School of Medicine

Department of Translational Medicine

Master's Degree in Medical Biotechnologies

The role of MDSCs and G8 assessment in elderly haematological patients enrolled in the ONCO-AGING clinical trial (NCT04478916)

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List of Abbreviations

BMI	Body Mass Index
CD	Cluster of Differentiation (e.g., CD3, CD14)
CGA	Comprehensive Geriatric Assessment
CK2	Casein Kinase 2
CRP	C-Reactive Protein
DCs	Dendritic Cells
DDR	DNA Damage Response
EORTC QLQ-C30	European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire Core
G8	Geriatric Screening Tool
HRQoL	Health-Related Quality of Life
iNOS	Inducible Nitric Oxide Synthase
IL	Interleukin
MDSCs	Myeloid-Derived Suppressor Cells
M-MDSCs	Monocytic Myeloid-Derived Suppressor Cells
NF-κB	Nuclear Factor Kappa-light-chain-enhancer of Activated B cells
NKT	Natural Killer T cells
OS	Overall Survival
PBMC	Peripheral Blood Mononucleated Cells
PD-1	Programmed Death-1
PD-L1	Programmed Death-Ligand 1

PFS	Progression Free Survival
PMN-MDSCs	Polymorphonuclear Myeloid-Derived Suppressor Cells
PRO	Patient-Reported Outcome
RT + CT	Radiation Therapy + Chemotherapy
SASP	Senescence-Associated Secretory Phenotype
STAT	Signal Transducer and Activator of Transcription
TCR	T Cell Receptor
TGF-β	Transforming Growth Factor Beta
Tregs	Regulatory T cells
VEGF	Vascular Endothelial Growth Factor

Summary

Age is considered one of the most important risk factors for many types of solid and hematological malignancies, as their incidence increases with age in parallel to the ever-growing elderly population. Moreover, cancer incidence is constantly increasing as a consequence of the increase in life. Therefore, it is critical to understand how aging processes and the biological role of Myeloid derived suppressor cells, may promote cancer growth and quality of life in elderly patients with hematological malignancies. Additionally, geriatric assessment has been increasingly recognized as a predictive and prognostic instrument to detect frailty in older adults with cancer. In particular, the G8 score is a simple and reproducible instrument to identify elderly patients who should undergo full geriatric evaluation. Due to their frailty, elderly patients may often be under-treated and a therapeutic choice also based on a comprehensive geriatric assessment (CGA) is recommended. We finally evaluated the role of G8 assessment in terms of quality of life using EORTC QLQ-C30 in patients with hematological malignancies or solid tumors.

In a monocentric cohort, 140 patients with cancer aged > 65 years, candidates to target directed agents or to RT + CT treatments are screened for frailty by the G8 test, 129 samples were collected at baseline for MDSC.

By univariate analysis we demonstrated that i) after a median follow-up of 24 months the percentages levels of M-MDSC and PMN-MDSCs were not associated with different clinical outcomes in terms of PFS and OS; iii) after a median follow-up of 24 months the G8 assessment with a median of 11 was associated with poor clinical outcomes in terms of OS. The use of G8

clinical assessment to assess the potential prognostic impact of quality of life is expected to contribute to the individualized care of elderly patients, resulting in a fine tuning of the therapeutic strategies.

Introduction

Ageing and Cellular Senescence

Ageing is a phenomenon experienced by most biological organisms, characterized by a gradual decline in function and an increased incidence of cancer. Williams hypothesized that ageing was a consequence of natural selection, suggesting that genes promoting early-life survival would also lead to late-life debility; he coined the concept of antagonistic pleiotropy[1].

Age-related degeneration gives rise to many well-known pathologies, including heart failure, osteoporosis, and neurodegeneration[2]. In multicellular organisms, ageing can also lead to genetic mutations that enable cells to proliferate inappropriately, colonize new tissues, and migrate, among other abnormalities[3]. Cancer is one of these ageing-related diseases despite its various and differing manifestations.

Recently, an increasing amount of evidence has connected numerous degenerative and hyperplastic age-related diseases to cellular senescence.

Cellular senescence is the irreversible cessation of cell proliferation in response to oncogenic stresses, there are no known physiological stimuli capable of stimulating senescent cells to reenter the cell cycle, although certain molecular biological manipulations can cause them to proliferate[4]. Cellular senescence was first described by Hayflick, who demonstrated that human fibroblasts do not proliferate indefinitely in culture, they instead had a finite replicative lifespan followed by an ageing stage[5]. The number of divisions that cells complete before reaching the end of their replicative lifespan is termed the Hayflick limit. This limit is defined by the finite

replicative lifespan of somatic cells, which is determined by telomere shortening with each division as cells replicate until they reach a critical length[6]. Telomeres shorten because the DNA replication machinery cannot fully copy the ends of linear DNA molecules and requires a labile primer for replication. In the absence of telomerase activity, which can replenish telomeric DNA, cells eventually exhaust their telomeres and trigger a DNA damage response (DDR). This damage response leads to activation of the tumor suppressor protein p53, halting cell division to prevent genomic instability[7]. When cells reach this state of irreversible growth arrest due to telomere shortening, they enter a state known as senescence - characterized by an inability to further divide. Senescent cells may accumulate in tissues over time, contributing to ageing and age-related diseases[8].

Cellular senescence can be triggered by telomere dysfunction, genomic damage, strong mitogenic signals, or epigenomic perturbations[9]. Senescent cells are identified by their permanent growth arrest; therefore, they lack proliferation markers; however, this alone is insufficient for identification. Senescent cells are often enlarged and adopt a flattened morphology if adherent - sometimes doubling the volume of somatic cells[10]. A commonly used marker for senescent cells is the detection of senescence-associated β-galactosidase[4], which is overexpressed in acidic lysosomes even at near-neutral pH. This staining has been instrumental in demonstrating the accumulation of senescent cells with age in tissues. Another widely utilized marker is the p16INK4a tumor suppressor protein whose expression increases in senescent cells induced by various stimuli compared to its low levels in normal cells[11].

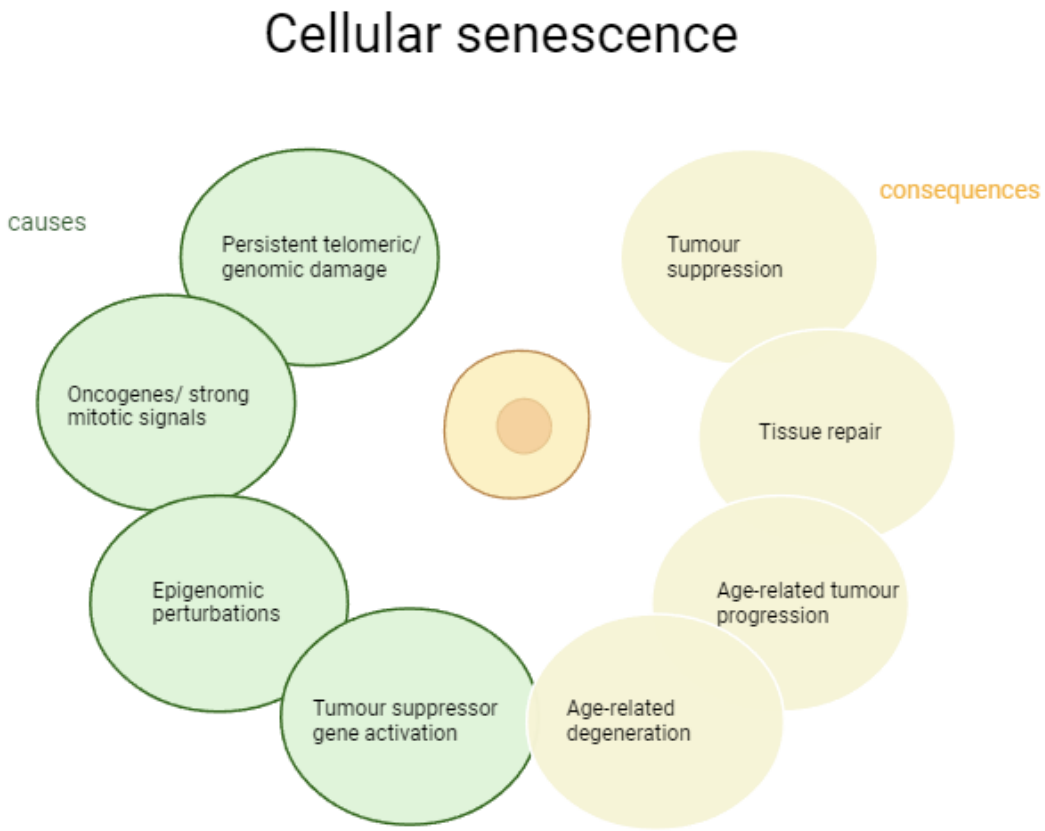

Figure 1: Cellular senescence is induced by various factors that can potentially lead to cancer, such as DNA damage (at telomeres or other genomic locations), strong signals promoting cell division (including those from activated oncogenes), epigenomic disruptions, and abnormal expression of certain tumor suppressor genes. The consequences of cellular senescence are complex: the growth arrest associated with senescence can prevent tumor formation, some characteristics of senescent cells can facilitate tissue repair, yet paradoxically, traits of senescent cells can also promote cancer development and accelerate the progression of age-related degenerative diseases[4].

Senescence may have evolved partly to suppress cancer development, since it is regulated and maintained by the p53/p21 and p16INK4a/pRB tumour suppressor pathways, acting as a barrier to malignant tumorigenesis[12]. Senescent cells exhibit modified chromatin organization, leading to changes in gene expression, including secretion of numerous proinflammatory cytokines, chemokines, growth factors, and proteases, collectively known as the senescence-associated secretory phenotype (SASP)[4].

The SASP represents a key feature of senescent cells with profound implications for ageing and age-related diseases. It encompasses a diverse array of cytokines, chemokines, growth factors, and proteases, which can have both beneficial and deleterious effects on tissues[13]. Certain components of the SASP stimulate cell proliferation, such as growth-regulated oncogenes (GROs) and amphiregulin, while others promote new blood vessel formation, including vascular endothelial growth factor (VEGF)[4]. However, the SASP also includes proteins with complex effects, like secreted frizzled related protein 1 (SFRP1)[14], IL-6 and IL-8, which can either stimulate or inhibit cell proliferation depending on context[15]. Importantly, many SASP factors promote inflammation directly or indirectly, contributing to chronic inflammation locally and possibly systemically[16]. This chronic inflammation is a major driver of age-related diseases, including various degenerative and hyperplastic conditions. The SASP is a plastic phenotype, meaning its composition can vary among cell types and in response to different stimuli. However, proinflammatory cytokines are consistently present, suggesting a fundamental role in senescence[17].

The SASP primarily arises in cells experiencing genomic damage or epigenomic perturbations, rather than simply through overexpression of certain proteins like p21 or p16INK4a. The DDR proteins ATM, NBS1, and CHK2 positively regulate the SASP, particularly after persistent DDR signaling has been established[18]. Additionally, transcription factors NF-κB and C/EBP-β also contribute to SASP regulation[19].

Interestingly, while p53 restrains the SASP, its inactivation leads to a hyperincrease in SASP factors, even in cells that resume proliferation[20]. Such cells pose a significant risk for malignant transformation due to their SASP expression and genomic instability.

The SASP has a powerful pro-regenerative activity which suggests that cellular senescence most likely evolved both to suppress cancer development and promote tissue repair in response to injury with its paracrine activity having varying effects based on context[4]. The SASP represents a complex network of signaling molecules with diverse effects on cell proliferation, tissue repair, and inflammation. While some SASP factors may promote tissue regeneration, others may contribute to age-related pathologies[21]. Understanding the dual role of cellular senescence and the SASP in cancer prevention, tissue repair, and ageing-related processes is essential for developing therapeutic strategies to target senescent cells and mitigate their deleterious effects while harnessing their potential benefits in tissue homeostasis[22].

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a sub-population of immature myeloid cells that can suppress the immune system[23]. They are generated in the bone marrow and in pathological conditions such as inflammation or cancer. MDSCs play a crucial role in immune evasion by inhibiting the activity of T cells, natural killer (NK) cells and dendritic cells (DCs)[24]. MDSCs play a pivotal role in modulating immune responses and are typically divided into two main groups: granulocytic polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) and monocytic myeloid-derived suppressor cells (M-MDSCs)[25]. PMN-MDSCs share similarities with

neutrophils, while M-MDSCs resemble monocytes. In cancer, PMN-MDSCs are typically the most common since they can constitute over 80% of all MDSCs, while M-MDSCs make up the remainder[26]. These cells are primarily found in bone marrow, peripheral blood, spleen, and tumors in various organs[27].

We know of multiple markers that are commonly used to identify MDSCs, we can divide them into three categories: those applicable to all MDSC subsets, those specific to granulocytic PMN-MDSCs and those for the monocytic MDSCs subsets. Markers applicable to all MDSCs include CD11b, Gr-1 (Ly6G/Ly6C), CD33, HLA-DR, CD124 (IL-4Rα), CD115 (CSF1R), CD80/CD86, and Nitric oxide synthase (iNOS)[28]. For PMN-MDSCs, CD15 serves as a specific marker, while M-MDSCs can be identified using CD14[29] (Figure 2).

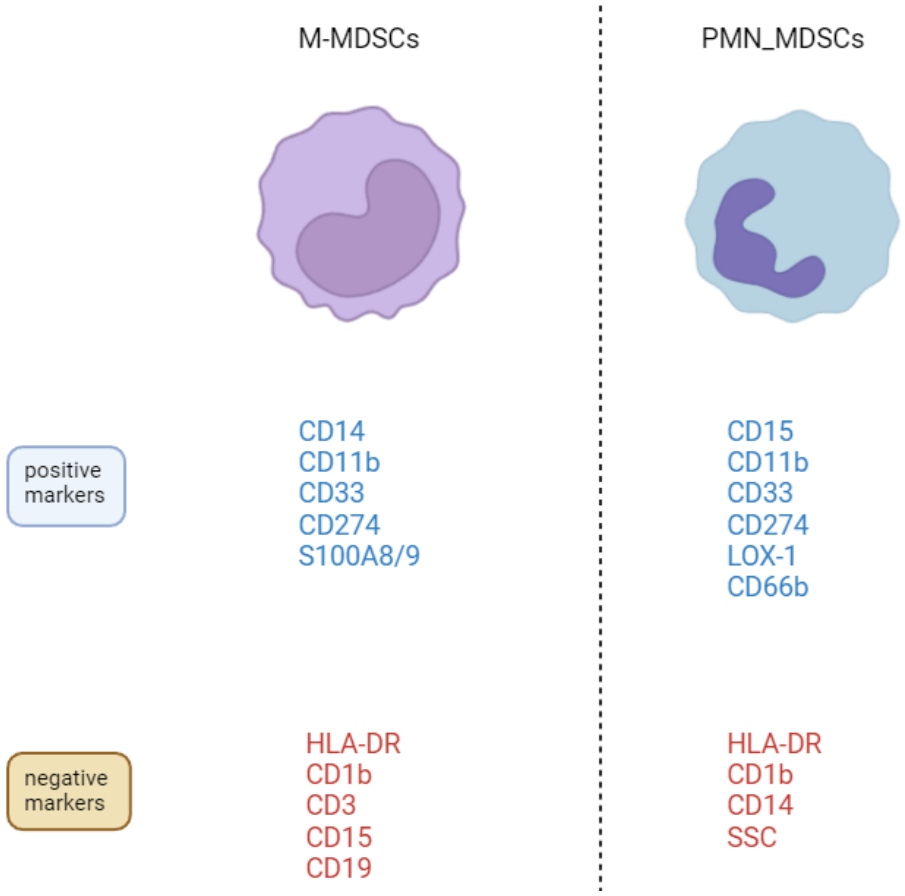

Figure 2: Myeloid-derived suppressor cell (MDSC) subtypes and characteristics. MDSCs have 2 subtypes: M-MDSCs, PMN-MDSCs. CD11b is expressed in all subtypes. M- and PMN-MDSCs are positive for CD14 and CD15, respectively[29].

MDSCs induce immune suppression primarily using molecules such as arginase, inducible nitric oxide synthase (iNOS), TGF-β, IL-10, COX2, indoleamine 2,3-dioxygenase (IDO) or by using mechanisms like cysteine sequestration and L-selectin expression reduction in T cells[26]. MDSCs can induce immunosuppression also through programmed death-ligand 1 (PD-L1)-mediated cellular contact, PD-L1 binds to PD-1 receptors on T cells, suppressing their function[30]. Predictably, the transcriptional regulation of MDSCs involves many signaling pathways and transcription factors that influence their function and expansion. Notch signaling plays a crucial role in the

accumulation of immature myeloid cells such as MDSCs[31]. Activation of Notch signaling can promote the expansion of MDSC populations by driving the differentiation of myeloid precursors into MDSCs rather than mature myeloid cells[32], such as dendritic cells or macrophages. Notch signaling can enhance the immunosuppressive function of MDSCs[31]. Notch activation in MDSCs can upregulate the expression of immunosuppressive molecules such as arginase-1, iNOS, and TGF-β, contributing to the inhibition of T cell responses and immune evasion by tumors[33]. Disruption of Notch transcriptional activity, mediated by abnormal enzymatic activity of casein kinase 2 (CK2), has been observed in tumor-derived MDSCs[34].

STAT proteins, particularly STAT3, regulate the expansion and immune suppressive activity of MDSCs upon activation by tumor-derived factors such as interleukin 6 (IL-6) and interleukin 10 (IL-10)[35]. STAT3 enhances MDSC proliferation and survival by upregulating various proteins involved in cell cycle progression and survival[36]. Additionally, STAT3 activation promotes the production of immunosuppressive factors like reactive oxygen species (ROS) and inhibits dendritic cell differentiation, therefore enhancing MDSC-mediated immune suppression[37]. STAT1 and STAT6 are also involved in promoting immune suppression. STAT1 mediates nitric oxide-induced immune suppression, while STAT6 signaling enhances the expression of immunosuppressive factors like arginase 1 and TGFβ in MDSCs and macrophages[35].

C/EBPβ regulates emergency granulopoiesis induced by cytokines and infections and is critical for the immunosuppressive behaviour exhibited by tumour-induced and bone marrow-derived MDSCs[38]. Loss of C/EBPβ reduces the expression of key immunosuppressive factors like arginase-1 and nitric oxide synthase (NOS), leading to reduced MDSC-mediated immune suppression and tumor growth[39].

MDSCs, chronic inflammation and cancer

Ageing is accompanied by a chronic, low-grade inflammatory state[40] and SASP contributes to fueling this state, which occurs when there is heightened inflammatory activity without overt infection or injury[41]. As we have previously discussed, inflammageing is characterized by increased levels of pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha (TNF-α), as well as acute-phase reactants like C-reactive protein (CRP)[42] (Figure 3). This inflammatory state is believed to arise from dysregulation of the immune system, particularly the innate immune system, and it is thought to contribute to the development and progression of age-related diseases, including cardiovascular disease, neurodegenerative disorders, and cancer[43]. This chronic inflammatory state is thought to result from a combination of factors, including cellular senescence, mitochondrial dysfunction, and activation of the immune system in response to various stressors over a lifetime[44].

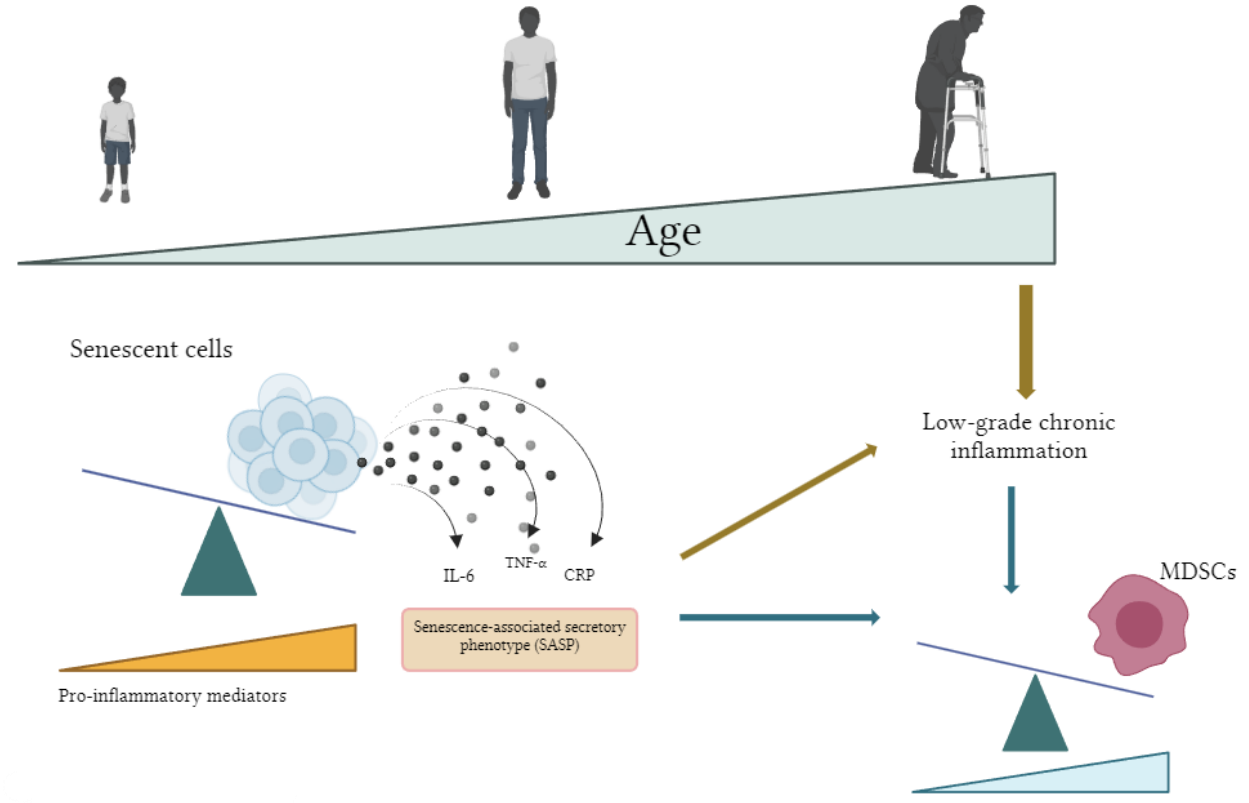

Figure 3: The connection between MDSCs, aging, and inflammation. With age, the number of senescent cells increases, and these senescent cells produce pro-inflammatory factors including IL-6, TNF-, or C- reactive protein (CRP), leading to the senescence-associated secretory phenotype (SASP)[41].

It has been found that MDSCs in healthy individuals and in conditions like pregnancy and obesity can have beneficial effects[45][46]. There's still ongoing research into the involvement of MDSCs in the ageing process, particularly in relation to inflammageing. This research suggests that elevated MDSC levels induced by inflammageing may contribute to immunosenescence and myeloid skewing observed in ageing[41].

As we know more than 50% of cancer patients are over 65 years old[47] but most of the literature that discusses the relationship between MDSCs and cancer focuses on lung, colorectal, prostate, and breast cancer[48], which are the most common tumors in developed countries. This literature

indicates an increased MDSC presence in the elderly suggesting heightened immune suppression favoring tumor development. Regardless, it is difficult to differentiate whether the increase in the presence and immune-suppressive action of MDSCs is caused by ageing or by the tumour itself[49].

MDSCs contribute to tumor progression by suppressing tumor-specific T cell responses, stimulating tumor angiogenesis, or facilitating tumor cell metastasis. The impaired efficacy of various cancer therapies has been attributed, at least in part, to MDSC accumulation and their contribution to an immunosuppressive microenvironment[29].

MDSCs and Immunosenescence

Immunosenescence denotes the gradual weakening of the immune system with age, impacting both adaptive and innate immunity. This decline encompasses reduced quantities of specific T and B cells, compromised T cell signaling, and diminished antibody production (Figure 4). Immunosenescence can undermine vaccine efficacy, heighten vulnerability to infections, and weaken the body's ability to combat cancer[50]. Similar immune deficiencies occur in inflammageing, where persistent inflammation further dampens immune responses. It is yet unclear whether immunosenescence arose as a significant defense mechanism against inflammageing or a detrimental consequence of it. Since inflammation is a consequence of ageing and not its original cause it is reasonable to think that inflammageing may cause the immune system to adapt to cope with the ageing microenvironment[51]. MDSCs play a pivotal role

in maintaining an immunosuppressive microenvironment, particularly in inflammatory conditions like cancer. They interact with other immunosuppressive cells such as regulatory T cells (Tregs) and regulatory B cells (Bregs) to establish an immunosuppressive milieu[50]. Active Tregs suppress the proliferation of T cells, while also stimulating the expansion and immunosuppressive activities of MDSCs, thus creating a positive feedback loop[52]. On the other hand, active Bregs can produce anti-inflammatory cytokines like IL-10 and TGF-β which inhibit immune reactions mediated by T helper type 1 (Th1 cells) and prevent autoimmune diseases[53]. Some Breg subsets have also been shown to be able to convert $CD4^+$ T cells into Tregs[54]. Ageing is also correlated to increased myelopoiesis and reduced lymphopoiesis, leading to MDSC accumulation and heightened immunosuppression[50]. While the exact relationship between immunosenescence and inflammation remains ambiguous, it is evident that ageing actively reshapes the immune system. In elderly patients, factors related to immunosenescence can influence the effectiveness of cancer treatment[55], underscoring the significance of comprehensive health assessments prior to treatment.

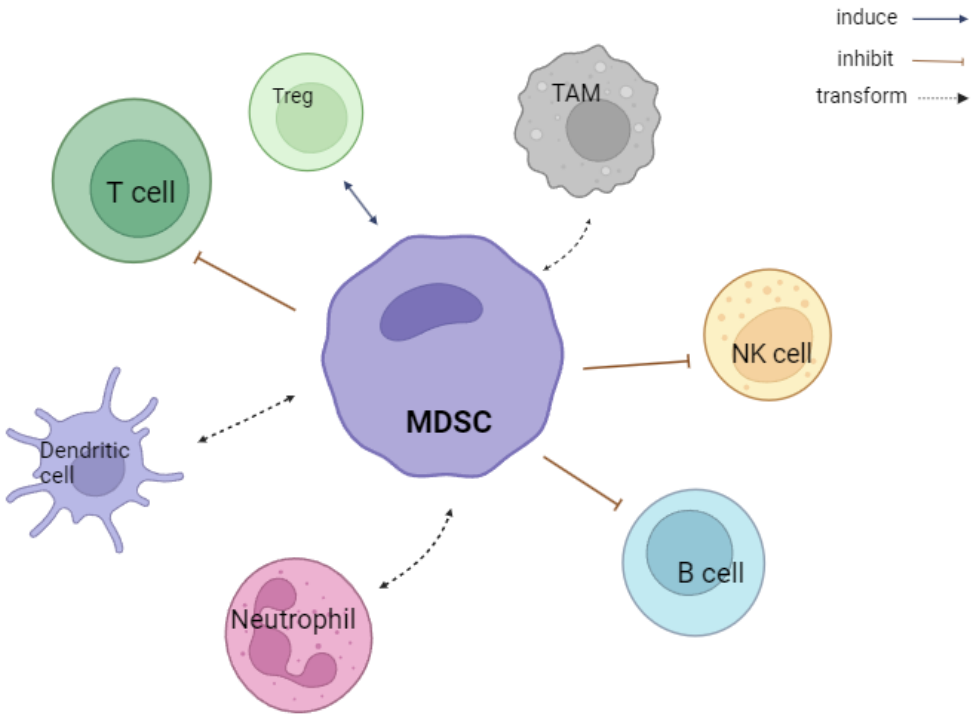

Figure 4: Overall view of the crosstalk between MDSCs and other immune cells[56].

MDSCs as a target for cancer immunotherapy

In the tumor microenvironment (TME), MDSCs orchestrate a myriad of immunosuppressive activities. They secrete various substances such as IL-10, iNOS, and arginase-1 (ARG1), which collectively inhibit T cell proliferation and function[26]. As we know, ARG1 depletes L-arginine, a crucial amino acid for T cell activation, exacerbating immunosuppression. Additionally, MDSCs release nitric oxide (NO), impairing T cell receptor (TCR) function, while also depriving T cells of essential amino acids necessary for their activation, further stifling immune responses[26].

MDSCs, crucial in cancer progression across various malignancies including GBM, melanoma, HCC, and metastatic CRC, correlate with poor prognoses and interact with CAFs, fostering immune evasion and disease advancement. Their recruitment and accumulation are shaped by factors like chronic inflammation and the TME, where hypoxia and oxidative stress influence their function and differentiation[56]. Notably, M-MDSCs can transform into tumour-associated macrophages (TAMs) with potent immunosuppressive properties, exacerbating immune evasion[57]. Overall, MDSCs represent a significant therapeutic target in cancer immunotherapy, warranting further investigation to develop effective strategies against their tumor-promoting effects.

Therapeutic strategies targeting MDSCs hold promise for disrupting their tumor-promoting functions and enhancing the efficacy of cancer therapy. Approaches such as chemotherapy, immune checkpoint inhibitors (ICI), and agents targeting specific MDSC-related pathways like COX-2/PGE2 and IDO have demonstrated encouraging results[58]. Moreover, assessing MDSC levels before initiating therapy may serve as a valuable prognostic marker, informing treatment decisions and predicting response to immunotherapy[32].

Quality of life in elderly haematological patients

Quality of life (QoL) is a complex and multifaceted concept that evaluates an individual's overall well-being in the context of their environment, cultural background, and social circumstances[59]. QoL is a valuable predictor of overall survival (OS) for patients affected by cancer, among many other diseases and conditions[60]. Given the expansion in the elderly

population over the years, ensuring the well-being of older adults and enhancing the quality of social services tailored to this demographic have become increasingly crucial. Particularly for dependent elderly individuals, social services are essential in facilitating an improved quality of life.[61]

Symptom burden has an extremely significant impact on the QoL of patients affected by haematological malignancies, it is characterized by symptoms such as fatigue, pain, insomnia, and diminished role function[62]. Psychological distress, social challenges, and cognitive impairment are also prevalent among haematological cancer patients. It is also known that advanced disease stages often correlate with poorer outcomes, including physical symptoms, psychological distress, social challenges, and cognitive impairment[63].

Therefore, prioritizing efforts to improve symptom management is especially important to uplift patients' quality of life and overall well-being. There are various predictors of symptom burden, encompassing age, gender, treatment status, and diagnosis, therefore healthcare providers should create personalized treatment plans that suit the patient's needs[64]. For instance, older patients may require additional support to manage symptoms effectively, while those undergoing active treatment may benefit from targeted interventions to alleviate treatment-related side effects[65]. Patients with lower education levels, on the other hand, may experience greater cognitive impairment which requires educational support tailored to their needs such as resources and interventions to help patients understand and cope with cognitive changes associated with their illness[62]. Aerobic exercise programs show promise in improving

physical and mental well-being[66], highlighting the importance of integrating interventions to enhance QoL into comprehensive care strategies.

Effectively managing the symptoms of haematological cancers requires an interdisciplinary approach. Through a collaborative effort patients would be able to receive comprehensive symptom relief, leading to better outcomes. Early QoL assessment and tailored interventions are essential for optimizing outcomes in elderly patients with haematological cancers[67]. Integrating these interventions into comprehensive care strategies can mitigate the impact of these diseases on patients' lives.

Quality of Life Assessments

The European Organization for Research and Treatment of Cancer (EORTC) launched a research initiative aimed at creating a comprehensive and adaptable framework for assessing the quality of life in patients involved in international clinical trials[68]. The Quality-of-Life Questionnaire Core (EORTC QLQ-C30), which is the organization's second-generation questionnaire, has become the most widely utilized patient-reported outcome (PRO) instrument in oncology research and clinical settings. The EORTC QLQ-C30 consists of 30 items, which cover nine symptom scales (pain, fatigue, nausea/vomiting, dyspnea, sleep disturbances, appetite loss, diarrhea, constipation, and financial difficulties), five functioning scales (social, physical, role, cognitive, and emotional functioning), and global health status/quality of life scales. In this 100-point system, higher scores on symptom scales denote a

higher symptom load, while higher scores on functioning scales and the global health status/quality of life scale signify a better health-related quality of life (HRQoL)[69].

The G8 screening tool

The G8 screening tool was developed to assess elderly cancer patients and serves as a valuable tool for detecting relevant geriatric impairments and predicting survival in elderly patients with haematological malignancies[70]. By assessing factors such as mobility, nutrition, and cognitive function, the G8 tool helps identify patients at risk of adverse outcomes, guiding prognosis and treatment decision-making. In haematological malignancies, which present unique challenges in older patients, this type of personalized care is crucial[71]. While Comprehensive Geriatric Assessments (CGA) are valuable and are recommended for older haematological patients[72], they can be time-consuming, making briefer screening tools like the G8 ideal for a quick identification of candidates for standard cancer treatment.

However, doubts exist regarding the G8's specificity, particularly in haematological cancer patients, as it may incorrectly identify patients without impairments as needing further assessment[73]. While the G8 tool may have limitations in accurately detecting impairments, it remains a strong predictor of mortality in this patient population. Incorporating the G8 screening tool into routine clinical practice can aid in optimizing care for elderly haematological patients, ensuring personalized treatment approaches tailored to individual needs[74].

Aim of thesis

Despite the advantages obtained in terms of PFS and OS thanks to modern therapies, currently, the quality of life in elderly cancer patients is still poor. This clinical need can be improved by identifying clinical and biological biomarkers that are able to predict the outcome of therapies. This thesis is divided into four sections. After a general introduction, Chapter one was based on the biological role of MDSC in patients with hematological malignancies. Chapter two covered several aspects concerning the implementation of geriatric screening assessment in patients withg hematological malignancies. Chapter three looked at the quality of life and the correlation between biological and clinical factors, we looked specifically at the role of G8 in patients with hematological malignancies. Finally, chapter four entails the general discussion and summary of the thesis.

The objectives of this study are to explore some of the aforementioned outstanding questions specifically: i) to evaluate the clinical impact of M-MDSCs and PMN-MDSCs found in patients treated with certain types of complex therapies within a prospective clinical trial in terms of PFS and OS; and ii) to evaluate the clinical impact of G8 assessment on quality of life in patients treated with certain types of complex therapies within a prospective clinical trial.

Materials and Methods

Participants and ONCOAGING study design

Before conducting the study, we have further screened patients from January 2020 and December 2022 using the G8 screening tool, aged >65 years, referred to our center for solid and hematological malignancies. G8 score was assessed at the time of first access.

This is a prospective, randomized clinical study. Patients older than 65 years, referred for treatment and follow-up to the Oncology, Hematology, and Radiation Oncology Department of the University Hospital Maggiore della Carità in Novara, Italy, and candidate to target agents or complex anti-neoplastic therapies will be screened for frailty by using the G8 questionnaire. Those patients classified as frail will be randomized to the following procedures: arm A, oncogeriatric evaluation by CGA; arm B, Control group. (Figure 5) shows the flowchart of the study. Blood samples will be collected for MDSC and cell senescence evaluation at baseline. Patients will be randomized by a REDCap (Research Electronic Data Capture) dedicated system with a 1:1 ratio; REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to standard statistical packages; and 4) procedures for data integration and interoperability with external sources. Patients randomized to CGA will undergo geriatric evaluation before treatment starts and every six months thereafter and

undergo geriatric intervention as required. Patients in arm B will be managed according to local clinical practice (Figure 5).

Inclusion criteria were patients with all types and stages of cancer; age ≥ 65 years; in need of the first line of treatment; an adequate understanding of the Italian language; and the ability to give informed consent. Exclusion criteria included severe known dementia, symptomatic brain metastases, and pre-existing major neurological or psychiatric problems.

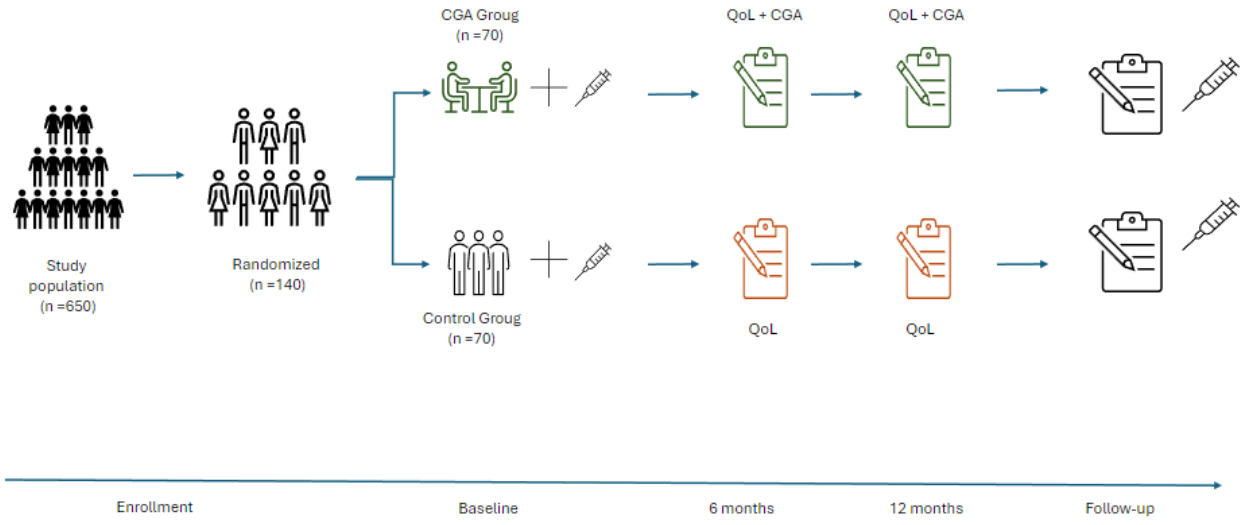

Figure 5: ONCCOAGING study design. Study assessment time points. Patients will be randomized into 2 arms after completion of the G8 screening phase; Arm A; will be evaluated by Comprehensive Geriatric Assessment (CGA) at baseline and would be re-evaluated after every 6 months. If necessary, an onco-geriatric follow-up will be carried out. Arm B is the Control group, no geriatric visit is scheduled. Both arms will be evaluated for their quality of life using the EORTC–QLQ-C30 questionnaire every 3 months. Blood samples from patients will be collected to evaluate T cells senescence and myeloid-derived suppressor cells at baseline and after 12 months or at disease progression.

G8 screening tool

G8 assessment was used as a screening tool to identify frail patients with scores equal to or lower than 14 (Figure 6). It was administered during the first visit with the clinician for patients aged 65 or more and eligible for target therapy as the first line. A score equal to or lower than 14 is an inclusion criterion of the study. It comprises eight questions, most of which come from the MNA questionnaire. Others are related to age and mobility.

The G8 questionnaire			
	Items	**Possible answers**	**Score**
A	Loss of appetite? Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?	0: severe anorexia 1: moderate anorexia 2: no anorexia	
B	Loss of weight during the last months	0: weight loss >3 kg 1: does not know 2: weight loss between 1 and 3 kg 3: no weight loss	
C	Mobility	0: bed or chair bound 1: able to get out bed/chair but not to go out 2: goes out	
E	Neuropsychological problems	0: severe dementia or depression 1: moderate dementia or depression 2: no psychological problem	
F	Body Mass Index	0: BMI < 18.5 1: BMI between 18.5 and <21 2: BMI 21 to <23 3: BMI ≥23	
H	Takes >3 prescription drugs per day	0: yes 1: no	
P	In comparison with other people with the same age, how do they consider their health status	0: not as good 0.5: does not know 1: as good 2: better	
	Age	0: >85 1: 80-85 2: <80	

Figure 6: G8 Geriatric Screening Tool. To Identifies elderly cancer patients who could benefit from comprehensive geriatric assessment (CGA)

EORTC QLQ-C30

The latest European Organization for Research and Treatment of Cancer (EORCT) Quality of Life Questionnaire (QLQ) is the EORTC QLQ-C30. It is composed of 30 questions to evaluate the oncologic patient's quality of life. Questions are divided into physical, cognitive, emotional, and social functions; moreover, symptoms are taken in evaluation, such as weakness, pain, nausea, and vomiting. Finally, the last two questions are related to the self-perception of health and quality of life. A total score is calculated, giving a result between 0, poor quality of life, and 100, excellent quality of life.

Peripheral Blood Mononucleated Cells (PMBC) isolation

Venous whole blood samples were collected in EDTA Vacutainer (BD Bioscience, Milan, IT) to prevent coagulation and processed within 4 hours after collection. To separate blood cells and allow PBMC collection, Ficoll-density gradient separation was performed (Figure 7). The blood sample was diluted in a 50 ml falcon tube with 30 ml physiological solution (NaCl 0,9%), then 15 ml of Ficoll-Paque (Lympholyte-H, Cedarlane, ND) solution was added into the tube by avoiding the mixing of blood and Ficoll. The tube was then centrifuged for 20 min at 800 RCF with an acceleration value of 7 and a break value of 0. The PBMCs were collected from the interphase between diluted plasma and separation medium, washed twice, and resuspended in physiological solution in the appropriate volume. The cells were then counted in a Burker chamber by diluting 1:10 with TURK solution.

CD3+ cells isolation

PBMCs pellet was resuspended in appropriate MACS buffer solution (phosphate-buffered saline 1x, -pH 7.2, 0.5% FBS and two mM EDTA) and CD3 MicroBeads (Miltenyi Biotech Bergisch Gladbach, Germany), according to manufacturer's instructions. According to the provided protocol, the cell suspension was loaded on a Column placed in the magnetic field of a suitable MACS Separator. We used two types of columns: MS for the isolation of fewer than 10 7 cells, and LS for more. The loaded 36 column was then washed with MACS Buffer. The magnetic column was removed from the magnetic separator and placed on a suitable collection tube. The CD3+ T-cells were with MACS buffer. Collected CD3+ T-cells were washed and counted with TURK solution. The purity of isolated CD3+ T-cells was determined by FACS analysis, labeling cells with APC conjugated anti-CD3 antibody (clone: UCHT1, eBioscence, USA). We considered a 95- 99% purity to proceed with RNA extraction (Figure 7).

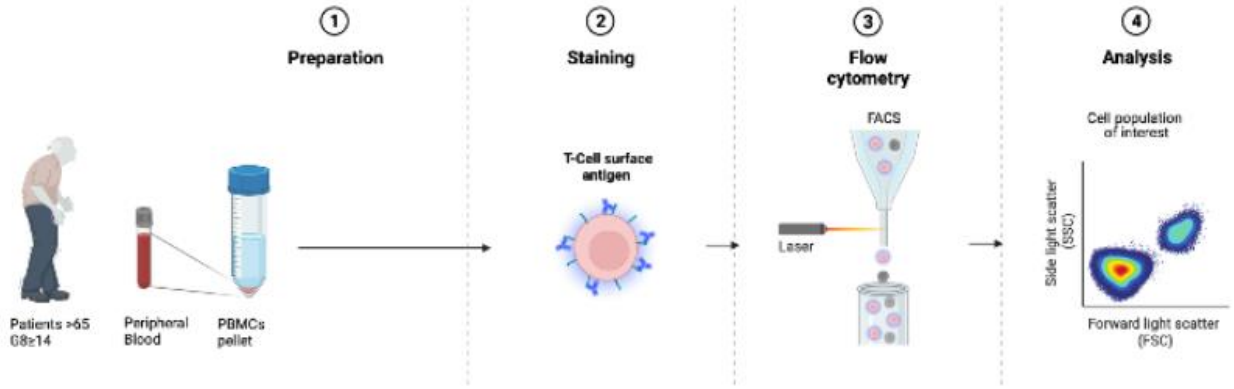

Figure 7: experimental workflow. Blood samples from enrolled patients will be collected to evaluate T cells senescence and myeloid-derived suppressor cells at baseline and after 12 months or at disease progression using flow cytometry.

Statistical Analysis

Normally distributed data were presented as mean and SD, whereas data following a non-normal distribution were presented as median and IQR. Categorical variables were summarized as counts and percentages. Differences in medians were evaluated using Mann–Whitney U test. The correlation between quantitative variables was assessed using the Spearman coefficient. PFS was measured from the date of treatment start to the date of progression (event) according to guidelines for the different disorders, death (event), or last follow-up (censoring). OS was measured from the date of initial presentation to the date of death from any cause (event) or last follow-up (censoring). Survival analysis was performed by the Kaplan-Meier method and compared between strata using the Log- rank test. Univariable Cox proportional hazards models were used to assess the impact of the factors on OS and PFS. The proportionality of hazards assumption was tested by visual inspection of the scaled Schoenfeld residuals plot and by the Grambsch and Therneau non-proportionality test. A two-sided P value of 0.05 was considered statistically significant. Associations between categorical variables were tested using the Pearson chi-square test or Fisher exact test as appropriate. A maximally selected rank statistic was used to determine the optimal cut-off for Mo_MDSc and G_MDSc based on the Log-rank statistics. The analysis was performed with the Statistical Package for the Social Sciences (SPSS) software v.24.0 (Chicago, IL, USA).

Ethical consideration

The Local Ethics Committee for medical and health research ethics in Novara as well as the Privacy Protection Representative at the University Hospital of Eastern Piedmont approved the

study. The inclusion in the study did not involve treatment leading to an increased risk of complications, and the comprehensive geriatric assessment did not have any consequences for treatment decisions. Extra blood was drawn at baseline and after 12 months or at progression of the disease. Ethical aspects included that the patients were subject to extra investigations and that sensitive information was obtained and stored. The inclusion was based on written informed consent, and the patients were informed that they might withdraw from the study at any time. The study investigator determined if the patient could provide written informed consent prior to the data collection. This was done by talking to the patient about their situation and describing the study. Patients who, to the best of the investigator's knowledge, seemed to understand the information were asked to sign the consent form. This decision was not changed even if the patient scored low on the QLQ C30 later. The data collection was terminated if the patient got tired or expressed distress when answering the questions, but this only happened on a few occasions.

Results

Patients' characteristics

From January 2020 to December 2022, a total of 140 eligible cancer patients were recruited at the University Hospital of Maggiore della Carità. Of these, 88 (62.86%) were male and 52 (37.14%) were female. Recruitment was distributed across departments as follows: 73 patients (52.14%) from hematology, 57 patients (40.71%) from oncology, and 10 patients (7.14%) from radiotherapy. Only four patients refused participation. Patient characteristics are summarized in Table 4. The

median age of patients was 75 years, ranging from 65 to 91 years. A significant majority (87.15%) were aged 70 or older, and 27.85% were aged 80 or older. All patients had newly diagnosed cancer, with 13.57% presenting with metastatic disease. Only 8 patients (5.71%) had no comorbidities other than cancer. In contrast, 68 patients (48.57%) had between one and three comorbidities, and 64 patients (45.72%) had four or more comorbidities. Most patients (73.57%) were using four or more concomitant medications due to their coexisting conditions, while 22.86% were using one to three additional medications alongside their cancer treatment. Only 3.57% of the patients did not use any concomitant medication apart from their cancer treatment.

	Total patients included	
	N° patients	%
Total N° pts	146	100
Age		
Median (range)	75 (65 - 91)	
Length (m)		
Median (range)	1.67 (1.45 - 1.90)	
Weight (kg)		
Median (range)	69 (34 – 114)	
BMI		
Median (range)	24.15 (14.0 - 39.4)	
Gender		
Female	88	62.86
Male	52	37.14
Tumour type		
Haematological malignancies	73	52.14
Solid tumours	67	47.86
Comorbidity		
None	5	3.57
1-3	32	22.86
≥4	103	73.57
Treatment		
Targeted therapy	105	75.0
Chemotherapy + targeted therapy	17	12.14
chemoimmunotherapy	8	5.72
Chemotherapy + radiotherapy	10	7.14

Table 1: Clinical features of patients at baseline. Patients' characteristics are written in the table. ECOG, Eastern Cooperative Oncology Group.

Overall Survival analysis (OS) and Progression Free Survival (PFS) analysis

Cancer patients are characterized by an increased vulnerability that makes them at elevated risk of death in comparison with healthy people. This is caused by the complex interaction between the host and the tumor. Indeed, the tumor can interact and disrupt different

physiological processes. Additionally, the median age of the study must be taken in mind since per se is a risk factor for death. We used the Kaplan-Meier Curve as a survival function to determine the outcome in terms of PFS (figure 8) and OS (figure 9) of enrolled individuals after 24 months.

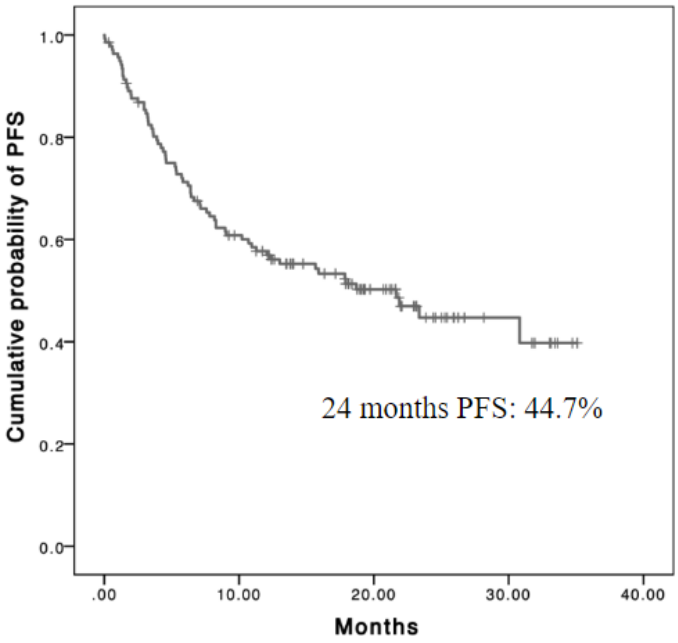

Figure 8: Kaplan-Meier estimates of progression-free survival (PFS) in the studied cohort. Log-rank statistics are plotted adjacent to the curves.

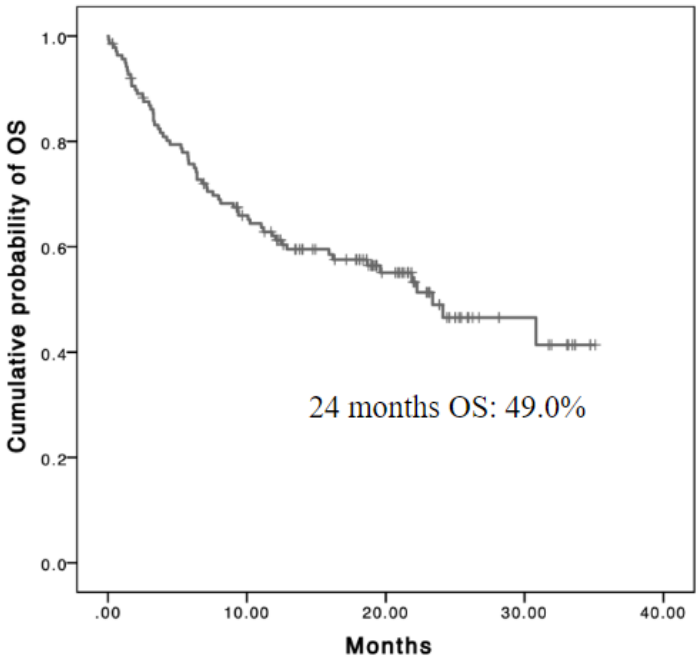

Figure 9: Kaplan-Meier estimates of overall survival in the studied cohort. Log-rank statistics are plotted adjacent to the curves.

We evaluated the outcomes of the 129 (92.14%) samples analyzed for MDSCs and 90 (64.29%) samples assessed for p16 from patients enrolled in this biological study, comparing them to patients not included in the study. The 24-month overall survival (OS) for patients included in the p16 analysis was 49.1%, compared to 35.2% for those not included (p=0.534) (Figure 10). Similarly, the 24-month OS for patients included in the MDSC analysis was 48.6%, while it was 23.9% for those not included in the molecular study (p=0.795) (Figure 10).

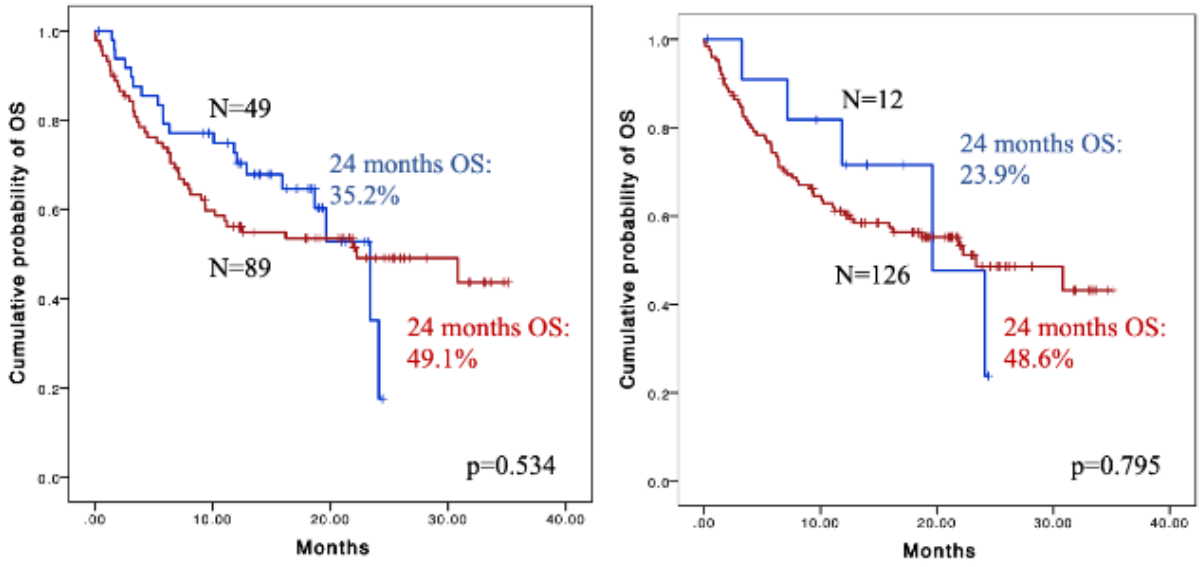

Figure 10: Kaplan-Meier estimates of progression-free survival in the studied cohort. Log-rank statistics are plotted adjacent to the curves.

Clinical impact of MDSCs

After a median follow-up of 24 months, we evaluated the clinical impact of MDSCs percentage in patients with hematological malignancies, in terms of PFS and OS. We conducted the univariate analysis considering the two subpopulations of MDSCs, Monocyte-MDSC (M-MDSC) and Granulocytes-MDSC (PMN-MDSCs). We demonstrated that the percentage of M-MDSCs in patients with hematological malignancies was not associated with poor clinical outcomes in terms of OS and PFS. We divided the hematological patients into two groups using as the cut off the calculated median of M-MDSCs (N=28 for higher percentage respect the median; N=40 for lower percentage respect the median). We showed that at 24 months, the OS of patients with a higher percentage of M-MDSCs with respect to the calculated median was 48.5% vs 63.6% (p=0.809) (Figure 11). Regarding the PFS, according to the same grouped patients used for OS

evaluation, we showed that the patients with higher percentage than the median were 42.7% vs 61.3% of the other group (p=0.601) (Figure 11).

We divided the patients in two groups on the basis of the median percentage of M-MDSCs (N=33 for higher percentage respect the median; N=25 for lower percentage respect the median) and demonstrated that at 24 months, the OS of patients with higher percentage of M-MDSCs respect to the median was 37.5% vs 47.0% for those with lower one (p=0.703) (Figure 11). Moreover, we demonstrated that there was no significant association with PFS considering patients with higher percentage of M-MDSCs with respect to median vs those patients with lower values (32.5% vs 40.3% respectively; p=0.550).

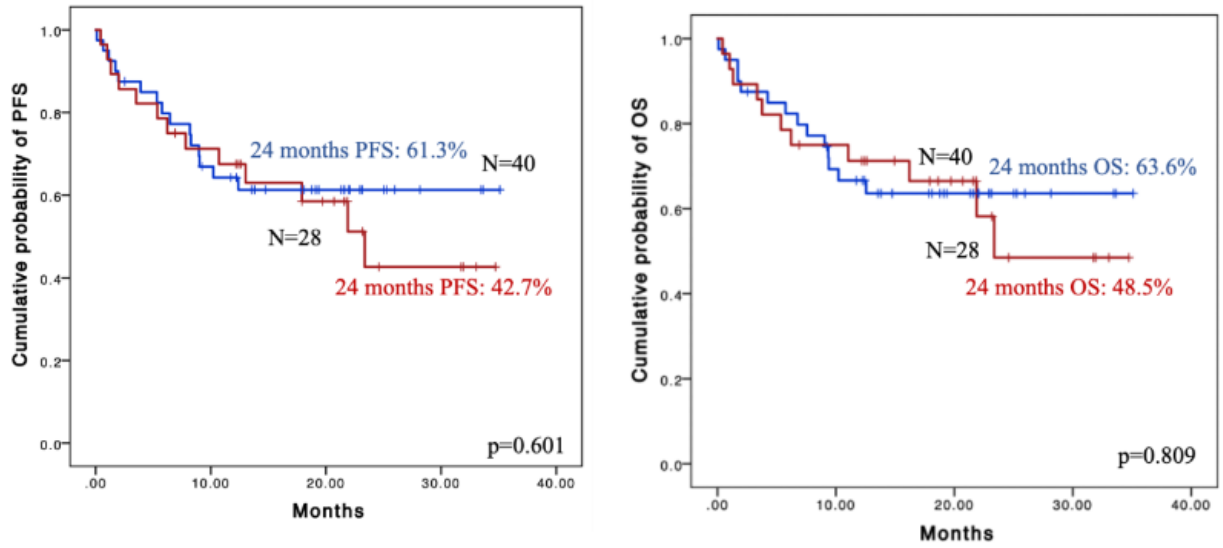

Figure 11: Kaplan-Meier estimates of progression-free survival and overall survival according to Mo-MDSCs percentage identified in the patients with haematological malignancies. Cases with elevated percentage from the median of M-MDSCs are represented by the red line. Cases equal to or less than the median percentage are represented by the blue line. Log-rank statistics are plotted adjacent to the curves.

Similarly, after a median follow-up of 24 months, the clinical impact of patients with hematological malignancies, we evaluated the PMN-MDSCs percentage in patients with haematological malignancies in terms of PFS and OS. Likewise, in the analysis performed for M-MDSC, we divided the patients into two groups using the median of PMN-MDSC percentage as the cut off (N=27 for higher percentage respect the median; N=41 for lower percentage respect the median). By univariate analysis, we showed that the percentage of PMN-MDSCs was not associated with poor clinical outcomes in terms of OS and PFS. Precisely, at 24 months, the OS of patients with higher percentage of PMN-MDSCs with respect to the median was 64.5% vs the patients with lower percentage 55.2% (p=0.197) (Figure 12). Regarding PFS, we demonstrated that patients with higher percentage of PMN-MDSCs with respect to the median were 64.5% vs 47.4% of patients with lower percentage (p=0.068; Figure 12).

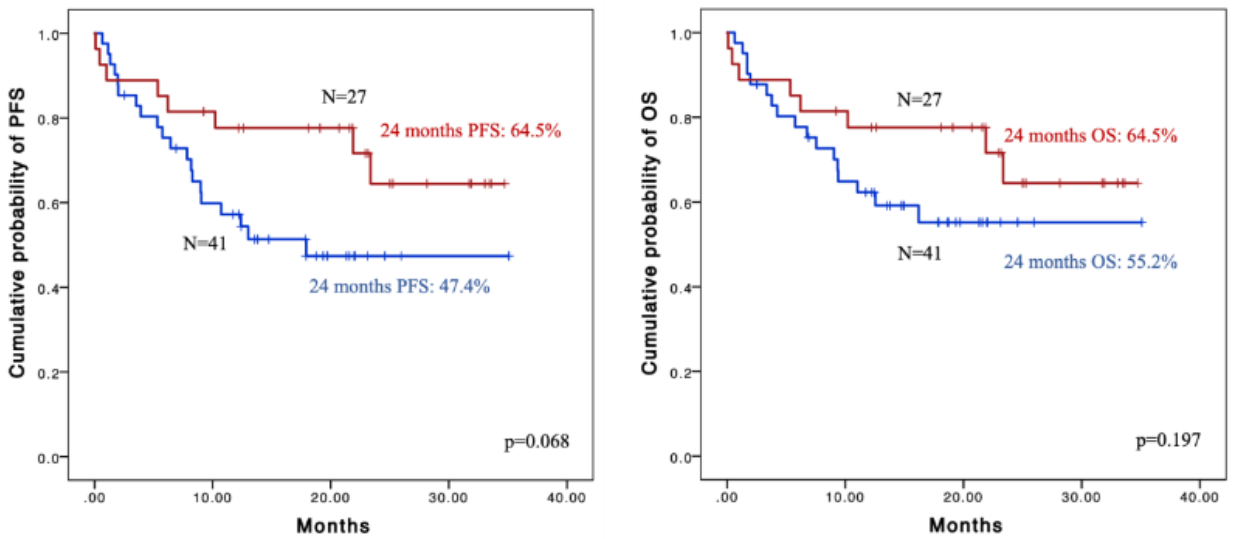

Figure 12: Kaplan-Meier estimates of progression-free survival and overall survival according to PMN-MDSCs expression level identified in the patients with hematological malignancies. Cases with higher percentage from the median of PMN-MDSCs are represented by the red line. Cases equal to or less than the median level are represented by the blue line. Log-rank statistics are plotted adjacent to the curves.

Predictive performance of the G8 questionnaire

After a median follow-up of 24 months, the clinical impact of G8 assessment was evaluated in terms of PFS. By univariate analysis, the G8 assessment was associated with poor clinical outcomes in terms of PFS. At 24 months, the PFS of patients with a G8 score over the median of 11 was 52.5% vs 30.9% below the cutoff cases (p=0.05) (Figure 13).

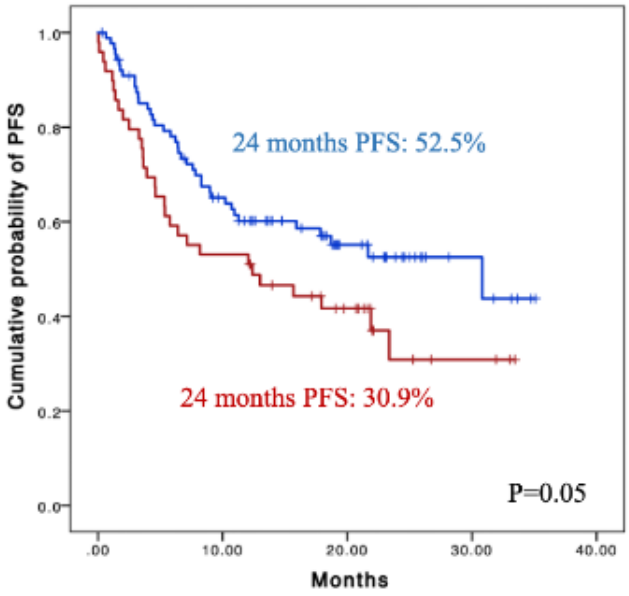

Figure 13: Kaplan-Meier estimates of progression-free survival according to G8 score in the patients with cancer. Cases with less than the median of 11 received targeted therapy are represented by the red line. Cases equal to or over the median of 11 and received targeted therapy are represented by the blue line. Log-rank statistics are plotted adjacent the curves.

Similarly, after a median follow-up of 24 months, the clinical impact of G8 assessment was evaluated in terms of OS. By univariate analysis, the G8 assessment was associated with poor clinical outcomes in terms of OS. At 24 months, the OS of patients with a G8 score over the median of 11 was 58.7% vs 32.4% below the cutoff cases (p=0.05) (Figure 14).

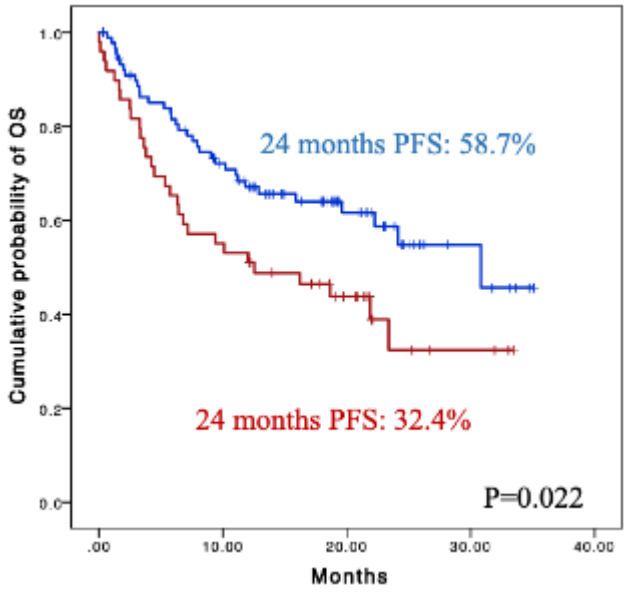

Figure 14: Kaplan-Meier estimates of Overall survival according to G8 score in the patients with cancer. Cases with less than the median of 11 received targeted therapy are represented by the red line. Cases equal to or over the median of 11 and received targeted therapy are represented by the blue line. Log-rank statistics are plotted adjacent the curves.

We investigated whether there was a correlation between G8 assessment and the quality of life of patients enrolled in the study. The impact of G8 assessment with the median of 11 was evaluated in terms of quality of life using EORTC QLQ-C30 with median Global Health status scores of 58.33. By univariate analysis, the low score of G8 assessment was associated with inferior quality of life outcomes in terms of Global Health status using the EORTC QLQ-C30 questionnaire (p=0.003) (Figure 15).

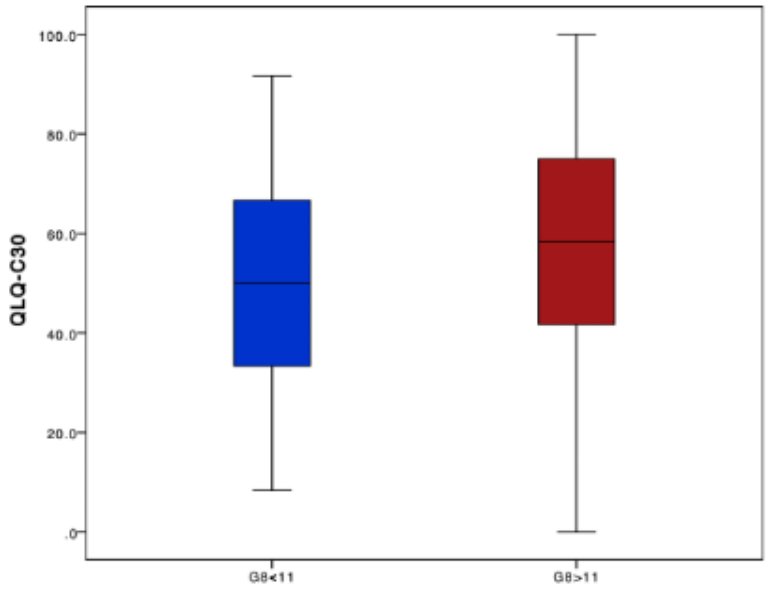

Figure 15: Boxplot of the scores for G8 assessment expression and patients on the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). Measuring Global Health status: the patient EORTC QLQ-C30 questionnaire scale scores range from 0 to 100. Higher scores indicate fewer symptoms of and higher quality of life.

Discussion

The study population consisted of 140 eligible cancer patients the median age of which was 75 years, with a majority (87.15%) aged 70 or older. As we know, age is the most important risk factor for most cancer types, which makes understanding the aging process and its impact on cancer development crucial. Notably, a significant portion of the patients (73.57%) were on multiple medications due to comorbidities, which reflects the complexity and challenges in managing older cancer patients. Comorbid conditions are associated with increased mortality rates in cancer patients. Studies have shown that the presence of multiple comorbidities can significantly reduce overall survival (OS) and progression-free survival (PFS) due to the compounded burden of managing multiple health issues alongside cancer treatment. This is

because comorbidities add to the overall symptom burden experienced by cancer patients, which we know exacerbates issues like fatigue, pain, and psychological distress[75]. This cumulative symptom burden can significantly deteriorate the quality of life (QoL)[76].

Overall Survival (OS) and Progression-Free Survival (PFS) were evaluated using Kaplan-Meier curves. At 24 months, the OS for patients included in the MDSC study was 48.6%, while those not included had an OS of 23.9%. This indicates a potential benefit in survival for those included in the MDSC analysis, although the difference was not statistically significant ($p=0.795$). These findings suggest that while biological studies may indicate trends towards better outcomes, larger sample sizes and further studies are needed to confirm these observations. The study also investigated the percentage of PMN-MDSCs in patients with hematological malignancies and their impact on clinical outcomes. Higher percentages of PMN-MDSCs were associated with better PFS (64.5% vs. 47.4%, $p=0.068$), although this was not statistically significant. This suggests a complex relationship between MDSCs and disease progression that warrants further investigation.

The G8 screening tool was effective in identifying frail patients. This tool, despite its brevity, helped predict poor outcomes, aligning with previous findings that underscore its value in clinical settings. However, the limitations in specificity suggest that while G8 is useful for initial screening, comprehensive assessments should follow to ensure accurate identification of frailty in elderly cancer patients.

The G8 assessment demonstrated a significant correlation with both PFS and OS. Patients with a G8 score above the median (11) had significantly better PFS (52.5% vs. 30.9%, p=0.05) and OS (58.7% vs. 32.4%, p=0.05) at 24 months. The impact of the G8 assessment on quality of life was significant. Patients with lower G8 scores reported inferior QoL outcomes, as measured by the EORTC QLQ-C30 questionnaire (p=0.003). This highlights the importance of early identification of frailty to not only improve survival outcomes but also enhance the quality of life for elderly patients. These results affirm the G8 tool's utility in predicting survival outcomes, supporting its integration into routine clinical practice for elderly cancer patients.

This study underscores the importance of comprehensive geriatric assessments and the integration of frailty screening tools like the G8 in managing older cancer patients. While the G8 tool effectively predicts poor outcomes and aligns with QoL measures[77], further research is needed to refine these tools and validate their findings in larger, more diverse patient populations. The role of MDSCs in survival outcomes presents an intriguing area for future research, potentially guiding more personalized and effective treatment strategies for hematological malignancies.

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