BMJ Oncology

Molecular and immune landscape of tumours in geriatric patients with non-small cell lung cancer, melanoma and renal cell carcinoma

Khalil Choucair ,[1] Andrew Elliott,[2] Matthew James Oberley,[2] Phillip Walker,[2] April K Salama,[3] Azhar Saeed,[4] Hirva Mamdani,[1] Dipesh Uprety,[1] Wafik S El-Deiry,[5] Himisha Beltran,[6] Stephen V Liu,[7] Chul Kim,[7] Abdul Rafeh Naqash,[8] Emil Lou,[9] Lujia Chen,[10] Anwaar Saeed[11]

To cite: Choucair K, Elliott A, Oberley MJ, *et al*. Molecular and immune landscape of tumours in geriatric patients with non-small cell lung cancer, melanoma and renal cell carcinoma. *BMJ Oncology* 2025;**4**:e000551. doi:10.1136/bmjonc-2024-000551

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjonc-2024-000551).

Received 23 July 2024
Accepted 02 January 2025

For numbered affiliations see end of article.

Correspondence to
Anwaar Saeed;
saeeda3@upmc.edu

ABSTRACT

Objective Cancer patients aged ≥80 years present unique characteristics affecting response to immune checkpoint inhibitors (ICIs), with unidentified molecular differences. This study aimed to explore potential biomarkers of response to ICI in patients ≥80 years.

Methods and analysis We analysed tumour samples (n=24 123) from patients ≥80 (versus<80) with non-small cell lung cancer (NSCLC), melanoma (MEL), and renal cell cancer (RCC). Using gene expression deconvolution, we investigated differences in tumour microenvironment (TIME) composition. Then, using next-generation sequencing and programmed death-ligand 1 (PD-L1) assessment, we evaluated gene expression differences between age groups and across tumour types, with a focus on ageing-related processes such as DNA damage response (DDR), immune checkpoint (IC) and metabolism-related genes. In a subset of patients ≥80 (n=1013), gene clustering and differential gene expression analyses were carried out to identify potential tumour-type specific expression patterns in responders to ICI.

Results Significant differences in TIME composition were seen in patients with NSCLC and MEL. In patients ≥80, tumour mutational burden was lower in patients with NSCLC, higher in MEL and RCC had fewer PD-L1+tumours. DDR, IC and metabolism-related gene enrichments were distinct in patients ≥80. In patients ≥80 treated with ICIs (n=1013), there were no significant differences in survival between gene clusters, but differential gene expression analysis identified potential tumour-type specific expression patterns in responders.

Conclusion Our findings reveal tumour type-specific expression profiles, TIMEs and response signatures to ICIs in patients ≥80, supporting further biomarker investigations in this population.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The participation of older patients in immune checkpoint inhibitors (ICIs) trials has been suboptimal. Recent efforts have revealed that patients over the age of 80 years with cancer treated with ICIs can have a favourable response and acceptable toxicity. However, the use of ICIs at any age is linked to the development of resistance as well as potential side effects. There are currently no known predictive molecular biomarkers of response to ICIs in patients at extreme ages that could select patients who might potentially respond while sparing others potential treatment-associated risks. This is particularly important in older, frailer patients with more comorbidity.

WHAT THIS STUDY ADDS

⇒ This is the first exploratory suggest molecular and immune differences in the ageing process across the three cancer types where ICIs are most used. Our work provides potential gene expression signatures of response to ICIs in patients ≥80 years and suggests potential immune and metabolic response signatures to ICIs.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The fi ndings of this study provide grounds for future biomarker discovery in this growing patient population. This analysis exclusively investigated molecular and immune changes in patients with cancer at extremes of age. The findings can have important implications in future biomarker research by providing an actionable framework to refine tumour-type specific, predictive panels of biomarkers, that take into account the underlying tumour immune microenvironment and co-occurring alterations. Ultimately, dedicated studies focused on refining our findings could thus be carried out in the future.

INTRODUCTION

Participation of older patients in immune checkpoint inhibitors (ICIs) trials has been suboptimal, particularly at the extremes of age, partially due to overall age-restrictive exclusion criteria.[1] A recent evaluation of a large, multicentre cohort of patients over the age of 80 years with cancer suggests that ICIs can have a favourable efficacy and toxicity profile in older adults.[2] Yet, up to two-thirds of patients, at any age, treated with ICIs

demonstrate resistance to these drugs, limiting their benefit while still exposing patients to risks of morbid or even life-threatening adverse events.[3 4] This is particularly important in the setting of older and frailer patients with more comorbidity.

In a recent publication from a multisite, international clinical database, our group has explored the role of inflammation as it relates to immunosenescence and chronic ageing-related inflammation, which we refer to as 'inflammaging', in impacting response to ICI, highlighting the role of low markers of inflammation in predicting response ICIs in this patients' population.[5] Immunosenescence, the age-related remodelling process of the immune system, results in immune dysregulation via both cellular and humoral immunity, with depletion of lymphocyte reserves, fewer CD4+ and CD8+ T cells, decreased variety of regulatory and memory T-cells and an overall increased proinflammatory state.[6–8] Inflammaging is reflected in the higher serum levels of interleukin-6 (IL-6), C reactive protein and tumour necrosis factor (TNF) observed in adults >70 years old.[9–11] On treatment with ICIs, a proinflammatory host state decreases response to these therapies, as higher levels of proinflammatory markers in the tumour immune microenvironment (TIME)[12 13] reduce tumour-infiltrating lymphocytes and confer worse survival.[14–16] Similarly, changes to molecular processes, such as DNA damage response (DDR),[17 18] glutamine and glucose metabolism[19–24] and expression of immune checkpoint (IC) genes,[25] have been tightly associated with the ageing process.

Given ageing-related biological differences, there is a need to better identify clinically relevant molecular differences and changes in TIME composition in patients with cancer at the extreme of age. Filling this gap in knowledge would delineate molecular biomarkers of response that can help with patient selection and monitoring response to ICIs in older patients with cancer. The objective of this study is to explore potential ageing-associated biomarkers of response to ICIs in older patients with cancer. To that end, we first investigated potential molecular and cellular TIME differences in a cohort of patients 80 years of age or older (≥80 year) in comparison to those younger than 80 (<80 year). To delineate potential biomarkers of ICI response in older patients, we next focused on potential gene sets with a differential expression between age groups and performed gene clustering analyses in a subset of patients ≥80 year specifically, to evaluate the impact of these clusters on survival.

RESULTS

Patient characteristics

A total of 24 123 tumours were analysed. Overall, 13.63% (n=3288) of patients were ≥80 year and 86.37% (n=20 835) were<80 year (table 1). The majority of patients had non-small cell lung cancer (NSCLC) (19 891; 82.46%), followed by melanoma (MEL) (2899; 12.02%) and renal cell cancer (RCC) (1333; 5.52%). Patients ≥80 year constituted 16.0%, 16.6% and 5.3% of patients with NSCLC, MEL and RCC samples, respectively. The majority of analysed patients were male (12 760; 52.89%) and white/

Table 1 Summary of patient samples submitted for molecular analysis

Tumour type >	NSCLC		MEL		RCC	
Age group (years)	**≥80**	**<80**	**≥80**	**<80**	**≥80**	**<80**
Total count (%)	2739 (16.0)	17 152 (84.0)	482 (19.9)	2417 (80.9)	67 (5.3)	1266 (94.7)
Median age (years)	83.0	67.0	84.0	65.0	81.0	63.0
Male (%)	50.6	50.3	67.6	60.9	77.6	70.9
Female (%)	49.4	49.7	32.4	39.1	22.4	29.1
Biopsy site						
Primary (%)	71.27	61.18	54.15	41.50	32.48	44.23
Metastatic (%)	28.22	38.02	37.34	48.70	67.16	55.77
Unclear (%)	0.51	0.80	8.51	9.81	0.00	0.00
Race						
White/Caucasians (%)	76.89	62.97	86.93	70.21	77.61	60.90
Black/African American (%)	8.03	12.90	2.49	3.52	4.48	9.95
Asian/Pacific Islander (%)	3.91	3.19	1.24	1.41	2.99	1.97
Other/unknown (%)	11.17	20.94	9.34	24.87	14.93	27.17
Timing of biopsy						
Pre-ICI initiation (%)	98.82	89.67	80.33	79.64	90.00	84.81
Post-ICI initiation (%)	7.18	10.33	19.67	20.63	10.00	15.19

ICI, immune checkpoint inhibitor; MEL, melanoma; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

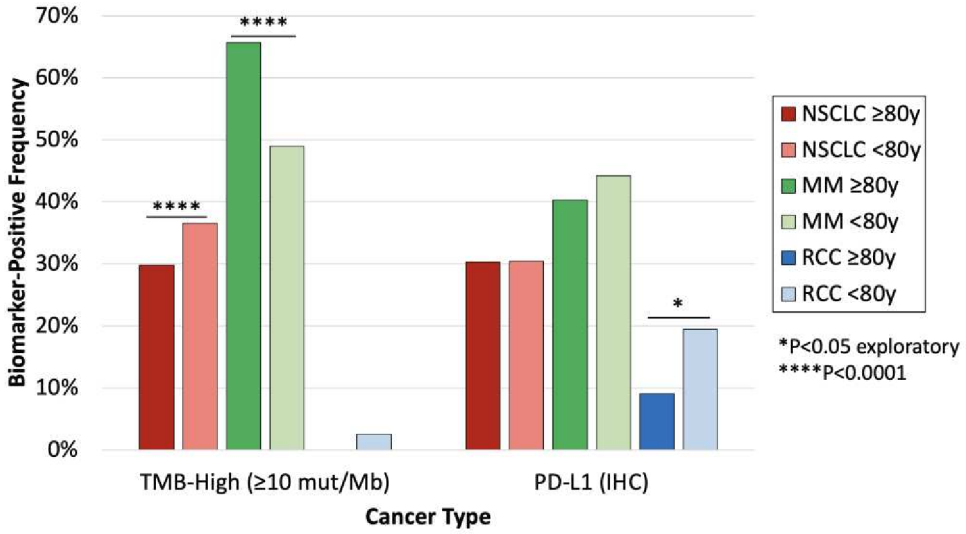

Figure 1 Comparison of the frequency of TMB-H and PD-L1+ (IHC) in patients <80 versus ≥80 year, across different tumour types. See methods for PD-L1 antibody and positive threshold details. IHC, immunohistochemistry; Mb, mega-base pair; MM, melanoma; mut, mutation; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; RCC, renal cell carcinoma; TMB-H, tumour mutational burden high.

Caucasians which was consistent across cancer types and age groups. Other information such as the site of tissue biopsy and its timing are further summarised in table 1.

Tumour mutational burden and PD-L1 analysis

For patients with NSCLC, tumour mutational burden high (TMB-H) was seen in 29.7% of patients ≥80 year and 36.5% of those <80 year (p<0.001). In contrast, TMB-H was more frequent in patients ≥80 year with MEL (vs <80 year: 65.7 vs 49.0%, p<0.01). Programmed death-ligand 1 (PD-L1)+tumours were less frequent among patients ≥80 year with RCC (9.0 vs 19.4%, exploratory-p<0.05), with no statistically significant difference by age group in patients with NSCLC and MEL (figure 1).

IC gene expression profiling

Increased expression of *PDCDL1G2* (PD-L2; 1.11-fold; p<0.05), *HAVCR2* (TIM-3; 1.11-fold; p<0.05) and CD80/86 (1.07/1.08-fold; p<0.05) was observed in patients ≥80 year with NSCLC, while *IL-6* expression was decreased (0.88-fold; p<0.05). The largest fold-change in IC gene expression was for *IL-6* (1.24-fold; not significant) in patients ≥80 year with MEL and *GZMB* (0.56-fold; p<0.05) in RCC.

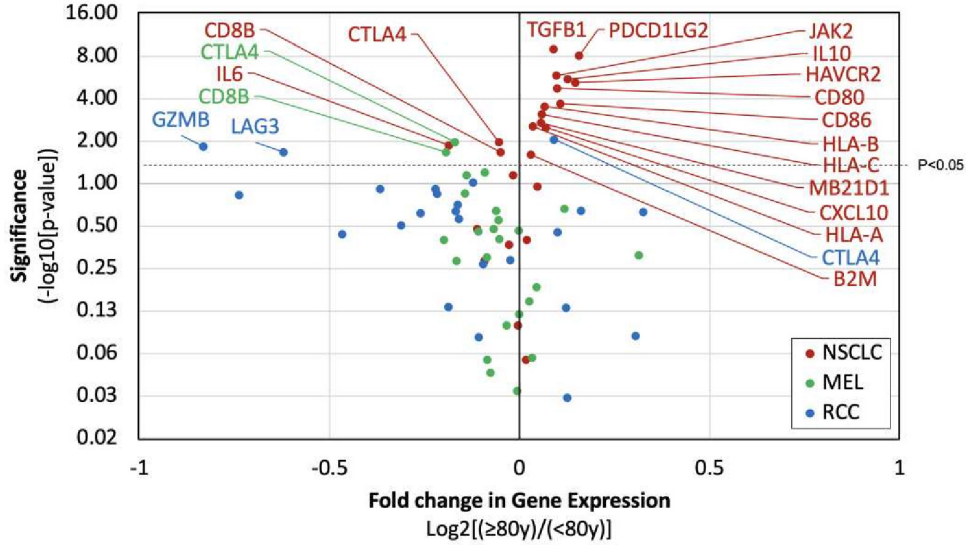

Figure 2 Volcano-plot depiction of fold-change in IC-related gene expression in patients <80 versus ≥80 year, across different tumour types. The dashed line represents the significance cut-off at p<0.05. NSCLC (red): non-small cell lung cancer; MEL (green): melanoma; RCC (blue): renal cell carcinoma. The x-axis reflects the Log2 fold change in gene expression between patients ≥80 year compared with <80 year (denominator). The y-axis depicts the change significance (–log10 of p value). IL-6, interleukin 6.

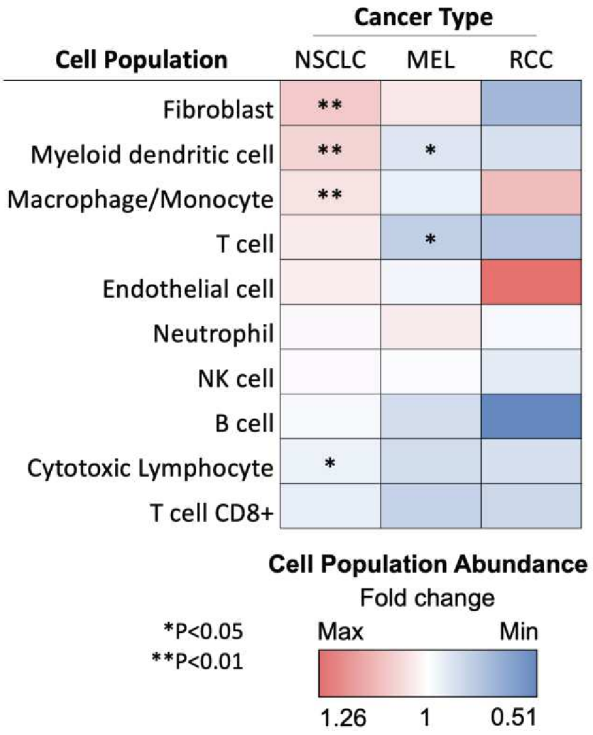

Figure 3 TIME cellular composition in patients <80 versus ≥80 year, across different tumour types. The matrix represents the fold-change cell population abundance in patients ≥80 year versus <80 year, with the highest abundance depicted in red and the lowest in blue. MEL, melanoma; NK cell, natural killer cell; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; TIME, tumour immune microenvironment.

Figure 2 details other IC genes including *CTLA4*, *TGFB1* and *LAG3*, among others.

Immune tumour microenvironment analysis

Estimated from bulk RNA sequencing, NSCLC ≥80 year had increased abundance of fibroblasts (1.09-fold, p<0.01), dendritic cells (1.07-fold, p<0.01) and macrophages (1.04-fold, p<0.01) in their TIME, while MEL ≥80 year patients had fewer infiltrating T-lymphocytes (0.82-fold, p<0.05) and dendritic cells (0.91-fold, p<0.05). There was no statistically significant age-related difference in the TIME composition of patients with RCC (figure 3).

DDR gene alterations analysis

Enrichment of DDR gene alterations among patients ≥80 year was distinct across cancer types. Compared with patients <80 year, patients ≥80 year with NSCLC and MEL had similar DDR gene mutation rates, except for *BRCA1* mutations which were more common in patients ≥80 year with MEL (2.10% vs 0.80% in <80 year; exploratory-p<0.05) (figure 4).

Glutamine and glucose metabolism-related gene expression profiling

Gene expression profiling of glutamine and glucose metabolism-related genes revealed increased expression of *SLC38A5* (1.17-fold; p<0.0001) and decreased *G6PC* expression (0.65-fold; p<0.01) in patients ≥80 year with NSCLC. While not statistically significant, patients ≥80 year with MEL and RCC had opposite trends for *SLC38A5* (0.96-fold and 0.77-fold, respectively) and *G6PC* expression (1.64-fold and 1.20-fold, respectively). Figure 5 details other glutamine and glucose metabolism-related genes and their respective fold changes in patients ≥80 year versus <80 year.

Gene set hierarchical clustering and survival analysis for patients ≥80 year

Given distinct expression profiles of IC and metabolism-related genes in patients ≥80 year across tumour types, we next performed a supervised hierarchical clustering analysis of both gene sets in this age group, across patients with NSCLC, RCC and MEL treated with ICIs for whom survival outcomes were available (n=1013) (figure 6). Despite

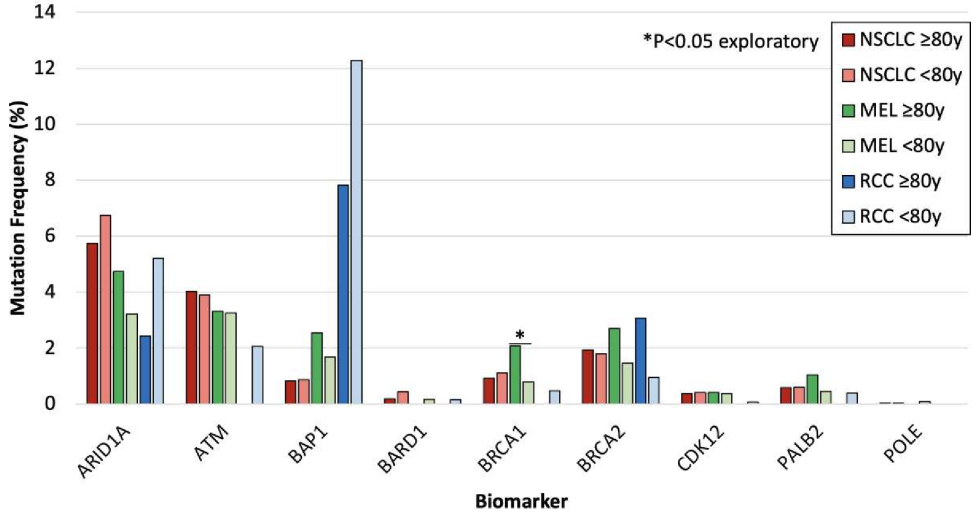

Figure 4 Comparison of DDR-related genes mutation frequency in patients <80 versus ≥80 year across different tumour types. The y-axis represents the per cent (%) mutation frequency in every age group, within specific histologies. DDR, DNA damage response; MM/MEL, melanoma; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

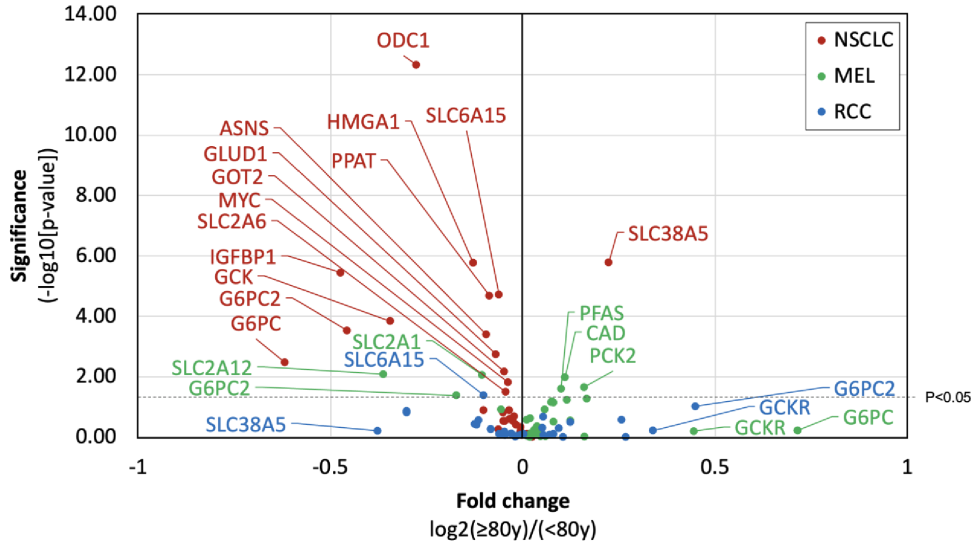

Figure 5 Volcano-plot depiction of fold-change in glutamine–glucose metabolism-related gene expression in patients <80 versus ≥80 year, across different tumour types. The dashed line represents the significance cut-off at p<0.05. MEL (green), melanoma; NSCLC (red), non-small cell lung cancer; RCC (blue), renal cell carcinoma. The x-axis reflects the Log2 fold change in gene expression between patients ≥80 year compared with <80 year (denominator). The y-axis depicts the change significance.

unique gene expression clusters within every tumour type, there were no statistically significant survival differences between the clusters (NSCLC: p=0.23; RCC: p=0.52; MEL: p=0.42). To identify potential specific patterns of gene expression in responders (vs non-responders), we subsequently performed an unsupervised differential gene clustering analysis between the two groups (segmented by median survival) for the gene sets of interest and across the three tumour types (figure 7). In patients with NSCLC, responders had significantly higher expression of certain IC genes (online supplemental table 1), notably IC receptors and ligands (*CTLA-4*, *LAG3*) and immune modulators/costimulatory molecules (*CD8B*, *IDO1*) (p<005). NSCLC responders also had higher expression

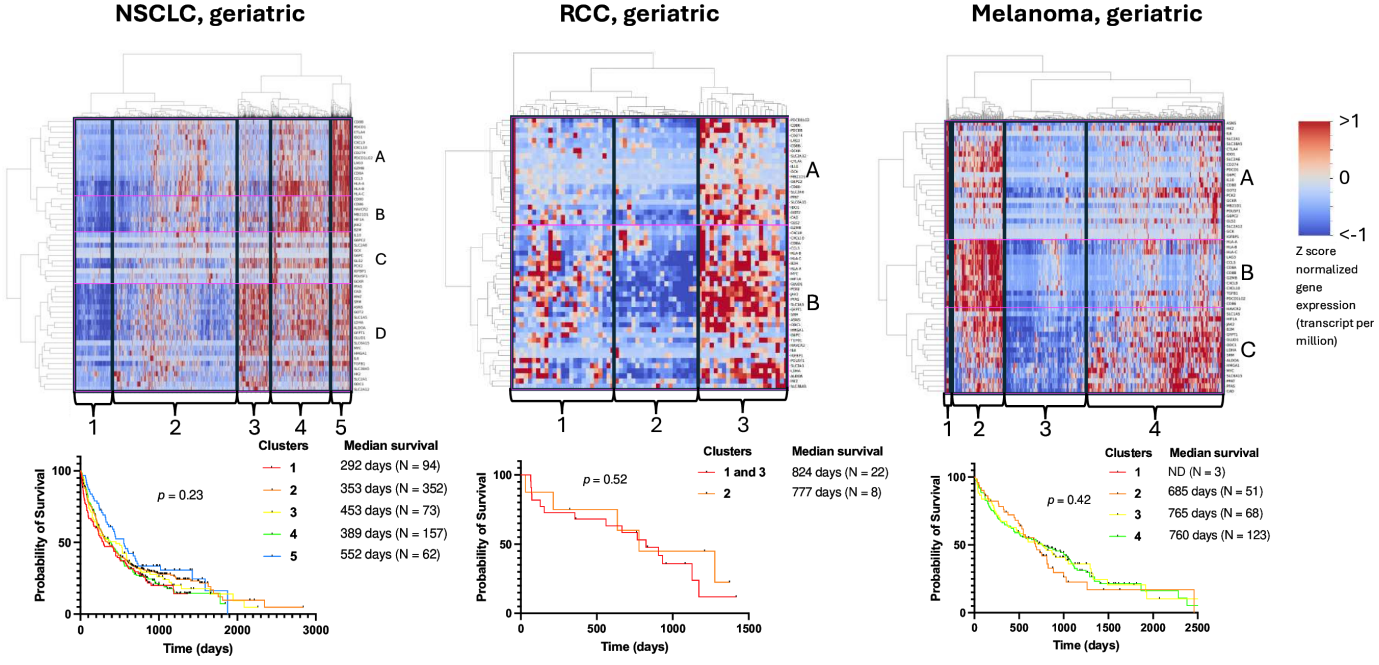

Figure 6 Hierarchical clustering of gene sets related to IC-related genes and glutamine–glucose metabolism-related genes in patients ≥80 year, across different tumour types. Gene expression (transcripts per million) was z-scored normalised. Heatmap rows represent genes and columns represent tumours. Heatmaps show hierarchical clustering of both genes and tumours. Column clusters are denoted by numbers (and black outlines) and row clusters by letters (and pink outlines). For tumours with outcomes data available, Kaplan-Meier curves show survival since the start of pembrolizumab, atezolizumab, nivolumab or ipilimumab for indicated clusters. NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

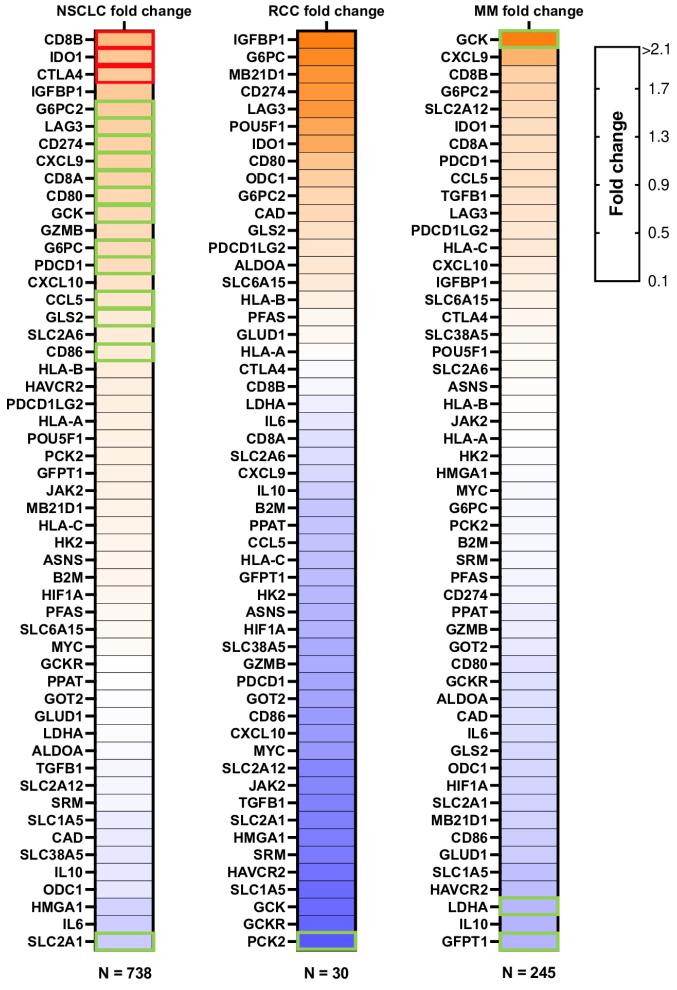

Figure 7 Differential gene expression analysis between responders and non-responders in patients ≥80 year, across different tumour types. Each tumour type was segmented as responders and non-responders, defined as ≥ or < median OS, respectively, since initiation of ICI (beginning of administration of pembrolizumab, atezolizumab, nivolumab or ipilimumab to last contact; NSCLC=348, RCC=764.5, MM=666 days). The fold change gene expression between responders and non-responders for the indicated genes and disease types are shown. A red box indicates a statistically significant difference (adjusted p<0.05). A green box indicates genes with a potential clinically relevant difference between both groups (p<0.05; adjusted p>0.05). IL, interleukin; MM, melanoma; NSCLC, non-small cell lung cancer; OS, overall survival; RCC, renal cell carcinoma.

of *GCK*, *GLS2* and *G6PC2* and lower expression of *SLC2A1* (exploratory-p<0.05). MEL and RCC responders did not have any significant change in the expression of any IC genes. MEL responders had higher expression of *GCK* (2.05-fold, exploratory-p=0.029) and lower expression of *LDHA* (0.75-fold; exploratory p=0.03) and *GFPT1* (0.74-fold; exploratory-p=0.031). After adjusting for multiple tests, these differences did not reach statistical significance (adjusted-p>0.05). RCC responders had lower expression of *PCK2* (0.51-fold; exploratory-p=0.038) with no other significant changes in the expression of any glutamine/glucose metabolism-related gene.

DISCUSSION

Determinants of response to ICIs in older patients with cancer remain poorly understood and biomarkers of ICI response in this population have not been explored before. Under-utilisation of genomic testing in this patient population could be a factor to explain this knowledge gap, although no published study has directly evaluated this aspect.[26 27]

Immunosenescence and inflammaging are inter-related biological processes that underlie ageing and carcinogenesis. They are hypothesised to share a common end product, immune suppression,[8 11 28] via dysregulation of cellular and humoral immunity[7 8] and modulation of the TIME composition.[13–16] In a recently published study, we showed that low levels of neutrophil-to-lymphocyte ratio (NLR) and systemic-immune-inflammatory index (SII) pre-ICI initiation were associated with significantly higher Objective response rates (ORR) in patients ≥80 year but not in younger patients and that low SII could independently predict improved response and survival in geriatric patients with cancer.[5]

In this current large-scale evaluation of patients ≥80 year, we show that this patient population has distinct molecular and cellular TIME associations, although in a cancer type-dependent fashion. NSCLC ≥80 year had an increased abundance of fibroblasts, dendritic cells and macrophages in their TIME. Macrophages play a proinflammatory role through the production of various cytokines and growth factors[29] and in the TIME promote the initiation and progression of human cancers, via M1 and M2 macrophages, respectively.[30 31] Similarly, fibroblasts in the TIME are potent inflammatory cells that enable chronic TIME inflammation via recruiting leukocytes.[32] They promote tumour growth, angiogenesis, invasion and metastasis by modulating the tumour extracellular matrix and exerting inhibitory effects on local dendritic cells that recruit and active T-cells antitumour immune responses.[33] Altogether, these findings suggest that in patients ≥80 year, proinflammatory changes in the TIME composition promote tumour progression. This is in line with our prior observation of a lower response rate to ICIs in patients ≥80 year with NLR-H and SII-H.[5] In contrast, patients ≥80 year with MEL had a significantly higher abundance of T-cell and cytotoxic T-lymphocytes in their TIME, with no significant changes in the abundance of the proinflammatory, tumour-promoting fibroblasts or macrophages. These differences—in the TIME across patients with NSCLC and MEL ≥80 year are also reflected in the differential frequency of TMB-H between both cancer types. These cellular and molecular associations suggest that older patients with MEL may have as good or even better responses to ICIs than younger patients when compared with NSCLC or RCC. Higher rates of primary resistance to ICIs in older patients with cancer have been observed in pivotal phase 3 clinical trials involving patients with NSCLC and RCC[34–37] but not in metastatic MEL. In patients with MEL, no age-associated response to these treatments has been observed and reported

clinical responses in subjects over 70 or 75 years of age have been comparable to younger subjects.[38–41] Notably, clinical responses have been reported in metastatic MEL after treatment with ICIs among patients over 90 years of age.[42 43] Thus, our findings provide cellular and molecular evidence suggestive of the biological explanation for clinical responses to ICIs in older patients with MEL.

A similar age-specific and histology-dependent pattern was also observed at the transcriptomic level of IC-related gene expression. Of significance, patients with NSCLC ≥80 year had a decreased expression of *IL-6* compared with patients<80 year, with an opposite trend for patients with MEL. IL-6 activates the acquired and innate immune response systems by promoting differentiation of naive CD4+T cells, modulation of inhibitory regulatory T-cells and differentiation of CD8+T cells into cytotoxic T-cells.[44] This age-specific and histology-dependent differential expression of *IL-6* suggests a molecular explanation for the above-reported clinical responses to ICIs in older patients with MEL. Lastly, patients ≥80 year with RCC had significantly lower expression of *GZMB* compared with patients <80 year. The *GZMB* gene encodes Granzyme B, a cytotoxic granule secreted by cytotoxic T lymphocytes and natural killer cells.[45] Lower expression of *GZMB* is likely a reflection of lower levels of CD8+T cells and NK cells in the TIME of patients ≥80 year with RCC. Along with a lower frequency of TMB-H and PD-L1+tumours, this potentially suggests a lower likelihood of response to ICIs in these patients. However, several reports support the clinical efficacy of ICIs in older adults with RCC (≥70 year),[46–48] suggesting the existence of different mechanisms of response to ICIs in patients with RCC than those described in this work. It is worth noting that despite many years of research, molecular profiling continues to be non-informative for the clinical management of the vast majority of patients with RCC, in contrast to NSCLC and MEL.

Metabolic changes in the glutamine pathway activation have also been linked to ageing and carcinogenesis.[19 21 24] Our results demonstrate distinct metabolic gene expression patterns in patients ≥80 year, across different tumour types. This suggests that, among older patients, different types of cancer capitalise on different sources of energy for their metabolic needs, with NSCLC relying more on glutamine metabolism, while MEL and RCC do not. Such observations have been linked to the differential expression of the MYC and MET oncoproteins.[23 49 50] The interaction between metabolic profiles and TIME remains to be explored.

Unlike previously published work,[18 51] our results did not reveal significant changes in the mutation frequency of DDR genes but revealed histology-specific patterns and a distinct enrichment of DDR gene alteration frequencies among patients ≥80 year. While the role of germline *BRCA1/2* variants in predisposition to MEL has been explored before (with controversial findings), somatic *BRCA* mutations in MEL have not been reported in the literature thus far. Our results, thus, highlight a new finding of significantly higher prevalence of somatic *BRCA1* mutations, in patients ≥80 year (vs <80 year; exploratory-p<0.05).

Given differences in IC-related and metabolism-related genes between patients <80 and ≥80 year, we next investigated the prognostic relevance of specific gene clusters within patients ≥80 year treated with ICIs and their potential impact on survival. Our supervised hierarchical gene clustering analysis identified distinct gene clusters across different tumour types (figure 6). While survival was overall not significantly different between clusters, they exhibited differences in clinical behaviour. For example, in patients with NSCLC, patients with gene cluster 1 had a median survival of 9.7 months (292 days) while those in cluster 5 had almost double the survival time with a median of 18.4 months (552 days). While differences in sample sizes could explain the lack of statistical significance, other factors need to be considered: first, the ageing factor itself, whereby ageing-related health comorbidities could play a dominant role in determining survival outcomes, thus negating the impact of the identified gene clusters. Second, in terms of the biological significance of these gene clusters, not all biological mechanisms directly translate into differences in survival outcomes. Gene expression represents one aspect of the intricate interplay of factors influencing cancer behaviour. Third, it may be that while genes may be clustered based on increased or decreased levels of expression, only the differential expression of a handful of genes within these clusters has functional implications and potential impact on survival in patients ≥80 year treated with ICIs. To address those concerns, we next performed an unsupervised differential gene analysis between responders and non-responders (using median survivals as cutoffs) for the gene sets of interest and across the three tumour types (figure 7). This approach identified unique gene expression patterns present exclusively in responders and again in a tumour-type specific fashion. For example, patients ≥80 year with NSCLC who responded to treatment had significantly higher expression of specific IC-related genes compared with non-responders. Similarly, geriatric NSCLC responders had a unique metabolic signature compared with non-responders (exploratory p-value<0.05), which, despite not reaching statistical significance when adjusting for multiple testing (q-values in online supplemental table 1), could carry potential clinical significance. Variance and heterogeneity between patients and the administered ICI-containing regimens could account for the loss of statistical significance after adjusting for multiple hypothesis testing. While no formal analysis was performed to compare the gene expression signature of responders between different tumour types, it appears that these immune and metabolic 'response signatures' are different in each tumour type (online supplemental table 1).

While our study provides valuable insights, it is important to acknowledge certain limitations. First, complete clinical data for the entire cohort of patients

(n=24 173) who underwent molecular analysis was not available. However, survival outcomes were accessible for 1013 patients ≥80 year, offering meaningful insights. Additionally, although the proportion of patients aged ≥80 years was relatively small, it is noteworthy that our study included a substantial cohort of this age group, with 3288 patients molecularly analysed and reported, representing the largest cohort in this demographic. The imbalance between age groups likely reflects the real-world context whereby a significantly larger proportion of patients who receive cancer care, including ICI therapy, are younger than 80 years.[52] Moreover, our cohort contained patients with NSCLC, MEL and RCC, three tumour types where ICIs are most commonly used. This molecular cohort is large enough to allow for reliable observations that pave the way for future investigations and discoveries in the field of age-associated biomarkers of response to ICI in older patients.

In conclusion, our exploratory analysis suggests differences in the ageing process across the three cancer types, as reflected by distinct molecular changes and composition of the immune tumour microenvironment. Our work further provides potential gene expression signatures of response to ICIs in patients ≥80 years. While a large body of literature provides a theoretical framework for our findings, our study is the first to exclusively investigate molecular and TIME cellular changes in patients with cancer at extremes of age and suggest potential immune and metabolic response signatures to ICIs, thus providing grounds for future biomarker discovery in this growing patient population. Our findings could become actionable in terms of refining a specific panel of biomarkers, for each of the described tumour types, that considers the underlying TIME and co-occurring alterations. For example, the clinical value of a limited biomarker panel for ICI response in MEL, containing BRCA1/2 somatic status, IL-6 expression, TIME cytotoxic T-cell composition and TMB levels could be explored. Dedicated studies focused on refining our findings could thus be carried out in the future.

MATERIAL AND METHODS

Patient samples

A total of 24 123 formalin-fixed paraffin-embedded (FFPE) samples from real-world patients with NSCLC (n=19 891), MEL (n=1333) and RCC (n=1333) were submitted for molecular profiling between 2015 and 2021 to a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Caris Life Sciences; Phoenix, Arizona, USA).

Information on therapy and outcomes was obtained through an honest broker tokenisation system, used to link insurance claims data and molecular data generated at Caris Life Sciences, such that all data remained irreversibly de-identified, in compliance with research use. De-identification was performed by a robust tokenisation process and the honest broker system was used to distribute the de-identified data between parties. Claims data included drug names and dates of billing for visits and treatments which were used to infer response to therapy.

DNA sequencing

Next-generation sequencing was performed on genomic DNA isolated from FFPE tumour samples using the NextSeq or NovaSeq platforms (Illumina, San Diego, California, USA) for a targeted gene panel or whole exome sequencing, respectively. The minimum purity allowed was 20%. A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, California, USA). All variants were detected with >99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of >500 and an analytic sensitivity to detect variants with a variant allele frequency of ≥5% for variant calling. Genetic variants identified were interpreted by board-certified molecular geneticists and categorised as 'pathogenic', 'presumed pathogenic', 'variant of unknown significance', 'presumed benign' or 'benign', according to the American College of Medical Genetics and Genomics standards. For analysis of mutation frequencies of individual genes, 'pathogenic' and 'presumed pathogenic' were counted as mutations. The DDR gene panel profile included *ARID1A*, *ATM*, *BAP1*, *BARD1*, *BRCA1*, *BRCA2*, *CDK12*, *PALB2* and *POLE*.

TMB was measured by counting all non-synonymous missense, non-sense, in-frame insertion/deletion and frameshift mutations found per tumour that had not been previously described as germline alterations according to the Database of Single Nucleotide Polymorphisms (dbSNP) and the 1000 Genomes Project (1KG) databases. TMB was adjusted by dividing by a factor of 1.2 to ensure that the fraction of TMB-H matched the observed published clinical data.[53] A cut-off of ≥10 mutations/MB was used based on the KEYNOTE-158 trial.[54]

RNA sequencing and gene expression profiling

For RNA sequencing, a minimum of 10% of tumour content in the area for microdissection from the FFPE sample was required to enable enrichment and extraction of tumour-specific RNA. Qiagen RNA FFPE tissue extraction kit was used for extraction and the RNA quality and quantity were determined using the Agilent TapeStation. Biotinylated RNA baits were hybridised to the synthesised/purified complementary DNA targets and the bait-target complexes were amplified in a post-capture PCR reaction. The Illumina NovaSeq 6500 was used to sequence the whole transcriptome from patients to an average of 60M reads. Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, California, USA) was used for fusion detection. Raw data were demultiplexed by Illumina Dragen Bio-IT accelerator, trimmed, counted, PCR-duplicates removed and aligned to human reference genome hg19 by STAR aligner. TPM (Transcripts Per Million Molecules) were generated using a Salmon aligner (1.10.2). The

IC-related gene panel included: *CXCL10, CXCL7, CCL5, CCL3, IL10, IL6, CGAS, CD8, CD3, CD274, LAG3, TIM3, CTLA4, IDO1, ICOS*, granzyme B (*GZMB*), human leucocyte antigen genes, beta-2 microglobulin *CD40, LAG3, IFN-γ*, TNF receptor superfamily member four gene (*TNFRSF4*, also known by the aliases *OX40* and *CD134*), TNF receptor superfamily member 9 gene (*4-1BB* and *CD137*), *HAVCR2, MB21D1, PPBP* and *BRD4*. The glutamine–glucose metabolism-related gene panel included: *MYC*, *HIF1A*, *HK2*, *LDHA*, *GOT2*, *PPAT*, *PFAS*, *GFPT1*, *CAD*, *GLS2*, *ASNS*, *ODC1*, *SRM*, *ALDOA*, *GLUD1*, *SLC1A5*, *SLC6A15*, *SLC38A5*, *SLC2A6*, *SLC2A1*, *SLC2A12*, *POU5F1*, *GCK*, *GCKR*, *PCK2*, *G6PC*, *IGFBP1*, *HMGA1* and *G6PC2*.

TIME analysis

The microenvironment cell population-counter was used to estimate the abundance of immune and stromal cell populations, as previously described.[55] Reported cell populations included: fibroblasts, myeloid dendritic cells, macrophage/monocyte, T cells, endothelial cells, neutrophils, natural killer cells, B-cells, cytotoxic lymphocytes and CD8+T cells. The relative abundance of cell populations was expressed as fold-change in patients ≥80 year versus <80 year for each of the cell populations described above.

Immunohistochemistry and PD-L1 assessment

Immunohistochemistry was performed on whole FFPE sections using automated staining techniques per the manufacturer's instructions. Staining was scored for intensity (0=no staining; 1+=weak staining; 2+=moderate staining; 3+=strong staining) and staining percentage (0–100%) by a board-certified pathologist. For NSCLC, positive expression of PD-L1 (22c3) on tumour cells was defined as tumour proportion score ≥1. For MEL and RCC, positive expression of PD-L1 (SP142; laboratory-developed test) on tumour cells was defined as ≥2+ stain intensity and ≥5% of cells stained.

Hierarchical clustering and outcomes data

Geriatric NSCLC, geriatric RCC or geriatric MEL tumours were clustered by the z-score normalised expression of the IC-related and the glutamine–glucose metabolism-related gene panels. Hierarchical clustering was done using the seaborn cluster map function (Ward's method, Euclidian distance metric). For tumours where data was available (n=1022), real-world overall survival was obtained from insurance claims and calculated from the start of treatment with pembrolizumab, atezolizumab, nivolumab or ipilimumab, to last contact. The Log-rank (Mantel-Cox) test was conducted, comparing the survival of the indicated clusters. We also performed a differential gene expression analysis between responders and non-responders, based on each group's median overall survival (mOS; responders≥mOS, vs non-responders<mOS).

Data availability

Raw data were generated at Caris Life Sciences (Caris Life Sciences; Phoenix, Arizona, USA) and are not publicly available due to commercial and legal restrictions but are available on reasonable request from the corresponding author. Processed data supporting the findings of this study are available from the corresponding author on request.

Statistical analysis

Patients were stratified into age subgroups of ≥80 and <80 year for comparison of DDR gene alterations, gene expression profiling (IC-related genes and glutamine–glucose metabolism-related genes) and TIME analysis. The prevalence of molecular alterations in different groups was compared using χ^2 or Fisher's exact tests. In addition, TMB and gene expression were compared among different cohorts using the Mann-Whitney U non-parametric test. P values were corrected for multiple comparisons using the Benjamini-Hochberg method to avoid type I error, unless noted as exploratory, with p values of<0.05 considered as significant differences. The Log-rank (Mantel-Cox) test was conducted to compare the survival of the different gene clusters.

Patients and public involvement

Patients were not involved in the research.

Author affiliations
[1]Department of Oncology, Barbara Ann Karmanos Cancer Institute, Detroit, Michigan, USA
[2]Caris Life Sciences, Phoenix, Arizona, USA
[3]Duke Cancer Institute, Durham, North Carolina, USA
[4]Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, Vermont, USA
[5]Medical Oncology, Brown University, Providence, Rhode Island, USA
[6]Dana-Farber Cancer Institute, Boston, Massachusetts, USA
[7]Georgetown University, Washington, Washington, USA
[8]Stephenson Cancer Center, University of Oklahoma, Oklahoma City, Oklahoma, USA
[9]Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA
[10]Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[11]Department of Medicine, UPMC Hillman Cancer Center, Pittsburgh, Pennsylvania, USA

X Khalil Choucair @KhalilChoucair8

Contributors KC wrote the first draft of the manuscript and all authors reviewed and edited the manuscript. The corresponding author (Anwaar Saeed) attests that all listed authors meet authorship criteria and that no authors meeting the criteria have been omitted. The lead author KC and the corresponding author Anwaar Saeed (guarantor) affirm that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted. KC, AE, LC and Anwaar Saeed contributed to study design and data interpretation. MJO, PW, AKS, Azhar Saeed, HM, DU, WSE-D, HB, SVL, CK, ARN and EL contributed to patients samples and information through the Caris database. KC, AE, LC and Anwaar Saeed contributed to data curation.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests Anwaar Saeed reports a leadership role with Autem therapeutics, Exelixis, KAHR medical and Bristol Myers Squibb; consulting or advisory board role with AstraZeneca, Bristol Myers Squibb, Merck, Exelixis, Pfizer, Xilio therapeutics, Taiho, Amgen, Autem therapeutics, KAHR medical and Daiichi Sankyo; institutional research funding from AstraZeneca, Bristol Myers Squibb, Merck, Clovis, Exelixis, Actuate therapeutics, Incyte Corporation, Daiichi Sankyo, Five prime therapeutics, Amgen, Innovent biologics, Dragonfly therapeutics, Oxford Biotherapeutics, Arcus therapeutics and KAHR medical; and participation as a data safety monitoring board chair for Arcus therapeutics. AKS reports research

funding (to institution) from Ascentage, Bristol Myers Squibb, IDEAYA, Immunocore, Merck, Olatec Therapeutics, Regeneron, Replimune and Seagen and consulting/advisory role for Bristol Myers Squibb, Iovance, Regeneron, Novartis and Pfizer. HM reports research funding (to the institution) from AstraZeneca and U CANCER VIVE Foundation and advisory roles for AstraZeneca, Genentech and Daiichi Sankyo. DU reports consulting and advisory roles with Daiichi Sankyo/AstraZeneca, AstraZeneca/Medimmune, Jazz Pharmaceuticals and Sanofi. HB has served as consultant/advisory board member for Janssen, Astellas, Merck, Pfizer, Roche, Harpoon, Amgen, Bayer, Daiichi Sankyo, Sanofi Genzyme, AstraZeneca and has received research funding (to institution) from Bristol Myers Squibb, Circle Pharma, Daiichi Sankyo and Novartis. CK reports research funding (to institution) from AstraZeneca, Novartis, Regeneron, Janssen, Genentech, Lyell, Daiichi Sankyo, Gilead, Macrogenics, Boehringer Ingelheim, Black Diamond Therapeutics and consulting fees from Arcus, AstraZeneca, Daiichi Sankyo, Eisai, Regeneron, Sanofi, Takeda, J&J and Pinetree. ARN reports research funding (to institution) from Loxo@Lilly, Surface Oncology, ADC Therapeutics, IGM Biosciences, EMD Serono, Aravive, Nikang Therapeutics, Inspirna, Exelexis, Revolution Medicine, Jacobio, Pionyr, Jazz Pharmaceuticals, NGM Biopharmaceuticals, Immunophotonics, Selexine, consultant editor compensation from JCO Precision Oncology, travel compensation from SITC/AACR/Conquer Cancer, Foundation/BinayTara Foundation and Foundation Med/Caris Life Sciences, Jazz Pharmaceuticals and grants from SWOG Hope Foundation, FDA Broad Agency Contract, with advisory Board and Speaking Engagement for Foundation Med. AE, MJO and PW are employees of Caris Life Sciences. All other authors declare no potential conflict of interest.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The present study was conducted in accordance with the guidelines of the Declaration of Helsinki, the Belmont Report and the US Common Rule. In compliance with policy 45 CFR 46.101(b), this study was conducted using retrospective, de-identified clinical data, patient consent was not required and the study was considered IRB-exempt.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Raw data were generated at Caris Life Sciences (Caris Life Sciences; Phoenix, Arizona, USA) and are not publicly available due to commercial and legal restrictions but are available upon reasonable request from the corresponding author. Processed data supporting the findings of this study are available from the corresponding author upon request.

ORCID iD

Khalil Choucair http://orcid.org/0000-0001-8145-3754

REFERENCES

1 Singh H, Kanapuru B, Smith C, *et al*. FDA analysis of enrollment of older adults in clinical trials for cancer drug registration: A 10-year experience by the U.S. Food and Drug Administration. *JCO* 2017;35:10009.
2 Nebhan CA, Cortellini A, Ma W, *et al*. Clinical Outcomes and Toxic Effects of Single-Agent Immune Checkpoint Inhibitors Among Patients Aged 80 Years or Older With Cancer: A Multicenter International Cohort Study. *JAMA Oncol* 2021;7:1856–61.
3 Martins F, Sofiya L, Sykiotis GP, *et al*. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol* 2019;16:563–80.
4 Wang DY, Salem J-E, Cohen JV, *et al*. Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis. *JAMA Oncol* 2018;4:1721–8.
5 Choucair K, Nebhan C, Cortellini A, *et al*. Characterization of Age-Associated, Neutrophil-to-Lymphocyte Ratio (NLR) and Systemic Immune-Inflammatory Index (SII) as Biomarkers of Inflammation in Geriatric Patients with Cancer Treated with Immune Checkpoint Inhibitors: Impact on Efficacy and Survival. *Cancers (Basel)* 2023;15:5052.
6 Nishijima TF, Muss HB, Shachar SS, *et al*. Comparison of efficacy of immune checkpoint inhibitors (ICIs) between younger and older patients: A systematic review and meta-analysis. *Cancer Treat Rev* 2016;45:30–7.
7 Fulop T, Le Page A, Fortin C, *et al*. Cellular signaling in the aging immune system. *Curr Opin Immunol* 2014;29:105–11.
8 Franceschi C, Bonafè M, Valensin S. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. *Vaccine (Auckl)* 2000;18:1717–20.
9 Wyczalkowska-Tomasik A, Czarkowska-Paczek B, Zielenkiewicz M, *et al*. Inflammatory Markers Change with Age, but do not Fall Beyond Reported Normal Ranges. *Arch Immunol Ther Exp* 2016;64:249–54.
10 Franceschi C, Bonafè M, Valensin S, *et al*. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. *Ann N Y Acad Sci* 2000;908:244–54.
11 Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014;69 Suppl 1:S4–9.
12 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
13 Llosa NJ, Cruise M, Tam A, *et al*. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015;5:43–51.
14 Kugel CH 3rd, Douglass SM, Webster MR, *et al*. Age Correlates with Response to Anti-PD1, Reflecting Age-Related Differences in Intratumoral Effector and Regulatory T-Cell Populations. *Clin Cancer Res* 2018;24:5347–56.
15 Ramirez AG, Wages NA, Hu Y, *et al*. Defining the effects of age and gender on immune response and outcomes to melanoma vaccination: a retrospective analysis of a single-institution clinical trials' experience. *Cancer Immunol Immunother* 2015;64:1531–9.
16 Grotz TE, Vaince F, Hieken TJ. Tumor-infiltrating lymphocyte response in cutaneous melanoma in the elderly predicts clinical outcomes. *Melanoma Res* 2013;23:132–7.
17 Ribezzo F, Shiloh Y, Schumacher B. Systemic DNA damage responses in aging and diseases. *Semin Cancer Biol* 2016;37–38:26–35.
18 Reinhardt HC, Schumacher B. The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends Genet* 2012;28:128–36.
19 Brewer RA, Gibbs VK, Smith DL. Targeting glucose metabolism for healthy aging. *Nutr Healthy Aging* 2016;4:31–46.
20 Shaw RJ. Glucose metabolism and cancer. *Curr Opin Cell Biol* 2006;18:598–608.
21 Wittig R, Coy JF. The Role of Glucose Metabolism and Glucose-Associated Signalling in Cancer. *Perspect Medicin Chem* 2007;1.
22 Meynial-Denis D. Glutamine metabolism in advanced age. *Nutr Rev* 2016;74:225–36.
23 Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer* 2016;16:619–34.
24 Li T, Le A. Glutamine Metabolism in Cancer. *Adv Exp Med Biol* 2018;1063:13–32.
25 Erbe R, Wang Z, Wu S, *et al*. Evaluating the impact of age on immune checkpoint therapy biomarkers. *Cell Rep* 2021;36:109599.
26 Foulkes WD, Knoppers BM, Turnbull C. Population genetic testing for cancer susceptibility: founder mutations to genomes. *Nat Rev Clin Oncol* 2016;13:41–54.
27 Waltz M, Cadigan RJ, Prince AER, *et al*. Age and perceived risks and benefits of preventive genomic screening. *Genet Med* 2018;20:1038–44.
28 Nikolich-Žugich J. The twilight of immunity: emerging concepts in aging of the immune system. *Nat Immunol* 2018;19:10–9.
29 Fujiwara N, Kobayashi K. Macrophages in Inflammation. *CDTIA* 2005;4:281–6.
30 Salmaninejad A, Valilou SF, Soltani A, *et al*. Tumor-associated macrophages: role in cancer development and therapeutic implications. *Cell Oncol* 2019;42:591–608.

31 Duan Z, Luo Y. Targeting macrophages in cancer immunotherapy. *Sig Transduct Target Ther* 2021;6:127.
32 Wei K, Nguyen HN, Brenner MB. Fibroblast pathology in inflammatory diseases. *J Clin Invest* 2021;131:e149538.
33 Ghorbaninezhad F, Asadzadeh Z, Masoumi J, *et al*. Dendritic cell-based cancer immunotherapy in the era of immune checkpoint inhibitors: From bench to bedside. *Life Sci* 2022;297:120466.
34 Borghaei H, Paz-Ares L, Horn L, *et al*. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* 2015;373:1627–39.
35 Brahmer J, Reckamp KL, Baas P, *et al*. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* 2015;373:123–35.
36 Motzer RJ, Tannir NM, McDermott DF, *et al*. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med* 2018;378:1277–90.
37 Landre T, Des Guetz G, Chouahnia K, *et al*. Immune Checkpoint Inhibitors for Patients Aged ≥ 75 Years with Advanced Cancer in First- and Second-Line Settings: A Meta-Analysis. *Drugs Aging* 2020;37:747–54.
38 Betof AS, Nipp RD, Giobbie-Hurder A, *et al*. Impact of Age on Outcomes with Immunotherapy for Patients with Melanoma. *The Oncol* 2017;22:963–71.
39 Rai R, McQuade JL, Wang DY, *et al*. Safety and efficacy of anti-PD-1 antibodies in elderly patients with metastatic melanoma. *Ann Oncol* 2016;27:vi381.
40 Robert C, Long GV, Brady B, *et al*. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372:320–30.
41 Chiarion Sileni V, Pigozzo J, Ascierto PA, *et al*. Efficacy and safety of ipilimumab in elderly patients with pretreated advanced melanoma treated at Italian centres through the expanded access programme. *J Exp Clin Cancer Res* 2014;33:30.
42 Johnpulle RAN, Conry RM, Sosman JA, *et al*. Responses to immune checkpoint inhibitors in nonagenarians. *Oncoimmunology* 2016;5:e1234572.
43 Ibrahim T, Mateus C, Baz M, *et al*. Older melanoma patients aged 75 and above retain responsiveness to anti-PD1 therapy: results of a retrospective single-institution cohort study. *Cancer Immunol Immunother* 2018;67:1571–8.
44 Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014;6:a016295.
45 Tibbs E, Cao X. Emerging Canonical and Non-Canonical Roles of Granzyme B in Health and Disease. *Cancers (Basel)* 2022;14:1436.
46 Nemoto Y, Ishihara H, Nakamura K, *et al*. Efficacy and safety of immune checkpoint inhibitors in elderly patients with metastatic renal cell carcinoma. *Int Urol Nephrol* 2022;54:47–54.
47 Araujo DV, Wells JC, Hansen AR, *et al*. Efficacy of immune-checkpoint inhibitors (ICI) in the treatment of older adults with metastatic renal cell carcinoma (mRCC) - an International mRCC Database Consortium (IMDC) analysis. *J Geriatr Oncol* 2021;12:820–6.
48 Hermansen CK, Donskov F. Outcomes based on age in patients with metastatic renal cell carcinoma treated with first line targeted therapy or checkpoint immunotherapy: Older patients more prone to toxicity. *J Geriatr Oncol* 2021;12:827–33.
49 Yuneva MO, Fan TWM, Allen TD, *et al*. The metabolic profile of tumors depends on both the responsible genetic lesion and tissue type. *Cell Metab* 2012;15:157–70.
50 Bott AJ, Peng I-C, Fan Y, *et al*. Oncogenic Myc Induces Expression of Glutamine Synthetase through Promoter Demethylation. *Cell Metab* 2015;22:1068–77.
51 Burgess RC, Misteli T, Oberdoerffer P. DNA damage, chromatin, and transcription: the trinity of aging. *Curr Opin Cell Biol* 2012;24:724–30.
52 White MC, Holman DM, Boehm JE, *et al*. Age and cancer risk: a potentially modifiable relationship. *Am J Prev Med* 2014;46:S7–15.
53 Chan TA, Yarchoan M, Jaffee E, *et al*. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* 2019;30:44–56.
54 Marabelle A, Le DT, Ascierto PA, *et al*. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair–Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *JCO* 2020;38:1–10.
55 Becht E, Giraldo NA, Lacroix L, *et al*. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol* 2016;17:218.