Title

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Journal

Human Immunology, 79(9)

ISSN

0198-8859

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Publication Date

2018-09-01

DOI

10.1016/j.humimm.2018.06.006

Peer reviewed

HHS Public Access

Author manuscript

Hum Immunol. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Hum Immunol. 2018 September ; 79(9): 659–667. doi:10.1016/j.humimm.2018.06.006.

Increased T Cell Immunosenescence and Accelerated Maturation Phenotypes in Older Kidney Transplant Recipients

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Abstract

Older kidney transplant recipients experience increased rates of infection and death, and less rejection, compared with younger patients. However, little is known about immune dysfunction in older compared with younger kidney transplant recipients and whether it is associated with infection. We evaluated T cell phenotypes including maturation, immune senescence, and exhaustion in a novel investigation into differences in older compared with younger patients receiving identical immune suppression regimens.

We evaluated PBMC from 60 kidney transplant recipients (23 older and 37 matched younger patients) by multiparameter immune phenotyping. Older kidney transplant recipients demonstrated decreased frequency of naïve CD4+ and CD8+ T cells, and increased frequency of terminally differentiated, immune senescent, and NK T cells expressing KLRG1. There was a trend towards increased frequency of T cell immune senescence in patients experiencing infection in the first year after transplantation, which reached statistical significance in a multivariate analysis.

This pilot study reveals immune dysfunction in older compared with younger transplant recipients, and suggests a likely mechanism for increased vulnerability to infection. The ability to assess T cell maturation and immune senescence in transplant recipients offers the potential for risk stratification and customization of immune suppression to prevent infection and rejection after transplantation.

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Keywords

T cell; immunosenescence; kidney transplantation; elderly; infection

1. Introduction

The numbers of older transplant recipients are growing, fueled by the aging population with more patients surviving longer with chronic diseases. In 2004, more than 20% of patients with end stage renal disease were older than 75 years, as compared with 7.6% in 1980 [1]. The percentage of solid organ recipients older than 65 has increased more than four-fold between 1988 and 2012, with 3315 patients over age 65 undergoing kidney transplantation in 2014 alone [1,2], and transplantation is considered the treatment of choice for end organ disease not amenable to medical therapy in both younger and older patients [3].

The clinical observation that older patients experience more infection than younger patients implies that immune senescence worsens outcomes in older transplant patients [4–6]. Invasive infections experienced by kidney transplant recipients include bacteremia, bacterial and fungal pneumonia, infections due to human herpes viruses, and community-acquired viral infections [7,8]. The impaired immunity and vulnerability to infection in older patients is likely exacerbated by the administration of immune suppressive medications to prevent rejection [9–11]. Older and younger transplant patients receive immunosuppressive medications dosed with similar goal drug levels in current clinical practice [3,12]. This ‘one size fits all’ approach may result in over-immune suppression in older patients [13–15]. Results from animal models of transplantation suggest that similar levels of calcineurin inhibitors exert greater immune suppression in older transplant recipients [16]. Little is known about optimal immune suppression regimens in older patients as the utility of these regimens has been established in clinical trials for which older patients are typically excluded. Simply administering lower amounts of drug in older patients, however, has not been effective, resulting in increased rates of rejection [14,17], suggesting that there is a need for a tool that measures the degree of immune dysfunction to guide dosing of immune suppression in older patients.

Age-associated immune dysfunction affects the adaptive immune system, with a consequent impaired ability to respond to pathogens and tumor cells, as well as impaired response to vaccination in elderly patients with immune senescence [18–20]. It is hypothesized that recurrent antigen exposure from acute and chronic infections including CMV, in concert with thymic involution, drives the immune dysfunction of aging, leading to impaired response to vaccination and increased susceptibility to infection [21–23]. Immune senescence affects all immune compartments, with the most striking changes seen in the CD4+ and CD8+ T cell lineage [20,24]. Chronic viral infections including CMV and HIV are associated with persistent inflammation and antigen exposure, and have been shown to accelerate the aging process [25–27]. A similar mechanism may be at work in the setting of transplantation with chronic exposure to allo-antigens as well as reactivation of CMV [28]. However, few studies have directly examined age-associated immunologic changes including T cell maturation and immune senescence in the older solid organ transplant

recipient. Given that both patient age and CMV serotype are known to influence immunologic aging, we evaluated in this study which patient characteristic had greater influence in the setting of immune suppression. Our previous studies have examined changes in the innate immune system and the existence of a pro-inflammatory cytokine profile in older compared with younger kidney transplant recipients [29]. In this study, we investigate the T cell phenotype of renal transplant recipients in relation to age, as well as to the adverse clinical outcome of infection. By defining a predictive profile of immune dysfunction, we present here a pilot study to investigate whether T cell changes can elucidate the mechanism of increased vulnerability to infection in the older transplant recipient,

2. Materials and Methods

2.1 Patients and samples

We enrolled kidney transplant recipients in the first month after transplantation at Ronald Reagan Medical Center. The UCLA Institutional Review Board approved this observational study. All patients signed informed consent. Blood was collected for peripheral blood mononuclear cell (PBMC) isolation at 3 months after transplantation during outpatient clinic visits. Using a similar patient cohort as reported previously [29], we identified 23 older patients over age 60 who had PBMC available for analysis; these were analyzed in comparison with 37 patients between the ages of 30 and 51, with a matched proportion of deceased versus living donor and ATG versus basiliximab induction therapy, for a total cohort of 60 patients. No patient was experiencing a significant infection or rejection episode at the time of PBMC collection. PBMC were isolated using previously published techniques [30,31], and frozen for storage until batched analysis could be performed.

Induction immunosuppression with antithymocyte globulin (ATG) was used for patients at increased risk for rejection (panel-reactive antibodies >20%, history of donor specific antibodies, positive crossmatch, cold ischemia time > 24 hours, or donation after cardiac death); patients not meeting these criteria received basiliximab. Maintenance immunosuppression was tacrolimus, mycophenolate mofetil (MMF), and prednisone. Mycophenolate mofetil and prednisone doses were similar in each patient, and tacrolimus was started at equivalent doses per kg body weight with similar target drug levels in each patient following the UCLA protocol. Patients received valganciclovir prophylaxis for cytomegalovirus for 6 months for high risk (donor positive, recipient negative) and 3 months for low risk (recipient positive) patients. Cotrimoxazole sulfate was administered for the first year after transplantation.

2.2 Flow cytometry

To discriminate between live and dead cells, a Fixable Aqua Dead Cell Stain (Invitrogen) that fluoresces in reaction to cellular amines was used to identify intact and alive lymphocytes (Supplemental Figure).

T cell maturation was assessed using a cocktail of fluorochrome-conjugated antibodies against CD3, CD4, CD8, CCR7, CD45RA to determine maturation phenotype. Naïve cells were defined as CCR7+/CD45RA+; effector memory as CCR7-/CD45RA-; and terminally

differentiated as CCR7-/CD45RA+ (antibodies obtained from BD Biosciences or Biolegend) (Supplemental Figure). In addition, patients were analyzed for exhaustion, senescence, and activation of T cells using KLRG1, CD57, CD38, CD28, and PD-1 [32,33]. T regulatory cells were defined as CD4+ CD25+CD127-, and were further subsetted by maturation subtype as above. Immunophenotyping of NK T cells was performed using fluorochrome-conjugated antibodies against CD56, CD3, CD4, CD8, and KLRG1. 10 patients (5 younger and 5 older) did not undergo analysis using both the CD57 and CD28 markers. A median of 89,000 events were analyzed for each patient sample, with a minimum number of events 15,000, Fluorescence from viable cells was measured by the BD LSRFortessa (BD Biosciences) with FCS Express software (DeNovo Software), for analysis.

2.3 Clinical data collection

Data was collected via review of the clinical record on immunosuppression induction type, living versus deceased donor, dialysis receipt and time on dialysis, pre-transplant diagnosis of diabetes mellitus, CMV antibody status, history of rejection using Banff criteria, and history of infection or CMV viremia using standard definitions, and death in the first year after transplantation [34–36]. Severe infection was defined as requiring intravenous antibiotic treatment and/or leading to extension of hospital stay or death.

2.4 Statistical analysis

For comparing the groups of interest, we first performed single variable analysis where we used the Mann-Whitney U-Test for continuous variables and the Fisher's exact test for categorical variables. In addition to the single variable analysis, we performed multivariable analysis on the immune cell proportions to adjust for possible confounders. Specifically, we used linear regression models and adjusted for factors including induction, donor type (deceased v. living) and CMV antibody status. In order to control for the False Discovery Rate (FDR), we calculated the FDR-adjusted p-values and used a threshold of 0.1 to indicate statistical significance, ensuring an FDR of 10% or less. Complete case analysis was used throughout. Finally, as an exploratory analysis, we used the Principal component analysis (PCA) on the markers that were available for all the subjects. We picked the PCs that were significantly associated with increased patient age (PC1, PC3), and visualized our data using these two PCs (Figure 3A, 3B). The first three PCs are shown in Table 5. Heat map was created using complete linkage and Euclidian distance. Statistical analysis was performed using JMP Pro 11 (SAS Software) and R software, v. 3.3.2 (http://www.r-project.org/).

3. Results

3.1 Patient characteristics in the older and younger cohorts

Twenty-two patients over age 60 who had undergone kidney transplantation at our center were matched based on transplant type (living versus deceased) and induction (ATG versus basiliximab) with 38 younger patients, ages 30 to 51 (Table 1). These older and younger patients had similar proportions in terms of sex, race, and ethnicity. There was a trend towards increased frequency of pre-transplant diabetes mellitus and receipt of dialysis in the older patient group, but these did not reach statistical significance. There were also no

statistically significant differences in immunosuppression regimen, doses, or drug levels (Table 1).

3. 2 Patient outcomes after transplantation by age group

Following clinical outcomes for the first year after transplant, younger patients experienced 6 episodes of rejection (16% incidence), ACR (n=5) and AMR (n=1), and older patients experienced only 2 episodes of rejection (8.7% incidence), both ACR, however, this difference was not statistically significant (Table 2). Frequency of BK viremia was similar between both groups. Although the number of CMV antibody positive and high risk (donor positive/recipient negative) patients was similar in both groups (Table 1), there was a trend towards increased incidence of CMV viremia in the older patients amongst patients at risk for CMV (p=0.091) (Table 2). The incidence of invasive infections was similar between groups, with 6 episodes (excluding urinary tract infection) (17%) in the younger patients, including osteomyelitis, bacterial sepsis, thrush, and viral gastroenteritis as compared with 4 episodes in the older patients (16%), which included bacterial pneumonia, pulmonary tuberculosis, *Clostridium difficile* associated bacteremia, and CMV colitis. Median time to invasive infection was 117 days for the younger patients and 154 days for the older patients, with no statistically significant difference by age, with the majority (>70%) occurring after the 3 month immunologic assessment. Analysis of a combined endpoint of invasive infection or CMV viremia over 250 IU/ml also revealed similar incidence in both patient groups, although there was a trend towards increased incidence in the older patients (Table 2). Of significant note, three of the four older patients experienced both CMV viremia and invasive infection, while none of the six younger patients with invasive infection demonstrated this pattern. These older patients all received basiliximab induction, and two were CMV antibody positive and one was donor CMV antibody positive, CMV antibody negative. One of the older patients, aged 60, died due to a likely cardiac arrest at day 292 after transplantation.

3.3 T cell phenotype and patient age

We analyzed T cell phenotype at three months post-transplant because this was the earliest time point at which there was sufficient numbers of cells for analysis in patients induced with ATG. There was no difference in the frequency of CD8+ and CD4+ T cells by patient age (Table 3). Older kidney transplant recipients had fewer naïve CD8+ T cells compared with younger patients, with a median frequency of 13% compared with 38% (p<0.001) (Figure 1A and Table 3). Older patients displayed an increased frequency of effector memory CD8+ T cells, with a median frequency of 32% compared with 19% (p=0.004) as well as terminally differentiated effector memory cells (TMRA) CD8+ T cells, cells with a median frequency of 44% as compared with 26% in younger patients (p=0.007) (Figure 1A and Table 3). Similarly, for CD4+ T cells, older patients had a decreased frequency of naïve T cells, with a median frequency of 16% compared with 37% for younger patients (p<0.001). Older patients also displayed an increased frequency of effector memory CD4+ T cells, with a median frequency of 39% compared with 22% in the younger patients (p=0.005) (Figure 1B and Table 3). However, there was no significant difference in the frequency of CD4+ TMRA T cells. There was no significant difference of CD4+/CD8+ ratio by patient age (p=0.513).

We also observed an increased frequency of senescent KLRG1+ CD4+ T cells in older (14%) compared with younger (8%) patients (p=0.007), as well as KLRG1+ CD8+ T cells (older 64% and younger 37%, p<0.001) (Figure 1C and Table 3). KLRG1+ NK T cells demonstrated a trend towards increased frequency in older (86%) compared with younger patients (71%) (p= 0.027). Subsetting CD8+ cells by CD57 and loss of CD28 as an alternate marker of senescence demonstrated increased frequency in older patients, with a median frequency of 32% in the older and 22% in the younger patients (p=0.036) (Figure 1D and Table 3). Similarly, there was a trend towards increased frequency of senescent CD8+CD57+KLRG1+ T cells in the older patients (26% compared with 16% in younger patients, p=0.064). There was no significant difference in the frequency of T regulatory cells or exhausted (PD-1+) CD4+ or CD8+ T cells. As indicated with an asterisk, for all of the associations described, FDR was <0.10, confirming statistical significance despite multiple testing performed (Table 3)

Multivariate analysis including both patient age and immunosuppression induction revealed similar findings, with patient age the dominant variable impacting immunologic parameters: For all CD8+ maturation phenotypes, patient age remained statistically significant while induction type was not significant in a multivariate model (CD8+ naïve p<0.001, effector memory p=0.003, and TMRA p=0.008 when corrected for induction type). CD4+ naïve T cells was the exception to this, with p=<0.001 for recipient age and p=0.007 in the multivariate analysis. However, for KLRG1+ CD8+, CD8+CD57+CD28-, and CD8+CD57+KLRG1+ T cells we observed a similar pattern of patient age remaining statistically significant and induction immunosuppression non-significant in multivariate analysis (p<0.001, p=0.008, and p=0.017 for patient age for the cell types listed above, respectively, and induction type not statistically significant). This reinforces our hypothesis that patient age is the most important predictor of immune phenotype.

3.3.1 Interaction between patient age and CMV antibody status—Given that both patient age and CMV antibody status are known to influence immunologic aging, we performed an additional multivariate analysis correcting for CMV antibody status as well as donor type (living versus deceased) and induction type (ATG versus basiliximab). The frequency of CD8+ naïve, effector memory, and TMRA T cells as well as CD4+ naïve and effector memory T cells remained significantly associated with age in the multivariable analysis (Table 3). This was also true for senescent phenotypes KLRG1+CD8+, KLRG1+CD4+ and CD8+CD57+CD28- T cells. Interestingly CD8+CD57+ and CD8+CD57+KLRG1+, which just missed statistical significance in the single variable analysis, were significant in the multivariable analysis (Table 3). As indicated with an asterisk, for all of the associations described, FDR was <0.10, confirming statistical significance with correction for multiple comparisons performed. This analysis reveals that age exerts a significant influence on immune phenotype for maturation and senescence regardless of CMV antibody status. In addition, these findings are independent of donor status and induction type.

3.3.2 T cell phenotype and clinical outcome of infection—Frequency of effector memory and TMRA CD8+ T cells trended higher in patients who experienced infection

(either an invasive infection or CMV viremia) in the first year after transplantation, but these findings did not reach statistical significance (Table 4). We also observed a similar trend towards increased frequency of CD8+CD57+ KLRG1+, with 32% frequency in patients with infection and 16% in those without, but this also did not reach statistical significance (p=0.063). There was also a trend towards increased frequency of senescent CD8+CD57+CD28- T cells, with a median frequency of 36% in those with infection as compared with 20% in those without (p=0.007) (Figure 2), but in the single variable analysis this analysis had an FDR>0.10 (Table 4). There was also a trend towards increased frequency of exhausted PD-1+ CD8+ T cells, with a median frequency of 11% in those with infection as compared with 5% in those without (p=0.048) (Figure 2). There was no difference in the frequency of T regulatory cells (Table 4). No associations were seen between acute cellular or antibody mediated rejection and immune phenotype (data not shown).

Under the multivariable analysis including CMV antibody status, age, living v. deceased donor, and induction type the senescent CD8+CD57+CD28- T cells was significantly associated with infection (p=0.002), with a FDR<0.10.

3.3.3 Simultaneous analysis of T cell phenotypes—Principal component (PC) analysis was performed for three iterations (PC1, 2, and 3) on the immune phenotype markers performed for every patient to integrate analysis of all T cell markers simultaneously (Table 5). PCA coefficients varied between −0.4 to 0.4, with similar coefficients indicating correlation between variables. PC1 and PC3, but not PC2, were associated with patient age. These PC were able to generate a separation of patients by age, with older patients clustering on the upper left (in blue) and younger patients (in pink) on the lower right of the graph (Figure 3A). Boxplot analyses also demonstrated a significant separation by patient age for both PC1 (p=0.006) and PC3 (p=0.001) (Figure 3B).

Similarly, unsupervised clustering of T cell phenotypes revealed a natural grouping of a subset of younger and older patients (Figure 4). Younger patients were clustered towards the top of heat map with increased frequency of both naïve CD8+ and CD4+ T cells, while older patients clustered towards the bottom of the heat map with increased frequency of KLRG1+, CD57+, and CD57+CD28- CD8+ and CD4+ T cells.

4. Discussion

In this study, we present one of the first investigations of the immune phenotype of renal transplant recipients in relation to age, as well as the adverse clinical outcome of infection, in the first year after transplantation. A difference in maturation state, immune senescence, and exhaustion has been previously described in older compared with younger healthy individuals.[20,21] However, this report represents, to our knowledge, the first investigation into these phenomena and their association with infectious complications in the immunosuppressed solid organ transplant population.

We observed a striking, and somewhat surprising, difference in immune phenotyping despite identical immunosuppression regimens with similar target tacrolimus levels, demonstrating that age-related immunologic differences are not abrogated by immunosuppression.

Notable differences observed in the older transplant recipients included a decrease in the frequency of naïve CD4+ and CD8+ T cells, which may explain the mechanism behind older patients' impaired immune response to new pathogens and vaccination as a result of the limited repertoire of naïve T cells available for activation and differentiation. Older patients also demonstrated an increase in the frequency of EM, TMRA, and senescent CD8+ T cells, which suggests that impaired ability of memory T cells to activate may lead to amnestic responses to a previously seen antigen. These findings were confirmed by applying a strict FDR threshold of <0.1 for statistical significance to correct for multiple testing (Table 3). In addition, multiple variable analysis correcting for potential confounders of the relationship between age and immune phenotype including CMV antibody status, donor type, and induction did not abrogate the associations observed between T cell maturation and senescence and patient age. Principal component analysis further underlines the differences between older and younger patients despite similar immune suppression regimens (Figure 3A and 3B).

Comparing these findings to previously published data in community-dwelling non-transplant recipients reveals differences in T cell maturation phenotype: Older kidney transplant recipients described here demonstrated a frequency of 13% naïve CD8+ T cells and 16% naïve CD4+ T cells, as compared with approximately 20% and 40%, respectively, in non-transplant patients of similar age [37]. One published study detailing the T cell maturation phenotypes in patients with end stage renal disease described naïve CD8+ and CD4+ frequencies more similar to non-transplant patients than the kidney transplant recipients described in our study [38]. In addition, the frequency of effector memory T cells was higher in transplant recipients reported here (32% for CD8+ T cells and 40% for CD4+) compared with non-transplant patients (approximately 20% for CD8+ and 30% for CD4+) [37], and patients with end stage renal disease were again more similar to non-transplant patients [38].

Another novel observation from this investigation is the possible link between immune dysfunction, specifically immune senescence and exhaustion, and infectious complications in transplant recipients. This suggests a mechanism for vulnerability to infection in patients on immune suppression, and possibly a negative impact of infection on the immune system. Development of these markers of immune dysfunction into tools to assess patient at risk, as well as guide adjustment in immune suppression medication, would represent a new approach for prevention or treatment infection. The burden of immune suppression on older patients may be further accentuated by differences in drug metabolism [6,16]. The three-month time point for immunologic assessment appears to have been an informative time point given the association seen between subsequent infection and immune phenotype.

Limitations of this study include the relative heterogeneity within the study cohort, although this issue is mitigated by the fact that older and younger patients received identical protocolized regimens in immune suppression dosing and patient care, as well as the cohort

matching based on donor type and induction. Extension to a larger cohort size would permit accrual of additional clinical outcomes of infection and death. The observation that age was not significantly associated with infection in this cohort suggests the limitations in analysis of low frequency events in a single center study.

Future studies will include analysis of pre transplant immune phenotype as well as longitudinal studies before and after transplant, and gene expression and DNA methylation analysis to determine mechanism of developing different immunologic states. These analyses will determine whether differences in immune phenotype prior to transplantation are predictive of clinical outcomes after transplant as well as whether there is a significant change in degree of immune dysfunction with the initiation of immune suppression. The inclusion of a larger patient cohort to serve as a validation dataset would permit confirmation of our findings as well as further adjustment for potential confounders. In addition, longer term follow-up of this patient cohort may reveal an association between T cell dysfunction phenotypes and development of malignancies and death.

Understanding the association between age and immune function sheds light into the mechanism of increased vulnerability to infection in older transplant recipients. These studies may also reveal the mechanism behind poor response to vaccination in transplant recipients [13]. Despite a large body of research on immune phenotypes including maturation, senescence, and exhaustion in healthy older individuals, we are just beginning to understand how these aspects of T cell immune dysfunction differ after solid organ transplantation in older as compared with younger individuals, and how T cell dysfunction might relate to a pro-inflammatory state in the innate immune system [13,29].

Developing methods for patient monitoring and measurement of levels of immune dysfunction may allow for prevention of both infection and rejection in older transplant recipients, and should make transplantation safer for the growing numbers of older patients with end stage organ disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Institutes of Health [R03AG050946], the IDSA ERF and NFID Young Investigator Award, the Older Americans Independence Center Career Development Award, and the NIH National Center for Advancing Translational Science (NCATS) [UL1TR001881].

Abbreviations:

PBMC	peripheral blood mononuclear cells
NK T cells	natural killer T cells
ATG	anti-thymocyte globulin
FDR	false discovery rate

IQR	interquartile range
TMRA	terminally differentiated RA+

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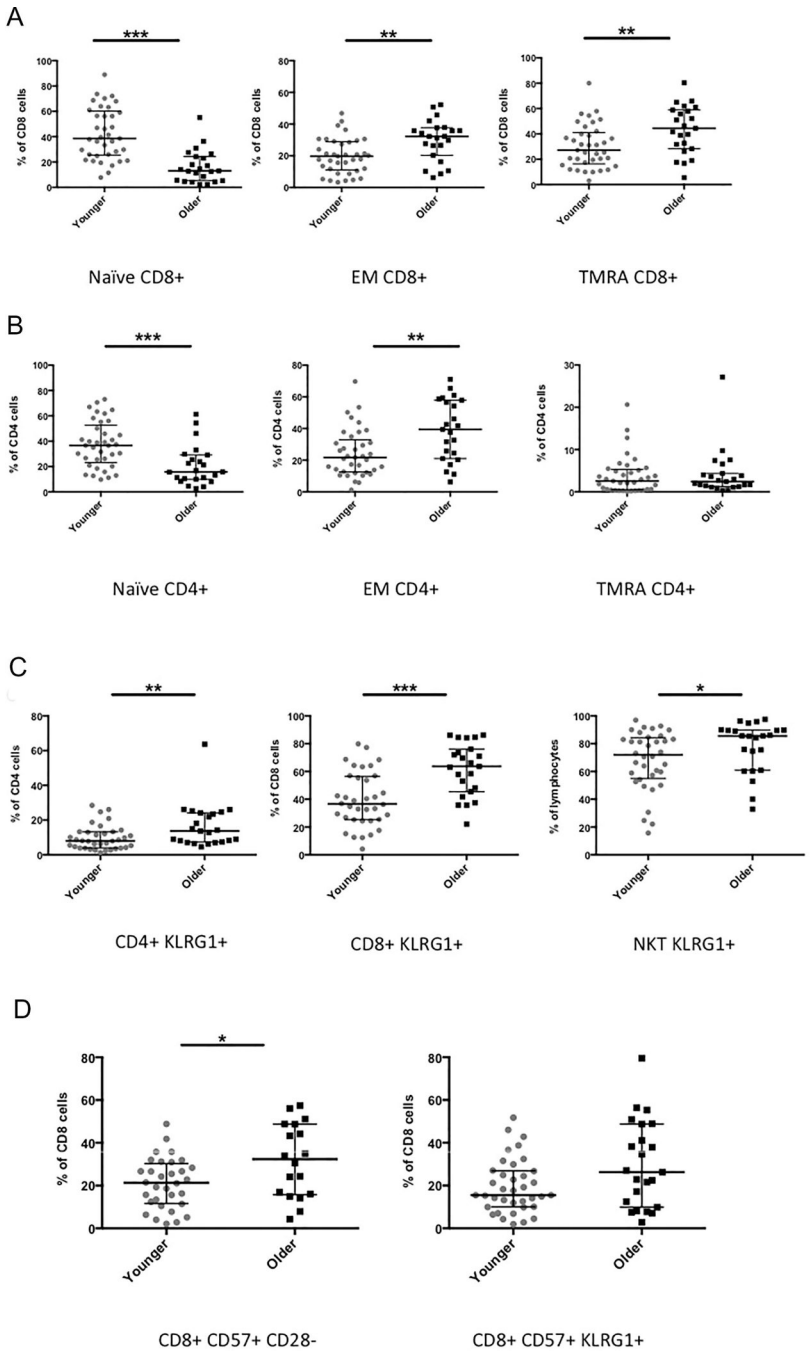

Figure 1:

Frequency of maturation subtypes by patient age. PBMC were analyzed for naive (CCR7+/CD45RA+), effector memory (EM) (CCR7-/CD45RA-), and terminally differentiated effector memory RA+ (TMRA) (CCR7-/CD45RA+) T cell content, or CD4+KLRG1+, CD8+ KLRG1+, and NKT KLRG1+ cells, or CD8+ CD57+CD28- and CD8+CD57+KLRG1+ T cells, as indicated, expressed as a percentage of the total number of CD8+ T cells or NKT cells Each dot corresponds to a sample; bars indicate median. *** indicates $p<0.001$, ** indicates $p<0.01$, by nonparametric test, and * indicates $p<0.05$ by

nonparametric test. **A**. CD8+ maturation subtypes by patient age. **B**. CD4+ maturation subtypes by patient age. **C**. Immune senescence as measured by KLRG1 expression by patient age. **D**. T cell activation and immune senescence as measured by CD57+ and CD28- and KLRG1+ by patient age.

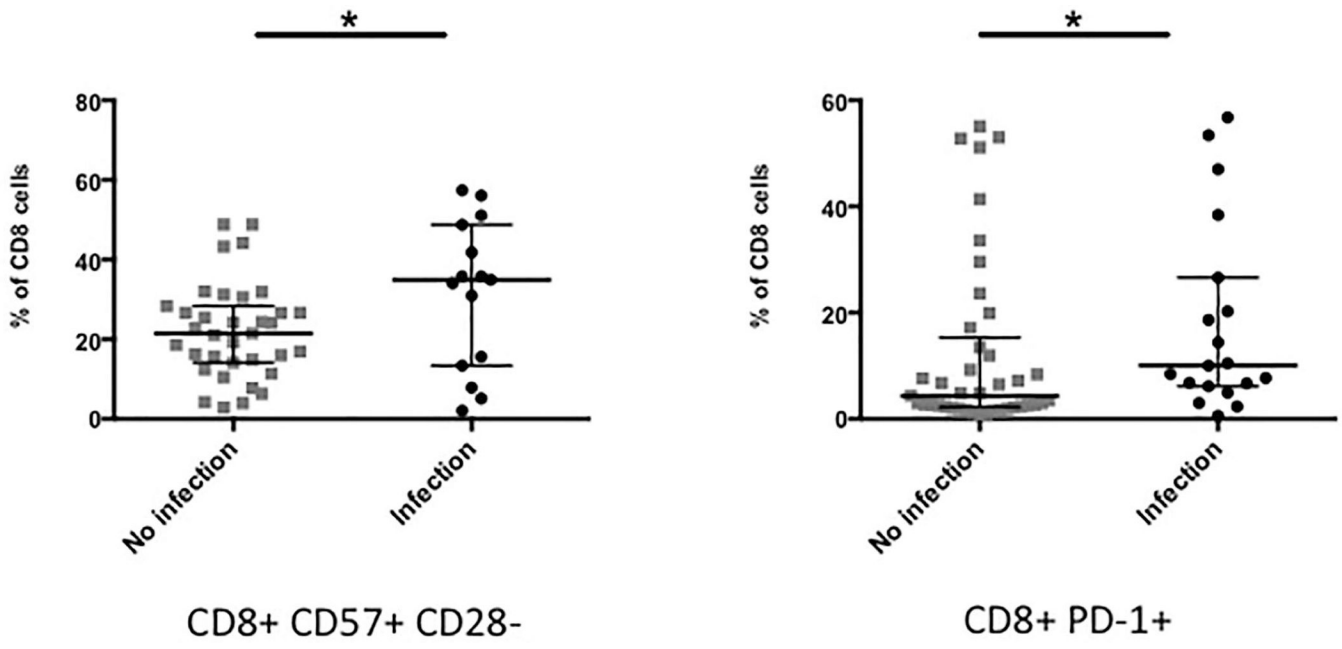

Figure 2:

Frequency of senescent and exhausted CD8+ T cells by post-transplant infection. PBMC were analyzed for senescent (CD57+CD28-) and exhausted PD-1+) CD8+ T cells, expressed as a percentage of the total number of CD8+ T cells. Each dot corresponds to a sample; bars indicate median. * indicates $p<0.05$ by nonparametric test.

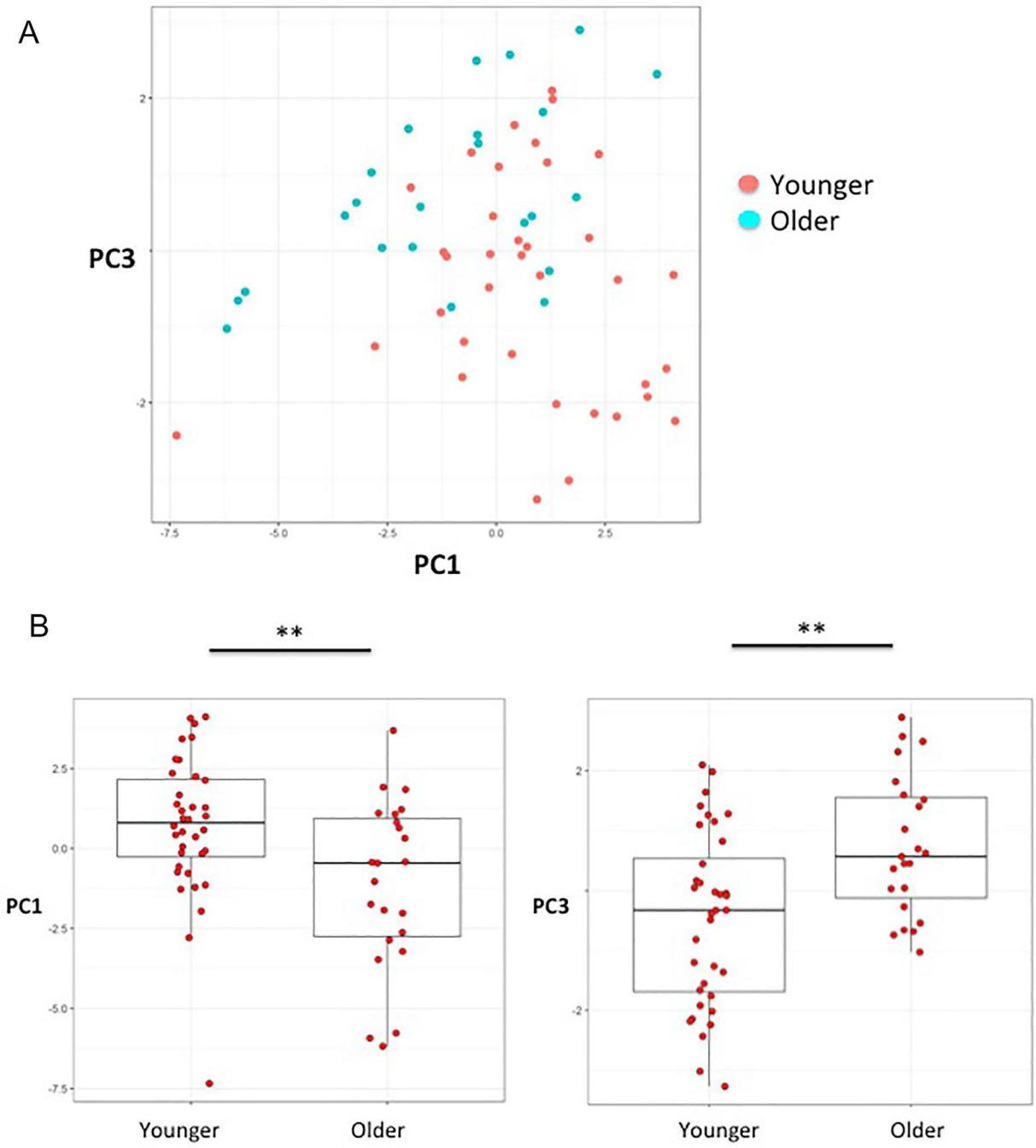

Figure 3:

Principal component (PC) analysis of T cell immune phenotypes by patient age. **A.** Scatter plot of older (blue dots) compared with younger (pink dots) graphed by two of the three PC analyses (PC1 and PC3) (Table 5). **B.** Boxplots of PC1 (left panel) and PC3 (right panel) in Older compared with Younger patients. ** indicates $p<0.01$ by t test,

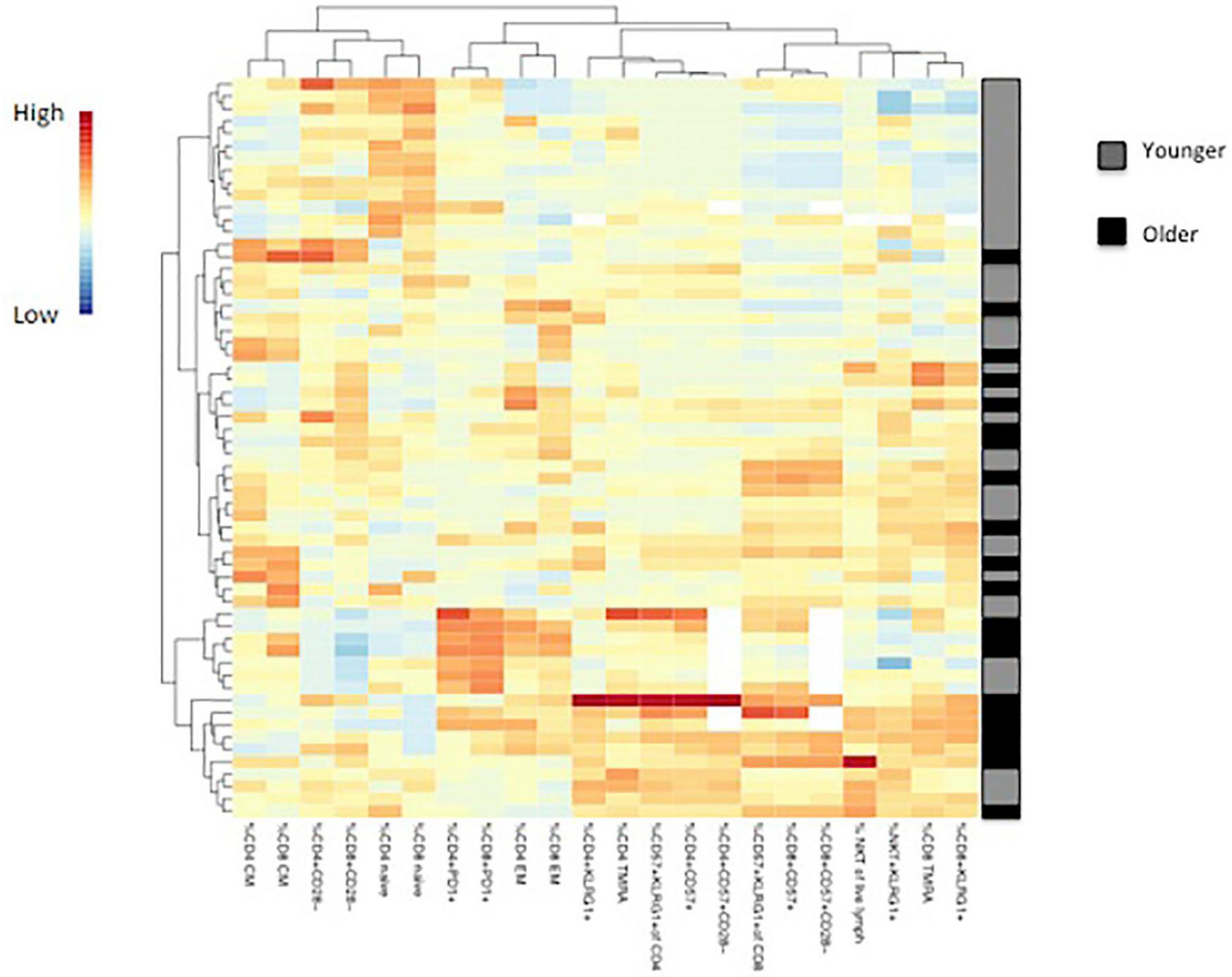

Figure 4:

Linkage heat map analysis of T cell subtype frequencies. Patient age is indicated on the right hand side of the diagram, black indicates older patients, and grey, younger patients. Increased frequency of T cell subsets is indicated in red to orange, and decreased frequency in dark and light blue.

Table 1.

Demographic and clinical characteristics and of older kidney transplant recipients and the younger cohort matched on transplant type and induction.

	Younger (<60) n=37	**Older (≥60) n=23**	
Median age (range)	43 (34–51)	67 (60–80)	N/A [*]
Male sex	60%	74%	0.282
White race	68%	65%	1.000
Hispanic	41%	35%	0.787
Dialysis	73%	91%	0.107
Diabetes pre-transplant	32%	57%	0.106
CMV Ab positive	70%	78%	0.561
CMV high risk[§]	14%	17%	0.722
Induction, ATG	30%	30%	1.000
Deceased donor	46%	44%	1.000
Tacrolimus Y/N[‡]	95%	83%	0.200
Tacrolimus trough (mean, SD)	9.7 (3.3)	10.1 (3.6)	0.806
MMF daily dose in g (mean, SD)[†]	1.4 (0.7)	1.2 (1.2)	0.395
Prednisone daily dose in mg (mean, SD)	5.3 (1.6)	5.6 (3.1)	0.901
Immune analysis, days post transplant (mean, SD)	83 (14.2)	90 (8.2)	0.099

[*] Not analyzed as groups defined based on age

[§] Defined as Donor CMV antibody positive, Recipient CMV antibody negative

[‡] Immunosuppression drugs and troughs assessed at the time of immune analysis. Of the 2 younger patients not on tacrolimus at 3 months, both were on cyclosporine, and of the 4 older patients not on tacrolimus at 3 months, 3 were on cyclosporine, and 1 was on sirolimus.

[†] One younger patient was on Myfortic at 3 months post transplant.

Table 2

Clinical outcomes of older kidney transplant recipients and the younger cohort matched on transplant type and induction during the first year after transplant.

Characteristic	Younger (<60) (n=38)	Older (≥60) (n=22)	p-value
Rejection (ACR or AMR)	16%	9%	0.698
BK viremia (any level)	22%	35%	0.369
CMV viremia (any level)	24%	48%	0.091
Invasive infection	17%	16%	1.000
Invasive infection or CMV viremia	24%	39%	0.257
Death	0	4%	

Table 3

Single variable analysis of association between indicated immune phenotyping markers by patient age (older compared with younger). Multivariate analysis adjusted for induction, donor type (deceased v. living), and CMV antibody status demonstrates the combined influence of patient age and CMV antibody status for each variable listed.

Immune phenotype*	Younger patients (n=36) Median (IQR)	Older patients (n=23) Median (IQR)	p-value, Single variable analysis	p-value, Multivariable analysis
CD8+ (of CD3)	33 (37–57)	29 (23–46)	0.792	0.626
CD4+ (of CD3)	50 (37–57)	45 (34–51)	0.171	0.191
CD8+ naive	38 (26–60)	13 (5–24)	**<0.001***	**<0.001***
CD8+ CM	7 (4–10)	5 (2–19)	0.816	0.168
CD8+ EM	19 (11–29)	32 (23–37)	**0.004***	**0.005***
CD8+ TMRA	26 (17–41)	44 (30–58)	**0.007***	**0.014***
CD4+ naive	37 (24–47)	16 (10–27)	**<0.001***	**0.001***
CD4+ CM	34 (22–45)	32 (22–46)	0.975	0.820
CD4+EM	22 (13–32)	39 (23–57)	**0.005***	**0.003***
CD4+ TMRA	3 (1–5)	2 (1–4)	0.975	0.954
KLRG1+CD8+	37 (25–56)	64 (47–75)	**<0.001***	**<0.001***
KLRG1+CD4+	8 (4–13)	14 (8–24)	**0.007***	**0.018***
NKT	3 (2–5)	4 (3–7)	0.063	0.151
KLRG1+NKT	71 (56 – 83)	86 (68 – 90)	**0.027***	0.087
CD8+CD57+	20 (12–31)	28 (14–48)	0.054	**0.014***
CD4+CD57+	3 (1–8)	9 (3–12)	**0.029***	0.056
CD8+CD28−	68 (54–78)	73 (55–85)	0.544	0.442
CD4+CD28−	34 (22–41)	32 (20–39)	0.652	0.528
CD8+CD57+CD28−	22 (12–30)	32 (16–48)	**0.036***	**0.011***
CD4+CD57+CD28−	2 (1–5)	6 (1–10)	0.051	0.073
CD8+CD57+KLRG1+	16 (10–27)	26 (11–45)	0.064	**0.025***
CD4+CD57+KLRG1+	2 (1–6)	7 (2–10)	**0.039***	0.087
CD4+ Treg	6 (4–12)	7 (4–11)	0.816	0.717

* Percentages are frequency of cell subtype out of CD3+, CD8+ or CD4+ T cells, or live lymphocytes for NKT cells. Data were summarized as Median (IQR). Abbreviations: CM, central memory; EM, effector memory; TMRA, terminally differentiated RA+ effector memory. Asterisks indicate comparisons with False Discovery Rate adjusted p-value <0.1.

Table 4

Single variable analysis of association between indicated immune phenotyping markers by infection in the first year. Multivariate analysis adjusted for patient age (old v. young), induction, donor type (deceased v. living), and CMV antibody status demonstrates the combined influence of patient age and CMV antibody status for each variable listed.

Immune phenotype*	No infection (n=42) Median (IQR)	Infection (n=17) Median (IQR)	p-value, Single variable analysis	p-value, Multivariable analysis
CD8+ (of CD3)	33 (24–41)	32 (21–36)	0.503	0.386
CD4+ (of CD3)	47 (35–56)	49 (37–60)	0.639	0.206
CD8+ naive	27 (17–54)	26 (5–36)	0.284	0.441
CD8+ CM	7 (4–13)	5 (2–9)	0.323	0.874
CD8+ EM	21 (11–32)	27 (16–32)	0.493	0.778
CD8+ TMRA	31 (19–44)	46 (27–54)	0.186	0.520
CD4+ naive	30 (14–44)	25 (16–37)	0.725	0.782
CD4+ CM	35 (27–45)	24 (18–45)	0.209	0.408
CD4+EM	24 (14–41)	35 (20–46)	0.284	0.775
CD4+ TMRA	2 (1–5)	3 (2–4)	0.124	0.357
KLRG1+CD8+	42 (33–64)	57 (36–72)	0.256	0.417
KLRG1+CD4+	9 (6–15)	9 (5–18)	0.639	0.383
NKT	3 (2–5)	3 (3–4)	0.585	0.679
KLRG1+NKT	81 (60 – 89)	76 (60 – 85)	0.831	0.549
CD8+CD57+	23 (13–28)	35 (18–48)	0.085	0.061
CD4+CD57+	4 (1–9)	6 (2–11)	0.173	0.162
CD8+CD28−	68 (55–79)	73 (55–85)	0.987	0.666
CD4+CD28−	34 (25–41)	28 (17–38)	0.203	0.389
CD8+CD57+CD28−	20 (13–27)	36 (31–49)	0.007	0.002*
CD4+CD57+CD28−	2 (1–7)	4 (1–10)	0.157	0.069
CD8+CD57+KLRG1+	16 (10–27)	32 (15–38)	0.063	0.037
CD4+CD57+KLRG1+	3 (1–8)	4 (2–9)	0.375	0.204
CD8+PD-1+	5 (1–13)	11 (6–27)	0.048	0.168
CD4+PD-1+	4 (2–12)	7 (6–31)	0.050	0.203
CD4+ Treg	6 (4–12)	7 (4–11)	0.816	0.717

*Markers were measured as percentages, which are frequency of cell subtype out of CD3+, CD8+ or CD4+ T cells, or live lymphocytes for NKT cells. Data were summarized as Median (IQR). Abbreviations: CM, central memory; EM, effector memory; TMRA, terminally differentiated RA+ effector memory. Asterisks indicate comparisons with False Discovery Rate adjusted p-value <0.1.

Table 5

Principle component (PC) analysis of association between indicated immune phenotyping markers by patient age (older compared with younger).

Immune phenotype	PC1	PC2	PC3
CD8+	−0.3	−0.1	0
CD4+	0.3	0.1	−0.1
CD8+ naive	0.3	−0.1	−0.3
CD8+ CM	0.1	−0.2	0.4
CD8+ EM	−0.2	−0.2	0.2
CD8+ TMRA	−0.2	0.3	0.1
CD4+ naive	0.2	0.1	−0.4
CD4+ CM	0.1	0	0.5
CD4+ EM	−0.3	−0.2	0
CD4+ TMRA	−0.3	0.2	−0.3
CD8+ CD57+	−0.2	0.3	0.1
CD4+ CD57+	−0.3	0.2	−0.1
CD8+ CD28-	0.1	0.4	0.1
CD4+ CD28-	0.1	0.3	0
CD8+ CD57+KLRG1+	−0.2	0.3	0.1
CD4+ CD57+KLRG1+	−0.3	0.2	−0.1
CD8+ PD1+	−0.2	−0.3	−0.2
CD4+ PD1+	−0.2	−0.3	−0.2
CD4+ Treg	−0.2	−0.2	0.1