoming year. Also, RibOB used simultaneous administration of CDK4/6 inhibitor ribociclib and letrozole as 'standard' first-line therapy in the older population. Hence, we cannot distinguish the contributions of either component to the immune-related effects of the combination treatment, especially in view of the versatile crosstalk between the endocrine system and immunity, which becomes even more complex in the context of aging[39,40]. The recently presented SONIA trial [41] suggests that it may be safe to use an aromatase inhibitor alone as first-line monotherapy, and only add the CDK4/6 inhibitors (to fulvestrant) in second line. We assume that the results would be comparable when CDK4/6 inhibitors would be used in second line, but this also requires further confirmation. Lastly, we did not assess the immunomodulatory effects of the other CDK4/6 inhibitors palbociclib and abemaciclib. As a result, we are unable to conclude whether or not the observed effects on host immunity are universal to CDK4/6 inhibition in general.

Nevertheless, this study has also major strengths. The study cohort was prospectively collected from a homogenous group of metastatic HR+/HER2- BC patients. Most importantly, this research has never been conducted before in the older HR/HER2- mBC population in order to understand what is happening with the immune system when older/clinically frail patients, most likely exhibiting some degree of immunosenescence, are treated for mBC with this standard treatment regimen. This study could be a landmark in the geriatric oncology field, as too few studies are conducted in the older BC population, including both fit and frail patients.

5. Conclusion

For the first time, we investigated whether CDK4/6 inhibitors in combination with ET affect the immune system in older HR+/HER2- mBC patients. Our results suggest that the CDK4/6 inhibitor ribociclib influences the anticancer immune response by shifting the balance from immunosuppression to immunological activation. Furthermore, CDK4/6 inhibitors combined with ET exerted a comparable immunomodulatory impact on fit and frail patients. However, to further validate these findings, there is a need for more extensive independent prospective studies involving larger cohorts of older BC patients (including fit and frail patients). In addition, apart from PBMC subsets and plasma immune markers, TCR sequencing analyses on PBMC could be an interesting technique to generate a more comprehensive picture of the dynamic changes in the immune environment during treatment.

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Conflict of interest

Hans Wildiers: his institution received financial compensation on his behalf for advisory boards, lecture fees and/or consultancy fees from Daiichi Sankyo, Gilead, Lilly, Pfizer, Novartis, MSD, Relay Therapeutics, PSI, Augustine Therapeutics, Astra Zeneca, Roche. He received travel support from Gilead, Daiichi Sankyo, Pfizer.

Authors' contributions

Conceptualization: HW – SH – CK – NP. Data curation: /. Formal analysis: AL. Funding acquisition: YL – HW. Investigation: YL – SH. Methodology: HW – SH – CK – NP – YL – LD – PN – EdA. Project administration: YL – SH – CK – HW. Resources: HW – PN – CK. Software: /. Supervision: HW – SH – CK – YL. Validation: /. Visualization: YL. Writing original draft: YL – SH – HW – GF. Writing – review & editing: All authors.

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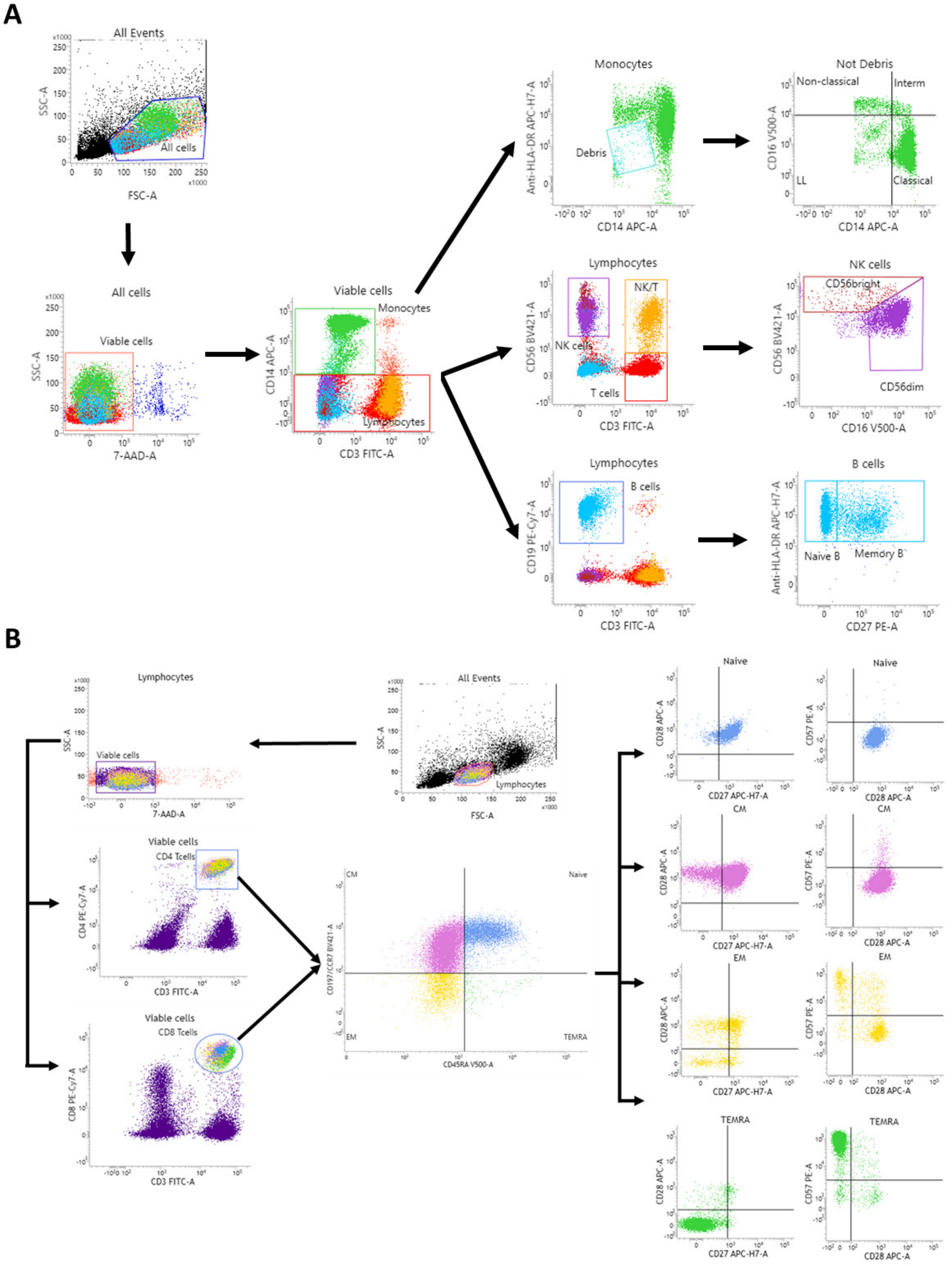
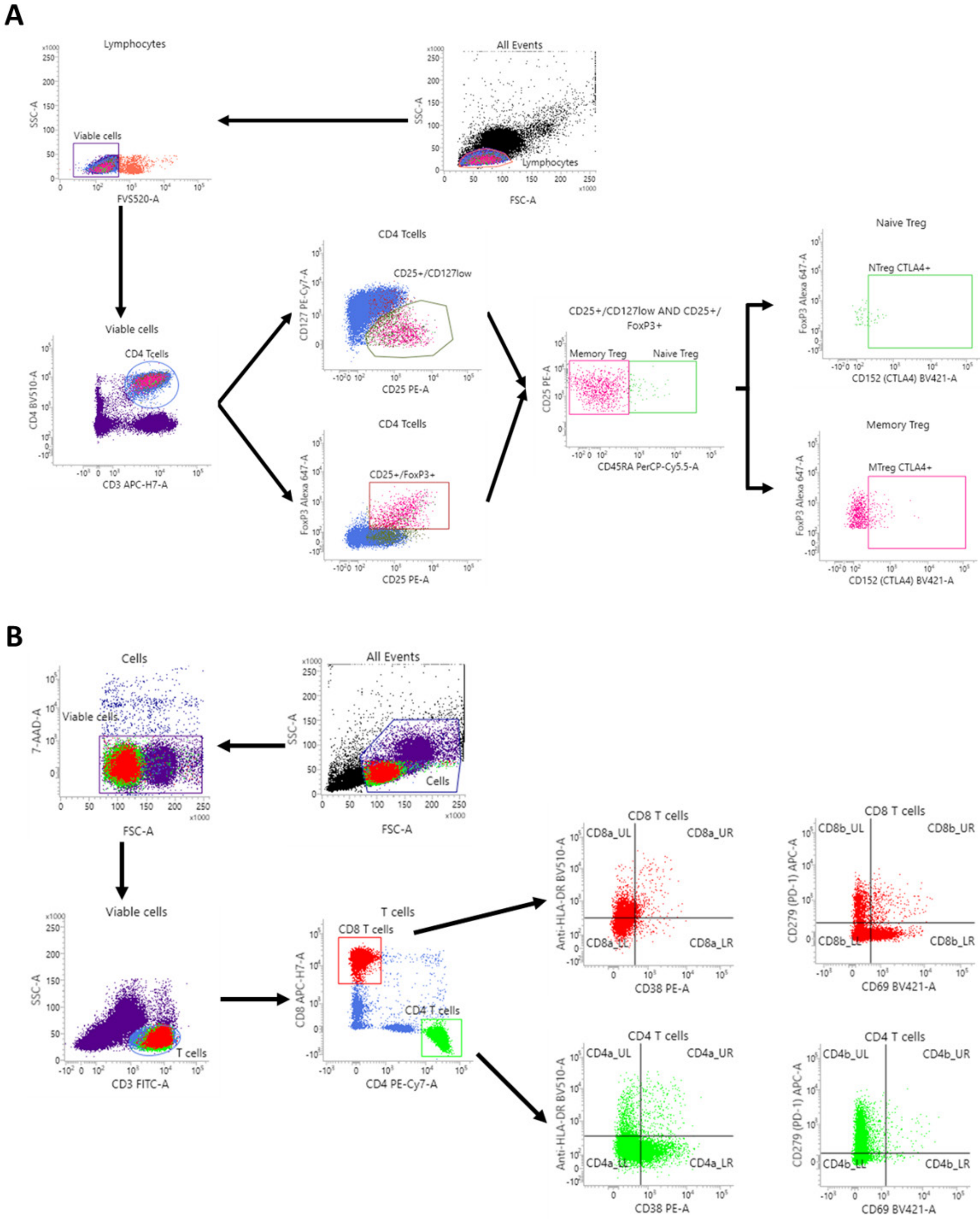
Supplementary Figure S1



Supplementary Figure S1: Schematic overview of the flow cytometry gating strategy applied for the (A) major PBMC

subsets, containing monocytes, NK-cells, B-cells, NK/T-cells, and total T-cells and (B) CD4+ and CD8+ T-cell subset profiling using 8-color flow cytometry. Fluorescence minus one (FMO) controls were used to discriminate between positive and negative signals and to aid gating of the different cell populations. **(A)** This staining panel included 7-AAD for dead cell exclusion (first gate). The major PBMC subsets (i.e., monocytes, NK-cells, B-cells, NK/T-cells, and total T-cells) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD14-APC, CD16-V500, CD19-PE.Cy7, CD27-PE, CD56-BV421, and HLA-DR-APC.H7. **(B)** This staining panel included 7-AAD for dead cell exclusion (first gate). The CD4+ T-cell subsets naïve, central memory (CM), effector memory (EM), and terminally differentiated effector memory (TEMRA) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD4-PE.CY7, CD27-APC.H7, CD28-APC, CD45RA-V500, CD57-PE, and CD197/CCR7-BV421. The CD8+ T-cell subsets naïve, central memory (CM), effector memory (EM), and terminally differentiated effector memory (TEMRA) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD8-PE.CY7, CD27-APC.H7, CD28-APC, CD45RA-V500, CD57-PE, and CD197/CCR7-BV421. CD: cluster of differentiation; Class MO: classical monocytes; Int MO: intermediate monocytes; Non-class MO: non-classical monocytes; MO: monocytes; NK: natural killer.

Supplementary Figure S2



Supplementary Figure S2: Schematic overview of the flow cytometry gating strategy applied for the (A) T-regulatory cell subset and (B) CD4+ and CD8+ T-cell activation status profiling using 8-color flow cytometry. Fluorescence minus one (FMO) controls were used to discriminate between positive and negative signals and to aid gating of the different cell populations. **(A)** First gating was applied on the lymphocyte population. Thereafter, this staining panel included FVS520 for dead cell exclusion (second gate). The T-regulatory cells including the naïve and memory subset were determined using a panel with the following antibody-fluorochrome combination: CD3-APC-H7, CD4-BV510, CD25-PE, CD45RA-PerCP-Cy5.5, CD127-PE-Cy7, CD152 (CTLA-4)-BV421, and FoxP3-AF647. **(B)** This staining panel included 7-AAD for dead cell exclusion (first gate). The CD4+ and CD8+ T-cell activation status subsets were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD4-PE.Cy7, CD8-APC.H7, CD38-PE, CD69-BV421, CD279 (PD-1)-APC, and HLA-DR-BV510. CD: cluster of differentiation; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; FVS: fixable viability stain; PD-1: programmed cell death protein 1; LL: lower left; LR: lower right; UL: upper left; UR: upper right.

Supplementary Table S3

Supplementary Table S3: Overview of antibody marker information in the PBMC staining panels used in the study cohort. Per PBMC subset, the antibody marker, clone, fluorochrome, company, and catalog number information is provided.

PBMC Subset	Marker	Clone	Fluorochrome	Company	Catalog number
Major Subsets					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD14	MφP9	APC	BD Biosciences	345787
	CD16	3G8	V500	BD Biosciences	561394
	CD19	HIB19	PE.Cy7	BioLegend	302216
	CD27	M-T271	PE	BioLegend	56406
	CD56	NCAM16.2	BV421	BD Biosciences	562751
	HLA-DR	G46-6	APC.H7	BD Biosciences	561358
	Brilliant Stain Buffer			BD Biosciences	563794
CD4 T-cells					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD4	SK3	PE.Cy7	BD Biosciences	557852
	CD27	MT271	APC.H7	BD Biosciences	560222
	CD28	CD28.2	APC	BioLegend	302912
	CD45RA	HI100	V500	BD Biosciences	561640
	CD57	HNK-1	PE	BioLegend	359612
	CD197/CCR7	G043H7	BV421	BioLegend	353208
	Brilliant Stain Buffer			BD Biosciences	563794
CD8 T-cells					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD8	SK1	PE.Cy7	BioLegend	344712
	CD27	MT271	APC.H7	BD Biosciences	560222
	CD28	CD28.2	APC	BioLegend	302912
	CD45RA	HI100	V500	BD Biosciences	561640
	CD57	HNK-1	PE	BioLegend	359612
	CD197/CCR7	G043H7	BV421	BioLegend	353208
	Brilliant Stain Buffer			BD Biosciences	563794
Tregs					
	FVS520			BD Biosciences	564407
	CD3	SK7	APC.H7	BD Biosciences	560275
	CD4	SK3	BV510	BD Biosciences	562971
	CD25	M-A251	PE	BD Biosciences	560989
	CD45RA	HI100	PerCP-Cy5.5	BD Biosciences	563429
	CD127	HIL-7R-M21	PE.Cy7	BD Biosciences	560822
	CD152 (CTLA-4)	BNI3	BV421	BD Biosciences	562743
	FoxP3	236A/E7	AF647	BD Biosciences	561184
	Brilliant Stain Buffer			BD Biosciences	563794

Continuation Table:

PBMC Subset	Marker	Clone	Fluorochrome	Company	Catalog number
T-cell activation status					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD4	SK3	PE.Cy7	BD Biosciences	557852
	CD8	SK1	APC.H7	BD Biosciences	560179
	CD38	HIT2	PE	BD Biosciences	555460
	CD69	FN50	BV421	BD Biosciences	562884
	CD279 (PD-1)	EH12.2H7	APC	BioLegend	329908
	HLA-DR	G46-6	BV510	BD Biosciences	563083
	Brilliant Stain Buffer			BD Biosciences	563794

Supplementary Table S4

Supplementary Table S4: Overview of the most common adverse events during the combination (ribociclib + letrozole) treatment in the patient cohort (n=20).

Adverse event	**Severity**		
	Grade 1-2	**Grade 3**	**Grade 4**
		number of patients (percent)	
Any adverse event	20 (100)	12 (60)	2(10)
Neutropenia	-	7 (35)	2 (10)
Hypertension	1 (5)	12 (60)	-
Pain	12 (60)	4 (20)	-
Fatigue	11 (55)	1 (5)	-
Infections	2 (10)	1 (5)	-
Dyspnea	11 (55)	-	-
Nausea	10 (50)	-	-
Anorexia	10 (50)	-	-
Constipation	9 (45)	-	-
Rash	9 (45)	-	-
Diarrhea	8 (40)	-	-
Dizziness	8 (40)	-	-
Edema	8 (40)	-	-
Dysgeusia	6 (30)	-	-
Cough	6 (30)	-	-
Alopecia	6 (30)	-	-
Vomiting	5 (25)	-	-
Itch	4 (20)	-	-
Hot flashes	4 (20)	-	-
Bloating	4 (20)	-	-
Palpitations	3 (15)	-	-
Myalgia	3 (15)	-	-
Epistaxis	3 (15)	-	-
Other AEs	10 (50)	8 (40)	-

Supplementary Table S5

Supplementary Table S5: Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the whole patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values were calculated using a paired Wilcoxon signed rank test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
PBMC - Major Subsets						
Viable cells (% of total cells)	20	98.99 [98.29;99.26]	20	99.19 [98.76;99.61]	0.182	0.327
Viable lymphocytes (% of total lymphocytes)	20	97.36 [93.46;98.17]	20	97.92 [96.94;98.59]	***0.019***	0.087
Total T-cells (% of viable lymphocytes)	20	62.16 [55.47;68.32]	20	61.97 [52.98;69.93]	0.841	0.946
Total T-cells (% of viable cells)	20	57.50 [45.84;62.30]	20	57.07 [46.77;64.40]	1.000	1.000
CD4+ T-cells (% of viable lymphocytes)	20	51.66 [39.48;56.80]	20	51.17 [41.65;56.88]	0.841	0.946
CD8+ T-cells (% of viable lymphocytes)	20	15.91 [11.36;18.93]	20	12.58 [9.09;18.00]	0.123	0.327
CD4/CD8 ratio (% CD4/CD8 ratio)	20	3.58 [2.42;4.65]	20	3.31 [2.62;6.73]	0.202	0.331
NK/T-cells (% of viable cells)	20	2.61 [1.49;5.78]	20	3.70 [1.78;5.62]	0.898	0.951
Total NK cells (% of viable cells)	20	7.86 [3.69;11.22]	20	9.63 [6.76;13.51]	0.154	0.327
CD56 bright CD16- (% of total NK cells)	20	4.66 [3.22;9.34]	20	5.90 [3.54;12.09]	0.414	0.622
CD56 dim CD16+ (% of total NK cells)	20	68.35 [47.60;82.71]	20	64.98 [50.43;88.52]	0.571	0.790
Total B-cells (% of viable cells)	20	8.65 [6.16;9.83]	20	8.28 [5.35;11.73]	0.622	0.799
Naive B-cells (% of total B-cells)	20	60.80 [50.18;74.87]	20	74.80 [63.53;80.94]	***0.001***	***0.012***
Memory B-cells (% of total B-cells)	20	37.94 [24.10;47.79]	20	24.48 [18.23;35.21]	***0.001***	***0.012***
Total monocytes (% of viable cells)	20	14.81 [10.16;23.10]	20	14.40 [7.36;19.17]	0.177	0.327
Non-classical monocytes (% of total monocytes)	20	4.23 [1.98;6.55]	20	6.16 [3.42;11.02]	0.154	0.327
Intermediate monocytes (% of total monocytes)	20	6.17 [4.46;7.04]	20	8.41 [6.23;11.84]	***0.002***	***0.012***
Classical monocytes (% of total monocytes)	20	80.89 [72.55;85.10]	20	73.97 [63.39;81.42]	***0.044***	0.159
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	20	42.15 [19.56;50.27]	20	45.14 [24.22;58.97]	***0.012***	***0.023***
CD27 expression in Naïve CD4+ (MFI)	20	1334.50 [1162.00;1668.50]	20	1733.00 [1476.50;2001.00]	***0.002***	***0.005***
CD28 expression in Naïve CD4+ (MFI)	20	686.50 [569.00;825.00]	20	806.00 [712.00;844.50]	***0.015***	***0.025***
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	20	91.71 [87.13;94.63]	20	96.29 [94.83;98.49]	***<0.001***	***0.005***
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	20	1424.50 [1265.00;1765.50]	20	1770.50 [1552.00;2023.50]	***0.005***	***0.010***
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	20	696.50 [590.00;829.00]	20	803.00 [717.50;841.50]	***0.023***	***0.032***
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	20	4.07 [1.48;11.63]	20	3.06 [0.78;8.97]	0.504	0.554
CM CD4+ (% of CD4+ T-cells)	20	53.77 [37.11;62.96]	20	46.21 [29.38;58.71]	***0.017***	***0.026***
CD27 expression in CM CD4+ (MFI)	19	1170.00 [885.00;1436.00]	19	1477.00 [1216.00;1758.00]	***0.001***	***0.005***
CD28 expression in CM CD4+ (MFI)	20	1180.00 [936.00;1290.50]	20	1224.00 [1098.50;1358.00]	***0.019***	***0.028***
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	20	76.72 [65.75;82.10]	20	85.23 [77.53;88.89]	***<0.001***	***0.004***
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	20	1386.50 [1222.00;1711.50]	20	1647.00 [1483.00;1917.00]	***0.001***	***0.005***

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	20	1185.50 [961.50;1263.50]	20	1211.00 [1103.00;1335.50]	***0.014***	***0.024***
CM CD4+ CD57+ (% of CM CD4+ T-cells)	20	2.47 [1.61;3.44]	20	1.95 [1.53;3.06]	0.335	0.394
EM CD4+ (% of CD4+ T-cells)	20	10.51 [6.70;15.07]	20	9.15 [5.77;13.73]	0.231	0.293
CD27 expression in EM CD4+ (MFI)	19	380.00 [280.00;522.00]	19	502.00 [368.00;793.00]	***0.001***	***0.005***
CD28 expression in EM CD4+ (MFI)	20	964.00 [834.50;1242.50]	20	1131.00 [1023.00;1260.00]	***0.012***	***0.023***
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	20	31.26 [21.65;41.04]	20	39.21 [32.27;52.50]	***0.001***	***0.005***
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	20	1146.00 [995.50;1310.00]	20	1336.50 [1252.00;1563.50]	***0.001***	***0.005***
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	20	1099.50 [960.00;1286.50]	20	1209.00 [1148.00;1374.50]	***0.002***	***0.007***
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	20	0.13 [0.00;0.40]	20	0.11 [0.04;0.27]	0.106	0.140
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	20	2.43 [1.23;8.52]	20	1.79 [0.69;5.37]	0.522	0.555
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	20	58.93 [53.20;67.31]	20	48.15 [44.20;64.09]	***0.003***	***0.007***
EM CD4+ CD57+ (% of EM CD4+ T-cells)	20	1.88 [1.13;8.33]	20	1.87 [1.20;4.17]	0.312	0.381
TEMRA CD4+(% of CD4+ T-cells)	20	0.48 [0.27;1.92]	20	0.49 [0.26;1.63]	0.735	0.758
CD27 expression in TEMRA CD4+ (MFI)	17	398.00 [154.00;545.00]	17	632.00 [346.00;891.00]	***0.003***	***0.008***
CD28 expression in TEMRA CD4+ (MFI)	20	692.50 [231.50;943.50]	20	963.00 [506.50;1171.00]	***0.012***	***0.023***
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	20	26.93 [11.58;42.57]	20	32.74 [20.24;53.91]	***0.002***	***0.005***
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	20	1092.50 [937.00;1301.50]	20	1493.00 [1172.00;1639.50]	***0.001***	***0.005***
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	20	1061.00 [887.00;1173.00]	20	1245.00 [975.00;1525.50]	***0.001***	***0.005***
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	20	1.63 [0.00;10.42]	20	3.00 [0.07;5.95]	0.782	0.782
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	20	18.64 [8.39;38.84]	20	10.10 [3.57;31.62]	***0.016***	***0.025***
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	20	34.32 [17.33;67.51]	20	28.59 [10.78;61.93]	0.368	0.419
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	20	17.62 [8.89;44.02]	20	21.62 [14.09;41.89]	***0.036***	0.085
CD27 expression in Naïve CD8+ (MFI)	20	1396.00 [1188.00;1865.00]	20	1921.00 [1434.50;2255.00]	***0.006***	***0.023***
CD28 expression in Naïve CD8+ (MFI)	20	577.50 [466.00;662.00]	20	584.00 [510.00;663.00]	0.257	0.339
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	20	72.14 [60.73;86.15]	20	88.64 [73.50;94.74]	***0.006***	***0.023***
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	20	1683.50 [1548.50;2036.00]	20	2057.00 [1755.50;2437.00]	***0.002***	***0.019***
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	20	605.50 [517.50;691.50]	20	610.50 [538.00;674.50]	0.231	0.331
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	20	4.13 [2.10;7.11]	20	2.40 [1.40;4.15]	0.316	0.373
CM CD8+ (% of CD8+ T-cells)	20	25.91 [17.36;33.04]	20	18.02 [15.82;28.72]	0.105	0.166
CD27 expression in CM CD8+ (MFI)	20	1059.00 [800.50;1284.00]	20	1313.00 [955.00;1638.00]	***0.002***	***0.019***

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD28 expression in CM CD8+ (MFI)	20	939.50 [769.50;1038.00]	20	947.00 [809.00;1111.00]	***0.040***	0.088
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	20	66.45 [51.44;73.09]	20	75.44 [61.14;85.70]	***0.011***	***0.035***
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	20	1416.50 [1221.00;1582.00]	20	1605.00 [1383.00;1874.50]	***0.002***	***0.019***
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	20	1006.00 [847.00;1079.50]	20	993.00 [882.00;1127.00]	0.058	0.113
CM CD8+ CD57+ (% of CM CD8+ T-cells)	20	10.78 [6.78;13.82]	20	10.64 [7.35;14.80]	0.701	0.746
EM CD8+ (% of CD8+ T-cells)	20	25.14 [14.66;34.27]	20	26.06 [12.65;33.73]	0.083	0.136
CD27 expression in EM CD8+ (MFI)	20	498.50 [390.50;658.00]	20	690.00 [485.00;877.50]	***0.003***	***0.020***
CD28 expression in EM CD8+ (MFI)	20	482.00 [372.00;605.50]	20	583.00 [354.00;703.00]	0.071	0.127
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	20	17.57 [11.12;27.87]	20	29.98 [15.76;37.23]	***0.006***	***0.023***
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	20	1329.00 [1241.50;1434.50]	20	1385.00 [1297.50;1636.50]	***0.012***	***0.036***
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	20	802.00 [752.50;919.00]	20	859.00 [679.00;1014.50]	0.273	0.347
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	20	3.01 [1.44;5.60]	20	3.48 [1.85;6.10]	0.058	0.113
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	20	25.63 [18.46;40.40]	20	24.14 [15.89;38.55]	0.312	0.373
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	20	46.55 [35.80;59.77]	20	37.81 [28.56;49.84]	***0.004***	***0.020***
EM CD8+ CD57+ (% of EM CD8+ T-cells)	20	44.77 [28.23;57.00]	20	48.30 [31.43;54.52]	0.956	0.956
TEMRA CD8+(% of CD8+ T-cells)	20	23.61 [11.02;33.31]	20	24.01 [10.82;31.82]	0.898	0.926
CD27 expression in TEMRA CD8+ (MFI)	20	266.50 [205.00;438.50]	20	291.50 [218.50;578.50]	***0.021***	0.058
CD28 expression in TEMRA CD8+ (MFI)	20	141.50 [76.00;225.50]	20	141.00 [81.00;228.50]	0.430	0.490
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	20	4.02 [1.95;5.575]	20	4.36 [2.34;10.53]	***0.033***	0.083
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	19	1515.00 [1345.00;1642.00]	19	1638.00 [1455.00;1767.00]	0.073	0.127
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	19	703.00 [653.00;791.00]	19	761.00 [633.00;827.00]	0.214	0.321
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	20	1.54 [0.52;2.82]	20	3.71 [1.35;5.48]	***0.001***	***0.019***
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	20	73.78 [59.53;84.14]	20	71.32 [57.63;84.78]	0.452	0.498
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	20	64.46 [56.19;77.01]	20	69.87 [59.59;79.18]	0.246	0.338
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	20	5.03 [3.88;6.89]	20	4.59 [3.34;5.36]	0.165	0.259
CD25 expression in Total Treg (MFI)	20	1606.00 [1185.00;2203.50]	20	2077.00 [1235.50;2947.50]	***0.033***	0.072
CTLA-4 expression in Total Treg (MFI)	20	250.50 [228.50;296.50]	20	284.00 [237.00;316.00]	0.153	0.259
Naïve Treg (% of total Tregs)	20	5.94 [3.67;9.51]	20	10.41 [5.20;17.12]	***<0.001***	***0.001***
CD25 expression of Naïve Treg (MFI)	20	1447.50 [835.00;1940.50]	20	2021.00 [1382.50;2454.50]	***0.011***	***0.039***
CTLA-4 expression of Naïve Treg (MFI)	20	229.00 [186.00;284.00]	20	268.50 [204.50;310.50]	0.245	0.337

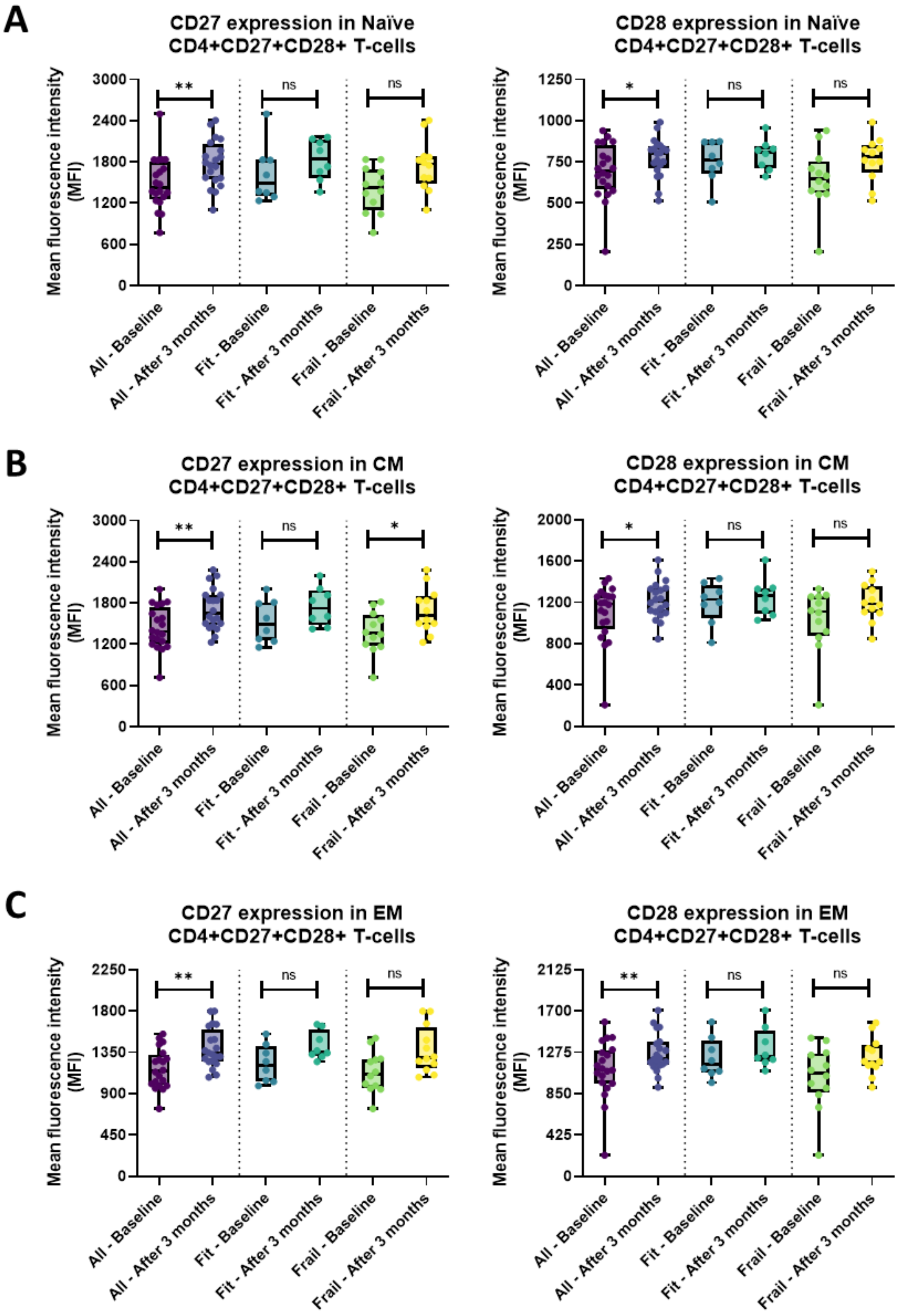
Continuation Table:

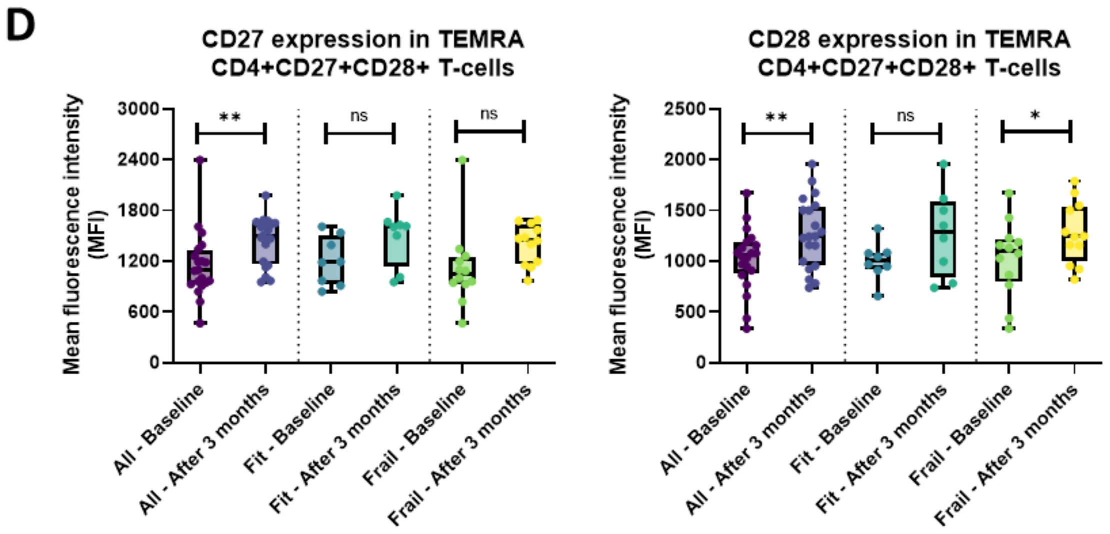
Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
Naïve Treg CTLA-4+ (% of Naïve Tregs)	20	23.25 [15.94;36.73]	20	27.44 [17.37;32.09]	0.953	0.953
Memory Treg (% of total Tregs)	20	93.77 [89.80;96.00]	20	88.90 [82.46;93.80]	***<0.001***	***0.001***
CD25 expression of Memory Treg (MFI)	20	1633.00 [1186.00;2178.00]	20	2057.00 [1255.50;2984.00]	***0.024***	0.066
CTLA-4 expression of Memory Treg (MFI)	20	255.00 [230.50;296.00]	20	273.00 [233.50;313.50]	0.316	0.384
Memory Treg CTLA-4+ (% of Memory Tregs)	20	18.10 [15.59;20.69]	20	18.50 [14.28;24.46]	0.349	0.384
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	20	1.55 [0.89;2.14]	20	1.32 [0.92;3.03]	0.738	0.876
PD1 expression in CD4+ (MFI)	20	201.50 [127.50;235.00]	20	196.00 [145.00;236.00]	0.335	0.669
CD4+ CD69+ (% of CD4+ T-cells)	20	31.94 [22.42;40.79]	20	28.54 [21.94;40.29]	0.546	0.819
CD69 expression in CD4+ (MFI)	20	253.50 [235.00;270.00]	20	254.50 [229.00;297.50]	0.373	0.689
CD4+ PD1+CD69+ (% of CD4+ T-cells)	20	0.93 [0.64;1.58]	20	0.90 [0.58;1.80]	0.985	0.985
CD4+ PD1-CD69- (% of CD4+ T-cells)	20	67.43 [58.65;76.96]	20	70.80 [58.65;77.06]	0.729	0.876
CD4+ HLADR+ (% of CD4+ T-cells)	20	14.41 [7.91;19.78]	20	15.46 [10.39;22.02]	0.114	0.455
HLADR expression in CD4+ (MFI)	20	401.00 [332.50;552.50]	20	421.50 [348.00;506.50]	0.674	0.876
CD4+ CD38+ (% of CD4+ T-cells)	20	31.90 [20.25;45.58]	20	34.62 [22.85;44.25]	0.177	0.472
CD38 expression in CD4+ (MFI)	20	653.50 [488.50;777.00]	20	655.00 [504.00;780.00]	0.877	0.957
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	20	3.08 [1.80;4.02]	20	3.99 [1.94;5.94]	0.066	0.364
CD4+ HLADR-CD38- (% of CD4+ T-cells)	20	54.84 [48.00;67.32]	20	54.62 [41.38;59.53]	0.133	0.455
CD8+ PD1+ (% of CD8+ T-cells)	20	21.31 [12.77;29.14]	20	23.81 [11.97;36.39]	0.430	0.738
PD1 expression in CD8+ (MFI)	20	270.50 [223.00;357.00]	20	292.50 [222.50;380.00]	0.766	0.876
CD8+ CD69+ (% of CD8+ T-cells)	20	31.63 [21.01;47.02]	20	37.83 [19.23;45.60]	0.701	0.876
CD69 expression in CD8+ (MFI)	20	390.50 [319.00;522.50]	20	470.50 [371.00;645.50]	0.154	0.461
CD8+ PD1+CD69+ (% of CD8+ T-cells)	20	7.85 [5.28;12.60]	20	8.60 [4.92;18.08]	0.294	0.642
CD8+ PD1-CD69- (% of CD8+ T-cells)	20	50.24 [39.40;65.04]	20	49.07 [39.66;62.29]	0.927	0.968
CD8+ HLADR+ (% of CD8+ T-cells)	20	40.22 [26.35;46.38]	20	41.86 [32.36;51.53]	0.076	0.364
HLADR expression in CD8+ (MFI)	20	505.50 [449.00;615.50]	20	513.00 [405.00;629.00]	0.515	0.819
CD8+ CD38+ (% of CD8+ T-cells)	20	11.97 [6.20;18.90]	20	12.11 [7.00;24.92]	***0.007***	0.172
CD38 expression in CD8+ (MFI)	20	280.00 [203.00;350.00]	20	274.50 [201.00;435.50]	0.273	0.642
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	20	5.13 [3.40;9.04]	20	6.51 [3.86;11.61]	0.064	0.364
CD8+ HLADR-CD38- (% of CD8+ T-cells)	20	53.63 [48.44;58.46]	20	48.05 [41.17;57.65]	***0.036***	0.364
Plasma - Essential Immune Response						
IL-4 (pg/mL)	20	0.06 [0.00;4.26]	20	0.33 [0.00;9.84]	0.890	0.898
IL-2 (pg/mL)	20	2.06 [0.96;4.02]	20	1.44 [0.08;6.75]	0.898	0.898
CXCL-10 (IP-10) (pg/mL)	20	218.23 [157.45;354.14]	20	391.26 [166.73;459.55]	***0.036***	0.217
IL-1 beta (pg/mL)	20	5.27 [0.77;14.48]	20	4.57 [1.16;13.83]	0.862	0.898
TNF alpha (pg/mL)	20	2.02 [1.53;3.43]	20	1.92 [0.86;3.38]	0.294	0.589
CCL2 (MCP-1) (pg/mL)	20	153.92 [115.28;207.57]	20	185.79 [148.30;248.67]	0.133	0.531

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
IL-17A (pg/mL)	20	2.55 [1.23;8.50]	20	2.33 [1.36;6.85]	0.196	0.587
IL-6 (pg/mL)	20	6.46 [2.88;9.01]	20	5.88 [2.73;10.83]	0.261	0.589
IL-10 (pg/mL)	20	1.85 [0.67;3.12]	20	0.61 [0.02;1.68]	***0.009***	0.101
IFN gamma (pg/mL)	20	1.29 [0.00;6.07]	20	2.33 [1.30;6.23]	0.799	0.898
IL-12p70 (pg/mL)	20	4.05 [1.81;6.93]	20	3.67 [2.08;12.22]	0.596	0.898
CXCL-8 (IL-8) (pg/mL)	20	4.30 [2.49;11.62]	20	5.47 [3.20;14.38]	0.648	0.898
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	20	1139.20 [837.97;1965.60]	20	1449.60 [994.13;2002.60]	***0.024***	0.096
4-1BB (pg/mL)	20	70.44 [54.40;156.36]	20	59.59 [43.26;128.39]	***0.048***	0.097
sCD27 (pg/mL)	20	55467.00 [48711.00;81131.00]	20	54618.00 [49390.00;71250.00]	***0.048***	0.097
B7.2 (CD86) (pg/mL)	20	543.30 [463.44;697.47]	20	368.31 [314.93;584.11]	***0.001***	***0.017***
Free Active TGF-B1 (pg/mL)	20	25.01 [18.60;35.43]	20	21.13 [8.38;35.87]	0.083	0.142
CTLA-4 (pg/mL)	20	8.84 [2.71;17.19]	20	7.90 [2.69;14.50]	***0.032***	0.097
PD-L1 (pg/mL)	20	200.04 [118.47;306.25]	20	195.22 [149.03;303.77]	0.956	0.956
PD-L2 (pg/mL)	20	12681.00 [10866.00;15195.00]	20	14435.00 [11462.00;16359.00]	0.123	0.185
PD-1 (pg/mL)	20	49.94 [16.51;151.25]	20	27.45 [16.80;98.56]	***0.005***	***0.029***
Tim-3 (pg/mL)	20	22351.00 [13890.00;33622.00]	20	19419.00 [14642.00;33057.00]	0.898	0.956
LAG-3 (pg/mL)	20	2905.30 [1775.20;7456.10]	20	2098.10 [1575.00;6071.20]	0.596	0.715
Gal-9 (pg/mL)	20	105359.00 [83066.00;150470.00]	20	118096.00 [93697.00;167533.00]	0.165	0.220

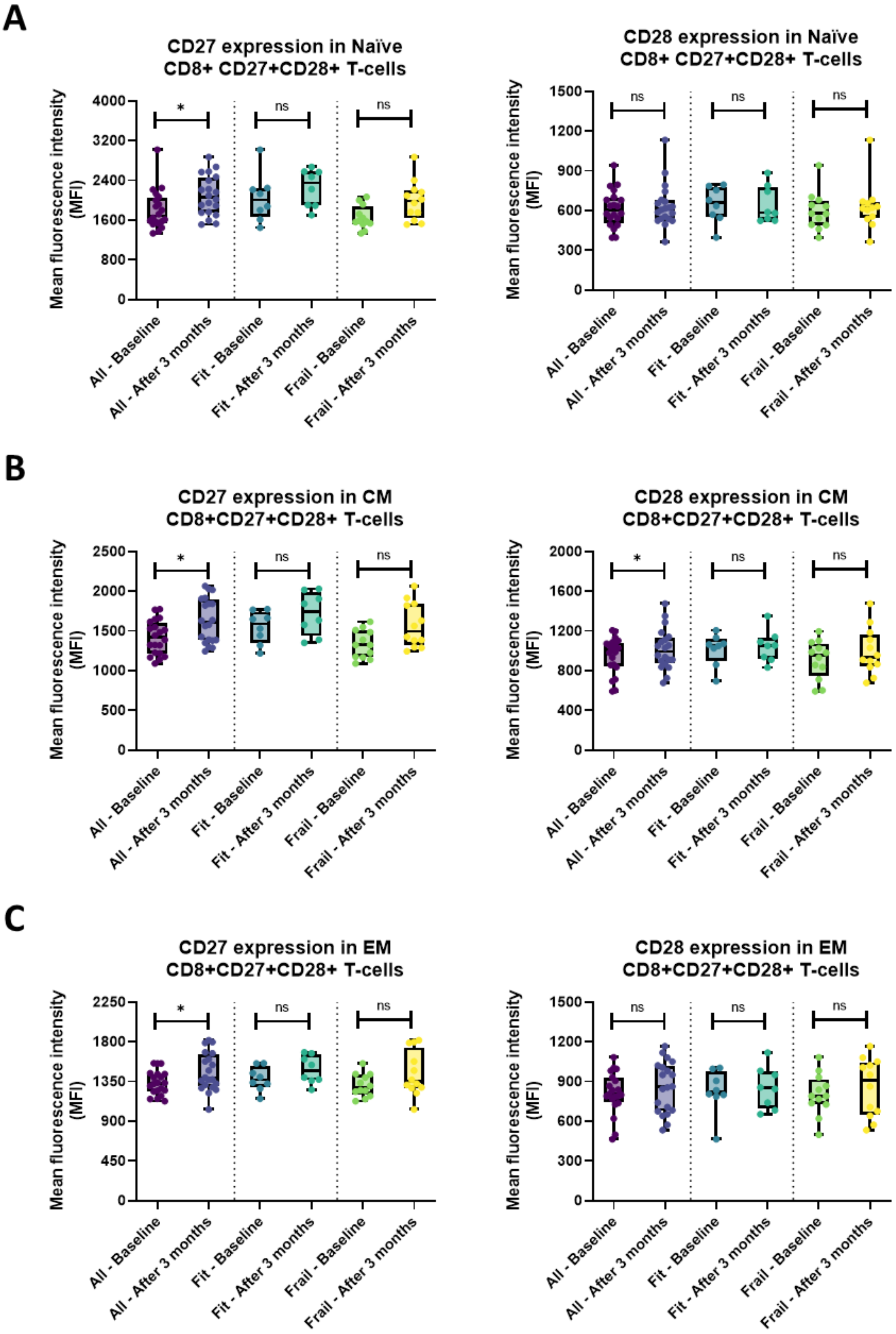
Supplementary Figure S6

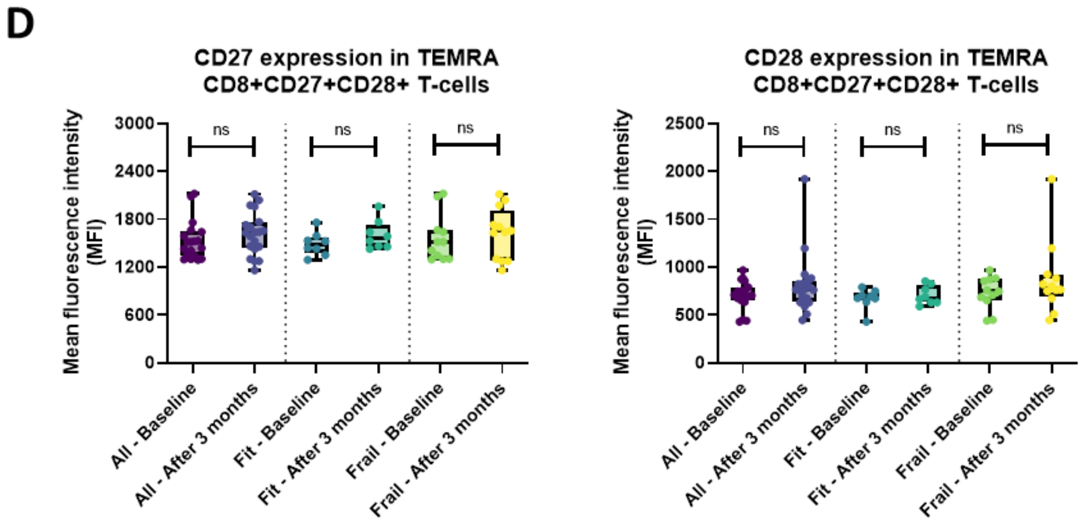




Supplementary Figure S6: Overview of the CD4+ T-cell subsets in the whole patient cohort and the fit and frail patient cohort separately. (A) The CD27 and CD28 expression of the double positive naïve CD4+CD27+CD28+ T-cells was significantly increased after three months of combination therapy in the whole cohort. No significant results were demonstrated in the fit and frail patient population. **(B)** A significantly increased expression of CD27 and CD28 of the CM CD4+CD27+CD28+ T-cells was noticed after three months of treatment in the whole patient cohort. Within the fit and frail population, no significant differences were found. **(C)** Within the entire patient population, the expression of the costimulatory receptors CD27 and CD28 of the EM CD4+CD27+CD28+ T-cell population was significantly increased after three months of treatment. Within the frail and fit population, no significant difference was found after three months of combination treatment. **(D)** In the whole cohort, after three months of treatment, the expression of CD27 and CD28 of the TEMRA CD4+CD27+CD28+ T-cells was significantly increased. In the frail population, a significant increase was shown in the CD28 expression of the TEMRA CD4+CD27+CD28+ T-cells. Within the fit population, no significant results were demonstrated. The boxplots represent the IQR of the percentage of the specific CD4+ T-cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leqslant 0.05$, with ** for $P \leqslant 0.01$, and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

Supplementary Figure S7





Supplementary Figure S7: Overview of the CD8+ T-cell subsets in the whole patient cohort and the fit and frail patient cohort separately. (A) In the entire cohort, the CD27 expression in the naïve CD8+CD27+ CD28+ T-cells was significantly elevated after three months of combination treatment. Within the frail and fit population, no significant differences were found. **(B)** In the CM CD8+ T-cell population, the CD27 and CD28 expression were significantly increased. No significant results were demonstrated in the fit and frail patient population. **(C)** The expression of CD27 in the double positive EM CD8+CD27+CD28+ T-cells was significantly increased after three months of treatment. No significant differences were found within the fit and frail patient population. **(D)** No significance was found in the expression of CD27 or CD28 in the double positive TEMRA CD8+CD27+CD28+ T-cells in the entire patient population and the fit and frail population. The boxplots represent the IQR of the percentage of the specific CD8+ T-cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leqslant 0.05$ and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

Supplementary Table S8

Supplementary Table S8: Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the fit patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values were calculated using a paired Wilcoxon signed rank test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.20 [96.94;99.51]	8	99.29 [98.88;99.61]	0.688	0.773
Viable lymphocytes (% of total lymphocytes)	8	98.15 [96.27;98.48]	8	97.79 [96.94;98.59]	0.547	0.703
Total T-cells (% of viable lymphocytes)	8	61.50 [56.82;71.20]	8	65.62 [56.74;72.41]	0.461	0.691
Total T-cells (% of viable cells)	8	59.39 [42.18;64.59]	8	59.69 [43.29;67.63]	0.383	0.689
CD4+ T-cells (% of viable lymphocytes)	8	52.70 [29.20;56.34]	8	52.78 [38.14;60.31]	0.250	0.500
CD8+ T-cells (% of viable lymphocytes)	8	16.44 [14.48;20.99]	8	15.24 [10.03;19.46]	0.078	0.201
CD4/CD8 ratio (% CD4/CD8 ratio)	8	3.16 [1.60;4.12]	8	2.78 [2.62;5.98]	0.078	0.201
NK/T-cells (% of viable cells)	8	4.46 [2.65;10.06]	8	4.74 [2.69;7.44]	0.461	0.691
Total NK cells (% of viable cells)	8	9.16 [5.54;12.23]	8	8.74 [6.76;11.65]	0.641	0.769
CD56 bright CD16- (% of total NK cells)	8	3.96 [2.64;12.78]	8	3.82 [2.55;14.38]	1.000	1.000
CD56 dim CD16+ (% of total NK cells)	8	82.71 [68.17;91.11]	8	71.87 [50.83;90.52]	0.148	0.334
Total B-cells (% of viable cells)	8	9.00 [7.47;10.06]	8	7.61 [5.91;9.87]	0.844	0.893
Naive B-cells (% of total B-cells)	8	58.95 [54.53;71.71]	8	71.81 [64.05;81.13]	***0.023***	0.084
Memory B-cells (% of total B-cells)	8	40.22 [27.47;44.21]	8	27.87 [17.79;35.21]	***0.008***	0.070
Total monocytes (% of viable cells)	8	13.77 [12.65;16.06]	8	12.89 [8.16;20.14]	0.547	0.703
Non-classical monocytes (% of total monocytes)	8	4.47 [3.04;5.03]	8	9.63 [7.70;12.98]	***0.008***	0.070
Intermediate monocytes (% of total monocytes)	8	5.76 [4.17;6.75]	8	8.63 [7.65;12.87]	***0.016***	0.070
Classical monocytes (% of total monocytes)	8	83.73 [80.18;86.12]	8	70.19 [64.80;78.07]	***0.016***	0.070
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	26.32 [18.89;49.10]	8	40.81 [18.93;47.80]	0.461	0.563
CD27 expression in Naïve CD4+ (MFI)	8	1458.50 [1248.50;1752.50]	8	1829.50 [1559.00;2084.50]	0.219	0.380
CD28 expression in Naïve CD4+ (MFI)	8	766.00 [666.00;864.00]	8	806.00 [712.00;838.50]	0.313	0.469
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	96.01 [90.34;98.05]	8	98.49 [97.00;98.68]	0.078	0.287
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	1491.00 [1323.50;1831.50]	8	1849.50 [1599.50;2109.50]	0.195	0.358
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	764.00 [690.50;871.00]	8	810.50 [717.50;840.50]	0.383	0.486
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	4.70 [2.43;18.71]	8	6.98 [1.03;10.63]	0.844	0.870
CM CD4+ (% of CD4+ T-cells)	8	60.90 [39.22;66.37]	8	48.86 [44.26;61.18]	0.148	0.327
CD27 expression in CM CD4+ (MFI)	8	1279.00 [970.00;1521.00]	8	1502.00 [1233.00;1767.00]	0.078	0.287
CD28 expression in CM CD4+ (MFI)	8	1265.00 [1068.50;1379.00]	8	1271.50 [1119.50;1358.00]	0.383	0.486
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	80.46 [74.87;83.76]	8	85.62 [82.75;90.89]	0.109	0.301
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	1490.00 [1261.50;1803.00]	8	1722.50 [1510.50;1962.00]	0.109	0.301

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	1227.50 [1092.50;1347.00]	8	1266.50 [1098.50;1335.50]	0.328	0.471
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	2.01 [1.53;3.10]	8	1.95 [1.64;2.49]	0.844	0.870
EM CD4+ (% of CD4+ T-cells)	8	10.51 [6.97;18.67]	8	11.79 [7.20;15.59]	0.844	0.870
CD27 expression in EM CD4+ (MFI)	8	456.00 [322.50;505.00]	8	691.00 [421.00;843.50]	***0.039***	0.287
CD28 expression in EM CD4+ (MFI)	8	1027.00 [890.00;1284.50]	8	1141.50 [996.00;1267.50]	0.148	0.327
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	8	34.08 [26.25;42.46]	8	51.89 [34.75;54.52]	***0.039***	0.287
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	1203.00 [1036.50;1389.00]	8	1355.50 [1316.50;1563.50]	0.055	0.287
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	1149.50 [1072.50;1365.50]	8	1232.50 [1187.50;1459.00]	0.078	0.287
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	0.18 [0.08;0.81]	8	0.13 [0.05;0.27]	0.148	0.327
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	4.47 [0.92;13.05]	8	1.46 [0.69;13.98]	0.383	0.486
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	53.20 [50.65;61.83]	8	45.53 [42.46;46.81]	0.055	0.287
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.54 [0.74;3.57]	8	1.74 [1.00;2.51]	0.945	0.945
TEMRA CD4+(% of CD4+ T-cells)	8	0.41 [0.20;1.47]	8	0.35 [0.24;0.91]	0.719	0.845
CD27 expression in TEMRA CD4+ (MFI)	8	500.50 [95.50;697.50]	8	546.00 [107.50;1033.50]	0.297	0.467
CD28 expression in TEMRA CD4+ (MFI)	8	519.00 [230.50;733.50]	8	766.50 [228.00;1233.50]	0.109	0.301
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	26.93 [11.58;48.75]	8	27.19 [12.42;59.22]	0.195	0.358
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1189.50 [939.50;1464.50]	8	1609.50 [1258.00;1646.50]	***0.008***	0.258
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1007.00 [929.00;1082.00]	8	1289.00 [889.00;1559.00]	***0.039***	0.287
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	10.42 [4.09;19.63]	8	5.21 [1.53;11.04]	0.250	0.413
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	24.13 [11.51;53.79]	8	16.57 [6.87;54.53]	0.195	0.358
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	47.02 [28.37;76.66]	8	45.56 [22.38;77.28]	0.742	0.845
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	8.89 [6.98;26.00]	8	21.62 [13.01;34.47]	0.109	0.490
CD27 expression in Naïve CD8+ (MFI)	8	1873.50 [1517.00;2110.50]	8	2255.00 [1848.50;2482.50]	0.195	0.496
CD28 expression in Naïve CD8+ (MFI)	8	642.50 [541.50;737.50]	8	554.00 [514.00;758.00]	0.641	0.729
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	88.32 [76.96;91.95]	8	94.74 [92.60;95.92]	0.148	0.490
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2005.00 [1706.50;2230.50]	8	2349.00 [1898.50;2572.50]	0.195	0.496
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	657.00 [558.00;770.00]	8	584.50 [531.50;766.50]	0.844	0.898
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	3.16 [1.80;7.34]	8	2.39 [1.77;3.50]	0.969	0.969
CM CD8+ (% of CD8+ T-cells)	8	17.90 [16.32;25.91]	8	17.98 [16.38;28.72]	0.742	0.816
CD27 expression in CM CD8+ (MFI)	8	1335.50 [1083.50;1461.00]	8	1566.00 [1222.50;1757.50]	0.148	0.490

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
CD28 expression in CM CD8+ (MFI)	8	1015.50 [896.00;1078.50]	8	1043.00 [844.50;1111.00]	0.547	0.722
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	74.34 [69.93;84.43]	8	86.13 [76.94;87.44]	0.195	0.496
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	1589.50 [1382.00;1715.00]	8	1740.00 [1484.50;1961.50]	0.148	0.490
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1057.50 [942.50;1103.00]	8	1052.00 [924.50;1116.00]	0.641	0.729
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.78 [8.77;12.74]	8	10.64 [8.03;12.77]	0.945	0.969
EM CD8+ (% of CD8+ T-cells)	8	30.36 [24.22;41.33]	8	27.05 [21.24;33.77]	***0.039***	0.490
CD27 expression in EM CD8+ (MFI)	8	631.00 [481.00;708.00]	8	769.00 [534.50;953.00]	0.078	0.490
CD28 expression in EM CD8+ (MFI)	8	474.50 [327.00;622.50]	8	519.00 [349.00;677.50]	0.266	0.548
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	23.70 [15.16;33.40]	8	34.13 [19.64;44.19]	0.078	0.490
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	1369.50 [1297.00;1500.00]	8	1467.00 [1367.00;1636.50]	0.078	0.490
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	819.50 [793.00;950.50]	8	849.50 [711.50;965.50]	0.547	0.722
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.95 [3.01;6.91]	8	4.96 [3.76;8.90]	0.250	0.548
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	27.68 [22.95;43.91]	8	23.53 [15.96;47.75]	0.641	0.729
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	36.82 [31.41;44.71]	8	28.56 [23.77;37.50]	0.078	0.490
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.74 [43.34;53.29]	8	44.96 [37.21;53.60]	0.641	0.729
TEMRA CD8+(% of CD8+ T-cells)	8	27.01 [18.18;39.86]	8	27.24 [14.57;32.13]	0.383	0.632
CD27 expression in TEMRA CD8+ (MFI)	8	257.50 [205.00;438.50]	8	234.00 [174.50;672.00]	0.313	0.573
CD28 expression in TEMRA CD8+ (MFI)	8	86.00 [67.00;132.50]	8	95.00 [51.00;171.50]	0.313	0.573
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	4.33 [2.76;5.20]	8	3.04 [2.48;10.97]	0.383	0.632
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	1481.50 [1392.00;1560.50]	8	1558.00 [1459.00;1702.50]	0.148	0.490
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	685.50 [655.00;726.00]	8	677.50 [632.50;797.50]	0.547	0.722
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	2.82 [2.26;4.50]	8	4.92 [1.29;5.52]	0.250	0.548
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	81.72 [72.29;85.97]	8	82.27 [62.76;86.50]	0.461	0.722
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	73.62 [64.18;77.01]	8	76.42 [62.60;82.14]	0.547	0.722
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	8	5.11 [4.28;6.19]	8	4.90 [3.90;6.14]	0.641	0.641
CD25 expression in Total Treg (MFI)	8	2203.50 [1610.00;2842.50]	8	2638.00 [2015.00;3325.00]	0.250	0.430
CTLA-4 expression in Total Treg (MFI)	8	228.50 [213.50;247.00]	8	264.00 [217.50;326.00]	***0.039***	0.143
Naïve Treg (% of total Tregs)	8	6.30 [4.76;8.05]	8	8.28 [4.24;15.33]	0.109	0.301
CD25 expression of Naïve Treg (MFI)	8	2048.50 [1173.50;2256.00]	8	2503.00 [1761.50;2730.50]	***0.039***	0.143
CTLA-4 expression of Naïve Treg (MFI)	8	186.00 [178.50;220.00]	8	204.50 [173.50;279.00]	0.297	0.430

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	15.94 [8.51;19.89]	8	17.37 [11.25;29.65]	0.383	0.468
Memory Treg (% of total Tregs)	8	93.51 [91.47;94.70]	8	91.09 [84.16;94.57]	***0.039***	0.143
CD25 expression of Memory Treg (MFI)	8	2178.00 [1627.50;2838.50]	8	2610.00 [1972.00;3329.00]	0.250	0.430
CTLA-4 expression of Memory Treg (MFI)	8	230.50 [215.50;256.50]	8	248.00 [205.00;293.50]	0.313	0.430
Memory Treg CTLA-4+ (% of Memory Tregs)	8	15.59 [8.43;17.68]	8	14.28 [6.42;21.34]	0.461	0.507
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	8	1.95 [1.69;2.89]	8	2.14 [1.32;2.78]	0.469	0.986
PD1 expression in CD4+ (MFI)	8	224.50 [160.50;297.00]	8	220.50 [180.00;289.50]	0.484	0.986
CD4+ CD69+ (% of CD4+ T-cells)	8	35.46 [25.93;42.24]	8	29.77 [24.87;40.96]	0.461	0.986
CD69 expression in CD4+ (MFI)	8	249.50 [236.00;290.50]	8	266.00 [243.00;289.50]	0.836	0.986
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.41 [0.98;2.13]	8	1.28 [0.90;1.47]	0.313	0.986
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	64.00 [56.19;73.22]	8	69.58 [58.10;74.39]	0.641	0.986
CD4+ HLADR+ (% of CD4+ T-cells)	8	8.37 [6.88;14.41]	8	12.19 [3.66;20.04]	0.078	0.625
HLADR expression in CD4+ (MFI)	8	333.00 [297.50;381.00]	8	416.50 [204.00;501.50]	0.250	0.986
CD4+ CD38+ (% of CD4+ T-cells)	8	33.65 [18.60;46.64]	8	34.62 [26.71;37.29]	0.945	0.986
CD38 expression in CD4+ (MFI)	8	671.00 [500.00;874.00]	8	622.00 [547.00;795.50]	0.547	0.986
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	2.41 [1.40;3.89]	8	3.91 [1.40;4.98]	0.688	0.986
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	59.02 [44.86;69.63]	8	54.98 [50.48;60.05]	0.641	0.986
CD8+ PD1+ (% of CD8+ T-cells)	8	29.14 [21.31;40.55]	8	26.38 [23.81;40.81]	1.000	1.000
PD1 expression in CD8+ (MFI)	8	325.00 [230.00;526.00]	8	310.50 [194.00;494.00]	***0.031***	0.469
CD8+ CD69+ (% of CD8+ T-cells)	8	36.86 [21.01;55.88]	8	31.47 [18.73;50.64]	***0.039***	0.469
CD69 expression in CD8+ (MFI)	8	513.00 [390.50;931.50]	8	565.00 [470.50;774.50]	0.844	0.986
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.68 [7.68;15.99]	8	11.39 [7.91;18.08]	0.742	0.986
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	36.20 [30.95;53.39]	8	41.03 [36.80;57.28]	0.109	0.656
CD8+ HLADR+ (% of CD8+ T-cells)	8	31.78 [16.88;42.36]	8	38.80 [9.82;46.05]	0.945	0.986
HLADR expression in CD8+ (MFI)	8	474.50 [341.00;488.00]	8	497.00 [284.00;540.50]	0.742	0.986
CD8+ CD38+ (% of CD8+ T-cells)	8	14.06 [7.27;17.44]	8	11.40 [8.02;22.77]	0.383	0.986
CD38 expression in CD8+ (MFI)	8	293.00 [230.50;342.50]	8	274.00 [227.50;403.00]	0.547	0.986
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	7.82 [1.71;10.11]	8	5.59 [2.36;11.15]	0.945	0.986
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	64.70 [38.71;77.09]	8	54.58 [45.98;78.05]	0.945	0.986
Plasma - Essential Immune Response						
IL-4 (pg/mL)	8	0.11 [0.00;28.48]	8	6.56 [0.31;17.75]	0.813	0.886
IL-2 (pg/mL)	8	3.34 [1.92;9.66]	8	2.35 [0.68;16.71]	0.461	0.820
CXCL-10 (IP-10) (pg/mL)	8	159.08 [125.71;355.70]	8	394.51 [100.25;495.51]	0.313	0.820
IL-1 beta (pg/mL)	8	6.06 [1.47;34.62]	8	5.96 [4.57;32.43]	0.547	0.820
TNF alpha (pg/mL)	8	1.81 [1.21;5.54]	8	1.14 [0.63;2.74]	0.109	0.594
CCL2 (MCP-1) (pg/mL)	8	193.48 [149.28;232.19]	8	202.58 [168.50;296.80]	0.461	0.820

Continuation Table:

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
IL-17A (pg/mL)	8	2.23 [1.18;22.16]	8	3.36 [1.45;11.39]	0.547	0.820
IL-6 (pg/mL)	8	8.40 [3.86;24.28]	8	5.80 [2.63;10.56]	0.641	0.854
IL-10 (pg/mL)	8	2.23 [1.54;3.82]	8	0.54 [0.00;1.69]	***0.016***	0.188
IFN gamma (pg/mL)	8	0.91 [0.00;35.63]	8	3.67 [1.90;7.15]	0.945	0.945
IL-12p70 (pg/mL)	8	6.93 [4.28;58.97]	8	12.22 [5.56;45.39]	0.742	0.886
CXCL-8 (IL-8) (pg/mL)	8	7.23 [3.55;25.42]	8	5.67 [2.23;22.07]	0.148	0.594
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	928.26 [798.87;1140.80]	8	1132.10 [955.26;1562.20]	0.195	0.335
4-1BB (pg/mL)	8	68.07 [50.57;119.55]	8	56.21 [38.70;88.13]	0.250	0.375
sCD27 (pg/mL)	8	52670.00 [45645.00;65276.00]	8	53535.00 [49080.00;62195.00]	0.383	0.459
B7.2 (CD86) (pg/mL)	8	524.80 [474.15;697.47]	8	411.43 [320.29;609.87]	0.109	0.263
Free Active TGF-B1 (pg/mL)	8	25.39 [22.12;32.09]	8	13.43 [7.00;28.59]	***0.023***	0.234
CTLA-4 (pg/mL)	8	10.14 [4.40;15.98]	8	7.36 [4.35;10.07]	0.195	0.335
PD-L1 (pg/mL)	8	279.06 [172.00;401.70]	8	265.37 [151.94;325.49]	0.313	0.417
PD-L2 (pg/mL)	8	11121.00 [10196.00;14318.00]	8	12081.00 [11067.00;16080.00]	0.078	0.234
PD-1 (pg/mL)	8	92.41 [16.51;201.92]	8	66.15 [19.51;101.02]	0.078	0.234
Tim-3 (pg/mL)	8	15345.00 [6568.40;26546.00]	8	18190.00 [8331.30;22119.00]	0.641	0.699
LAG-3 (pg/mL)	8	5456.70 [2198.80;16183.00]	8	3388.50 [2008.30;18530.00]	1.000	1.000
Gal-9 (pg/mL)	8	102158.00 [76361.00;127351.00]	8	113572.00 [87969.00;171239.00]	0.055	0.234

Supplementary Table S9

Supplementary Table S9: Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the frail patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values were calculated using a paired Wilcoxon signed rank test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	12	98.65 [98.29;99.18]	12	99.19 [98.76;99.61]	0.339	0.747
Viable lymphocytes (% of total lymphocytes)	12	96.73 [90.71;97.76]	12	97.92 [96.82;98.60]	***0.034***	0.235
Total T-cells (% of viable lymphocytes)	12	62.16 [53.54;64.67]	12	60.47 [49.61;66.66]	0.519	0.747
Total T-cells (% of viable cells)	12	54.68 [47.77;61.56]	12	56.93 [47.61;60.10]	0.622	0.747
CD4+ T-cells (% of viable lymphocytes)	12	50.22 [44.03;57.76]	12	49.89 [41.65;55.80]	0.569	0.747
CD8+ T-cells (% of viable lymphocytes)	12	13.96 [8.49;18.23]	12	10.72 [7.78;16.06]	0.569	0.747
CD4/CD8 ratio (% CD4/CD8 ratio)	12	3.99 [2.61;6.86]	12	4.13 [2.80;7.17]	0.733	0.777
NK/T-cells (% of viable cells)	12	1.81 [1.43;3.70]	12	2.82 [1.52;4.51]	0.850	0.850
Total NK cells (% of viable cells)	12	6.52 [3.69;10.64]	12	11.08 [6.38;15.27]	***0.043***	0.235
CD56 bright CD16- (% of total NK cells)	12	4.74 [4.00;8.47]	12	6.54 [4.76;10.49]	0.569	0.747
CD56 dim CD16+ (% of total NK cells)	12	58.52 [19.32;76.44]	12	64.98 [43.70;88.52]	0.110	0.330
Total B-cells (% of viable cells)	12	7.51 [5.58;9.54]	12	9.29 [4.52;12.36]	0.622	0.747
Naive B-cells (% of total B-cells)	12	62.99 [42.09;75.50]	12	74.80 [62.54;80.94]	***0.021***	0.235
Memory B-cells (% of total B-cells)	12	35.87 [23.20;56.53]	12	24.48 [18.23;36.41]	0.052	0.235
Total monocytes (% of viable cells)	12	18.11 [8.34;24.38]	12	15.94 [5.82;19.00]	0.266	0.684
Non-classical monocytes (% of total monocytes)	12	3.41 [1.19;9.72]	12	4.79 [2.77;7.91]	0.733	0.777
Intermediate monocytes (% of total monocytes)	12	6.17 [4.74;7.32]	12	8.36 [5.13;10.63]	0.077	0.278
Classical monocytes (% of total monocytes)	12	79.92 [68.76;83.23]	12	75.53 [59.36;82.19]	0.622	0.747
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	12	44.61 [22.85;50.60]	12	54.15 [30.13;63.86]	***0.012***	***0.045***
CD27 expression in Naïve CD4+ (MFI)	12	1312.00 [914.50;1596.50]	12	1717.50 [1410.50;1818.00]	***0.007***	***0.038***
CD28 expression in Naïve CD4+ (MFI)	12	614.00 [548.50;726.50]	12	791.00 [676.50;850.00]	***0.033***	0.063
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	12	88.82 [71.31;92.93]	12	95.25 [91.67;96.83]	***0.002***	***0.032***
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	12	1424.50 [1134.00;1677.50]	12	1770.50 [1509.50;1875.00]	***0.021***	0.053
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	12	649.50 [565.50;739.50]	12	783.50 [705.00;845.00]	***0.034***	0.063
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	12	2.68 [1.35;11.63]	12	1.81 [0.47;5.06]	0.274	0.312
CM CD4+ (% of CD4+ T-cells)	12	47.86 [33.74;59.69]	12	38.75 [27.56;57.95]	0.052	0.078
CD27 expression in CM CD4+ (MFI)	11	1006.00 [817.00;1326.00]	11	1477.00 [1135.00;1758.00]	***0.005***	***0.032***
CD28 expression in CM CD4+ (MFI)	12	1089.50 [868.00;1247.50]	12	1198.00 [1098.50;1370.50]	***0.027***	0.059
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	12	70.12 [59.29;79.39]	12	85.03 [72.43;87.00]	***0.001***	***0.016***
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	12	1359.50 [1183.50;1601.00]	12	1621.00 [1483.00;1878.50]	***0.009***	***0.038***

Continuation Table:

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	12	1111.00 [888.50;1256.50]	12	1186.00 [1103.00;1342.00]	***0.016***	0.053
CM CD4+ CD57+ (% of CM CD4+ T-cells)	12	2.87 [1.96;4.05]	12	2.17 [1.37;3.41]	0.151	0.192
EM CD4+ (% of CD4+ T-cells)	12	10.35 [5.42;14.76]	12	7.59 [4.40;10.94]	0.176	0.215
CD27 expression in EM CD4+ (MFI)	11	371.00 [134.00;579.00]	11	490.00 [333.00;793.00]	***0.042***	0.070
CD28 expression in EM CD4+ (MFI)	12	955.00 [802.50;1201.50]	12	1103.50 [1023.00;1260.00]	0.052	0.078
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	12	31.17 [17.30;38.43]	12	38.07 [30.48;44.77]	***0.021***	0.053
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	12	1114.00 [971.00;1274.00]	12	1292.00 [1192.50;1573.00]	***0.027***	0.059
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	12	1062.00 [874.00;1257.50]	12	1179.50 [1135.50;1339.50]	***0.021***	0.053
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	12	0.04 [0.00;0.32]	12	0.11 [0.00;0.40]	0.445	0.474
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	12	2.19 [1.45;4.06]	12	2.16 [0.57;3.75]	1.000	1.000
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	12	62.95 [56.89;72.30]	12	57.76 [47.14;64.68]	***0.043***	0.070
EM CD4+ CD57+ (% of EM CD4+ T-cells)	12	2.14 [1.37;11.21]	12	2.33 [1.33;5.32]	0.204	0.240
TEMRA CD4+(% of CD4+ T-cells)	12	0.62 [0.33;2.60]	12	0.56 [0.38;2.25]	0.733	0.756
CD27 expression in TEMRA CD4+ (MFI)	9	396.00 [201.00;418.00]	9	632.00 [573.00;878.00]	***0.004***	***0.032***
CD28 expression in TEMRA CD4+ (MFI)	12	799.50 [455.00;1068.50]	12	1008.00 [629.00;1171.00]	0.110	0.151
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	12	27.59 [13.65;35.75]	12	32.86 [25.25;53.91]	***0.005***	***0.032***
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	12	1044.50 [937.00;1229.00]	12	1454.50 [1172.00;1609.00]	***0.034***	0.063
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	12	1104.00 [816.00;1208.00]	12	1245.00 [1055.00;1525.50]	***0.009***	***0.038***
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	12	0.00 [0.00;1.88]	12	1.15 [0.00;5.68]	0.129	0.170
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	12	12.95 [5.33;35.81]	12	4.82 [2.48;26.06]	0.067	0.097
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	12	27.99 [8.55;53.71]	12	22.99 [9.38;44.65]	0.424	0.466
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	12	20.00 [14.10;48.67]	12	21.17 [14.13;52.44]	0.266	0.398
CD27 expression in Naïve CD8+ (MFI)	12	1229.50 [1014.00;1579.00]	12	1676.00 [1227.50;2032.00]	***0.016***	0.099
CD28 expression in Naïve CD8+ (MFI)	12	538.00 [436.00;641.00]	12	599.50 [499.00;641.00]	0.301	0.398
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	12	66.27 [46.33;77.72]	12	81.19 [61.98;88.81]	***0.027***	0.111
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	12	1579.00 [1526.50;1820.50]	12	1983.50 [1671.00;2189.00]	***0.003***	0.056
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	12	576.50 [491.50;661.50]	12	619.00 [546.50;661.00]	0.151	0.278
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	12	5.19 [2.72;6.86]	12	2.40 [1.33;4.85]	0.266	0.398
CM CD8+ (% of CD8+ T-cells)	12	29.27 [22.16;39.40]	12	18.44 [14.94;32.76]	0.052	0.144
CD27 expression in CM CD8+ (MFI)	12	905.50 [694.50;1069.00]	12	1178.00 [876.50;1488.50]	***0.009***	0.077

Continuation Table:

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD28 expression in CM CD8+ (MFI)	12	878.50 [691.00;1015.00]	12	867.50 [795.00;1126.00]	0.064	0.151
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	12	56.80 [45.18;66.45]	12	70.19 [53.41;75.44]	***0.021***	0.099
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	12	1323.50 [1172.00;1497.50]	12	1495.00 [1338.00;1832.00]	***0.009***	0.077
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	12	956.50 [775.00;1057.00]	12	933.00 [851.50;1148.50]	0.052	0.144
CM CD8+ CD57+ (% of CM CD8+ T-cells)	12	9.92 [6.56;14.91]	12	10.62 [5.94;16.58]	0.910	0.938
EM CD8+ (% of CD8+ T-cells)	12	17.33 [10.72;28.47]	12	17.09 [10.91;31.89]	0.622	0.708
CD27 expression in EM CD8+ (MFI)	12	437.50 [357.00;578.50]	12	638.50 [478.00;822.00]	***0.021***	0.099
CD28 expression in EM CD8+ (MFI)	12	497.50 [396.50;591.00]	12	586.00 [354.00;722.50]	0.129	0.251
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	12	15.30 [8.64;23.33]	12	26.96 [15.76;32.24]	0.052	0.144
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	12	1274.50 [1211.00;1414.50]	12	1347.50 [1279.50;1682.50]	0.129	0.251
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	12	791.00 [732.00;899.50]	12	906.50 [658.50;1036.50]	0.258	0.398
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	12	2.16 [0.85;3.14]	12	1.96 [1.65;4.51]	0.176	0.306
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	12	24.00 [12.54;40.40]	12	24.14 [14.47;32.75]	0.470	0.554
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	12	56.06 [42.69;64.97]	12	49.03 [35.57;54.79]	***0.043***	0.144
EM CD8+ CD57+ (% of EM CD8+ T-cells)	12	49.35 [15.50;60.11]	12	49.91 [21.78;54.52]	0.970	0.970
TEMRA CD8+(% of CD8+ T-cells)	12	22.32 [6.91;26.87]	12	18.43 [7.98;30.10]	0.339	0.415
CD27 expression in TEMRA CD8+ (MFI)	12	268.50 [195.50;386.00]	12	318.50 [238.50;469.50]	0.064	0.151
CD28 expression in TEMRA CD8+ (MFI)	12	188.00 [105.00;354.00]	12	188.00 [113.00;330.00]	0.850	0.905
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	12	3.60 [1.80;6.91]	12	6.09 [1.99;10.27]	0.077	0.170
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	11	1515.00 [1303.00;1664.00]	11	1657.00 [1300.00;1977.00]	0.278	0.398
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	11	747.00 [653.00;873.00]	11	765.00 [674.00;925.00]	0.320	0.407
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	12	1.30 [0.42;1.54]	12	3.28 [1.42;4.08]	***0.001***	***0.032***
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	12	65.24 [43.68;81.82]	12	66.02 [50.96;78.16]	0.850	0.905
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	12	61.92 [39.21;76.18]	12	67.67 [46.39;78.59]	0.301	0.398
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	12	4.57 [3.88;6.90]	12	3.97 [3.34;5.32]	0.233	0.428
CD25 expression in Total Treg (MFI)	12	1293.50 [1096.50;1816.50]	12	1535.50 [1124.50;2303.00]	0.092	0.242
CTLA-4 expression in Total Treg (MFI)	12	272.50 [246.50;301.00]	12	292.50 [258.00;314.00]	0.807	0.807
Naïve Treg (% of total Tregs)	12	5.15 [3.04;13.94]	12	11.08 [7.28;23.51]	***0.003***	***0.019***
CD25 expression of Naïve Treg (MFI)	12	1279.50 [827.50;1556.50]	12	1773.50 [1169.50;2216.50]	0.110	0.242
CTLA-4 expression of Naïve Treg (MFI)	12	252.50 [216.50;318.50]	12	292.50 [242.50;313.50]	0.636	0.699

Continuation Table:

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
Naïve Treg CTLA-4+ (% of Naïve Tregs)	12	33.18 [23.25;40.31]	12	30.24 [24.04;32.85]	0.365	0.574
Memory Treg (% of total Tregs)	12	94.74 [85.54;96.79]	12	88.49 [76.07;92.20]	***0.003***	***0.019***
CD25 expression of Memory Treg (MFI)	12	1345.00 [1094.50;1845.50]	12	1555.00 [1173.00;2373.50]	0.064	0.235
CTLA-4 expression of Memory Treg (MFI)	12	271.00 [248.00;305.00]	12	287.50 [255.50;331.50]	0.583	0.699
Memory Treg CTLA-4+ (% of Memory Tregs)	12	19.74 [16.52;21.61]	12	19.98 [18.26;24.46]	0.622	0.699
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	12	1.26 [0.88;1.62]	12	1.12 [0.84;3.21]	0.380	0.593
PD1 expression in CD4+ (MFI)	12	195.50 [99.50;216.00]	12	165.50 [120.50;236.00]	0.424	0.593
CD4+ CD69+ (% of CD4+ T-cells)	12	31.54 [17.07;38.34]	12	27.01 [16.96;37.41]	0.910	0.949
CD69 expression in CD4+ (MFI)	12	262.00 [226.50;270.00]	12	245.50 [216.00;305.50]	0.183	0.487
CD4+ PD1+CD69+ (% of CD4+ T-cells)	12	0.74 [0.50;1.04]	12	0.66 [0.37;2.15]	0.470	0.593
CD4+ PD1-CD69- (% of CD4+ T-cells)	12	67.83 [61.28;82.28]	12	72.24 [61.23;82.71]	0.970	0.970
CD4+ HLADR+ (% of CD4+ T-cells)	12	16.98 [11.69;22.80]	12	17.99 [11.79;22.56]	0.424	0.593
HLADR expression in CD4+ (MFI)	12	459.00 [388.00;627.00]	12	442.50 [380.00;522.00]	0.622	0.711
CD4+ CD38+ (% of CD4+ T-cells)	12	28.78 [20.25;45.58]	12	34.99 [22.85;46.95]	0.077	0.309
CD38 expression in CD4+ (MFI)	12	644.50 [488.50;777.00]	12	686.00 [481.00;780.00]	0.692	0.755
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	12	3.47 [2.14;4.28]	12	4.04 [2.57;7.23]	0.064	0.307
CD4+ HLADR-CD38- (% of CD4+ T-cells)	12	51.84 [48.00;63.98]	12	46.41 [38.73;59.53]	0.129	0.388
CD8+ PD1+ (% of CD8+ T-cells)	12	15.24 [9.90;23.11]	12	15.11 [8.96;32.54]	0.470	0.593
PD1 expression in CD8+ (MFI)	12	267.50 [203.50;323.50]	12	292.50 [233.00;361.00]	0.424	0.593
CD8+ CD69+ (% of CD8+ T-cells)	12	31.63 [22.80;42.70]	12	37.83 [24.52;41.97]	0.266	0.581
CD69 expression in CD8+ (MFI)	12	342.50 [279.50;437.00]	12	391.00 [292.00;541.50]	0.092	0.316
CD8+ PD1+CD69+ (% of CD8+ T-cells)	12	6.17 [5.13;9.99]	12	7.28 [4.22;16.06]	0.470	0.593
CD8+ PD1-CD69- (% of CD8+ T-cells)	12	58.50 [47.46;69.09]	12	52.81 [42.74;67.57]	0.233	0.560
CD8+ HLADR+ (% of CD8+ T-cells)	12	41.85 [33.45;47.17]	12	45.47 [36.28;60.72]	***0.021***	0.161
HLADR expression in CD8+ (MFI)	12	531.50 [489.50;709.00]	12	535.50 [439.00;806.00]	0.622	0.711
CD8+ CD38+ (% of CD8+ T-cells)	12	8.47 [6.20;21.45]	12	13.74 [6.99;29.79]	***0.002***	***0.047***
CD38 expression in CD8+ (MFI)	12	243.50 [192.50;355.00]	12	282.50 [180.00;456.50]	0.458	0.593
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	12	4.98 [4.12;6.99]	12	6.96 [4.14;11.61]	***0.027***	0.161
CD8+ HLADR-CD38- (% of CD8+ T-cells)	12	52.45 [48.44;55.66]	12	45.36 [34.90;51.94]	***0.009***	0.111
Plasma - Essential Immune Response						
IL-4 (pg/mL)	12	0.06 [0.00;1.38]	12	0.00 [0.00;3.83]	0.844	0.922
IL-2 (pg/mL)	12	1.80 [0.67;2.24]	12	0.95 [0.00;4.18]	0.622	0.829
CXCL-10 (IP-10) (pg/mL)	12	311.58 [176.37;354.14]	12	370.47 [189.67;459.55]	0.092	0.369
IL-1 beta (pg/mL)	12	5.27 [0.77;11.90]	12	3.02 [0.75;9.23]	0.470	0.705
TNF alpha (pg/mL)	12	2.07 [1.63;3.43]	12	2.43 [1.38;3.60]	0.910	0.922
CCL2 (MCP-1) (pg/mL)	12	133.65 [97.65;174.79]	12	165.28 [140.36;248.67]	0.176	0.470

Continuation Table:

Biomarker	**Baseline**		**After 3 months**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
IL-17A (pg/mL)	12	2.78 [1.35;8.50]	12	2.31 [1.36;5.32]	0.240	0.470
IL-6 (pg/mL)	12	3.86 [2.88;8.14]	12	6.16 [3.75;10.83]	***0.034***	0.314
IL-10 (pg/mL)	12	1.37 [0.62;2.63]	12	0.70 [0.09;1.68]	0.274	0.470
IFN gamma (pg/mL)	12	1.32 [0.00;6.07]	12	1.94 [0.91;4.83]	0.922	0.922
IL-12p70 (pg/mL)	12	2.05 [0.71;4.19]	12	2.75 [1.30;5.44]	0.266	0.470
CXCL-8 (IL-8) (pg/mL)	12	3.77 [1.94;11.62]	12	5.28 [3.78;9.94]	0.052	0.314
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	12	1504.05 [978.83;2268.00]	12	1689.80 [1086.20;2303.20]	0.129	0.349
4-1BB (pg/mL)	12	80.31 [54.40;156.65]	12	72.05 [51.50;143.09]	0.204	0.349
sCD27 (pg/mL)	12	66274.00 [50880.00;87801.00]	12	62981.00 [49390.00;73961.00]	0.064	0.256
B7.2 (CD86) (pg/mL)	12	582.31 [452.28;700.38]	12	360.66 [300.49;565.35]	***0.007***	0.082
Free Active TGF-B1 (pg/mL)	12	23.19 [11.09;42.99]	12	25.66 [11.16;41.20]	0.677	0.813
CTLA-4 (pg/mL)	12	8.84 [2.25;27.96]	12	8.88 [1.04;23.83]	0.148	0.349
PD-L1 (pg/mL)	12	165.63 [113.50;213.15]	12	187.01 [149.03;223.63]	0.204	0.349
PD-L2 (pg/mL)	12	13370.00 [11972.00;16020.00]	12	14498.00 [12619.00;16398.00]	0.569	0.759
PD-1 (pg/mL)	12	33.25 [16.94;118.63]	12	24.74 [14.23;95.23]	0.052	0.256
Tim-3 (pg/mL)	12	26074.00 [18660.00;38595.00]	12	29643.00 [16492.00;37149.00]	0.791	0.850
LAG-3 (pg/mL)	12	2560.40 [1596.90;3621.40]	12	2042.20 [1398.00;4275.60]	0.569	0.759
Gal-9 (pg/mL)	12	105868.00 [90925.00;159163.00]	12	118096.00 [97476.00;167533.00]	0.850	0.850

Supplementary Table S10

Supplementary Table S10: Interaction analysis between time and frailty status for PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers. A change score was calculated between patients' values at three months and baseline (positive values indicating increase). The difference in change score was tested using a Mann-Whitney U test. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
PBMC - Major Subsets						
Viable cells (% of total cells)	8	-0.01 [-0.08;2.39]	12	0.35 [-0.04;1.02]	0.729	0.937
Viable lymphocytes (% of total lymphocytes)	8	0.23 [-0.45;0.91]	12	0.99 [0.30;2.01]	0.263	0.527
Total T-cells (% of viable lymphocytes)	8	3.81 [-2.26;6.06]	12	-0.85 [-9.24;1.74]	0.203	0.527
Total T-cells (% of viable cells)	8	2.28 [-0.87;5.90]	12	-3.07 [-5.87;4.56]	0.263	0.527
CD4+ T-cells (% of viable lymphocytes)	8	1.51 [-0.63;8.54]	12	-1.37 [-7.94;3.19]	0.298	0.536
CD8+ T-cells (% of viable lymphocytes)	8	-2.24 [-4.90;-0.20]	12	-0.67 [-2.97;0.63]	0.375	0.614
CD4/CD8 ratio (% CD4/CD8 ratio)	8	0.94 [0.01;1.93]	12	0.09 [-1.38;1.46]	0.263	0.527
NK/T-cells (% of viable cells)	8	-0.35 [-1.48;0.61]	12	-0.03 [-0.29;1.33]	0.418	0.627
Total NK cells (% of viable cells)	8	-1.02 [-2.55;2.26]	12	5.07 [0.37;7.44]	0.114	0.527
CD56 bright CD16- (% of total NK cells)	8	-0.32 [-0.70;0.93]	12	1.73 [-2.36;5.06]	0.512	0.709
CD56 dim CD16+ (% of total NK cells)	8	-9.64 [-16.30;-2.25]	12	10.79 [2.18;22.49]	***0.049***	0.442
Total B-cells (% of viable cells)	8	-0.98 [-1.52;1.96]	12	0.76 [-2.47;4.11]	0.969	0.969
Naive B-cells (% of total B-cells)	8	9.37 [2.97;11.87]	12	9.04 [2.03;14.08]	0.847	0.953
Memory B-cells (% of total B-cells)	8	-9.25 [-10.63;-3.24]	12	-8.87 [-13.01;-2.05]	0.908	0.961
Total monocytes (% of viable cells)	8	-2.59 [-5.57;2.52]	12	-2.22 [-10.92;2.83]	0.787	0.945
Non-classical monocytes (% of total monocytes)	8	5.39 [3.29;9.00]	12	-0.42 [-5.55;2.41]	***0.010***	0.176
Intermediate monocytes (% of total monocytes)	8	4.04 [2.26;5.38]	12	1.95 [-0.32;4.61]	0.203	0.527
Classical monocytes (% of total monocytes)	8	-10.08 [-16.99;-6.40]	12	-0.89 [-11.98;6.74]	0.203	0.527
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	2.82 [-1.65;14.32]	12	7.13 [3.12;11.87]	0.512	0.925
CD27 expression in Naïve CD4+ (MFI)	8	339.50 [94.00;481.00]	12	196.00 [34.00;888.00]	0.787	0.932
CD28 expression in Naïve CD4+ (MFI)	8	60.00 [-34.00;114.00]	12	105.00 [-3.50;214.50]	0.487	0.925
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	1.10 [0.71;8.12]	12	3.88 [1.58;13.85]	0.464	0.925
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	317.00 [88.00;397.50]	12	180.00 [27.00;729.50]	1.000	1.000
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	48.50 [-29.00;102.50]	12	88.00 [-3.00;182.50]	0.375	0.925
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	0.10 [-1.40;3.47]	12	-0.34 [-1.16;0.27]	0.563	0.925
CM CD4+ (% of CD4+ T-cells)	8	-0.83 [-10.94;-0.16]	12	-5.08 [-10.21;-0.68]	0.671	0.925
CD27 expression in CM CD4+ (MFI)	8	322.00 [96.00;483.00]	11	176.00 [92.00;743.00]	0.837	0.932
CD28 expression in CM CD4+ (MFI)	8	91.50 [-74.50;177.00]	12	134.00 [12.50;280.00]	0.335	0.925
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	4.08 [2.21;12.56]	12	10.13 [5.46;15.48]	0.154	0.925
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	262.50 [72.50;407.50]	12	145.50 [60.50;691.00]	0.847	0.932
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	70.00 [-60.00;180.00]	12	95.50 [13.00;249.00]	0.464	0.925

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	0.38 [-0.71;0.56]	12	-0.41 [-1.16;0.17]	0.335	0.925
EM CD4+ (% of CD4+ T-cells)	8	0.50 [-3.42;2.34]	12	-1.94 [-3.41;0.13]	0.375	0.925
CD27 expression in EM CD4+ (MFI)	8	248.00 [86.50;359.50]	11	172.00 [-74.00;254.00]	0.386	0.925
CD28 expression in EM CD4+ (MFI)	8	165.00 [-17.00;226.50]	12	159.50 [-32.00;226.00]	0.969	1.000
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	8	9.65 [3.73;18.21]	12	7.90 [2.71;15.97]	0.671	0.925
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	252.00 [100.00;347.50]	12	329.00 [-9.50;456.50]	0.512	0.925
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	115.00 [14.00;196.50]	12	189.50 [-60.00;345.50]	0.418	0.925
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	-0.11 [-0.59;0.03]	12	-0.01 [-0.20;0.03]	0.374	0.925
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	-0.29 [-2.52;0.54]	12	-0.37 [-0.81;1.22]	0.616	0.925
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	-8.04 [-11.64;-3.94]	12	-7.74 [-13.09;2.64]	1.000	1.000
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	0.04 [-0.30;0.37]	12	-0.30 [-4.79;0.44]	0.418	0.925
TEMRA CD4+(% of CD4+ T-cells)	8	0.02 [-0.06;0.04]	12	0.01 [-0.13;0.45]	0.847	0.932
CD27 expression in TEMRA CD4+ (MFI)	8	240.00 [-17.00;351.00]	9	316.00 [192.00;422.00]	0.361	0.925
CD28 expression in TEMRA CD4+ (MFI)	8	234.50 [-6.00;470.00]	12	150.00 [-15.00;330.50]	0.563	0.925
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	3.68 [-0.08;11.75]	12	11.93 [2.64;20.72]	0.232	0.925
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	278.00 [107.00;393.00]	12	321.00 [110.00;467.50]	0.729	0.925
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	182.00 [54.50;488.50]	12	95.50 [46.50;452.50]	0.729	0.925
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	-1.46 [-4.93;0.50]	12	0.07 [-0.38;5.68]	0.082	0.925
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	-2.13 [-5.89;0.05]	12	-3.71 [-12.99;-0.57]	0.464	0.925
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	-2.48 [-14.55;8.90]	12	-1.31 [-15.41;5.08]	0.729	0.925
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	4.66 [1.18;12.36]	12	2.50 [-2.32;8.18]	0.418	0.999
CD27 expression in Naïve CD8+ (MFI)	8	372.00 [70.50;785.00]	12	248.50 [45.50;881.00]	0.787	0.999
CD28 expression in Naïve CD8+ (MFI)	8	27.00 [-36.50;82.00]	12	48.00 [-26.00;112.00]	0.589	0.999
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	3.48 [1.07;16.91]	12	9.99 [2.78;23.19]	0.375	0.999
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	342.00 [59.00;657.50]	12	209.00 [64.00;672.50]	0.908	0.999
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	13.50 [-49.50;76.50]	12	47.50 [-34.00;100.00]	0.563	0.999
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	0.00 [-2.94;0.98]	12	-0.93 [-2.90;0.26]	0.671	0.999
CM CD8+ (% of CD8+ T-cells)	8	0.74 [-1.55;2.29]	12	-5.30 [-9.86;-1.56]	***0.049***	0.999
CD27 expression in CM CD8+ (MFI)	8	263.50 [73.00;488.00]	12	228.50 [46.00;583.00]	0.908	0.999
CD28 expression in CM CD8+ (MFI)	8	40.50 [-43.00;136.00]	12	90.00 [3.50;283.00]	0.464	0.999
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	4.51 [2.42;16.05]	12	13.63 [-0.02;21.95]	0.464	0.999

Continuation Table:

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	234.00 [54.00;368.00]	12	183.00 [47.00;486.00]	0.729	0.999
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	32.50 [-53.00;111.50]	12	63.00 [11.50;228.50]	0.464	0.999
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	1.15 [-3.86;2.83]	12	-0.15 [-2.13;2.59]	0.908	0.999
EM CD8+ (% of CD8+ T-cells)	8	-3.25 [-4.58;-0.88]	12	-0.77 [-2.57;1.39]	0.097	0.999
CD27 expression in EM CD8+ (MFI)	8	79.00 [41.50;265.50]	12	165.00 [20.50;370.00]	0.512	0.999
CD28 expression in EM CD8+ (MFI)	8	55.00 [-43.50;130.50]	12	61.50 [-45.50;227.50]	0.563	0.999
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	3.24 [1.81;15.12]	12	8.36 [-0.11;19.98]	0.729	0.999
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	157.00 [29.50;203.50]	12	56.00 [-21.50;331.00]	0.969	1.000
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	30.00 [-34.50;83.50]	12	7.00 [-90.50;199.00]	0.969	1.000
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	1.89 [-0.38;2.27]	12	0.66 [-0.20;2.34]	0.847	0.999
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	-3.60 [-6.12;3.68]	12	-3.90 [-9.66;4.78]	0.847	0.999
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	-7.79 [-13.54;0.46]	12	-8.87 [-15.78;2.93]	0.787	0.999
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	1.18 [-1.91;3.53]	12	-0.40 [-7.18;4.76]	0.847	0.999
TEMRA CD8+(% of CD8+ T-cells)	8	-1.45 [-6.26;0.94]	12	1.61 [-1.30;6.37]	0.133	0.999
CD27 expression in TEMRA CD8+ (MFI)	8	17.50 [-23.50;176.00]	12	110.00 [-5.00;158.00]	0.487	0.999
CD28 expression in TEMRA CD8+ (MFI)	8	29.50 [-16.00;52.00]	12	-1.50 [-35.00;55.50]	0.787	0.999
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	0.94 [-1.30;6.69]	12	3.14 [-0.43;5.13]	0.847	0.999
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	125.50 [-32.50;204.00]	11	41.00 [-109.00;361.00]	0.710	0.999
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	29.00 [-6.50;84.50]	11	14.00 [-62.00;323.00]	1.000	1.000
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	0.42 [-0.55;3.01]	12	1.80 [1.06;2.59]	0.298	0.999
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	-2.15 [-8.27;2.86]	12	-1.20 [-6.26;4.30]	0.671	0.999
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	0.64 [-1.76;4.40]	12	1.52 [-3.25;9.60]	0.847	0.999
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	8	-0.10 [-0.50;0.14]	12	-0.95 [-1.85;0.12]	0.375	0.766
CD25 expression in Total Treg (MFI)	8	196.50 [-57.00;1125.50]	12	229.50 [-42.50;767.00]	0.787	0.932
CTLA-4 expression in Total Treg (MFI)	8	35.50 [5.00;64.00]	12	-6.00 [-32.50;43.00]	0.165	0.766
Naïve Treg (% of total Tregs)	8	3.12 [-0.51;3.84]	12	4.84 [1.84;8.89]	0.298	0.766
CD25 expression of Naïve Treg (MFI)	8	355.50 [44.00;672.00]	12	322.50 [-174.00;951.50]	0.616	0.932
CTLA-4 expression of Naïve Treg (MFI)	8	20.00 [4.00;69.00]	12	3.00 [-15.50;34.50]	0.418	0.766
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	1.74 [-0.49;10.56]	12	-0.73 [-16.63;3.83]	0.232	0.766
Memory Treg (% of total Tregs)	8	-3.28 [-4.41;-0.12]	12	-4.97 [-8.73;-1.76]	0.263	0.766
CD25 expression of Memory Treg (MFI)	8	214.50 [-95.50;1111.00]	12	204.00 [64.00;762.00]	0.969	0.969
CTLA-4 expression of Memory Treg (MFI)	8	18.50 [-7.50;48.00]	12	1.50 [-37.00;46.00]	0.700	0.932
Memory Treg CTLA-4+ (% of Memory Tregs)	8	0.81 [-0.71;5.38]	12	1.08 [-3.34;3.82]	0.847	0.932

Continuation Table:

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	8	-0.31 [-0.81;0.39]	12	0.15 [-0.20;1.47]	0.298	0.794
PD1 expression in CD4+ (MFI)	8	15.00 [-6.00;22.50]	12	16.50 [-33.50;53.50]	0.847	0.884
CD4+ CD69+ (% of CD4+ T-cells)	8	-0.32 [-6.04;3.50]	12	0.03 [-4.91;3.84]	0.729	0.884
CD69 expression in CD4+ (MFI)	8	-8.50 [-18.50;33.00]	12	13.50 [-4.50;43.50]	0.375	0.836
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	-0.57 [-0.81;0.32]	12	0.03 [-0.09;0.80]	0.203	0.695
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	-0.04 [-3.61;6.86]	12	-0.10 [-4.21;3.97]	0.616	0.884
CD4+ HLADR+ (% of CD4+ T-cells)	8	4.03 [-1.75;4.49]	12	0.90 [-1.75;5.80]	0.847	0.884
HLADR expression in CD4+ (MFI)	8	35.50 [-36.50;171.50]	12	-17.00 [-86.00;35.50]	0.177	0.695
CD4+ CD38+ (% of CD4+ T-cells)	8	-0.35 [-3.58;6.10]	12	1.95 [-0.22;8.70]	0.418	0.836
CD38 expression in CD4+ (MFI)	8	-48.00 [-85.00;98.00]	12	15.00 [-52.00;109.00]	0.512	0.878
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	0.31 [-0.51;1.98]	12	0.85 [0.31;1.81]	0.418	0.836
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	-1.07 [-5.49;2.06]	12	-2.68 [-12.19;1.44]	0.464	0.856
CD8+ PD1+ (% of CD8+ T-cells)	8	1.28 [-9.20;8.50]	12	0.07 [-0.99;8.24]	0.729	0.884
PD1 expression in CD8+ (MFI)	8	-15.50 [-37.00;-5.00]	12	-4.50 [-24.50;56.50]	0.153	0.695
CD8+ CD69+ (% of CD8+ T-cells)	8	-2.89 [-6.68;-0.49]	12	0.94 [-2.55;8.64]	0.070	0.695
CD69 expression in CD8+ (MFI)	8	60.00 [-209.50;133.50]	12	27.50 [-0.50;146.50]	0.729	0.884
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	1.15 [-2.99;2.37]	12	0.06 [-0.48;4.16]	0.969	0.969
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	1.62 [0.36;13.59]	12	-1.51 [-10.33;3.14]	0.232	0.695
CD8+ HLADR+ (% of CD8+ T-cells)	8	-1.67 [-5.26;5.20]	12	4.14 [1.26;14.62]	0.083	0.695
HLADR expression in CD8+ (MFI)	8	18.50 [-34.00;51.50]	12	36.50 [-66.50;208.00]	0.758	0.884
CD8+ CD38+ (% of CD8+ T-cells)	8	2.53 [-0.90;8.00]	12	1.83 [0.94;7.01]	0.787	0.884
CD38 expression in CD8+ (MFI)	8	39.00 [-20.00;95.00]	12	7.50 [-36.00;82.50]	0.787	0.884
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	-0.07 [-1.08;3.42]	12	1.91 [0.28;4.75]	0.203	0.695
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	0.96 [-8.41;4.39]	12	-6.17 [-17.26;-3.46]	0.133	0.695
Plasma - Essential Immune Response						
IL-4 (pg/mL)	8	0.29 [-1.15;6.22]	12	0.00 [-0.44;0.50]	0.560	0.840
IL-2 (pg/mL)	8	0.31 [-1.48;7.05]	12	-0.15 [-1.83;0.83]	0.464	0.835
CXCL-10 (IP-10) (pg/mL)	8	99.44 [-26.50;259.46]	12	74.28 [11.17;197.84]	0.908	0.969
IL-1 beta (pg/mL)	8	3.19 [-10.07;18.89]	12	-3.19 [-8.79;3.59]	0.487	0.835
TNF alpha (pg/mL)	8	-1.11 [-3.32;0.15]	12	0.11 [-1.00;1.13]	0.133	0.397
CCL2 (MCP-1) (pg/mL)	8	21.50 [-59.56;132.87]	12	38.48 [-13.15;95.82]	0.908	0.969
IL-17A (pg/mL)	8	-1.08 [-10.87;2.35]	12	-0.90 [-1.51;0.56]	0.787	0.969
IL-6 (pg/mL)	8	-0.57 [-9.71;2.34]	12	1.43 [-0.07;5.02]	0.097	0.397
IL-10 (pg/mL)	8	-1.87 [-2.28;-1.21]	12	-0.89 [-1.81;0.70]	0.114	0.397
IFN gamma (pg/mL)	8	0.47 [-24.19;2.61]	12	0.00 [-3.44;1.90]	0.969	0.969
IL-12p70 (pg/mL)	8	-1.61 [-14.13;4.29]	12	1.30 [-1.48;4.03]	0.298	0.714
CXCL-8 (IL-8) (pg/mL)	8	-1.56 [-8.51;-0.44]	12	1.47 [0.51;3.24]	***0.023***	0.274
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	142.73 [51.07;345.70]	12	65.99 [-40.75;226.39]	0.464	0.795
4-1BB (pg/mL)	8	-19.40 [-39.76;7.14]	12	-4.20 [-17.44;2.91]	0.464	0.795
sCD27 (pg/mL)	8	-599.80 [-3829.00;846.70]	12	-5006.00 [-17099.00;1198.10]	0.203	0.609
B7.2 (CD86) (pg/mL)	8	-143.10 [-203.50;-55.31]	12	-108.20 [-134.00;-35.32]	0.616	0.924

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
Free Active TGF-B1 (pg/mL)	8	-14.20 [-18.56;-5.11]	12	-8.73 [-11.22;9.84]	0.133	0.609
CTLA-4 (pg/mL)	8	-3.70 [-8.00;1.19]	12	-2.03 [-4.54;0.70]	0.787	0.990
PD-L1 (pg/mL)	8	-50.06 [-106.10;38.33]	12	15.21 [-13.97;40.45]	0.154	0.609
PD-L2 (pg/mL)	8	1297.00 [-126.30;1712.80]	12	495.34 [-276.10;2131.10]	0.847	0.990
PD-1 (pg/mL)	8	-26.26 [-100.90;0.81]	12	-9.40 [-42.33;2.62]	0.464	0.795
Tim-3 (pg/mL)	8	815.64 [-1557.00;2143.90]	12	93.20 [-4254.00;2849.00]	0.908	0.990
LAG-3 (pg/mL)	8	-408.80 [-1412.00;2921.60]	12	-347.70 [-593.80;448.45]	1.000	1.000
Gal-9 (pg/mL)	8	12127.00 [2463.40;35168.00]	12	3440.50 [-10789.00;18306.00]	0.203	0.609

Supplementary Table S11

Supplementary Table S11: Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers between the fit and frail patients at baseline. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values were calculated using an unpaired Mann-Whitney U test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.20 [96.94;99.51]	12	98.65 [98.29;99.18]	0.232	0.596
Viable lymphocytes (% of total lymphocytes)	8	98.12 [96.27;98.48]	12	96.73 [90.71;97.76]	0.154	0.531
Total T-cells (% of viable lymphocytes)	8	61.50 [56.82;71.20]	12	62.16 [53.54;64.67]	0.643	0.930
Total T-cells (% of viable cells)	8	59.39 [42.18;64.59]	12	54.68 [47.77;61.56]	0.908	0.969
CD4+ T-cells (% of viable lymphocytes)	8	52.70 [29.20;56.34]	12	50.22 [44.03;57.76]	0.671	0.930
CD8+ T-cells (% of viable lymphocytes)	8	16.44 [14.48;20.99]	12	13.96 [8.49;18.23]	0.177	0.531
CD4/CD8 ratio (% CD4/CD8 ratio)	8	3.16 [1.60;4.12]	12	3.99 [2.61;6.86]	0.154	0.531
NK/T-cells (% of viable cells)	8	4.46 [2.65;10.06]	12	1.81 [1.43;3.70]	***0.049***	0.442
Total NK cells (% of viable cells)	8	9.16 [5.54;12.23]	12	6.52 [3.69;10.64]	0.375	0.836
CD56 bright CD16- (% of total NK cells)	8	3.96 [2.64;12.78]	12	4.74 [4.00;8.47]	0.563	0.921
CD56 dim CD16+ (% of total NK cells)	8	82.71 [68.17;91.11]	12	58.52 [19.32;76.44]	***0.015***	0.272
Total B-cells (% of viable cells)	8	9.00 [7.47;10.06]	12	7.51 [5.58;9.54]	0.418	0.836
Naive B-cells (% of total B-cells)	8	58.95 [54.53;71.71]	12	62.99 [42.09;75.50]	0.969	0.969
Memory B-cells (% of total B-cells)	8	40.22 [27.47;44.21]	12	35.87 [23.20;56.53]	0.969	0.969
Total monocytes (% of viable cells)	8	13.77 [12.65;16.06]	12	18.11 [8.34;24.38]	0.512	0.921
Non-classical monocytes (% of total monocytes)	8	4.47 [3.04;5.03]	12	3.41 [1.19;9.72]	0.969	0.969
Intermediate monocytes (% of total monocytes)	8	5.76 [4.17;6.75]	12	6.17 [4.74;7.32]	0.787	0.969
Classical monocytes (% of total monocytes)	8	83.73 [80.18;86.12]	12	79.92 [68.76;83.23]	0.154	0.531
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	26.32 [18.89;49.10]	12	44.61 [22.85;50.60]	0.335	0.567
CD27 expression in Naïve CD4+ (MFI)	8	1458.50 [1248.50;1752.50]	12	1312.00 [914.50;1596.50]	0.232	0.497
CD28 expression in Naïve CD4+ (MFI)	8	766.00 [666.00;864.00]	12	614.00 [548.50;726.50]	0.203	0.497
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	96.01 [90.34;98.05]	12	88.82 [71.31;92.93]	***0.019***	0.307
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	1491.00 [1323.50;1831.50]	12	1424.50 [1134.00;1677.50]	0.464	0.567
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	764.00 [690.50;871.00]	12	649.50 [565.50;739.50]	0.177	0.497
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	4.70 [2.43;18.71]	12	2.68 [1.35;11.63]	0.563	0.641
CM CD4+ (% of CD4+ T-cells)	8	60.90 [39.22;66.37]	12	47.86 [33.74;59.69]	0.203	0.497
CD27 expression in CM CD4+ (MFI)	8	1279.00 [970.00;1521.00]	11	1006.00 [817.00;1326.00]	0.231	0.497
CD28 expression in CM CD4+ (MFI)	8	1265.00 [1068.50;1379.00]	12	1089.50 [868.00;1247.50]	0.133	0.497
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	80.46 [74.87;83.76]	12	70.12 [59.29;79.39]	0.114	0.497

Continuation Table:

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	1490.00 [1261.50;1803.00]	12	1359.50 [1183.50;1601.00]	0.375	0.567
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	1227.50 [1092.50;1347.00]	12	1111.00 [888.50;1256.50]	0.217	0.497
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	2.01 [1.53;3.10]	12	2.87 [1.96;4.05]	0.232	0.497
EM CD4+ (% of CD4+ T-cells)	8	10.51 [6.97;18.67]	12	10.35 [5.42;14.76]	0.464	0.567
CD27 expression in EM CD4+ (MFI)	8	456.00 [322.50;505.00]	11	371.00 [134.00;579.00]	0.536	0.631
CD28 expression in EM CD4+ (MFI)	8	1027.00 [890.00;1284.50]	12	955.00 [802.50;1201.50]	0.464	0.567
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	8	34.08 [26.25;42.46]	12	31.17 [17.30;38.43]	0.464	0.567
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	1203.00 [1036.50;1389.00]	12	1114.00 [971.00;1274.00]	0.335	0.567
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	1149.50 [1072.50;1365.50]	12	1062.00 [874.00;1257.50]	0.177	0.497
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	0.18 [0.08;0.81]	12	0.04 [0.00;0.32]	0.241	0.497
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	4.47 [0.92;13.05]	12	2.19 [1.45;4.06]	0.700	0.721
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	53.20 [50.65;61.83]	12	62.95 [56.89;72.30]	0.083	0.497
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.54 [0.74;3.57]	12	2.14 [1.37;11.21]	0.203	0.497
TEMRA CD4+(% of CD4+ T-cells)	8	0.41 [0.20;1.47]	12	0.62 [0.33;2.60]	0.375	0.567
CD27 expression in TEMRA CD4+ (MFI)	8	500.50 [95.500;697.50]	9	396.00 [201.00;418.00]	0.597	0.656
CD28 expression in TEMRA CD4+ (MFI)	8	519.00 [230.50;733.50]	12	799.50 [455.00;1068.50]	0.177	0.497
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	26.93[11.58;48.75]	12	27.59 [13.65;35.75]	0.728	0.728
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1189.50 [939.50;1464.50]	12	1044.50 [937.00;1229.00]	0.464	0.567
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1007.00 [929.00;1082.00]	12	1104.00 [816.00;1208.00]	0.616	0.656
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	10.42 [4.09;19.63]	12	0.00 [0.00;1.88]	***0.009***	0.283
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	24.13 [11.51;53.79]	12	12.95 [5.33;35.81]	0.418	0.567
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	47.02 [28.37;76.66]	12	27.99 [8.55;53.71]	0.263	0.511
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	8.89 [6.98;26.00]	12	20.00 [14.10;48.67]	0.083	0.195
CD27 expression in Naïve CD8+ (MFI)	8	1873.50 [1517.00;2110.50]	12	1229.50 [1014.00;1579.00]	***0.010***	0.064
CD28 expression in Naïve CD8+ (MFI)	8	642.50 [541.50;737.50]	12	538.00 [436.00;641.00]	0.177	0.278
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	88.32 [76.96;91.95]	12	66.27 [46.33;77.72]	***0.004***	0.064
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2005.00 [1706.50;2230.50]	12	1579.00 [1526.50;1820.50]	***0.041***	0.148
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	657.00 [558.00;770.00]	12	576.50 [491.50;661.50]	0.335	0.442
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	3.16 [1.80;7.34]	12	5.19 [2.72;6.86]	0.671	0.821
CM CD8+ (% of CD8+ T-cells)	8	17.90 [16.32;25.91]	12	29.27 [22.16;39.40]	0.059	0.176

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD27 expression in CM CD8+ (MFI)	8	1335.50 [1083.50;1461.00]	12	905.50 [694.50;1069.00]	***0.006***	0.064
CD28 expression in CM CD8+ (MFI)	8	1015.50 [896.00;1078.50]	12	878.50 [691.00;1015.00]	0.177	0.278
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	74.34 [69.93;84.43]	12	56.80 [45.18;66.45]	***0.002***	0.064
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	1589.50 [1382.00;1715.00]	12	1323.50 [1172.00;1497.50]	***0.041***	0.148
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1057.50 [942.50;1103.00]	12	956.50 [775.00;1057.00]	0.203	0.305
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.78 [8.77;12.74]	12	9.92 [6.56;14.91]	0.729	0.859
EM CD8+ (% of CD8+ T-cells)	8	30.36 [24.22;41.33]	12	17.33 [10.72;28.47]	0.070	0.192
CD27 expression in EM CD8+ (MFI)	8	631.00 [481.00;708.00]	12	437.50 [357.00;578.50]	0.097	0.214
CD28 expression in EM CD8+ (MFI)	8	474.50 [327.00;622.50]	12	497.50 [396.50;591.00]	0.847	0.932
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	23.70 [15.16;33.40]	12	15.30 [8.64;23.33]	0.083	0.195
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	1369.50 [1297.00;1500.00]	12	1274.50 [1211.00;1414.50]	0.165	0.278
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	819.50 [793.00;950.50]	12	791.00 [732.00;899.50]	0.512	0.650
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.95 [3.01;6.91]	12	2.16 [0.85;3.14]	***0.028***	0.131
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	27.68 [22.95;43.91]	12	24.00 [12.54;40.40]	0.335	0.442
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	36.82 [31.41;44.71]	12	56.06 [42.69;64.97]	***0.012***	0.067
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.74 [43.34;53.29]	12	49.35 [15.50;60.11]	0.847	0.932
TEMRA CD8+(% of CD8+ T-cells)	8	27.01 [18.18;39.86]	12	22.32 [6.91;26.87]	0.177	0.278
CD27 expression in TEMRA CD8+ (MFI)	8	257.50 [205.00;438.50]	12	268.50 [195.50;386.00]	0.877	0.934
CD28 expression in TEMRA CD8+ (MFI)	8	86.00 [67.00;132.50]	12	188.00 [105.00;354.00]	***0.045***	0.148
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	4.33 [2.76;5.20]	12	3.60 [1.80;6.91]	0.908	0.936
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	1481.50 [1392.00;1560.50]	11	1515.00 [1303.00;1664.00]	1.000	1.000
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	685.50 [655.00;726.00]	11	747.00 [653.00;873.00]	0.231	0.332
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	2.82 [2.26;4.50]	12	1.30 [0.42;1.54]	***0.010***	0.064
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	81.72 [72.29;85.97]	12	65.24 [43.68;81.82]	0.133	0.273
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	73.62 [64.18;77.01]	12	61.92 [39.21;76.18]	0.154	0.278
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	8	5.11 [4.28;6.19]	12	4.57 [3.88;6.90]	***0.787***	0.787
CD25 expression in Total Treg (MFI)	8	2203.50 [1610.00;2842.50]	12	1293.50 [1096.50;1816.50]	***0.041***	0.075
CTLA-4 expression in Total Treg (MFI)	8	228.50 [213.50;247.00]	12	272.50 [246.50;301.00]	0.031	0.075
Naïve Treg (% of total Tregs)	8	6.30 [4.76;8.05]	12	5.15 [3.04;13.94]	0.787	0.787
CD25 expression of Naïve Treg (MFI)	8	2048.50 [1173.50;2256.00]	12	1279.50 [827.50;1556.50]	0.059	0.081

Continuation Table:

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CTLA-4 expression of Naïve Treg (MFI)	8	186.00 [178.50;220.00]	12	252.50 [216.50;318.50]	***0.025***	0.075
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	15.94 [8.51;19.89]	12	33.18 [23.25;40.31]	***0.025***	0.075
Memory Treg (% of total Tregs)	8	93.51 [91.47;94.70]	12	94.74 [85.54;96.79]	0.787	0.787
CD25 expression of Memory Treg (MFI)	8	2178.00 [1627.50;2838.50]	12	1345.00 [1094.50;1845.50]	***0.037***	0.075
CTLA-4 expression of Memory Treg (MFI)	8	230.50 [215.50;256.50]	12	271.00 [248.00;305.00]	***0.049***	0.077
Memory Treg CTLA-4+ (% of Memory Tregs)	8	15.59 [8.43;17.68]	12	19.74 [16.52;21.61]	***0.019***	0.075
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	8	1.95 [1.69;2.89]	12	1.26 [0.88;1.62]	***0.049***	0.147
PD1 expression in CD4+ (MFI)	8	224.50 [160.50;297.00]	12	195.50 [99.50;216.00]	0.133	0.289
CD4+ CD69+ (% of CD4+ T-cells)	8	35.46 [25.93;42.24]	12	31.54 [17.07;38.34]	0.177	0.327
CD69 expression in CD4+ (MFI)	8	249.50 [236.00;290.50]	12	262.00 [226.50;270.00]	0.877	0.908
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.41 [0.98;2.13]	12	0.74 [0.50;1.04]	***0.023***	0.110
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	64.00 [56.19;73.22]	12	67.83 [61.28;82.28]	0.177	0.327
CD4+ HLADR+ (% of CD4+ T-cells)	8	8.37 [6.88;14.41]	12	16.98 [11.69;22.80]	***0.034***	0.136
HLADR expression in CD4+ (MFI)	8	333.00 [297.50;381.00]	12	459.00 [388.00;627.00]	***0.023***	0.110
CD4+ CD38+ (% of CD4+ T-cells)	8	33.65 [18.60;46.64]	12	28.78 [20.25;45.58]	0.908	0.908
CD38 expression in CD4+ (MFI)	8	671.00 [500.00;874.00]	12	644.50 [488.50;777.00]	0.671	0.732
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	2.41 [1.40;3.89]	12	3.47 [2.14;4.28]	0.354	0.532
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	59.02 [44.86;69.63]	12	51.84 [48.00;63.98]	0.563	0.711
CD8+ PD1+ (% of CD8+ T-cells)	8	29.14 [21.31;40.55]	12	15.24 [9.90;23.11]	***0.015***	0.110
PD1 expression in CD8+ (MFI)	8	325.00 [230.00;526.00]	12	267.50 [203.50;323.50]	0.263	0.421
CD8+ CD69+ (% of CD8+ T-cells)	8	36.86 [21.01;55.88]	12	31.63 [22.80;42.70]	0.464	0.655
CD69 expression in CD8+ (MFI)	8	513.00 [390.50;931.50]	12	342.50 [279.50;437.00]	***0.015***	0.110
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.68 [7.68;15.99]	12	6.17 [5.13;9.99]	0.076	0.202
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	36.20 [30.95;53.39]	12	58.50 [47.46;69.09]	***0.023***	0.110
CD8+ HLADR+ (% of CD8+ T-cells)	8	31.78 [16.88;42.36]	12	41.85 [33.45;47.17]	0.133	0.289
HLADR expression in CD8+ (MFI)	8	474.50 [341.00;488.00]	12	531.50 [489.50;709.00]	***0.041***	0.140
CD8+ CD38+ (% of CD8+ T-cells)	8	14.06 [7.27;17.44]	12	8.47 [6.20;21.45]	0.671	0.732
CD38 expression in CD8+ (MFI)	8	293.00 [230.50;342.50]	12	243.50 [192.50;355.00]	0.563	0.711
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	7.82 [1.71;10.11]	12	4.98 [4.12;6.99]	0.671	0.732
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	64.70 [38.71;77.09]	12	52.45 [48.44;55.66]	0.203	0.348
Plasma - Essential Immune Response						
IL-4 (pg/mL)	8	0.11 [0.00;28.48]	12	0.06 [0.00;1.38]	0.773	0.928
IL-2 (pg/mL)	8	3.34 [1.92;9.66]	12	1.80 [0.67;2.24]	0.154	0.423
CXCL-10 (IP-10) (pg/mL)	8	159.08 [125.71;355.70]	12	311.58 [176.37;354.14]	0.232	0.423
IL-1 beta (pg/mL)	8	6.06 [1.47;34.62]	12	5.27 [0.77;11.90]	0.616	0.858
TNF alpha (pg/mL)	8	1.81 [1.21;5.54]	12	2.07 [1.63;3.43]	0.643	0.858

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CCL2 (MCP-1) (pg/mL)	8	193.48 [149.28;232.19]	12	133.65 [97.65;174.79]	0.070	0.419
IL-17A (pg/mL)	8	2.23 [1.18;22.16]	12	2.78 [1.35;8.50]	0.969	0.969
IL-6 (pg/mL)	8	8.40 [3.86;24.28]	12	3.86 [2.88;8.14]	0.203	0.423
IL-10 (pg/mL)	8	2.23 [1.54;3.82]	12	1.37 [0.62;2.63]	0.247	0.423
IFN gamma (pg/mL)	8	0.91 [0.00;35.63]	12	1.32 [0.00;6.07]	0.905	0.969
IL-12p70 (pg/mL)	8	6.93 [4.28;58.97]	12	2.05 [0.71;4.19]	***0.008***	0.093
CXCL-8 (IL-8) (pg/mL)	8	7.23 [3.55;25.42]	12	3.77 [1.94;11.62]	0.232	0.423
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	928.26 [798.87;1140.80]	12	1504.50 [978.83;2268.00]	0.133	0.531
4-1BB (pg/mL)	8	68.07 [50.57;119.55]	12	80.31 [54.40;156.65]	0.464	0.695
sCD27 (pg/mL)	8	52670.00 [45645.00;65276.00]	12	66274.00 [50880.00;87801.00]	0.298	0.595
B7.2 (CD86) (pg/mL)	8	524.80 [474.15;697.47]	12	582.31 [452.28;700.38]	0.969	0.969
Free Active TGF-B1 (pg/mL)	8	25.39 [22.12;32.09]	12	23.19 [11.09;42.99]	0.616	0.821
CTLA-4 (pg/mL)	8	10.14 [4.40;15.98]	12	8.84 [2.25;27.96]	0.847	0.924
PD-L1 (pg/mL)	8	279.06 [172.00;401.70]	12	165.63 [113.50;213.15]	0.154	0.531
PD-L2 (pg/mL)	8	11121.00 [10196.00;14318.00]	12	13370.00 [11972.00;16020.00]	0.177	0.531
PD-1 (pg/mL)	8	92.41 [16.51;201.92]	12	33.25 [16.94;118.63]	0.787	0.924
Tim-3 (pg/mL)	8	15345.00 [6568.40;26546.00]	12	26074.00 [18660.00;38595.00]	0.114	0.531
LAG-3 (pg/mL)	8	5456.70 [2198.80;16183.00]	12	2560.40 [1596.90;3621.40]	0.263	0.595
Gal-9 (pg/mL)	8	102158.00 [76361.00;127351.00]	12	1058680.00 [90925.00;159163.00]	0.375	0.643

Supplementary Table S12

Supplementary Table S12: Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers between the fit and frail patients after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The p-values were calculated using an unpaired Mann-Whitney U test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.29 [98.88;99.61]	12	99.19 [98.76;99.61]	0.758	1.000
Viable lymphocytes (% of total lymphocytes)	8	97.79 [96.94;98.59]	12	97.92 [96.82;98.60]	0.969	1.000
Total T-cells (% of viable lymphocytes)	8	65.62 [56.74;72.41]	12	60.47 [49.61;66.66]	0.375	1.000
Total T-cells (% of viable cells)	8	59.69 [43.29;67.63]	12	56.93 [47.61;60.10]	0.908	1.000
CD4+ T-cells (% of viable lymphocytes)	8	52.78 [38.14;60.31]	12	49.89 [41.65;55.80]	0.671	1.000
CD8+ T-cells (% of viable lymphocytes)	8	15.24 [10.03;19.46]	12	10.72 [7.78;16.06]	0.203	1.000
CD4/CD8 ratio (% CD4/CD8 ratio)	8	2.78 [2.62;5.98]	12	4.13 [2.80;7.17]	0.418	1.000
NK/T-cells (% of viable cells)	8	4.74 [2.69;7.44]	12	2.82 [1.52;4.51]	0.132	1.000
Total NK cells (% of viable cells)	8	8.74 [6.76;11.65]	12	11.08 [6.38;15.27]	0.464	1.000
CD56 bright CD16- (% of total NK cells)	8	3.82 [2.55;14.38]	12	6.54 [4.76;10.49]	0.616	1.000
CD56 dim CD16+ (% of total NK cells)	8	71.87 [50.83;90.52]	12	64.98 [43.70;88.52]	0.671	1.000
Total B-cells (% of viable cells)	8	7.61 [5.91;9.87]	12	9.29 [4.52;12.36]	0.671	1.000
Naive B-cells (% of total B-cells)	8	71.81 [64.05;81.13]	12	74.80 [62.54;80.94]	0.787	1.000
Memory B-cells (% of total B-cells)	8	27.87 [17.79;35.21]	12	24.48 [18.23;36.41]	1.000	1.000
Total monocytes (% of viable cells)	8	12.89 [8.16;20.14]	12	15.94 [5.82;19.00]	0.908	1.000
Non-classical monocytes (% of total monocytes)	8	9.63 [7.70;12.98]	12	4.79 [2.77;7.91]	0.083	1.000
Intermediate monocytes (% of total monocytes)	8	8.63 [7.65;12.87]	12	8.36 [5.13;10.63]	0.512	1.000
Classical monocytes (% of total monocytes)	8	70.19 [64.80;78.07]	12	75.53 [59.36;82.19]	0.847	1.000
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	40.81 [18.93;47.80]	12	54.15 [30.13;63.86]	0.177	0.845
CD27 expression in Naïve CD4+ (MFI)	8	1829.50 [1559.00;2084.50]	12	1717.50 [1410.50;1818.00]	0.512	0.845
CD28 expression in Naïve CD4+ (MFI)	8	806.00 [712.00;838.50]	12	791.00 [676.50;850.00]	0.939	0.968
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	98.49 [97.00; 98.68]	12	95.25 [91.67; 96.83]	***0.028***	0.845
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	1849.50 [1599.50;2109.50]	12	1770.50 [1509.50;1875.00]	0.671	0.886
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	810.50 [717.50;840.50]	12	783.50 [705.00;845.00]	0.969	0.969
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	6.98 [1.03;10.63]	12	1.81 [0.47;5.06]	0.232	0.845
CM CD4+ (% of CD4+ T-cells)	8	48.86 [44.26;61.18]	12	38.75 [27.56;57.95]	0.203	0.845
CD27 expression in CM CD4+ (MFI)	8	1502.00 [1233.00;1767.00]	12	1404.00 [1117.00;1698.00]	0.616	0.884
CD28 expression in CM CD4+ (MFI)	8	1271.50 [1119.50;1358.00]	12	1198.00 [1098.50;1370.50]	0.616	0.884
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	85.62 [82.75;90.89]	12	85.03 [72.43;87.00]	0.464	0.845
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	1722.50 [1510.50;1962.00]	12	1621.00 [1483.00;1878.50]	0.616	0.884

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	1266.50 [1098.50;1335.50]	12	1186.00 [1103.0;1342.00]	0.671	0.886
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	1.95 [1.64;2.49]	12	2.17 [1.37;3.41]	0.908	0.966
EM CD4+ (% of CD4+ T-cells)	8	11.79 [7.20;15.59]	12	7.59 [4.40;10.94]	0.203	0.845
CD27 expression in EM CD4+ (MFI)	8	691.00 [421.00;843.50]	12	481.00 [379.50;733.50]	0.512	0.845
CD28 expression in EM CD4+ (MFI)	8	1141.50 [996.00;1267.50]	12	1103.05 [1023.00;1260.00]	0.729	0.890
EM CD4+ CD27+ CD28+(% of EM CD4+ T-cells)	8	51.89 [34.75;54.52]	12	38.07 [30.48;44.77]	0.335	0.845
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	1355.50 [1316.50;1563.50]	12	1292.00 [1192.50;1573.00]	0.418	0.845
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	1232.50 [1187.50;1459.00]	12	1179.50 [1135.50;1339.50]	0.335	0.845
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	0.13 [0.05;0.27]	12	0.11 [0.00;0.40]	0.510	0.845
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	1.46 [0.69;13.98]	12	2.16 [0.57;3.75]	0.787	0.896
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	45.53 [42.46;46.81]	12	57.76 [47.14;64.68]	0.114	0.845
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.74 [1.00;2.51]	12	2.33 [1.33;5.32]	0.232	0.845
TEMRA CD4+(% of CD4+ T-cells)	8	0.35 [0.24;0.91]	12	0.56 [0.38;2.25]	0.335	0.845
CD27 expression in TEMRA CD4+ (MFI)	8	546.00 [107.50;1033.50]	11	576.00 [376.00;878.00]	0.773	0.896
CD28 expression in TEMRA CD4+ (MFI)	8	766.50 [228.00;1233.50]	12	1008.00 [629.00;1171.00]	0.729	0.890
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	27.19 [12.42;59.22]	12	32.86 [25.25;53.91]	0.512	0.845
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1609.50 [1258.00;1646.50]	12	1454.50 [1172.00;1609.00]	0.464	0.845
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1289.00 [889.00;1559.00]	12	1245.00 [1055.00;1525.50]	0.908	0.966
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	5.21 [1.53;11.04]	12	1.15 [0.00;5.68]	0.260	0.845
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	16.57 [6.87;54.53]	12	4.82 [2.48;26.06]	0.232	0.845
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	45.56 [22.38;77.28]	12	22.99 [9.38;44.65]	0.154	0.845
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	21.62 [13.01;34.47]	12	21.17 [14.13;52.44]	0.671	0.886
CD27 expression in Naïve CD8+ (MFI)	8	2255.00 [1848.50;2482.50]	12	1676.00 [1227.50;2032.00]	0.070	0.461
CD28 expression in Naïve CD8+ (MFI)	8	554.00 [514.00;758.00]	12	599.50 [499.00;641.00]	0.616	0.884
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	94.74 [92.60;95.92]	12	81.19 [61.98;88.81]	***0.019***	0.204
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2349.00 [1898.50;2572.50]	12	1983.50 [1671.00;2189.00]	0.154	0.633
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	584.50 [531.50;766.50]	12	619.00 [546.50;661.00]	1.000	1.000
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	2.39 [1.77;3.50]	12	2.40 [1.33;4.85]	0.787	0.962
CM CD8+ (% of CD8+ T-cells)	8	17.98 [16.38;28.72]	12	18.44 [14.94;32.76]	0.969	1.000
CD27 expression in CM CD8+ (MFI)	8	1566.00 [1222.50;1757.50]	12	1178.00 [876.50;1488.50]	0.105	0.578

Continuation Table:

Biomarker	**Fit**		**Frail**		**P-values**	
	N	**Median [IQR]**	**N**	**Median [IQR]**	**Raw**	**FDR**
CD28 expression in CM CD8+ (MFI)	8	1043.00 [844.50;1111.00]	12	867.50 [795.00;1126.00]	0.512	0.805
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	86.13 [76.94;87.44]	12	70.19 [53.41;75.44]	***0.012***	0.201
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	1740.00 [1484.50;1961.50]	12	1495.00 [1338.00;1832.00]	0.217	0.789
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1052.00 [924.50;1116.00]	12	933.00 [851.50;1148.50]	0.464	0.805
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.64 [8.03;12.77]	12	10.62 [5.94;16.58]	0.847	0.998
EM CD8+ (% of CD8+ T-cells)	8	27.05 [21.24;33.77]	12	17.09 [10.91;31.89]	0.298	0.789
CD27 expression in EM CD8+ (MFI)	8	769.00 [534.50;953.00]	12	638.50 [478.00;822.00]	0.512	0.805
CD28 expression in EM CD8+ (MFI)	8	519.00 [349.00;677.50]	12	586.00 [354.00;722.50]	0.671	0.886
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	34.13 [19.64;44.19]	12	26.96 [15.76;32.24	0.375	0.805
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	1467.00 [1367.00;1636.50]	12	1347.50 [1279.50;1682.50]	0.512	0.805
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	849.50 [711.50;965.50]	12	906.50 [658.50;1036.50]	0.939	1.000
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.96 [3.76;8.90]	12	1.96 [1.65;4.51]	***0.041***	0.337
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	23.53 [15.96;47.75]	12	24.14 [14.47;32.75]	0.563	0.844
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	28.56 [23.77;37.50]	12	49.03 [35.57;54.79]	***0.010***	0.201
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.96 [37.21;53.60]	12	49.91 [21.78;54.52]	0.969	1.000
TEMRA CD8+(% of CD8+ T-cells)	8	27.24 [14.57;32.13]	12	18.43 [7.98;30.10]	0.335	0.789
CD27 expression in TEMRA CD8+ (MFI)	8	234.00 [174.50;672.00]	12	318.50 [238.50;469.50]	0.463	0.805
CD28 expression in TEMRA CD8+ (MFI)	8	95.00 [51.00;171.50]	12	188.00 [113.00;330.00]	0.154	0.633
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	3.04 [2.48;10.97]	12	6.09 [1.99;10.27]	0.969	1.000
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	1558.00 [1459.00;1702.50]	12	1649.50 [1287.50;1852.50]	0.729	0.925
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	677.50 [632.50;797.50]	12	781.50 [712.50;904.50]	0.263	0.789
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	4.92 [1.29;5.52]	12	3.28 [1.42;4.08]	0.512	0.805
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	82.27 [62.76;86.50]	12	66.02 [50.96;78.16]	0.263	0.789
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	76.42 [62.60;82.14]	12	67.67 [46.39;78.59]	0.335	0.789
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	8	4.90 [3.90;6.14]	12	3.97 [3.34;5.32]	0.418	0.563
CD25 expression in Total Treg (MFI)	8	2638.00 [2015.00;3325.00]	12	1535.50 [1124.50;2303.00]	***0.041***	0.151
CTLA-4 expression in Total Treg (MFI)	8	264.00 [217.50;326.00]	12	292.50 [258.00;314.00]	0.616	0.616
Naïve Treg (% of total Tregs)	8	8.28 [4.24;15.33]	12	11.08 [7.28;23.51]	0.512	0.563
CD25 expression of Naïve Treg (MFI)	8	2503.00 [1761.50;2730.50]	12	1773.50 [1169.50;2216.50]	0.059	0.151
CTLA-4 expression of Naïve Treg (MFI)	8	204.50 [173.50;279.00]	12	292.50 [242.50;313.50]	0.083	0.151

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	17.37 [11.25;29.65]	12	30.24 [24.04;32.85]	0.083	0.151
Memory Treg (% of total Tregs)	8	91.09 [84.16;94.57]	12	88.49 [76.07;92.20]	0.512	0.563
CD25 expression of Memory Treg (MFI)	8	2610.00 [1972.00;3329.00]	12	1555.00 [1173.00;2373.50]	0.059	0.151
CTLA-4 expression of Memory Treg (MFI)	8	248.00 [205.00;293.50]	12	287.50 [255.50;331.50]	0.177	0.278
Memory Treg CTLA-4+ (% of Memory Tregs)	8	14.28 [6.42;21.34]	12	19.98 [18.26;24.46]	0.070	0.151
PBMC – T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	8	2.14 [1.32;2.78]	12	1.12 [0.84;3.21]	0.354	0.607
PD1 expression in CD4+ (MFI)	8	220.50 [180.00;289.50]	12	165.50 [120.50;236.00]	0.298	0.595
CD4+ CD69+ (% of CD4+ T-cells)	8	29.77 [24.87;40.96]	12	27.01 [16.96;37.41]	0.263	0.595
CD69 expression in CD4+ (MFI)	8	266.00 [243.00;289.50]	12	245.50 [216.00;305.50]	0.643	0.848
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.28 [0.90;1.47]	12	0.66 [0.37;2.15]	0.298	0.595
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	69.58 [58.10;74.39]	12	72.24 [61.23;82.71]	0.263	0.595
CD4+ HLADR+ (% of CD4+ T-cells)	8	12.19 [3.66;20.04]	12	17.99 [11.79;22.56]	0.232	0.595
HLADR expression in CD4+ (MFI)	8	416.50 [204.00;501.50]	12	442.50 [380.00;522.00]	0.418	0.669
CD4+ CD38+ (% of CD4+ T-cells)	8	34.62 [26.71;37.29]	12	34.99 [22.85;46.95]	0.847	0.968
CD38 expression in CD4+ (MFI)	8	622.00 [547.00;795.50]	12	686.00 [481.00;780.00]	0.908	0.969
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	3.91 [1.40;4.98]	12	4.04 [2.57;7.23]	0.335	0.607
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	54.98 [50.48;60.05]	12	46.41 [38.73;59.53]	0.464	0.695
CD8+ PD1+ (% of CD8+ T-cells)	8	26.38 [23.81;40.81]	12	15.11 [8.96;32.54]	***0.049***	0.590
PD1 expression in CD8+ (MFI)	8	310.50 [194.00;494.00]	12	292.50 [233.00;361.00]	0.671	0.848
CD8+ CD69+ (% of CD8+ T-cells)	8	31.47 [18.73;50.64]	12	37.83 [24.52;41.97]	0.969	0.969
CD69 expression in CD8+ (MFI)	8	565.00 [470.50;774.50]	12	391.00 [292.00;541.50]	***0.037***	0.590
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.39 [7.91;18.08]	12	7.28 [4.22;16.06]	0.298	0.595
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	41.03 [36.80;57.28]	12	52.81 [42.74;67.57]	0.203	0.595
CD8+ HLADR+ (% of CD8+ T-cells)	8	38.80 [9.82;46.05]	12	45.47 [36.28;60.72]	0.232	0.595
HLADR expression in CD8+ (MFI)	8	497.00 [284.00;540.50]	12	535.50 [439.00;806.00]	0.203	0.595
CD8+ CD38+ (% of CD8+ T-cells)	8	11.40 [8.02;22.77]	12	13.74 [6.99;29.79]	0.729	0.874
CD38 expression in CD8+ (MFI)	8	274.00 [227.50;403.00]	12	282.50 [180.00;456.50]	0.969	0.969
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	5.59 [2.36;11.15]	12	6.96 [4.14;11.61]	0.512	0.723
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	54.58 [45.98;78.05]	12	45.36 [34.90;51.94]	0.097	0.595
Plasma - Essential Immune Response						
IL-4 (pg/mL)	8	6.56 [0.31;17.75]	12	0.00 [0.00;3.83]	0.080	0.330
IL-2 (pg/mL)	8	2.35 [0.68;16.71]	12	0.95 [0.00;4.18]	0.260	0.510
CXCL-10 (IP-10) (pg/mL)	8	394.51 [100.25;495.51]	12	370.47 [189.67;459.55]	0.616	0.824
IL-1 beta (pg/mL)	8	5.96 [4.57;32.43]	12	3.02 [0.75;9.23]	0.083	0.330
TNF alpha (pg/mL)	8	1.14 [0.63;2.74]	12	2.43 [1.38;3.60]	0.143	0.428
CCL2 (MCP-1) (pg/mL)	8	202.58 [168.50;296.80]	12	165.28 [140.36;248.67]	0.298	0.510

Continuation Table:

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
IL-17A (pg/mL)	8	3.36 [1.45;11.39]	12	2.31 [1.36;5.32]	0.729	0.824
IL-6 (pg/mL)	8	5.80 [2.63;10.56]	12	6.16 [3.75;10.83]	0.729	0.824
IL-10 (pg/mL)	8	0.54 [0.00;1.69]	12	0.70 [0.09;1.68]	0.756	0.824
IFN gamma (pg/mL)	8	3.67 [1.90;7.15]	12	1.94 [0.91;4.83]	0.297	0.510
IL-12p70 (pg/mL)	8	12.22 [5.56;45.39]	12	2.75 [1.30;5.44]	***0.015***	0.181
CXCL-8 (IL-8) (pg/mL)	8	5.67 [2.23;22.07]	12	5.28 [3.78;9.94]	1.000	1.000
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	1132.10 [955.26;1562.20]	12	1689.80 [1086.20;2303.20]	0.263	0.632
4-1BB (pg/mL)	8	56.21 [38.70;88.13]	12	72.05 [51.50;143.09]	0.263	0.632
sCD27 (pg/mL)	8	53535.00 [49080.00;62195.00]	12	62981.00 [49390.00;73961.00]	0.616	0.732
B7.2 (CD86) (pg/mL)	8	411.43 [320.29;609.87]	12	360.66 [300.49;565.35]	0.787	0.787
Free Active TGF-B1 (pg/mL)	8	13.43 [7.00;28.59]	12	25.66 [11.16;41.20]	0.263	0.632
CTLA-4 (pg/mL)	8	7.36 [4.35;10.07]	12	8.88 [1.04;23.83]	0.616	0.732
PD-L1 (pg/mL)	8	265.37 [151.94;325.49]	12	187.01 [149.03;223.63]	0.512	0.732
PD-L2 (pg/mL)	8	12081.00 [11067.00;16080.00]	12	14498.00 [12619.00;16398.00]	0.512	0.732
PD-1 (pg/mL)	8	66.15 [19.51;101.02]	12	24.74 [14.23;95.23]	0.512	0.732
Tim-3 (pg/mL)	8	18190.00 [8331.30;22119.00]	12	29643.00 [16492.00;37149.00]	0.133	0.632
LAG-3 (pg/mL)	8	3388.50 [2008.30;18530.00]	12	2042.20 [1398.00;4275.60]	0.154	0.632
Gal-9 (pg/mL)	8	113572.00 [87969.00;171239.00]	12	118096.00 [97476.00;167533.00]	0.671	0.732