Immunity & Ageing

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CD57$^+$ EMRA CD8$^+$ T cells in cancer patients over 70: associations with prior chemotherapy and response to anti-PD-1/PD-L1 therapy

Cécile Gonnin[1,2], Michelle Leemans[3], Florence Canoui-Poitrine[3,4], Morgane Lebraud[2], Aurélien Corneau[5], Louise Roquebert[2], Philippe Caillet[6], Pierre Gay[6], Johanna Canovas[6], Axelle Histe[3,4], Catherine Blanc[7], Carine El-Sissy[2,8,9], Anis Larbi[10,11], Johanne Poisson[12,13], Pauline Ober[2], Pascaline Boudou-Rouquette[14], Pierre-André Natella[15], Hélène Vallet[16,17], Besma Saadaoui[6], Richard Layese[3,4], Eric Tartour[1,2], Elena Paillaud[6,18,19*†] and Clémence Granier[1,2*†]

Abstract

Background Immune ageing complicates cancer treatment in older individuals. While immunotherapy targeting the PD-1/PD-L1 pathway can reinvigorate T cells, these cells tend to become senescent with age. This study investigates different CD8$^+$ T cell subsets usually associated with senescence, in cancer patients over 70 years old who are undergoing anti-PD-1/PD-L1 immunotherapy, and examines the relationship between these senescent cells and prior chemotherapy exposure. We analyzed data from the Elderly Cancer Patient (ELCAPA) cohort, which included 35 patients enrolled between March 2018 and March 2021.

Results Flow cytometry and unsupervised analysis were employed to characterize Effector Memory CD45RA$^+$ (EMRA) and CD8$^+$ T cell senescence at baseline, before initiating PD-1/PD-L1 therapy. EMRA cells were found to overexpress CD57 and KLRG1 compared to overall CD8$^+$ T cells. Chemotherapy prior to anti-PD-1/PD-L1 was associated with an increased proportion of CD57$^+$ EMRA CD8$^+$ T cells ($p=0.009$) and its granzyme B (GRZB) subset ($p=0.007$). Using a 10% cut-off to define positivity, the six-month non-response tends to be associated with the CD57$^+$ GRZB$^+$ EMRA positivity ($p=0.097$). Other CD8$^+$ T cell subsets (EMRA, CD57$^+$, or KLRG1$^+$), usually associated with senescence, showed no significant association with previous chemotherapy or response to anti-PD-1/anti-PD-L1 therapy.

Conclusions These findings underscore the impact of prior chemotherapy on expanding the pool of senescent T cells, particularly CD57$^+$ EMRA CD8$^+$ T and CD57$^+$ GRZB$^+$ EMRA CD8$^+$ T cells, whose expansion could potentially affect the effectiveness of anti-PD-1/PD-L1 immunotherapy in elderly patients. This highlights the need for tailored approaches in this population.

Keywords Ageing, Senescence, Immune-ageing, Cancer, Tumour, Geriatrics, CD8-positive T-lymphocytes, EMRA, CD57, PD-1, Immunotherapy

†Elena Paillaud and Clémence Granier contributed equally to this work.

*Correspondence:
Elena Paillaud
elena.paillaud@aphp.fr
Clémence Granier
clemence.granier@aphp.fr; clemence.granier@chu-orleans.fr
Full list of author information is available at the end of the article

Background

Immune checkpoint inhibitors (ICI), such as anti-PD-1/PD-L1 and anti-CTLA-4, emerged around 2010 as a new standard of care for several cancers. Today, they are prescribed for more than 20 indications, including melanoma, lung, renal, bladder, and head and neck cancers. Certain biomarkers, such as PD-L1 tumor proportion score ≥ 50%, guide the use anti-PD-1 pembrolizumab as a first-line treatment for metastatic non-small-cell lung cancer [1, 2]. The improved response, survival rates, quality of life, and reduced side effects with ICIs, compared to chemotherapy, make them particularly appealing for treating older cancer patients. Older adults are more likely to have severe chemotherapy toxicities than younger ones [3]. However, treatment decisions in older adults remain more complex than in younger ones, due to the heterogeneity of their health status and lack of evidence-based data in this population [4]. As ICIs reached a growing place in the therapeutic arsenal to treat older cancer patients, several studies suggest that the benefits and toxicities in this population are comparable to those in younger patients [5]. Nonetheless, factors influencing this population's response need to be precisely determined.

First, data on the impact of immunological ageing on ICI treatment response and toxicities are lacking. Immune ageing is a complex, multifactorial process characterized by functional and structural alterations in the immune system that occur with ageing. These modifications include a decline in the lymphocytes proliferative capacity, impaired antigen-presenting cell function, reduced diversity of the lymphocyte repertoire, and alterations in the inflammatory responses [6].

Ageing induces well-characterized phenotypic changes in T lymphocytes [7–9]. Differentiated T lymphocytes are characterized by the loss of expression of the costimulatory molecules CD27 and CD28 ($CD27^{-}CD28^{-}$). CD45RA, a protein tyrosine phosphatase receptor, typically found on naïve T cells (while memory T cells express CD45RO), is re-expressed by the most differentiated T lymphocytes, referred to as effector memory $CD45RA^{+}$ (EMRA) cells. $CD8^{+}$ T cells tend to have a higher proportion of EMRA cells compared to $CD4^{+}$ T cells, and this pool increases with ageing. The percentages of EMRA and $CD27^{-}CD28^{-}$ among $CD8^{+}$ T cells increase with age [10]. The overexpression of receptors usually expressed by NK cells (that we will refer to as NK receptors), such as CD57 and KLRG1, as well as NKG2D, NKG2A, and other members of the KIR/KAR receptor family, accumulates on the differentiated T cells such as EMRA. The threshold for T cell activation via the T cell receptor (TCR) is lower in the elderly [11, 12].

Additionally, senescent T lymphocytes may express DAP-12, a protein involved in NK cell signaling through its immunoreceptor tyrosine-based activation motif (ITAM). These findings suggest that senescent T lymphocytes shift from TCR to NK signaling, a process seemingly regulated by stress proteins called sestrins. Sestrin-2 accumulates in T cells with senescence in a complex of MAP kinases p38, Erk and JnK [12] [13]. Furthermore, as dysfunctional $CD8^{+}$ T cells accumulate in the tumour microenvironment, ICIs are known to reinvigorate the $CD8^{+}$ T cell proliferation and functions (cytokine secretion and cytotoxic functions), by disrupting CTLA-4/CD80-86 or PD-1/PD-L1 inhibitory signals. CD28 plays a key role in the effective mechanism of action of ICI targeting PD-1 [14]. In this context, since $CD8^{+}$ T cell senescence involves loss of CD28 and the accumulation of EMRA cells, which have less proliferative potential and exhibit less specific responses, the $CD8^{+}$ T cell senescence may affect the efficacy of anti-PD-1/anti-PD-L1 therapy.

Secondly, the relationship between immune ageing and chemotherapy, and its clinical relevance, needs further exploration. In non-Hodgkin lymphoma (NHL), chemotherapy has been shown to shorten the telomere length in $CD8^{+}$ T cells [15], suggesting that chemotherapy induces $CD8^{+}$ T cell senescence. Despite the supposed underlying mechanism of cellular senescence induction by chemotherapy is via DNA damage, the relationship between T cell senescence and chemotherapy is not well explored with clinical relevance. This raises questions about the efficacy of ICI treatment in patients who have previously received chemotherapy.

The characterization of $CD8^{+}$ T cell senescence with both EMRA and NK receptors and sestrin-2 has not yet been done in older cancer patients. These biomarkers could provide insight into immune ageing and its association with chemotherapy and response to ICI treatment.

In this study, we aimed to characterize CD8 + T cell senescence in older cancer patients treated with anti-PD-1/PD-L1 using unsupervised-guided cytometry analysis and to examine its association with previous chemotherapy treatment and response to ICI therapy.

Materials and methods

Study design

Patients

We analyzed data from the ELderly CAncer PAtient (ELCAPA) cohort study. ELCAPA is a French, prospective observational, multicenter cohort of patients aged 70 years or older with newly diagnosed cancer at any stage. All patients included in the ELCAPA study were referred to a geriatrician for a Comprehensive Geriatric

Assessment (CGA) prior to treatment initiation, at one of 19 centers in the Ile-de-France region [16].

For the present study, we included patients with an ICI treatment intent and a peripheral blood mononuclear cells (PBMC) blood sample available. These patients were enrolled in the ELCAPA cohort between March 2018 and March 2021 at two centers: Georges Pompidou European Hospital and Cochin Hospital, Paris, France. Blood samples were collected just before the initiation of ICI treatment, regardless of the previous treatment line.

The immunotherapy agents used for cancer treatment included anti-PD1 (pembrolizumab, nivolumab) and anti-PD-L1 (durvalumab, avelumab). Prior chemotherapy regimens included combinations of carboplatin and taxol, carboplatin and gemcitabin, or taxol alone.

Of the 44 patients originally enrolled in this study, 35 were eligible for cytometry analysis.

The ELCAPA study protocol received approval from the institutional ethical committee (*CPP Ile-de-France I*, Paris, France; reference: 2019 mai-MS121) and was registered on ClinicalTrials.gov (NCT: 02884375). All patients were provided with detailed information about the study's objectives and procedures and gave verbal consent for the clinical aspects and written informed consent for the biological sample analysis.

Endpoints

Tumor response was evaluated by RECIST v1.1 criteria [17] and classified in complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The primary endpoint is the Overall Response Rate (ORR) at 6 months, defined as CR, PR, or SD at 6 months, *versus* non-response, defined as PD at 6 months, or death within 6 months. Secondary endpoints were time until death (overall survival) at 3 and 6 months. The index date was the date of CGA.

Clinical and biological characteristics

Clinical characteristics Demographic variables (age and sex) and oncological variables (primary tumour site and metastatic status) were collected at inclusion. The global functional status was assessed using the Eastern Cooperative Oncology Group Performance Status (ECOG-PS) scale [18]. ECOG-PS was categorized into three levels: 0–1 (fully active or restricted in strenuous activity) and 2, and 3–4 (increasing disability).

The CGA at inclusion, performed by a geriatrician with expertise in oncology, included several parameters: autonomy (activities of daily living (ADL (Katz, 6 items)) score, abnormal if score $\leq$ 5/6; instrumental ADL (IADL (Lawton, 8 items)) score, abnormal if score $\leq$ 7/8), mobility and fall risk (timed get up and go test, abnormal if unable to perform, or time to perform > 20s), nutritional status (body mass index in kg/m^2 (BMI); weight loss $\geq$ 10% in the last 6 months), cognitive status (mini-mental state examination (MMSE), abnormal is score $\leq$ 24/30), mood (mini geriatric depression scale (GdS) score, risk for depression if score $\geq$ 1/4), and comorbidities (cumulative illness rating scale for geriatrics (CIRS-G), 5-point scale ranging from 0 (no dysfunction) to 4 (extremely severe dysfunction)) [16].

Biological characteristics Routine laboratory tests, including lactate dehydrogenase (LDH), complete blood count (CBC), C-reactive protein (CRP) testing were performed at the centre of inclusion and extracted from a secured and standardized electronic case report.

Characterization of T cell senescence by flow cytometry

Experiment protocol

Senescence phenotyping of CD8$^+$ T cells was performed on the biological samples provided by the Biological Resources Center and Tumor Bank Platform (BB-0033–00063). Mononuclear cells were separated using a Ficoll density gradient and a numeration was performed. Five to ten million mononuclear cells were frozen per vial in 1 mL of a serum-free medium containing 10% DMSO (CryoMaxx, PAA, Pasching, Austria). After homogenization, the cryotubes were placed in a freezing container (Nalgene Mr. Frosty, Thermo Fisher Scientific, MA, USA). The samples were stored overnight at −80 °C, and the frozen vials were then transferred to liquid nitrogen until further analysis. The frozen human PBMC were thawed and incubated with flow cytometry antibodies. Cells were stained with a fixable viability stain and labelled for surface (CD3, CD4, CD8, CD27, CD45RA, CD28, CD57, KLRG1, CD56, NKG2A, NKG2D) and intracellular (granzyme-B (GRZB), Ki-67, Sestrin-2, and Dap-12) markers (see Table S1 for markers, clones and brands). All staining except for sestrin-2, was direct. Sestrin-2 expression was assessed after fixation and permeabilization (BioLegend FoxP3 Perm and Fix). After labelling, red blood cells were lysed using VersaLyse lysing solution (Beckman Coulter, Inc., USA) according to the manufacturer's recommendations. Surface and intracellular markers were analyzed by flow cytometry using a CytoFLEX LX flow cytometer (Beckman Coulter, Inc., USA). Calibration of the cytometer was performed daily with Flow-Set and Flow-Check fluorosphere (Beckman Coulter, Inc., USA). Fluorescence minus one (FMO) controls were used to verify the absence of spillover after applying the compensation matrix and as gating controls.

Additionally, isotype controls were used for Sestin-2, Dap-12, GRZB, NKG2A and NKG2D.

Gating strategy and T cell differentiation identification

Singlet & viable cells were selected before analyzing cell subsets.This study focused on $CD8^+$ T cells. $CD4^-$ $CD8^+$ $CD3^+$ cells were selected. CD27, CD28, and CD45RA were employed to classify the maturational stages of $CD8^+$ T cells. Naïve T cells express CD45RA while memory T cells are $CD45RA^-$ ($CD45RO^+$). Naïve and central memory T cells coexpress the costimulatory molecules CD27 and CD28, while effector memory T cells are predominantly $CD28^-$ and $CD27^-$. The most differentiated T cells reexpress CD45RA [9, 10]. We considered the $CD45RA^+CD27^+$ as naïve, $CD45RA^-CD27^+$ as central memory (CM) and $CD45RA^-CD27^-$ as effector memory (EM). As $CD8^+$ T cell tend to lose CD28 before losing CD27 [19], $CD27^-$ $CD45RA^+$ CD8 + T cell were mainly $CD28^-$ (Figure S1). $CD27^-CD28^-CD45RA^+$, the most differentiated effector $CD8^+$ T cell subset, was referred to as EMRA in the present study [8, 9].

CD57, CD56, NKG2A, NKG2D, Sestrin-2, and Dap-12 were used to assess senescence, while the cytotoxicity of GRZB was also investigated. The results were studied in percentage of these markers among total $CD8^+$ T cells. Absolute count was not available in our study. The gating strategy is shown in Figure S1.

Bioinformatics and statistical analysis

Unsupervised analysis by opt-SNE

Flow cytometry data were extracted from Kaluza Flow Cytometry Software v2.2 (Beckman Coulter).

Dimensionality reduction of flow cytometry data was performed using the opt-SNE, an optimized t-SNE algorithm, a modified version of t-SNE (t-distributed stochastic neighbor embedding) [20] on the OMIQ (Domatics) platform.

After establishing the compensation matrix, $CD3^+CD8^+CD4^-$ T cells were extracted and an arcsinh transformation was applied. After a preliminary cleaning step was conducted, data from 25 out of 35 patients were suitable for analysis. The OptSNE algorithm was therefore applied to 25 patients, with analysis performed on a range of 1500 to 3000 $CD3^+$ cells per sample. This subsample was merged for analysis. Identification of the target population was conducted based on a two-dimensional representation, and patients excluded during the cleaning step were recovered using the classical gating method.

CD27, CD45RA, CD28, CD57, KLRG1, CD56, NKG2A, NKG2D, GZRB, Ki-67, Sestrin-2 and Dap-12 were integrated in the algorithm, with the following setup for max iterations: 1000, optSNE end: 5000, perplexity: 30, theta: 0.5, components: 2, random seed: 4478, verbosity: 25.

Statistical analysis

Descriptive statistics included numbers (n) (%), mean ± SD (standard deviation), or median interquartile range (IQR) [1st quartile—3rd quartile] for baseline clinical and biological characteristics. Comparison between groups was conducted using Pearson's Chi-square test, Fischer, or Student T-test, Wilcoxon as appropriate. ORR was expressed as n (%) at 6 months. Overall survival was estimated by using the Kaplan–Meier curve.

A paired Wilcoxon signed rank test was performed to compare the median fluorescence intensity of ses-2, granzyme B and Ki-67 between differentiation stage types, taking into account the clustered structure of the data. *P* values from the Wilcoxon signed rank test were corrected for multiple comparisons using the false discovery rate method.The association between $CD8^+$ T cell senescence and response was analyzed using Firth's logistic regression due to the small sample size. This analysis was conducted in both univariate form and with adjustment for cancer site (lung *versus* other locations) and prior chemotherapy. No imputation was performed for missing data. The significance threshold was set at 0.05, and all tests were two-tailed.

Statistical analysis was performed by using Stata SE v17.0 (College Station, TX, USA).

Results

Clinical characteristics (Table 1)

A total of 35 patients were included in this study. The median follow-up was 24.9 months [95% CI: 16.8; 33.8]. The median age of the cohort was 79 years [74–84]. At 6 months, the overall response rate (ORR) was 36.7% (11/30), and the stable disease (SD) and partial response (PR) rate being 16.7% (5/30) and 20% (6/30), respectively [missing data: $n=5$] (Table 2). Overall survival (OS) at 3 and 6 months were 67.7% (CI 95%: 49.2—80.6) and 64.6% (CI 95%: 46.1—78.1), respectively (Figure S2).

The main patient characteristics are listed in Table 1. Among the 35 patients, 17 (48%) had previously received chemotherapy, while 18 (52%) received ICI as a first-line treatment (including 2 patients who received ICI in combination with chemotherapy). There was no significant difference in age between these two groups. The male-to-female ratio tended to be lower in the chemotherapy pretreated group ($p=0.060$). Tumour types were mainly lung, renal, and bladder cancer, with no difference in cancer types between the two groups. At the time of study inclusion, the three patients without metastasis were all in the chemotherapy-pretreated group.

Table 1 Patients characteristics for the whole population and according to first-line chemotherapy prior to anti-PD-1/PD-L1

Baseline characteristics	Data available	Total ($n=35$)	No chemotherapy prior to ICI ($n=18$)	Chemotherapy prior to ICI ($n=17$)	*P*-value
Age	35	79 [74 – 84]	78.5 [73—84]	79 [77—84]	0.894
Sex (male)	35	26 (74.3%)	16 (88.9%)	10 (58.8%)	0.060
Tumour type	35				0.097
Colon		1 (2.9%)	0 (0%)	1 (5.9%)	
Stomach		1 (2.9%)	0 (0%)	1 (5.9%)	
Bladder		5 (14.3%)	2 (11.1%)	3 (17.6%)	
Kidney		5 (14.3%)	5 (27.8%)	0 (0%)	
Lung		21 (60.0%)	11 (61.1%)	10 (58.8%)	
ENT		1 (2.9%)	0	1 (5.9%)	
Mesothelioma		1 (2.9%)	0 (0%)	1 (5.9%)	
Metastases	31				0.081
No		2 (6.5%)	0	2 (14.3%)	
Yes		28 (90.3%)	17 (100%)	11 (78.6%)	
unknown		1 (3.2%)	0 (0%)	1 (7.1%)	
ECOG-PS	28				> 0.99
0		2 (7.1%)	1 (5.9%)	1 (9.1%)	
1		11 (39.3%)	7 (41.2%)	4 (36.4%)	
2		14 (50.0%)	8 (47.1%)	6 (54.5%)	
3		1 (3.6%)	1 (5.9%)	0 (0%)	
ADL score (/6)	30	6 [5.5 – 6]	6 [5.5—6]	6 [5.5—6]	0.816
IADL score (/8)	28	6.5 [3 – 8]	5.5 [3 – 9]	7.5 [3.5—8]	0.442
TUG (≤ 20s)	20	16 (80.0%)	11 (84.6%)	5 (71.4%)	0.587
BMI	28	23.4 [21.2 – 25.3]	23.3 [20.1 – 24.5]	24.8 [21.3 – 26.0]	0.218
Weight loss over 6 months	28	7 (25.0%)	3 (17.7%)	4 (36.4%)	0.381
MMSE score (/30)	17	27 [25 – 30]	28 [27 – 30]	24.5 [21.5 – 28.5]	0.063
Mini-GDS score (/4)	25	0 [0 – 1]	0 [0 – 2]	0 [0—1]	0.735
CIRS-G	19	10 [6 – 13]	8 [2 – 16]	10 [7 – 10]	0.967
LDH (UI/L)	12	214 [190 – 235]	202.5 [177.5 – 235]	218 [213 – 550]	0.307
Lymphocyte count (/mm^3)	22	1230 [1040 – 1570]	1295 [870 – 1570]	1230 [1085 – 1595]	0.710
Neutrophils (/mm^3)	24	5940 [4070 – 7080]	6175 [4206.5—7690]	5205 [4070—6220]	0.426
Ratio NLR	22	3.8 [2.6—7.5]	6.6 [3.5 – 8.2]	3.5 [2.5 – 4.3]	0.166
CRP	28	13.9 [5.8 – 22.8]	14.4 [5.6 – 29.7]	12.6 [7.0 – 22.8]	0.837
Ratio CRP/ALBU	19	0.37 [0.18 – 0.58]	0.29 [0.13 – 0.58]	0.45 [0.25 – 0.57]	0.539
ICI line	35				NR
1		18 (51.4%)	18 (100%)	0	
2		15 (42.9%)	0	15 (88.2%)	
> 2		2 (5.7%)	0	2 (11.8%)	
ICI molecule	35				0.034
Anti-PD1		23 (65.7%)	11 (61.1%)	12 (70.6%)	
Anti-PD-L1		5 (14.3%)	1 (5.6%)	4 (23.5%)	
Anti-PD1 + anti-CTLA-4		4 (11.4%)	4 (22.2%)	0	
Chemotherapy + anti-PD-1		3 (8.6%)	2 (11.1%)	1 (5.9%)	

Results are presented as numbers (%) or median [1st quartile—3rd quartile]. The global functional status was assessed using the Eastern Cooperative Oncology Group Performance Status (ECOG-PS) scale, as described by [18]. ECOG-PS was categorized into three levels (0–1, 2, and 3–4), where scores of 0 and 1 indicate fully active or restricted in strenuous activity to scores of 2 and higher indicating increasing disability. The comprehensive geriatric assessment included the following variables: autonomy (activities of daily living (ADL) score, abnormal if score ≤ 5/6; instrumental ADL (IADL) score, abnormal if score ≤ 7/8), mobility and fall risk (timed get up and go test, abnormal if unable to perform, or time to perform > 20s), nutritional status (body mass index in kg/m^2 (BMI); absence or presence of weight loss ≥ 10% in the last 6 months), cognitive status (mini-mental state examination (MMSE), abnormal is score ≤ 24/30), mood (mini geriatric depression scale (GdS) score, risk for depression if score ≥ 1/4), and comorbidities (cumulative illness rating scale for geriatrics (CIRS-G), 5-point scale ranging from 0 (no dysfunction) to 4 (extremely severe dysfunction)) [16]

ENT ear nose and throat, *BMI* Body Mass Index, *Mini- GDS* Mini-Geriatric Depression Scale, *MMSE* Mini-Mental State Examination, *TUG* Time Up and Go, *LDH* Lactate Dehydrogenase, *NLR* neutrophil lymphocytes ratio, *CRP* C reactive protein, *ALBU* albumin, *ICI* immune checkpoint inhibitor, *NR* not relevant. Molecules: anti-PD1: pembrolizumab, nivolumab; anti-PD-L1: durvalumab, avelumab; chemotherapy prior to ICI: carboplatin and taxol, carboplatin and gemcitabin or taxol alone

Table 2 Overall response rate and mortality according to CD57$^+$EMRA CD8$^+$ T cell positivity

Outcome	Available data	Total n=35	CD57$^+$EMRA< 10 *n* = 16	D57$^+$EMRA ≥ 10 *n* = 19
ORR at 6 months	29			
Complete response		0	0	0
Partial response		6/29 (21%)	3/12 (25%)	3/17 (18%)
Stable disease		5/29 (17%)	2/12 (17%)	3/17 (18%)
Progressive disease		6/29 (21%)	1/12 (8%)	5/17 (29%)
Death		12/29 (41%)	6/12 (50%)	6/17 (35%)
Global death	35	23/35	11/17	12/17

The 6-month overall response rate and mortality are shown for the entire population and stratified by CD57$^+$ EMRA positivity (using a 10% cut-off among CD8$^+$ T cells)

There were no differences between patients with or without prior chemotherapy before ICI treatment in functional status (ECOG-PS, ADL, IADL, TGUG), nutritional status (BMI, weight loss in the past 6 months), cognitive status, mood and comorbidity (CIRS-G). Additionally, the results of routine lab tests for LDH, lymphocytes and neutrophils count, and CRP/albumin were not different between the 2 groups (Table 1).

Senescence-associated CD8$^+$ T cell subsets in the cohort

CD8$^+$ T cell senescence phenotyping was performed by flow cytometry at baseline (i.e. prior to anti-PD-1/PD-L1 treatment). Among the 35 patients, data from 25 patients were eligible for dimensionality reduction and clustering and the 35 were eligible for conventional analysis.

Identification and prevalence of senescence-associated CD8$^+$ T cell subsets

The proportion of EMRA CD8$^+$ T cells varied among individuals, ranging from 2.8% to 38.2% with a mean ± SD of 13.7 ± 8.6 of the total CD8$^+$ T cells in the overall cohort. In this cohort of patients aged over 70 years old, the percentage of EMRA did not correlate significantly with age or sex ratio (data not shown). As shown in Fig. 1, within the entire CD8$^+$ T cell population: (i) CD57 expression was predominantly expressed by CD28$^-$ cells (77.4% ± 17.7), and (ii) GRZB expression was observed mainly in CD28$^-$ cells (84.1 ± 17.6).

Unsupervised analysis using optSNE allowed the identification of CD8$^+$ T cell differentiation stages according to CD45RA, CD27, and CD28 expression. CD57 was mainly expressed by effector T cells (EM and EMRA). KLRG1 was mainly expressed by effector T cells (EM and EMRA), less so by CM T cells, and was weakly expressed, if at all, by naïve T cells (Fig. 1). The expression of NKG2A among CD8$^+$ T cells was low, averaging around 5%. NKG2D and CD56 expressions were very weak, at less than 1% among the CD8$^+$ T cells in the cohort (Figure S3). Quantification of these markers as percentages among CD8$^+$ T cells is reported in Table S2.

Characterization of EMRA CD8$^+$ T cell

Senescence markers

NK receptors expression by EMRA CD8$^+$ T cell Among EMRA CD8$^+$ T cells, the majority expressed KLRG1, with a mean ± SD of 86.0 ± 16.6%, compared to 66.1 ± 17.3% in the whole CD8$^+$ T cell population ($p < 0.0001$). Two clusters emerged based on CD57 expression: the predominant EMRA cluster was CD57$^+$ (CD57 expression of 80.5 ± 18.8% *versus* 52.1 ± 20.4% in the whole CD8$^+$ T cells, $p < 0.0001$), while a smaller but significant subset of EMRA was CD57$^-$ (19.5 ± 18.8% *versus* 47.9 ± 20.3% in the whole CD8$^+$ T cells, $p < 0.0001$). EMRA CD8$^+$ T cells were predominantly CD57$^+$ KLRG1$^+$ (63.9 ± 16.7% *versus* 45.7 ± 20.0% in the whole CD8$^+$ T cells, $p = 0.0004$). To note, a minor subset of CD57$^+$ EMRA cells expressed high levels of the signaling adaptor molecule DAP-12, which is typically expressed in activated NK cells (Fig. 1). The expression of NKG2A in EMRA CD8$^+$ T cells averaged under 5%. Given the very low or absent expression of CD56 and NKG2D on CD8$^+$ T cells in this cohort, their quantification on EMRA cells was not deemed relevant (Table S2).

Sestrin expression The stress-responsive protein sestrin-2, which accumulates with senescence, showed higher expression in more differentiated CD8$^+$ T cell subsets compared to naïve cells (Fig. 2 and Table S3: naïve *versus* CM: $p = 0.002$, naïve *versus* EM: $p = 0.002$, naïve *versus* EMRA: $p = 0.002$). Its expression did not vary significantly between EMRA subsets according to CD57 expression (Fig. 2, Table S3: p = ns).

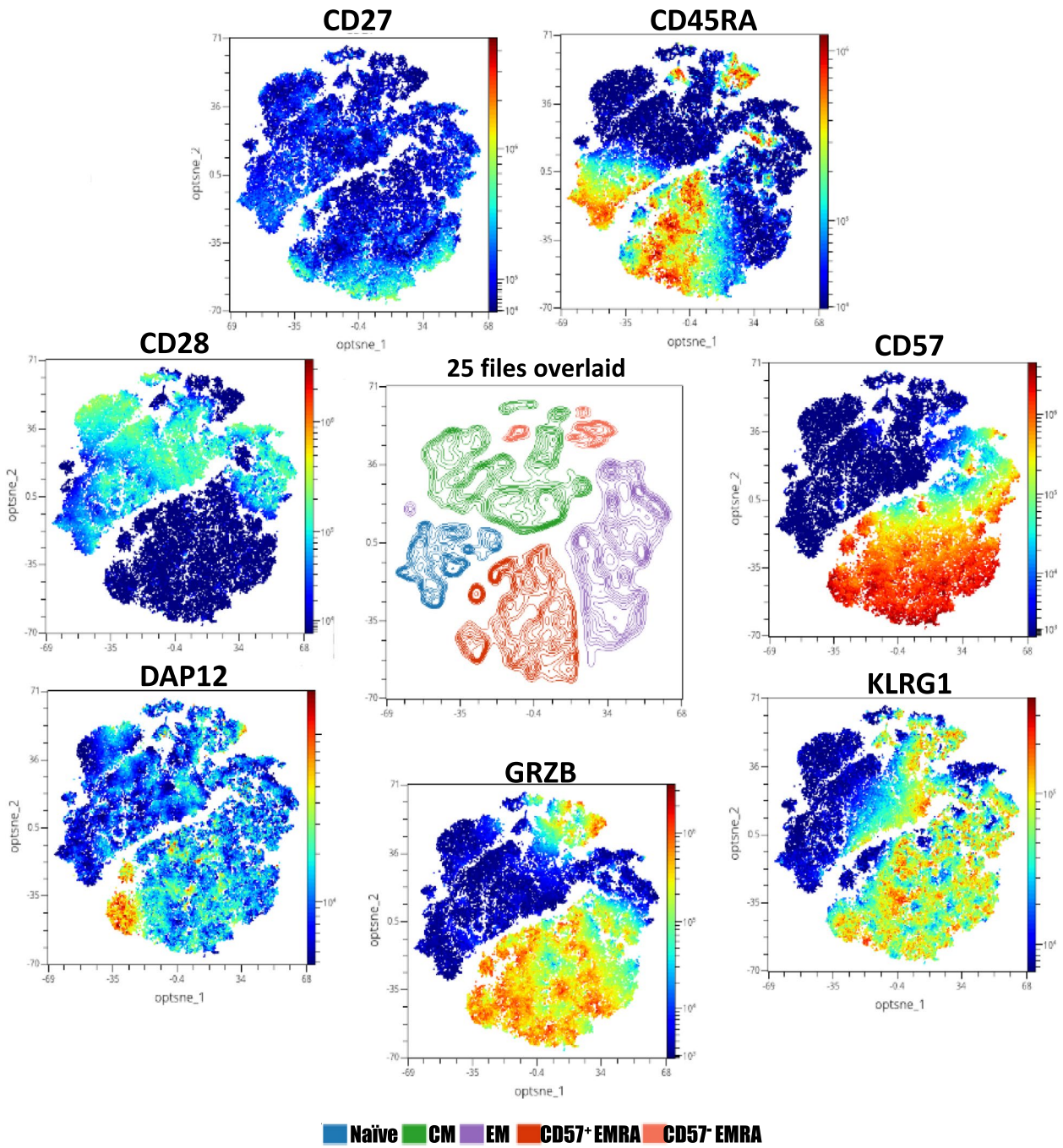

Fig. 1 Optimized t-distributed stochastic neighbor embedding (opt-SNE) plots of $CD8^+$ T cells from 25 cohort patients. Opt-SNE plots of CD8 + T cells from 25 eligible patients' data were generated, with overlaid to illustrate the expression of selected markers. In the scatterplots, marker expressions is represented by a continuous color gradient, ranging from dark blue (indicating minimal expression) to dark red (indicating maximal expression). The central optSNE1/optSNE2 plot reveals 5 distinct clusters corresponding to the differentiation stages, identified through CD27, CD28, CD45RA expression levels as naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory (EMRA). The expression of NK receptors (CD57 KLRG1) and granzyme B functional marker expressions characterize mainly effector memory cells (EM and EMRA). DAP12 is highly expressed in CD57hi CD8 + T cells. Granzyme B + CD8 + T cells are mainly CD28-

Granzyme B and Ki-67 expression

EMRA and $CD57^+$ EMRA $CD8^+$ T cells highly expressed GRZB, with means ± SD of 86.1 ± 11.1% and 80.7 ± 10.7% respectively. The more differentiated was the $CD8^+$ T cell, the more it expressed GRZB, in particular, $CD57^+$ EMRA expressed more GRZB compared to the $CD57^-$ EMRA

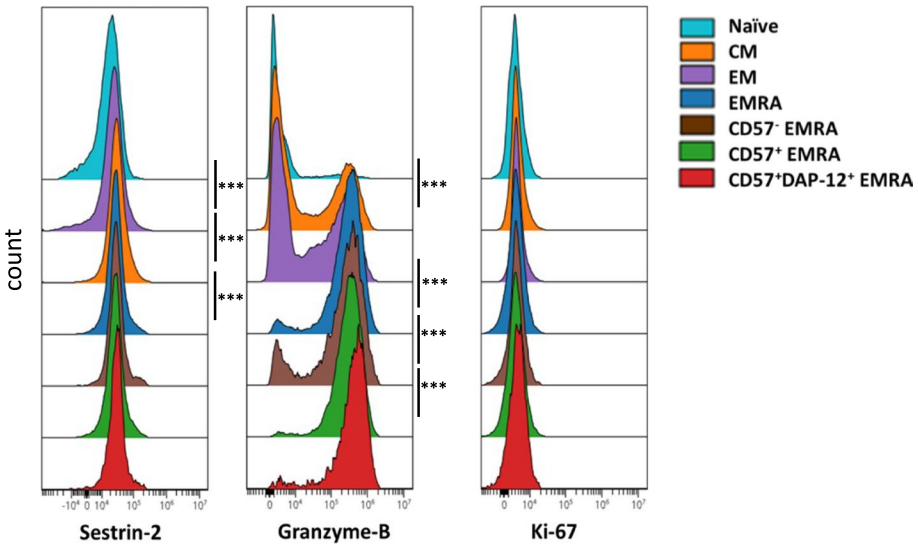

Fig. 2 Mean fluorescence intensity of Sestrin-2, granzyme B, and Ki-67 across $CD8^+$ T cells maturation stages. The mean fluorescence intensity (MFI) of Sestrin-2, granzyme B, and Ki-67 was measured across different stages of CD8 + T cell maturation, ranging from the least differentiated (naïve) to the most differentiated (CD57 + DAP12 + EMRA) cells ($n = 35$). CM: central memory; EM: effector memory. Differences between the two groups were tested using a two-sided unpaired t-test. The p-values are indicated by asterisks as follows: < 0.05*, < 0.01**, < 0.001***, < 0.0001****

$CD8^+$ T cells ($p < 0.0001$) (Fig. 2). In contrast, Ki-67 expression did not vary significantly across CD8 + T cell differentiation stages (Fig. 2).

$CD8^+$ T cell senescence and its association with prior chemotherapy and anti-PD-1/PD-L1 treatment outcomes

Association with prior chemotherapy

$CD57^+$ EMRA $CD8^+$ T cells and its $GRZB^+$ subset, but not total EMRA or $CD57^-$ EMRA or $CD57^+$ or $KLRG1^+$ $CD8^+$ subsets, were significantly associated with prior chemotherapy treatment. In the group of patients who received chemotherapy before ICI, the median percentage among $CD8^+$ T cells [IQR] of $CD57^+$ EMRA and $CD57^+$ $GRZB^+$ EMRA $CD8^+$ T cells were 12.5 [10.1—18.3] and 9.6 [8.6—13.8], respectively. These values were significantly higher compared to the chemotherapy-naïve group, which had median values of 7.3 [3.9—10.3] and 5.8 [2.6—8.3], respectively ($p = 0.009$ and $p = 0.007$, Fig. 3A and Table S4). These associations remained significant after adjusting for sex and cancer type (Table S4).

Using a 10% threshold to define $CD57^+$ EMRA $CD8^+$ T cells positivity, 13 out of 17 patients (76.5%) in the pre-chemotherapy group were positive, compared to 6 out of 18 (33.3%) in the chemotherapy-naïve group who received anti-PD-1/PD-L1 as a first-line treatment ($p = 0.010$, adjusted $p = 0.013$) (Fig. 3B).

Association with response to anti-PD-1/PD-L1 treatment

When using a 10% threshold to define $CD57^+$ $GRZB^+$ EMRA $CD8^+$ T cells positivity, 8/10 (80%) positive patients were non-responders (PD/Death), while only 2 out of 10 (20%) responders (SD/PR) (Fig. 3C). A proportion of $CD57^+GRZB^+$EMRA $CD8^+$ T cells ≥ 10% of the total $CD8^+$ T cells population tended to be associated with non-response at six months (adjusted $p = 0.097$, Table S5).

Using a 10% cutoff to define $CD57^+$ EMRA $CD8^+$ T cells positivity, 11/17 (64.7%) positive patients were non-responders, compared to 6 out of 17 (35.3%), responders (adjusted $p = 0.488$, positive predictive value = 58%) (Table 2 & Fig. 3C). Other $CD8^+$ T cells subsets, including the previously described Senescence Immunological Profile (SIP) i.e. $CD28^-CD57^+KLRG1^+$ [21], did not allow discrimination between responder and non-responders to the anti-PD-1/PD-L1 therapy (Table S5).

Discussion

The aim of the study was to characterize $CD8^+$ T cell senescence in a cohort of patients aged over 70 years old receiving ICI and to study the association between prior chemotherapy exposure and cancer outcomes. We focused on EMRA T cells, NK-markers (CD57, KLRG1, CD56, NKG2D, Dap-12), and sestrin-2 expression, which are recognized markers of senescent $CD8^+$ T cells. The percentage of EMRA among $CD8^+$ T cells varied significantly between individuals, illustrating the inter-individual variability even after 70 years old. CD57 and KLRG-1 were mainly expressed on differentiated T cells, whereas CD56, NKG2A, and NKG2D were weakly expressed.

The more differentiated these $CD8^+$ T cells were, the more they expressed Dap-12, a signaling adaptor typically

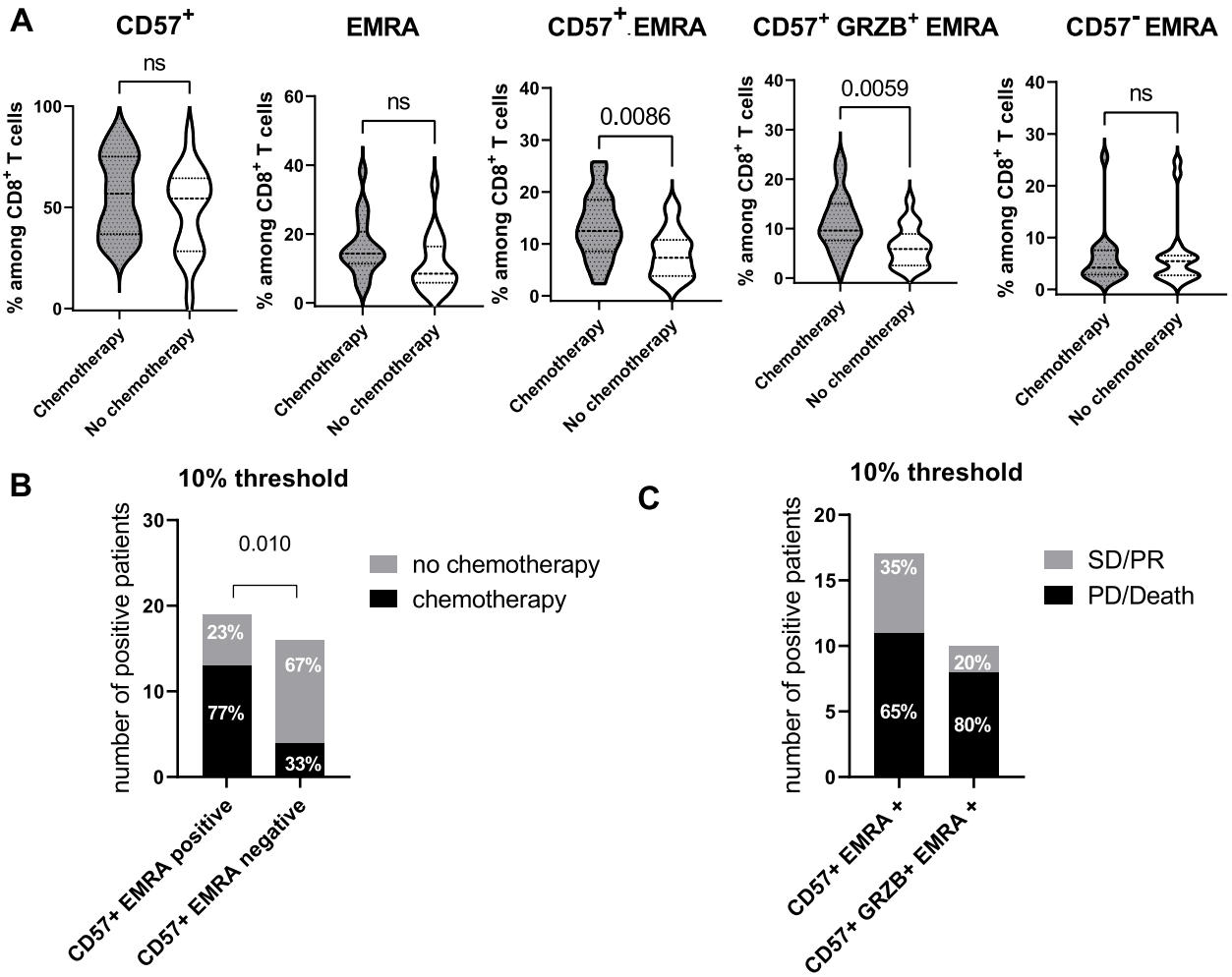

Fig. 3 Association of $CD8^+$ T cells senescence with prior chemotherapy and response to anti-PD-1/PD-L1 therapy: **A** The senescence of $CD8^+$ T cells, indicated by EMRA, CD57, and granzyme B (GRZB) expression, is analyzed in relation to prior chemotherapy exposure. The data are presented as a proportion of the total $CD8^+$ T cell population. **B** The frequency of $CD57^+$ EMRA $CD8^+$ T cells (cut-off 10%) is compared based on pre-exposure to chemotherapy. **C** The proportion of $CD57^+$ EMRA and $CD57^+$ $GRZB^+$ EMRA CD8.$^+$ T cells (cut-off 10%) is shown in relation to treatment response, categorized as progressive disease (PD), partial response (PR), or stable disease (SD)

found in NK cells. Notably, EMRA $CD8^+$ T cells (i) overexpressed CD57 and KLRG1 compared to the overall $CD8^+$ T cell population and (ii) were predominantly $KLRG1^+$ and/or $CD57^+$ suggesting that these highly differentiated T cells exhibit NK-like behavior in terms of NK receptors expression and signaling.

We identified two subsets of EMRA $CD8^+$ T cell that emerged according to CD57 expression: a predominant $CD57^+$ subset and a smaller $CD57^-$ subset. Importantly, the $CD57^+$ EMRA $CD8^+$ T cell subset, but not total EMRA population or other subsets such as $CD57^+$ or KLRG-1^+, was associated with prior chemotherapy exposure. This suggests that chemotherapy may induce T cell senescence, likely through mechanisms related to DNA damage. This finding aligns with previous studies showing reduced telomere length of T cells of NHL patients receiving chemotherapy [15, 22]. Another study in a non-geriatric cohort demonstrated an increase in the proportion of the senescent subset $CD28^-$ $CD57^+$ $CD8^+$ T cells in lung cancer patients, with differential reconstitution of the $CD8^+$ pool depending on CD28 and CD57 expression after chemotherapy [23].

We observed that the overall response to anti-PD-1/PD-L1 therapy (stable disease and partial response *versus* progression or death) tended to be inversely associated with $CD57^+$ $GRZB^+$EMRA $CD8^+$ T cell positivity ($p=0.097$). Even if the difference was not statistically significant, patients with progressive disease were more prone to exhibit $CD57^+$ EMRA $CD8^+$ T cell positivity. Previous studies have demonstrated that terminally differentiated $CD8^+$ T cells possess high functional and cytotoxic potential capacities and highly secrete the GRZB [21, 24]. However, this GRZB expression is not necessarily linked

to specific cytotoxicity. In a previous study, $CD28^-$ $CD57^+$ $KLRG1^+$ $CD8^+$ T cells (called Senescence Immunological Profile (SIP)) was associated with response to anti-PD-1/PD-L1 (using an optimal cut-off of 39.5% in the discovery cohort or 28% in the validation cohort), in an adult non-geriatric cohort [21]. However, in our study of patients aged 70 and over, SIP was not significantly associated with the ORR. It is possible that $CD57^+$ EMRA subset is more relevant to this geriatric oncology population. The markers used in the SIP are closely related to those of $CD57^+$ EMRA (SIP: $CD28^-$ $CD57^+$ $KLRG1^+$ compared to $CD57^+$ EMRA: $CD45RA^+$ $CD27^-$ $CD28^-$ $CD57^+$), reinforcing the relevance of $CD57^+$ and $CD28^-$ as key markers in the clinical characterization of T cell senescence.

CD57 expression by $CD8^+$ T cells is known to lead to replicative senescence and impaired proliferation. $CD28^-$ $CD57^+$ $CD8^+$ T cells have been characterized in the context of chronic antigen stimulation, such as HIV infection as well as chronic inflammation and cancer [25]. These cells may represent chronically stimulated cells with diminished functionality such as reduced perforin secretion [26]. Although EMRA $CD8^+$ T cells have high cytotoxic and cytokine secretion potential [24, 27], they may have difficulties in activation and proliferation following specific stimulation [13]. One considers that they have a NK-like behaviour [12]. There are some differences in their characterisation, depending on the study.

Our study has some limitations. The cohort was heterogeneous in terms of cancer type and prior treatment, which may introduce confounding factors. We made statistical adjustments to limit this heterogeneity. Furthermore, this is an exploratory study with a small cohort and the results need to be confirmed in larger studies that include geriatric parameters to assess frailty in older patients. Moreover, due to the unavailability of lymphocyte count at the time of sampling and the use of PBMC, we could not analyze the absolute count data. Due to technical limits, we did not study the PD-1 expression or perform a functional characterization of the EMRA depending on their CD57 expression. Interestingly, in the literature, Verma et al. showed that, after stimulation, $CD57^+$ EMRA $CD8^+$ T cells are less prone to activation (decreased proliferation capacities and IFNɣ secretion) and had longer telomeres compared to the $CD57^-$ counterpart [28]. This suggests that CD57 could potentially discriminate senescent EMRAs.

Despite the above-mentioned limitations, our study is important as it is one of the first to focus on patients aged 70 and older. To our knowledge, this cohort is unique in the literature and addresses a clinically relevant question. Given that the median age at cancer diagnosis is around 65–70 years [29], there is a need for specific studies in the older population to identify relevant and appropriate biomarkers.

Conclusions

In summary, our findings in older cancer patients suggest that EMRA $CD8^+$ T cells can be subdivided into two subsets based on CD57 expression, with $CD57^+$ EMRA being predominant and associated with prior chemotherapy exposure. $GRZB^+$ ± $CD57^+$ EMRA subsets appear to be relevant markers to investigate in elderly patients receiving anti-PD-1/PD-L1 therapy, particularly following first-line chemotherapy. This suggests that (i) $CD57^+$EMRA $CD8^+$ T cell could serve as a marker of chemotherapy-induced senescence and (ii) first-line chemotherapy might be reconsidered before ICI in cases where it induces $CD57^+$ EMRA $CD8^+$ T cells. These preliminary results pave the way for larger clinico-biological studies aimed at identifying biomarkers to guide decision-making in older cancer patients eligible for immunotherapy.

Abbreviations

ADL	Activities of daily living
ALK	Anaplastic lymphoma kinase
BMI	Body mass index
CBC	Complete blood count
CGA	Comprehensive geriatric assessment
CIRS-G	Cumulative illness rating scale-geriatric
CKD	Chronic kidney disease
CI	Confidence interval
CR	Complete response
CRP	C-reactive protein
ECOG-PS	Eastern cooperative oncology group performance status
ELCAPA	ELderly cancer patient
EMRA	Effector memory re-expressing cd45ra
ERK	Extracellular signal-regulated kinase
EMRA	Effector memory re-expressing cd45ra
HAS	French national authority for health
IADL	Instrumental activities of daily living
FMO	Fluorescence minus one
FoxP3	Forkhead box p3
ICI	Immune checkpoint inhibitors
JNK	C-jun n-terminal kinases
KAR	NK cell activating receptors
KIR	NK cell inhibitory receptors
KLRG1	Killer cell lectin-like receptor g1
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinases
Mini-GDS	Mini-geriatric depression scale
MMSE	Mini-mental state examination
NKG2A	Natural killer group 2a receptor
NKG2D	Natural killer group 2d receptor
NHL	Non-hodgkin lymphoma
NK cells	Natural killer cells
NLR	Neutrophil-to-lymphocyte ratio
optSNE	Optimized t-distributed stochastic neighbor embedding
OR	Odds ratio / aOR adjusted odds ratio
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cells
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PR	Partial response
Ses-2	Sestrin-2
SIP	Senescence immunological profile
TCR	T cell receptor
TMB	Tumour mutational burden

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-024-00487-4.

Supplementary Material 1.

Acknowledgements
We thank the pole de ressources biologiques (PBR HEGP and Cochin hospital) for sample collection and extraction. The biological samples have been provided by the Biological Resources Center and Tumor Bank Platform (BB-0033-00063). We thank the patients for their participation.

Authors' contributions
CGO, MLEE, FC, MLEB, LR, RL, EP and CGR conceived and designed the experiments. PC, PG, JC, AH, JP, PBR, PAN, BS, HV and EP facilitated procurement of the remnant blood samples and data from patients. CGO, MLEE, FC, MLEB, LR, PO, RL and CGR performed the experiments CGO, MLEE, FC, MLEB, AC, LR, CB, CE, AL, JPO, PO, RL, ET, EP and CGR analysed the data. CGO, MLEE, FC, LR, RL, ET, EP and CGR performed the statistical analyses. CGO, MLEE, FC, CE, PC, HV, ET, EP and CGR wrote the paper. All authors have read and approved the final draft of the manuscript.

Funding
This work was supported by Canceropôle Ile de France (Emergence 2020 grant).

Data availability
The data supporting the findings of this study are available from the corresponding authors upon written reasonable request.

Declarations

Ethics approval and consent to participate
The local Ethical Committee approved this study: the ELCAPA study protocol received approval from the institutional ethical committee (*CPP Ile-de-France I*, Paris, France; reference: 2019 mai-MS121) and was registered on ClinicalTrials.gov (NCT: 02884375).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Université Paris Cité, INSERM, PARCC, Paris, France. [2]Department of Immunology, APHP, Hôpital Européen Georges Pompidou (HEGP), Paris, France. [3]Université Paris-Est Créteil, INSERM, IMRB, Créteil F-94010, France. [4]AP-HP, Hopital Henri-Mondor, Public Health Department and Clinical Research Unit (URC Mondor), Créteil F-94010, France. [5]Sorbonne Université, Centre de recherche de Saint Antoine, CISA, Paris F-75012, France. [6]Department of Geriatric Medicine, Hôpital Europeen Georges Pompidou, AP-HP, Paris, France. [7]Plateforme de Cytométrie de la Pitié-Salpêtrière (CyPS) in Paris, Paris, France. [8]INSERM, Laboratory of Integrative Cancer Immunology, Paris, France. [9]Cordeliers Research Center, Sorbonne University, University Paris Cité, Paris, France. [10]Medical and Scientific Affairs, Beckman Coulter Life Sciences, Paris, France. [11]Department of Medicine, Division of Geriatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada. [12]Université Paris Cité, Department of Geriatrics, European Georges Pompidou Hospital, Paris Cancer Institute CARPEM, Assistance-Publique Hôpitaux de Paris (AP-HP), Paris F-75015, France. [13]Université Paris-Cité, INSERM, Centre de recherche sur l'inflammation, UMR 1149, Paris F-75018, France. [14]Department of medical Oncology, Ariane program, Cochin hospital, Paris Cancer Institute CARPEM, Assistance-Publique Hôpitaux de Paris (AP-HP), Paris F-75014, France. [15]AP-HP, Hopital Henri-Mondor, Hopital Henri-Mondor, Public Health Department and Clinical Research Unit (URC Mondor), Créteil F-94010, France. [16]Sorbonne Université, Institut National de la Santé et de la Recherche Médicale (INSERM), UMRS 1135, Centre d'immunologie et de Maladies Infectieuses (CIMI), Paris, France. [17]Department of Geriatrics, Saint Antoine hospital, Assistance Publique Hôpitaux de Paris (AP-HP), Paris, France. [18]Université de Paris Cité, Paris, France. [19]Univ. Paris Est Créteil, Inserm U955, IMRB, Créteil, France.

Received: 2 September 2024 Accepted: 12 November 2024
Published online: 27 December 2024

References

1. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N Engl J Med. 2016;375:1823–33.
2. Subbiah V, Solit DB, Chan TA, Kurzrock R. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) ≥10: a decision centered on empowering patients and their physicians. Ann Oncol. 2020;31:1115–8.
3. Beinse G, Reitter D, Segaux L, Carvahlo-Verlinde M, Rousseau B, Tournigand C, et al. Potential drug-drug interactions and risk of unplanned hospitalization in older patients with cancer: A survey of the prospective ELCAPA (ELderly CAncer PAtients) cohort. Journal of Geriatric Oncology. 2020;11:586–92.
4. Soubeyran P, Bellera C, Paillaud E. Achieving harmony in oncological geriatric assessment – Should we agree on a best set of tools? Journal of Geriatric Oncology. 2023;14: 101482.
5. Pawelec G. Does patient age influence anti-cancer immunity? Semin Immunopathol. 2019;41:125–31.
6. Liu Z, Liang Q, Ren Y, Guo C, Ge X, Wang L, et al. Immunosenescence: molecular mechanisms and diseases. Sig Transduct Target Ther. 2023;8:1–16.
7. Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. T memory stem cells in health and disease. Nat Med. 2017;23:18–27.
8. Koch S, Larbi A, Derhovanessian E, Özcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. Immun Ageing. 2008;5:1–12.
9. Romero P, Zippelius A, Kurth I, Pittet MJ, Touvrey C, Iancu EM, et al. Four Functionally Distinct Populations of Human Effector-Memory CD8 $^+$ T Lymphocytes. J Immunol. 2007;178:4112–9.
10. Callender LA, Carroll EC, Bober EA, Akbar AN, Solito E, Henson SM. Mitochondrial mass governs the extent of human T cell senescence. Aging Cell. 2020;19: e13067.
11. Granier C, Gey A, Roncelin S, Weiss L, Paillaud E, Tartour E. Immunotherapy in older patients with cancer. Biomedical Journal. 2021;44:260–71.
12. Pereira BI, De Maeyer RPH, Covre LP, Nehar-Belaid D, Lanna A, Ward S, et al. Sestrins induce natural killer function in senescent-like CD8 + T cells. Nature Immunology. 2020;21(6):684-94.
13. Lanna A, Gomes DCO, Muller-Durovic B, McDonnell T, Escors D, Gilroy DW, et al. A sestrin-dependent Erk/Jnk/p38 MAPK activation complex inhibits immunity during ageing. Nat Immunol. 2017;18:354–63.
14. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1–mediated inhibition. Science. 2017;355:1428–33.
15. Lee J-J, Nam C-E, Cho S-H, Park K-S, Chung I-J, Kim H-J. Telomere length shortening in non-Hodgkin's lymphoma patients undergoing chemotherapy. Ann Hematol. 2003;82:492–5.
16. Caillet P, Canoui-Poitrine F, Vouriot J, Berle M, Reinald N, Krypciak S, et al. Comprehensive Geriatric Assessment in the Decision-Making Process in Elderly Patients With Cancer: ELCAPA Study. JCO. 2011;29:3636–42.
17. Tazdait M, Mezquita L, Lahmar J, Ferrara R, Bidault F, Ammari S, et al. Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. European Journal of Cancer. 2018;88:38–47.
18. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649–55.
19. Larbi A, Fulop T. From, "truly naïve" to "exhausted senescent" T cells: When markers predict functionality. Cytometry A. 2014;85:25–35.
20. Belkina AC, Ciccolella CO, Anno R, Halpert R, Spidlen J, Snyder-Cappione JE. Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. Nat Commun. 2019;10:5415.
21. Ferrara R, Naigeon M, Auclin E, Duchemann B, Cassard L, Jouniaux JM, et al. Circulating T-cell immunosenescence in advanced non-small cell

lung cancer patients treated with single agent PD-1/PD-L1 inhibitors or platinum-based chemotherapy. Clin Cancer Res. 2020;27(2):492-503.
22. Li P, Hou M, Lou F, Björkholm M, Xu D. Telomere dysfunction induced by chemotherapeutic agents and radiation in normal human cells. Int J Biochem Cell Biol. 2012;44:1531–40.
23. Onyema OO, Decoster L, Njemini R, Forti LN, Bautmans I, De Waele M, et al. Shifts in subsets of CD8+ T-cells as evidence of immunosenescence in patients with cancers affecting the lungs: an observational case-control study. BMC Cancer. 2015;15:1016.
24. Seidel JA, Vukmanovic-Stejic M, Muller-Durovic B, Patel N, Fuentes-Duculan J, Henson SM, et al. Skin resident memory CD8+ T cells are phenotypically and functionally distinct from circulating populations and lack immediate cytotoxic function. Clin Exp Immunol. 2018;194:79–92.
25. Strioga M, Pasukoniene V, Characiejus D. CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease. Immunology. 2011;134:17–32.
26. Huff WX, Kwon JH, Henriquez M, Fetcko K, Dey M. The Evolving Role of CD8+CD28− Immunosenescent T Cells in Cancer Immunology. Int J Mol Sci. 2019;20:2810.
27. Henson SM, Lanna A, Riddell NE, Franzese O, Macaulay R, Griffiths SJ, et al. p38 signaling inhibits mTORC1-independent autophagy in senescent human CD8+ T cells. American Society for Clinical Investigation; 2014 [cited 2022 Jun 27]. Available from: http://www.jci.org/articles/view/75051/pdf.
28. Verma K, Ogonek J, Varanasi PR, Luther S, Bünting I, Thomay K, et al. Human CD8+ CD57- TEMRA cells: Too young to be called "old." PLoS ONE. 2017;12: e0177405.
29. Van Herck Y, Feyaerts A, Alibhai S, Papamichael D, Decoster L, Lambrechts Y, et al. Is cancer biology different in older patients? The Lancet Healthy Longevity. 2021;2:e663–77.

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