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Review

Risk of infection in elderly patients with AML and MDS treated with hypomethylating agents

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Summary. The aim of this small volume is to raise awareness among Italian hematologists using hypomethylating drugs about risk - and even more important about "risk management" - and the treatment of the infectious events that may arise during therapy with these drugs. Since we wanted this review to be an extremely practical tool, we have included the most recent clinical case studies, the indications provided by the guidelines and expert opinions. Infectious complications are among the most common adverse events that can arise during treatment with hypomethylating drugs. For this reason, a large portion of the chapters of this small volume has been dedicated to a detailed description (on the basis of an attentive review of the literature) of what the hematologist can expect to encounter in terms of risk of infection, in patients treated with azacitidine or decitabine, and to the preventive investigations to carry out and the active prophylaxis measures recommended before commencing treatment with these drugs. What led us to write this book was the conviction that the critical sharing of the studies published in literature and of our personal experiences could contribute to prompting reflection on how we operate and that this, in turn, would lead to the best possible management of these treatments, both in patients with myelodysplasia and in patients with acute myeloid leukemia, preventing - and efficiently managing - infectious events - during therapy. We should not be misled by the fact that the treatments are prevalently administered on an outpatient basis; these patients due to their disease and, in particular, to their age, are extremely frail, and infectious and hemorrhagic complications are the main causes of their admissions to hospital. But expert knowledge and management of the risks of infection can guarantee better management of their needs on an outpatient basis, with undeniable advantages for the clinic but, first and foremost, for the patients. (www.actabiomedica.it)

Key words: AML, MDS, hypomethylating agents

Introduction

Neoplastic disease in elderly patients is a major problem given the fact that in western countries nowadays around 25% of the general population is over 65 years of age and the prevalence of oncological diseases in advanced age is around 60%, making it one of the main causes of death. hematological neoplasms (in-

cluding myelodysplasias and acute (particularly myeloid) leukemia are among the most frequent types in patients within this age group. Their treatment involves numerous problems, which are often extremely complex, essentially connected with: low tolerability to aggressive chemotherapy treatments; concomitant, frequently debilitating, chronic diseases which often destroy natural barriers; a lower capacity of the bone marrow to recover after chemotherapy, exposing the patients to prolonged periods of neutropenia. Additionally, these blood disorders also involve the tissues and cells responsible for the body's natural immune defences, so their onset determines a state of severe immunodeficiency leading to greater susceptibility to infective complications of various kinds and degrees of seriousness. This is an aspect of major importance nowadays, since often these complications can have a decisive effect on the clinical development and prognosis of the disease.

Standard or high-dosage chemotherapy treatments produce a pathophysiological condition called neutropenia (neutrophil granulocyte count <1000/mm3), of duration and intensity, which, as already mentioned, in the elderly patient can be compounded by the difficulty of recovering normal blood crasis, due to the natural progressive replacement of hematopoietic bone marrow with fatty tissue. This condition, associated with a considerable reduction in the phenomena of chemotaxis and phagocytosis, is one of the main causes of increased exposure to the risk of bacterial or fungal infectious diseases, which, in a relatively frequent percentage of cases can lead to the patient's exitus.

From the epidemiological standpoint, the most important infective problems for elderly neutropenic patients include:

a) gradually increasing incidence of infections and bacteremia due to multi-resistant Gram-negative germs.
b) progressive increase of fungal infections, particularly filamentous fungi (aspergillosis and mucormycosis)
c) Onset of viral infections, of variable etiology and clinical expression.

The introduction of hypomethylating agents has changed the clinical outcome for elderly patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) over the last decade or so. In the past those patients eligible for chemotherapy were treated either with standard chemotherapy protocols, which were often too aggressive (3+7) or with very bland treatments (i.e. low dose cytarabine), with unsatisfactory or limited results.

With azacitidine and decitabine, there was a change from an extremely medicalized form of management, involving admission to hospital (especially for acute leukemia) during the induction phase, to an essentially outpatient-based form of management, giving priority to quality of life and good results in terms of prognosis.

Literature on the risk of infection with standard treatments is full of data reporting a high risk of infection, for the most part fungal or bacterial. On the contrary, the risk of infection with hypomethylating agents has not been quantified, particularly in patients with acute myeloid leukemia. So it is essential to figure out whether this risk exists and to identify the phases of treatment during which it is at its highest, which types of infections should be considered the most frequent and what diagnostic and therapeutic (prophylaxis and treatment) strategies are the most suitable.

In absence of controlled prospective studies on this problem, a panel of experts is required in order to give instructions that are useful and applicable in "*real life*", until studies able to provide the necessary answers are carried out.

The aim of this publication is to discuss the risk of infection in myelodysplasias and acute myeloid leukemia in the elderly, with a special focus on treatments with hypomethylating agents, and to examine whether these drugs can lead to increased risks. Figures, risk factors and advice will also be provided on the management of the infections and therapy in patients with infectious diseases of different etiology.

High risk myelodysplastic syndromes and acute leukemia in the elderly

General considerations on elderly hematological patients

Myeloid neoplasms (MDS and AML) are diseases of the elderly. In fact, approx. 50% of cases de-

velop in the population over 65 years of age, i.e. 12% of the total population. In Europe it is estimated that by 2030 the elderly will account for 20% of the overall population and 70% of all patients with neoplasia. The management of neoplasms in the elderly patient is rapidly becoming one of the most frequent problems encountered in hematology (1).

Aging

Aging brings about a reduction in life expectancy and of the functional reserves of the various organs and systems, bringing about a more limited tolerance to stress. For this reason, as the patient ages, the benefits of treatments based on standard chemotherapy drugs decrease and the risks connected with their tolerability increase. Aging is an individualized process: people of the same age can have a different life expectancy and a totally different tolerance to stress. For this reason, it is important to determine the physiological age of the patient, in order to be able to make a benefit/risk assessment of the therapy (2,3).

From the clinical standpoint, the best validated determination of physiological age can be obtained through the *Comprehensive Geriatric Assessment* - CGA. Based on the CGA, it was possible to build mortality risk prediction models for elderly patients (70 years and over) affected by a variety of clinical conditions. In the oncohematological field, some studies demonstrated a correlation between dependence for one or more ADLs (*Activity of Daily Living*), advanced degree of comorbidity and chemotherapeutic complications. The CGA is very useful in the planning of the anti-neoplastic treatment in the elderly patient, for the following reasons: 1) it gives an estimate of life expectancy and of the tolerability of the treatment; 2) it enables the identification of conditions such as overlooked reversible comorbidities, malnutrition risk, absence of a proper *caregiver*, which can impair the success of the treatment; 3) it enables the use of a common language in the description of the elderly patient (4-6).

For example, a lack of autonomy pinpointed by the ADL scale, the presence of one or more geriatric syndromes or a serious comorbidity threatening the survival of the patient are contraindications to any form of active therapy, since these patients have a very limited life expectancy and tolerance to treatment. Additionally, it is very important to recognize that a lack of functional autonomy means an incapacity to compensate for one's own functional losses (7). Moreover, for patients taking 8 or more drugs per day, the risk of encountering at least one adverse pharmacological interaction is very high, and the risk of pharmacological interactions is responsible for a 60% increase in the risk of chemotherapy complications. Lastly, the evaluation of the *caregiver* is a key factor for the success of the treatment (8). Very often the *caregiver* of the elderly patient is another elderly person, usually the spouse, who also has serious health problems. The ideal *caregiver* should be able to assist the patient at home, take them to a treatment centre in the case of emergency and provide emotional support. Among the organic functional deficits, it should be considered that impaired glomerular filtration is almost universal with aging. Many drugs give rise to toxic metabolites, the toxicity of which can increase in the event of reduced renal function (9).

Aging and frailty are two concepts that have always gone hand in hand. There is virtually universal agreement on the importance of frailty as a condition of extreme susceptibility to stress, so that even minor stress (such as elective surgery), can cause a progressive functional decline. A practical definition of frail has been defined on the basis of the results of the *Cardiovascular Health Study* (CHS), where basing themselves of 5 parameters, researchers defined three groups of individuals: not frail (all parameters normal); pre-frail (abnormality of 1 or 2 parameters) and frail (abnormality of 3 or more parameters) (10,11). These three groups have a different life expectancy and a different risk of hospitalization and functional decline. The five parameters are:

- unintentional loss of at least 4.5 kg in the 6 months leading up to the evaluation;
- decreased strength of manual pressure;
- decreased physical activity;
- decreased maximum walking speed;
- decreased energy level.

When this was established, it constituted the reference for frailty studies. Of particular interest is the fact that recent studies have demonstrated how asthenia, a common symptom both in the elderly and in the

cancer patient, heralds the development of frailty in over 70% of cases (11). Although the relations between frailty, cancer and anti-neoplastic treatment need to be further defined, a benefit-risk assessment applied to the treatment of the elderly patient affected by myleoid neoplastic disease must be based both on the nature of the pathology and on the patient's CGA. In addition to providing an estimate of life expectancy and tolerance to the treatment, the CGA also makes it possible to pinpoint unfavourable but reversible conditions that could jeopardize the results of the treatment and use a common language to classify the elderly patient.

Acute myeloid leukemia in the elderly

AML is a disease typically found in the elderly. In fact, its incidence increases progressively from approx. 1 case per 100,000 inhabitants at the age of 40, to more than 15 cases per 100,000 in the age *range* 75-80, so over 50% of AML cases are diagnosed in patients over the age of 60 (12). This has led to growing interest in the disease in the elderly, not only from the clinical standpoint, through retrospective and prospective studies specifically designed for the elderly, but also from a strictly biological standpoint, with a view to investigating age-related molecular and cytogenetic differences.

Despite this, treatment results remain historically unsatisfactory in terms of achieving complete remission (CR) and, even more so, long-term survival (13). It should also be considered that the majority of the results in literature derive from multi-centre cooperative *trials*, from which at least 40% of elderly patients are excluded due to age >75 years and/or the presence of one or more comorbidities at the time of diagnosis. Other selection factors include the attitude of the patient and the doctor, the scientific interest of the latter, the availability of beds in the healthcare institution and, on occasion, the geographical distance from the hospital. All this determines a significant selection of patients deemed *unfit*, who only receive supportive care or bland treatments managed, whenever possible, on an outpatient basis. The consequence of this is that a large proportion of patients are treated with therapeutic strategies that do not have the power to change the natural course of the disease (14) (Table 1).

Table 1. Most frequent causes for the exclusion of elderly patients with AML from aggressive chemotherapy.

Causes for exclusion
• Age >75 years
• Performance status ≥ 2-3
• Attitude of patient and family members
• Attitude of doctor
• Scientific interest of the doctor in a given therapy
• Lack of a caregiver
• Distance from treatment centre
• Availability of beds

This proportion is now over 80% in patients aged between 60 and 70 years, while it is still largely under 50% in those over 70 years of age. These considerations prompt reflection on a redefinition of the age limit on the basis of which a patient with AML should be placed in the "elderly" category; today this limit should probably be raised above 70 years of age.

Prognostic factors

The most important prognostic factors in AML in elderly patients include age, cytogenetics and the presence of comorbidities. Most of the information derives from clinical *trials* based on the provision of intensive induction treatment followed by consolidation, since the prognosis of patients treated with supportive care alone is generally unfavourable, irrespective of the initial biological characteristics.

Cytogenetics and molecular changes

The criteria for cytogenetic prognostic classification of AML in elderly patients are shown in Table 2 and derive from the studies of the British research group*Medical Research Council* (MRC) (15).

In elderly patients the frequency of chromosomal abnormalities with a prognostically unfavourable significance is greater compared with young adults and translates, on a clinical level, to a lower percentage of CR and in a shorter duration. Additionally, t(8;21) and inv(16) are rare over 60 years of age and, in any case,

Table 2. Cytogenetic prognostic classification of AML in elderly patients

Prognostic group	**CR (%)**	**Survival at 5 years (%)**
Favourable karyotype: *t(8;21), inv(16), t(16;16)*	75	35
Intermediate Karyotype: *normal karyotype, non-complex karyotype, no abnormalities of chromosomes 5 and 7*	60	15
Unfavourable karyotype: *complex karyotype, abnormalities of chromosomes 5 and 7*	20	5

they are associated with less satisfactory therapeutic results (16).

Cases of AML preceded by MDS or Myeloproliferative Neoplasms (MPNs) are significantly more frequent in elderly patients compared to younger patients (30% vs. 10%). The prognostic impact of the diagnosis of MDS prior to the onset of AML is generally considered unfavourable. Moreover, among the unfavourable criteria, it is important to highlight the frequency with which an elderly patient is diagnosed with AML associated with signs of morphological myelodysplasia, an entity that has been recognized in the WHO classification of myeloid neoplasms. It is plausible that undiagnosed MDS may have preceded the onset of AML in many of these patients, suggesting a suspected diagnosis of secondary leukemia (17).

The favourable prognostic significance of NPM1 mutations in absence of FLT3/ITD mutations in young adult patients with a normal karyotype, has been confirmed by numerous studies, to such an extent that patients with AML-NPM1+/FLT3- and a normal karyotype are excluded from the transplant programmes at the first CR. Recently, an analysis of CALGB (Cancer and Leukemia Group B) confirmed these data also in elderly patients, even although globally the *outcome* for the latter was less favourable than that of their young counterparts (18). As regards FLT3 mutations, associated with a worse prognosis in patients aged <60 years, they do not seem to exert a prognostically significant effect in elderly patients (19).

Comorbidity

Very advanced age and the presence of one or more comorbidities are unfavourable prognostic factors in elderly patients with AML and represent one of the most frequent reasons for their exclusion from intensive chemotherapy regimens aimed at achieving CR. Special *geriatric assessment* scales have been proposed for patient evaluation, in order to render clinical assessment more uniform and mitigate the subjectivity of the doctor when judging a patient eligible or otherwise for a given choice of therapy (20). The use of these multi-dimensional evaluations should undoubtedly be encouraged, particularly in clinical *trials* with a view to rendering the study populations more homogeneous and the results of the trials themselves more reproducible. On the other hand, it must also be considered that not infrequently the compromising of *performance status* (PS) is exclusively or prevalently due to the leukemic disease and that clinical conditions can improve considerably after appropriate supportive care (transfusions, antibiotics, hydration), so that a patient ineligible at the outset could become eligible after the aforementioned care (21).

Supportive care and chemotherapy

The only randomized trial published in literature which compared chemotherapy with supportive care was conducted by EORTC (European Organization for Research and Treatment of Cancer) in 1989. In a cohort of patients aged over 65 years a comparison was made between intensive standard chemotherapy and a *watch and wait* strategy, based on supportive care and bland cytoreduction therapy only in the case of leukocytosis and/or symptoms associated with the hematological disease. The *outcome* proved to be better for the patients who underwent intensive chemotherapy compared to those of the group treated exclusively

with supportive care (median survival time: 21 weeks against 11) (22).

For over 20 years, standard induction chemotherapy of AML has been based on the association of daunorubicin (DNR) and ARA-C. Lowenberg et al. demonstrated that an increase of more than 45 mg/m^2 of DNR during induction improves clinical results in terms of the achievement and duration of CR, but the benefit is limited to patients aged 65-70 years with intermediate cytogenetics. If age >60 years is an unfavourable prognostic factor in patients at the outset, it is even more so after the onset of a relapse. In a trial that recruited 50 elderly patients with AML during its first relapse, the percentage of the second CR was lower than 40% for the 100 patients selected to receive intensive salvage therapy (23).

Therapy for "unfit" elderly patients

In oncology, the term *unfit* defines patients affected by neoplasms, generally (but not always) elderly, who are unable to tolerate intensive treatment, and therefore require modified or blander treatments which, in the majority of cases, do not aim at changing the natural course of the underlying neoplastic disease. With regard to AML, this definition covers the existence of two different categories of *unfit* patient: one which can be subjected to a less intensive therapeutic regimen which tends, in any case, to achieve CR; the second in which the patient can only be given the best supportive care and cytostatic therapy for leukocytosis control. The latter subgroup includes the vast majority of patients aged 75-80 years, independently of the PS and existing comorbidities (24.)

The first category is limited almost exclusively to patients aged >70 <80 years and is the one with the greatest margin of uncertainty, and hence the one in which the attitude of the clinician plays a fundamental role in the final therapeutic choice. Over and above possible recourse to more or less complex multidimensional geriatric evaluations, simply determining the PS through score systems such as that of the *Eastern Cooperative Oncology Group* (ECOG) or Karnofsky supplies information of clinical importance, that may be independent of associated comorbidities. The clinical relevance of PS in AML at diagnosis is clearly demonstrated by the close correlation between poor PS and mortality within the first 30 days of the start of induction chemotherapy. Additionally, in an extensive Swedish study on non selected patients, it was demonstrated that mortality among the elderly people (>75 years) with a good PS can be lower than that in a younger population with a poorer PS. A potential cause of the erroneous interpretation of clinical conditions is that of thinking that the poor PS depends strictly on the hematological disease. In other words, the determination of the PS does not distinguish between the decline in general conditions due to AML, reversible with supportive care, and the irreversible comorbidities that are not related to it. In this case, the *score* of the comorbidities would be more effective for the definition of the *unfit* condition and hence for the therapeutic decision in relation to the PS, which could, in turn, be reconsidered after correcting anemia, controlling sepsis and any other complications directly correlated to AML (24).

In conclusion, although age on its own is not sufficient to be able to predict early mortality during induction and response to therapy, every effort should be made to define uniform and universally accepted criteria for defining a patient with AML as *unfit*. A PS >2, non-reversible after supportive care, could represent a contraindication for the administration of intensive chemotherapy and, in general, of therapies aiming to achieve CR. An integrated evaluation of PS and comorbidities might therefore be useful in identifying patients that might not benefit from a standard treatment.

In addition to the clinical factors correlated to the conditions of the patient, various studies have definitively demonstrated that the biological characteristics of AML are different in the elderly patient as opposed to the young one. In elderly patients the disease is more often preceded by a phase of MDS and more frequently it is characterized by unfavourable cytogenetics. As suggested in a recent edition of the WHO classification and even more recently in the guidelines of the *European Leukemia Net*, the karyotype evaluation at diagnosis is obligatory for the purpose of predicting the *outcome* and could also be used for stratifying the patients in three different treatment lines (25). Elderly patients with unfavourable cytoge-

netics, in particular those with an abnormality of chromosomes 5 and 7and/or a complex karyotype, usually have a percentage of CR lower than 30% and a median survival rate of a few months. Since almost all elderly patients diagnosed with AML need substantial supportive care, postponing the induction therapy for a few days in order to acquire the cytogenetic result seems reasonable in order to avoid toxicity that could also be fatal. The effect of the time interval between the diagnosis of AML and the start of induction therapy has recently been evaluated on an extensive study population by Sekeres et al. (26). Contrary to what was observed in young people/adults, postponing the treatment by 5-7 days does not worsen the prognosis in terms of achieving CR and prolonging survival, it actually improves cytogenetic stratification. In conclusion, a modern therapeutic approach should envisage the acquisition of the cytogenetic results with all possible haste (not more than 5-7 days): in the presence of an unfavourable karyotype, the possibility of giving the patient access to clinical *trials* using experimental drugs should be considered, since in this group of patients, even if deemed *fit*, the potential toxicity of the intensive chemotherapy is not compensated by an advantage in terms of survival. Similarly, AML secondary to previous MDS, especially of a duration >6 months, identifies elderly patients who are biologically *unfit* and who therefore share the same negative prognosis as those with the unfavourable karyotype. Hence the clinico-therapeutic considerations stated above apply to them as well. Having said this, it is nonetheless important to underline the availability of an extensive arsenal of new drugs that could, potentially, be used in the context of clinical *trials* for the treatment of elderly patients who are clinically or biologically unfit (27).

Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are a group of disorders of the hematopoietic stem cell, classified as myeloid neoplasms, characterized by the presence of dysplastic hematopoiesis, cytopenia of one or more of the peripheral blood chains and an increased risk of leukemic evolution. The incidence of this condition is approx. 5 cases out of 100,000 persons per year in the general population, but it increases to 50 cases out of 100,000 persons per year after the age of 60. The median age of diagnosis is around 65 - 70 years, with a predominance among males. This means that approx. 3,000 diagnoses of MDS per year can be expected in the Italian population; due to the progressive aging of the population, this disease is on the increase. The incidence could even be higher than that reported in the international registries, considering that many cases are not diagnosed due to unclear symptoms, or due to the fact that the patient is not eligible for bone marrow aspiration. Excluding forms correlated with therapies (28, 29), onset before 50 years of age is rare.

Table 3. WHO MDS Classification

MDS Classification
• MDS with single lineage dysplasia (MDS-SLD)
• MDS with multilineage dysplasia (MDS-SLD)
• MDS with ring sideroblasts (MDS-RS) with single lineage dysplasia
• MDS with ring sideroblasts (MDS-RS) with multilineage dysplasia
• MDS with excess blasts 1 (MDS-EB-1)
• MDS with excess blasts 2 (MDS-EB-2)
• Myelodysplastic syndrome associated with isolated del(5q)
• Unclassifiable myelodysplastic syndrome (MDS-U)

The integration of morphological, histopathological and cytogenetic evaluations makes it possible to make a diagnosis of MDS in accordance with the current WHO classification of 2017 (Table 3).

This classification is a useful tool for the definition of the various subtypes, which have different prognoses.

Prognosis

MDSs are an extremely heterogeneous group of disorders in terms of clinical outcome. In fact, they comprise low-risk, indolent forms (approx. 60-70% of cases) with a life expectancy similar to that of the general population, and high-risk forms (approx. 30% of cases), more similar to AML (30). Prognostic factors can be subdivided into factors correlated to the characteristics of the disease (percentage of blasts, presence of abnormal chromosomes, degree of bone

marrow failure) and factors linked to the characteristics of the patient (age, comorbidities, transfusional requirements).

Prognostic factors linked to the disease

In order to define the risk correlated to the characteristics of the disease, prognostic systems combining a wide range of clinical characteristics with hematological variables are used. 1997 saw the introduction of the IPSS (*International Prognostic Scoring System*), which is based on the evaluation of the percentage of blasts in the bone marrow, of cytogenetic abnormalities and the number of peripheral cytopenias (Table 4). On the basis of this system, 4 classes of risk are identified: (low, intermediate-1, intermediate-2 and high) with different average survival rates and probabilities of evolution into AML (31).

For many years the IPSS has been the reference system for clinical decisions and for the study design of numerous important clinical *trials*. It was subsequently observed that other factors could have an important prognostic significance, such as multilineage dysplasia, severe anemia or dependency on transfusions and the presence of bone marrow fibrosis.

More recently, the *International Working Group* for the prognosis of MDSs reviewed the IPSS system (IPSS-R) on the basis of the analysis of an extensive cohort of non-treated patients. This analysis made it possible to attribute a prognostic meaning even to the rarest cytogenetic abnormalities, identifying 5 groups of cytogenetic risk, rather than the three identified by the IPSS system; the cytogenetic abnormalities together with the evaluation of the bone marrow blasts and the peripheral cytopenias form the basis underlying the new system IPSS-R (32) (Table 5). There are calculation systems available online for defining the IPSS-R risk category (http://www.ipss-r.com). The patients included in the very high risk group have a median survival of only 0.8 years, while the average survival of the very low risk patients is 8.8 years, in any case very different from the life expectancy of individuals of the same age belonging to the general population.

Even although new factors with prognostic importance have been identified and new *scoring* systems have been introduced in order to improve the stratification of patients with MDS, the majority of clinical trials conducted so far to evaluated the efficacy and safety of the therapeutic agents have been chiefly based on the IPSS system. As a result, all the current recommendations based on therapeutic evidence refer to patients who were stratified according to the IPSS system. For this reason, it is currently recommended that all patients

Table 4. International Prognostic Scoring System (IPSS)

Variables	**Score**				
	0	**0.5**	**1**	**1.5**	**2**
Bone marrow blasts	<5%	5-10%	-	11-20	21-30
Karyotype*	Favourable	Intermediate	Unfavourable	-	-
Cytopenia*	0/1	2/3	-	-	-

Risk groups
SCORE 0= low (median survival 5.7 years - risk of leukemic evolution 25% at 9.4 years)
SCORE 0.5 -1= intermediate - 1 (median survival 3.5 years - risk of leukemic evolution 25% at 3.3 years)
SCORE > 1.5-2= high (median survival 1.2 years - risk of leukemic evolution 25% at 1.2 years)
SCORE > 2= high (median survival 0.4 years - risk of leukemic evolution 25% at 0.2 years)

*Favourable: normal, del(5q) (isolated alteration), del(20q) (isolated alteration), - Y (isolated alteration)
*Intermediate: other abnormalities;
*Unfavourable: complex karyotype (≥3 abnormalities), chromosome 7 abnormalities;

Cytopenia: hemoglobin <10 g/dL, platelets < 100x109/L, neutrophils > 1.8x109/L

Table 5. International Prognostic Scoring System-Revised (IPSS-R)

Variables	Score						
	0	**0.5**	**1**	**1.5**	**2**	**3**	3
Karyotype*	Very favourable		Favourable	-	Intermediate	Unfavourable	Very unfavourable
% Blasts	0-2	-	3-4	-	5-10	>10	-
hemoglobin	≥10		8-9	<8	-	-	-
Platelets	≥100	50-99	<50	-	-	-	-
ANC	≥0.8	<0.8	-	-	-	-	-

Risk Groups
SCORE ≤1.5 = **Very low** (median of survival 9.3 years - risk of leukemic evolution rare)
SCORE 2-3 = **Low** (median of survival 6.3 years - risk of leukemic evolution 25% at 10.8 years)
SCORE 4-4.5 = **Intermediate** (median of survival 3.4 years - risk of leukemic evolution 25% at 3.2 years)
SCORE 5-6 = **High** (median of survival 1.2 years - risk of leukemic evolution 25% at 1.4 years)
SCORE >6 = **Very High** (median of survival 0.6 years - risk of leukemic evolution 25% at 0.7 years)

*Very favourable: Y, del(11q)
*Favourable: normal, del(5q), del(12q), double change that includes del(5q)
*Intermediate: del(7q), +8, +17, i(17q), other abnormalities;
*Unfavourable: complex karyotype (3 abnormalities), double change that includes -7/del(7q); inv(3)/t(3q)/del(3q),
* Very unfavourable: complex karyotype (>3 abnormalities)

be stratified for risk according to this system. However, some clinical trials and prospective registries also include the stratification of patients according to the revised IPSS-R system.

Recently, it has been observed that also immunophenotyping analysis using flow cytometry can be useful for identifying subgroups of patients with different clinical characteristics and response to therapeutical treatments (33). Additionally, the spread of massive sequencing technologies has made it possible to identify new somatic mutations which could be added in the prognostic *scores*, allowing a more accurate stratification of patient risk. For five genetic abnormalities (mutations of genes *TP53*, *ETV6*, *RUNX1*, *ASXL1*, *EZH2*) an unfavourable prognostic significance has been defined (34). Gene *SF3B1* mutations are associated with anemia and the presence of ring sideroblasts and identify a sub-portion of patients with a favourable prognosis (35).

Prognostic factors linked to the patient

Various factors linked to the general state of health of the patient affect the clinical outcome and the management of the patient. Included among these factors are age, comorbidities, functional abilities (the PS), nutrition conditions and cognitive state. Advanced age is an independent unfavourable prognostic factor that affects the probability of survival and has been added to various prognostic *scores*. In patients affected by MDS a high incidence of comorbidity has been observed; on diagnosis more than half of the patients present one or more comorbidities with a significant impact on survival. The most frequent comorbidity is heart disease and a significant increase in cardiac complications has been reported in patients with severe anemia or transfusion dependence. The problems clinked to the existence of comorbidities when low- and high-risk MDS patient categories are considered. In low-risk patients the comorbidities affect prognosis by directly increasing the risk of death for causes unconnected with leukemic evolution. On the contrary, in high-risk patients the clinical relevance of slight to moderate comorbidities is eclipsed by the severity of the disease; but in these patients the comorbidities still affect clinical outcome by reducing tolerance to the therapeutic treatment.

The prognostic relevance of the comorbidities can have important implications in the management of

patients and greatly improve their risk stratification in relation to the criteria linked to the disease. This particularly applies to the low-risk patient group. In particular, two comorbidity *scores* potentially useful at a clinical level are the *Myelodysplastic Syndrome-specific Comorbidity Index* (MDS-CI) (36), developed for the general population of MDS patients receiving, above all, supportive care, and the *Hematopoietic Cell Transplantation-specific Comorbidity* index (37), developed for patients considered who are candidates for allogeneic hematopoietic stem cell transplantation. The two *scores* have a common basis for defining the degree of severity of the individual diseases and define a separate risk category on the basis of the comorbidity *burden* (Table 6).

Therapy of MDS

The therapeutic strategy depends on the evaluation of prognostic factors connected both with the type of disease and with patient-related variables.

Table 6. Myelodysplastic Syndrome-specific Comorbidity Index (MDS-CI) e Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI)

Comorbidity	**Score definition**	**HCT- CI**	**MDS-CI**
Heart	Atrial fibrillation arrhythmia, sick sinus syndrome, ventricular arrhythmia	1	-
	Any of the following heart diseases: coronary heart disease, myocardial infarct, congestive heart failure, FEV <50%	1	2
	Valvular diseases with the exception of mitral valve prolapse	3	-
Cerebrovascular	Disease Cerebrovascular TIA or stroke	1	-
Lung	Moderate pulmonary disease. Dyspnea on exertion or DLCO and/or FEV1 66%-80%	2	-
	Severe pulmonary disease. Dyspnea at rest or requiring oxygen therapy or DLCO and/or FEV1 <65%	3	1
Liver	Slight liver disease. Chronic liver disease with persistently altered bilirubin over 1.5 times normal values or AST or ALT up to 2.5 times normal values. Moderate-to-severe liver disease. Chronic liver disease with persistently altered bilirubin over 1.5 times normal values or AST or ALT over 2.5 times normal values.	1 3	- 1
Kidney	Moderate-to-severe kidney disease. Creatinine >2mg/dl, dialysis, transplant.	2	1
Tumours	Solid neoplasm in the past. Any previous treatment, excluding skin cancer.	3	1
Rheumatic diseases	Rheumatological diseases, SLE, rheumatoid arthritis, mixed connective tissue disease, rheumatic polymyalgia.	2	-
Gastroenteric	Peptic ulcer Treatment request	2	-
Diabetes	Diabetes treated with insulin or oral anti-diabetics (not just diet)	1	-
Obesity	Obesity BMI >35kg/m3	1	-
Psychiatric	Psychiatric disorders. Depression, anxiety requiring treatment.	1	-
Infections	Infections requiring antibiotics after day 0. To be filled in only for patients subjected to a transplant.	1	-

SCORE	**Risk Groups**
0	LOW
1-2	INTERMEDIATE
≥3	HIGH

The modulation and personalization of the treatment is based on the following essential elements: WHO diagnosis, evaluation of the karyotype and consequent calculation of the IPSS risk score, age, PS and comorbidities. With this information, the ideal type of therapy can be defined and provided while taking into consideration the therapeutic aggressiveness that can be tolerated by the patient in question.

For high-risk patients (IPSS intermediate-2 and high), characterized by a high tendency of leukemic progression, an attempt is made, where possible, to change the course of the disease using hypomethylating agents, reserving the allogeneic stem cell transplantation option for the few patients able to tolerate the procedure. In low-risk patients (IPSS low and intermediate-1), where leukemic progression is less frequent and death may often be connected with heart problems aggravated by anemia, the priority is to correct the cytopenias and improve the patient's quality of life.

The therapeutic orientation recommended by most guidelines may be roughly summarized as follows:

• IPSS score low or int-1. No treatment is envisaged until the level of anemia drops below Hb levels of 10 gr/dl. When the anemia is symptomatic or the Hb level is below 10 gr/dl, Erythropoiesis-stimulating agents (ESA) may be used. When cases with an abnormality of 5q- isolated or in association with other karyotype alterations - become transfusion-dependent, they can successfully benefit from therapy with lenalidomide. Allogeneic transplantation, for cases involving relatively young people without comorbidities, should generally be taken into consideration, not in the early phases but when the anemia starts to progress and the need for transfusional support arises.

• IPSS score int-2 or high. The possibility of allogeneic transplantation should be considered in the early stages in cases that would benefit from it due to their age and lack of comorbidities, when a donor is available. Most patients in this risk bracket are potential candidates for hypomethylating therapy with azacitidine for at least 4-6 cycles, to be continued if a positive response is obtained.

Hypomethylating therapy in myelodysplastic syndromes and acute myeloid leukemia

The high-risk MDSs which, according to the *National Comprehensive Cancer Network* (NCCN) guidelines include patients with IPSS scores intermediate-2 and high, or IPSS-R scores high-very high and intermediate in cases resistant to a previous line of treatment (for the most part erythropoietin), present median survival rates not much different from those of AML.

For these diseases, the only curative therapeutic option is allogeneic transplantation, which can, however, only be practised in less than 10% of patients, for reasons connected with age and comorbidities. For many years hypomethylating therapy, including azacitidine and decitabine, has been available for these patients.

In particular, azacitidine (Vidaza, AZA, Celgene) at the standard dose of 75 mg/m^2/7 days per month is recommended in Italy for MDSs with intermediate-2 and high IPSS scores and for chronic myelomonocytic leukemia (CMML) with 10-29% bone marrow blasts without myeloproliferative disorder. Having been approved in the context of IPSS, which was based on the FAB classification, AZA was originally indicated also for acute myeloid leukemia with 20-29% bone marrow blasts and multilineage dysplasia. This year it has been approved in Italy also for patients with AML who are not eligible for hematopoietic cell transplant with over 30% blasts, with multilineage dysplasia. In patients under 65 years of age, AZA is reimbursable in cases with unfavourable cytogenetic risk, while for patients over 65 years of age it is approved irrespective of the cytogenetic risk.

Decitabine (DAC, Dacogen, Janssen) is, on the other hand, indicated in adult patients newly diagnosed with "*de novo*" or secondary AML, on the basis if the WHO classification, who are not candidates for standard induction chemotherapy.

This discussion will also include the most extensive studies on the approved use of AZA and DAC.

Hypomethylating therapy with azacitidine and decitabine

The efficacy of hypomethylating drugs for MDS and AML is probably linked to the high levels of

methylation which perform an important pathogenetic role in these diseases, blocked by the hypomethylating effect of the therapy. To this is added the cytotoxic action, more evident in the case of decitabine, due both to a direct mechanism and to the induction of proapoptotic gene expression (38). The hypomethylating mechanism justifies the need to carry out at least 4-6 cycles before a response to the treatment is observed along with fewer adverse effects compared with the standard induction chemotherapy. In particular, the frequency of hematological adverse effects (neutropenia, anemia and thrombocytopenia) and other adverse effects (especially nausea, vomiting and diarrhea) is higher during the first cycle of treatment and tends to gradually subside. This reinforces the concept that if the treatment is interrupted too early, the patient is exposed to the adverse effects without being able to experience the hematological benefits which appear, on average, after approx. 4-6 months. The reversibility of the hypomethylating effect also explains the need to continue the treatment until time to progression in responsive patients. In fact, the interruption of the treatment inevitably leads to the progression of the disease and frequently the death of the patients after a median period of approx. 5 months (39, 40). This is connected with the fact that the disease is not eradicated, as shown by the persistence of the carrier clones of specific somatic mutations, especially at stem cell level, irrespective of whether a clinical response has been obtained (41).

Hypomethylating therapy of MDS

In MDS, after the phase III trial that demonstrated the possibility of inducing a hematological response and an overall survival advantage in patients treated with azacitidine compared to standard therapy regimens (n=358 pts., median survival rate: 24 vs15 months) (42), the efficacy of this treatment was confirmed in numerous *real life* clinical experiences (Table 7). In particular, a study carried out by the cooperative group GROM-L demonstrated that AZA induced overall responses in 56% of the patients (including CR in 18.5%, partial remission (PR) in 17% and hematological improvement (HI) in 21%) and an overall median survival time of 17.1 months (43). Among the prognostic factors, the low comorbidity index and the response to the treatment (not only including CR/PR/HI, but also stability of the disease, observed in 28.5% of the patients) proved to be factors independently associated with prolongation of survival. In the AZA-001 trial, moreover, it was confirmed that continuation with the treatment after the achievement of hematological improvement is associated in 48% of cases with an improvement in the quality of the response after a median period of 3 cycles (*range*: 1-11) (44). This finding has also been confirmed by numerous *real life* studies, which show how prolonged treatment lasting more than 6 cycles can induce responses in a further 20-25% of cases (43).

Hypomethylating therapy of AML

AML is a disease typically found in the elderly, with a constantly increasing incidence after the age of 60. In these patients, the prognosis is particularly unfavourable, due to factors connected both with the patient and with the biological characteristics of the disease. The efficacy of AZA in AML was already evident in the phase III study of 2009, in which the FAB classification was applied and 113 patients with 20-29% of blasts were included (45). In these patients the probability of complete remission was 18% and was similar to that of the patients receiving standard therapy (16%). Survival proved to be significantly longer in the AZA arm of the trial (24.5 vs 16 months, p=0.005), with results similar to those observed in high-risk MDSs. One of the criticisms of this trial was that the survival of the control group was also long, compared to "typical" cases, which generally only last a few months. This was probably linked to the selection of the patients in the context of a multicentre clinical protocol, but the advantage of survival with AZA was, in any case, evident and was associated with a shorter stay as a hospital in-patient (45).

In the trial that followed, AZA-AML-001, 241 elderly patients with AML were randomized to receive either AZA or a conventional care regime (CCR), among which low dose cytarabine (n= 158), intensive chemotherapy (n=44), or supportive care (n=45) (46). Since approx. 13% of the patients in the CCR arm were receiving azacitidine as a salvage therapy, the

Table 7. Hypomethylating therapy of MDS and AML real life studies

References	Therapy	Patients (n)	Responses (%)	Responses time median (range)	Median OS (months)	Median PFS (months)
MDS						
Fenaux, 2009 (42)	Azacitidine 75 mg/m²/7days	179 pts	17% CR/ 12% PR/ 10% HI	2 cycles (1-16)	24,5	17,8
Voso, 2013 (40)		196 pts	19% CR/ 17% PR/ 21% HI	4,5 cycles (7-15)	17,1	-
Sebert, 2017 (53)		702 pts MDS/AML	44% ORR/ 15% CR	-	14,9	-
AML						
Dombret, 2015 (46)	Azacitidine 75 mg/m²/7days	236 pz	28% CR/CRi	-	10,4 12,1*	9,3
Pleyer, 2017 (58)		193 pz	19% CR/CRi/PR	-	10,7	13,8
Kantarjian, 2012 (49)	Decitabine 20 mg/m²/5days	242 pz	18% CR/CRp	4,3 months	7,7	8,5
Blum, 2010 (50)	Decitabine 20 mg/m²/10days	53 pz	64% CR/CRi	3 cycles (1-6)	14	12

* Post-hoc analysis: time to the next treatment
CR = complete remission; CRi = CR with complete haematological recovery; PR = partial remission; HI = haematological improvement; OS = overall survival; PFS: progression-free survival

prolongment of survival in the AZA arm only became evident in a *post-hoc* analysis in which the patients were registered at the moment in which they changed therapy (OS: 12.1 months with AZA vs 6.9 months with CCR, p=0,019). Other *real life* studies have confirmed these data. Among these, the Austrian registry has reported the results of treatment with AZA in 302 patients affected by AML, 79 of whom with 20-30% blasts and 172 of whom with more than 30% blasts (47). Responses were observed after an average of 3 months, in percentages similar to those reported for MDS (48% of the total) and with at least 31% of improvement in the quality of the response at subsequent moments. Unfortunately, the median duration of the response was limited (3.4 months) and survival was 9.6 months from the start of treatment.

Treatment with decitabine proved positive in AML after various attempts with variable doses. In fact, the first trial, which included cases of high-risk MDSs, chronic myelomonocytic leukemia and AML, envisaged a dosage of 15 mg/m² i.v./every 8 hours/day in three administrations, not very practical in an outpatients' setting, with a maximum of 10 cycles for patients in CR (48). Later studies, on the other hand, introduced the regimen of 20 mg/m²/day i.v. for 5 or 10 days per month. An international, multicentre randomized open phase III trial compared the efficacy and safety of decitabine (20 mg/m²/day

for 5 consecutive days every 4 weeks) vs cytarabine or the best supportive care, in a cohort of 485 patients with an average age of 73 years (64-91 years), newly diagnosed with *de novo* or secondary AML, and low or intermediate risk cytogenetics (49). The percentage of CR or CR with incomplete blood count recovery (CRi) was 17.8% with decitabine vs 7.8% in the control arm (p=0.001) and OS was significantly longer in the decitabine arm, considering the *cut-off* extended to 2010. The benefit of decitabine was more evident in patients ≥70 years, with *de novo* AML, more than 30% bone marrow blasts, low to intermediate risk cytogenetics and ECOG PS ≥2.

In order to try to increase the efficacy of the treatment, in a phase II clinical study it was decided to explore the following treatment plan: DAC 20 mg/m^2/day for 10 days in patients over the age of 60, with previously untreated AML (50). The overall response rate (ORR) was 64%, including 47% of CR and 17% of CRi, without differences on the basis of karyotype and toxicity similar to the 5-day plan. Similar data were reported by Ritchie et al. (51) out of 52 patients treated with decitabine for 10 days, for at least one induction cycle. After achieving CR, most of the patients continued with the plan for 5 days, until the appearance of toxicity or progression of the disease. The CR rate was 46% and the median OS of 11 months, while an average of 2 cycles were needed to obtain a response (1-4 cycles). So in this study, the 10-day treatment plan was well-tolerated, with toxicity similar to the 5-day schedule.

The possibility of prolonging survival evident also in patients with AML able to maintain the stability of the disease with hypomethylating therapy, made it possible to include stability of the disease among the response criteria provided for by the recently published ELN guidelines, at least in the context of clinical trials (52).

In general, the median survival time of patients responsive to hypomethylating therapy is approx.13-15 months, with relapses in the vast majority of patients. In order to obtain more prolonged responses of better "quality", various attempts were made at therapeutic combinations with inhibitors of histone deacetylase, lenalidomide and immune*check-point* inhibitors. At the moment although the combinations have significantly increased the adverse effects, particularly cycopenias and infection complications, they do not seem to have improved the responses. Probably new treatment schedules will have to be developed with a view to reducing the occurrence of adverse events.

Prognostic factors

In general, the factors that affect the outcome of the treatment with hypomethylating agents are largely unknown (53). Since this remains a "challenging" treatment which requires several months before demonstrating its potential benefits, there is great interest in the study of predictive response factors. Among these, the doubling of the platelet count at the beginning of the second cycle of therapy has been reported as a favourable prognostic factor for azacitidine and decitabine. This is a phenomenon observed in approx. 20% of patients, but its biological bases are unknown(54).

The *Groupe Francophone des Myelodysplasies* (GFM) has developed a clinical *score* that includes ECOG PS ≥ 2, existence of circulating blasts, transfusion dependence (≥ 4 units of Red Blood Cells/8 weeks) and unfavourable intermediate karyotype (55). This *score* identifies three groups characterized by a median survival time of 6 and 15 months in the groups with high and intermediate risk and not reached in the favourable group.

Over recent years numerous somatic mutations which play a prognostic role have been identified in MDS and AML. In particular, the number of genes mutated (over 2) is, in itself, an unfavourable prognostic factor both in MDS and in AML (56,57). In the context of hypomethylating therapy, mutations of *TET2* play a favourable role in the majority of the trials, while mutations of *TP53* play an unfavourable role also in the context of an allogeneic transplant after a*bridge* with azacitidine. Decitabine used for 10 days seems, on the other hand, to delete the TP53-mutated clone and its unfavourable effect (58), although these data require confirmation on a larger scale.

Neutropenia, neuropathy and other risk factors in the elderly patient with myelodysplastic syndrome and acute myeloid leukemia

Infections are one of the main causes of morbidity and mortality in elderly patients affected by MDS and AML (60-62). The risk factors for infection in these patients do not substantially differ between the young and the old, but in the latter type of patient PS, which is often poor, and comorbidities that increase exponentially as age increases play an important role also in predisposing the patient towards infection (14). The risk of infection in elderly patients with AML and with MDS is highly variable and depends on the interaction between the state of immunosuppression due to the underlying disease or to the treatments carried out, and possible organ dysfunction, more frequent in the elderly patient, and exposure to opportunistic pathogens (63,64).

Although neutropenia is the main predisposing factor in these diseases, various other immune disorders have been reported in MDS and AML, evolved from a previous phase of myelodysplasia. Among these impairment of the neutrophil function, defects in B,T and NK cells and the possible consequences of iron overload could underlie the increased risk of infection in this type of patient (Figure 1).

Generally speaking, we can distinguish infectious risk factors connected with the underlying disease, factors connected with the patient and factors connected with the therapy administered to treat the underlying disease.

Neutropenia

Severe and prolonged neutropenia with polymorphonucleates (PMN) <500/mm3 is undoubtedly the main risk factor for the development of infections in the elderly patient with AML and MDS at diagnosis. Neutropenia occurs in approx. 50% of patients with newly diagnosed MDS, including 70-80% of patients with high-risk MDS and 15-20% of patients with low-risk MDS (65). In the GROM retrospective registry the incidence of severe neutropenia at diagnosis

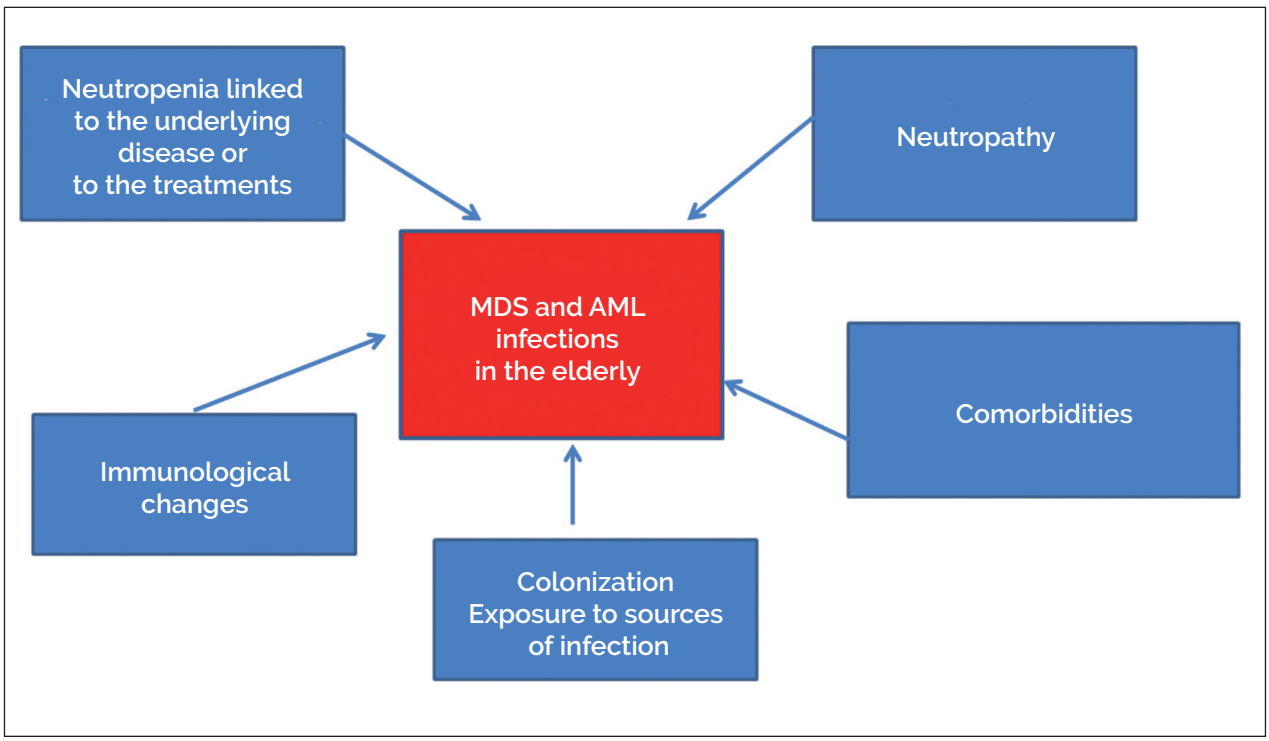

Figure 1. Infectious risk factors in the elderly patient with MDS and AML

in patients with an IPSS score of intermediate 2 /high was 60% (66).

In advanced MDS and AML, neutropenia is part of a more general process of bone marrow failure which combines altered differentiation, resistance to apoptosis and leukemic proliferation. In low-risk MDS on the other hand, it is mainly due to an increase in apoptosis (67,68) or in some cases to the intervention of immune mechanisms through T cell-mediated inhibition of hematopoiesis or autologous granulocytes. This latter T cell-mediated mechanism is probably associated with the increase observed in the plasma levels of TNF-α and IFN-γ and is potentially reversible through the administration of an immunosuppressive treatment (69,70).

That neutropenia is the main but not the only cause of infection in MDS is confirmed by the fact that the absolute neutrophil granulocyte count was not found to be related to survival rates following episodes of infection, at least in a historical series of patients with MDS in various risk categories (61). It is also important to point out how the risk and severity of infectious episodes in elderly patients can be increased by other factors such as their frequent comorbidities and the use of drugs such as hypomethylating agents, which are effective in the treatment of MDS and AML but which can transiently worsen the neutropenia, especially during the initial treatment cycles (42,71-78). Neutropenia in itself does not, however, preclude either the starting and/or continuing hypomethylating therapy, also in consideration of the fact that in responsive patients the neutropenia becomes less severe, or has even cleared up by the third or fourth cycle of treatment.

The effects of hypomethylating agents on the risk of infection is scarcely and heterogeneously documented, with a percentage in the various *trials* of infectious events or febrile neutropenia varying from 1% to over 50% in some studies (42,71-78).

Neutropathy and other immunological disorders

In MDS a differentiation defect of the multipotent stem cell can lead - as regards the myeloid lineage - not only granulocytopenia, but also in an abnormal function of the polymorphonuclear neutrophils, whose task it is to defend the body against pathogenic microorganisms through the mechanism of phagocytosis.

Normally, the response of the neutrophils, crucial for the eradication of the invading bacteria, can be broken down into the following steps: adhesion and movement in the endothelium of the vessel wall, migration towards the site of inflammation, diapedesis through the endothelial layer, degranulation and lastly phagocytosis of the bacteria through the so-called respiratory "*burst*" with the production of reactive oxygen species (ROS) bactericides and the action of lysosomal enzymes.

The neutrophil granulocytes are attracted to the sites of infection by mediators of inflammation produced in these sites, among which, of particular importance, is interleukin-8 *or CXC chemochine ligand 8* (IL-8 or CXCL8) produced by monocytes and endothelial cells, which activates the *CXC chemokine receptors 1 and 2* (CXCR1 and CXCR2) and the *growth-related oncogene* (GROα)/CXCL1 (79,80). On meeting the bacteria, the neutrophils enclose the microorganisms inside the phagosome, which fuses with the intracellular granules, forming the phagolysosome. In the latter, the bacteria are killed by exposure to enzymes, antimicrobial peptides and ROS species (81).

Various neutrophil defects have been recorded in patients with MDS or with AML with dysplasia, often associated with morphological abnormalities (82). Many of these are involved in the process of attracting the polymorphonuclear neutrophils, towards the site of inflammation and killing of the pathogenic bacteria:

- Deficit in the contents of the primary granules, the main enzyme of which is myeloperoxidase (MPO) which normally plays a role in the *killing* of the bacteria (82-85).
- Deficit in lactoferrin and protease antibiotics, such as elastase and cathepsin G, and deficiencies in the glycoproteins of the granular membrane (83,86-88).
- Alteration in the production of ROS, essential for the bactericide function of the neutrophils during the respiratory *burst* (89-91).
- Reduction of the cellular membrane expression of the complex CD11c/CD18, which regulates adhesion, migration and diapedesis (92-94).
- Deficit in the chemotaxis of the neutrophils (93-95).
- Reduction of the migration towards gradient formed by IL-8/GROα, essential for the re-

cruitment and migration of the neutrophils towards the site of infection, compared to healthy donors (96).

Reduced bactericidal and fungicidal activity of dysplastic neutrophils was demonstrated in a study that evaluated *in vitro*cytocidal effector functional capacity against Gram-positive and Gram-negative bacteria and against yeasts, polymorphonuclear neutrophils isolated from patients affected by myeloidysplastic syndrome and compared with those separated from healthy volunteers (97).

In addition to neutropenia and neutropathy, immunological changes have been frequently reported, particularly in MDS. The impact of B-cell impairment in MDS on infectious risk has not been well defined. In a retrospective trial on 284 patients with MDS, the absolute numbers of peripheral B lymphocytes were found to be reduced in patients with MDS compared to controls (98). Hypergammaglobulinemia and hypogammaglobulinemia were found in 39% and 8% of the patients with MDS, respectively (99,100). The majority of patients with MDS also present with lymphocytopenia, mainly due to a decrease in T-helper lymphocyte counts. (98,99). It is not known, however, whether such imbalances affect the risk of infections in MDS. Anomalies of regulatory T (Treg) cells have also been described in patients with MDS, in particular a reduced number of Treg cells in high-risk MDS, and impaired Treg function, notwithstanding a normal absolute count in low-risk MDS (101).

With regard to NK cells in patients with MDS, a reduced expression of NK receptors such as NKG2D has been observed with lower levels of IL-32 in the NK cells themselves; both of these aspects could contribute to the impairment of the cytolytic function of the NK cells observed in these patients (102-104).

Colonization and environmental exposure

Admission to hospital and exposure to potential sources of fungi are two very important aspects for the risk of bacterial infections (hospital-acquired often due to multi-resistant germs) and fungal infections, respectively. The risk of colonization with multi-resistant germs is, however, greater in patients with AML who are admitted to hospital for intensive chemotherapy (105,106). For elderly patients with AML or MDS who receive hypomethylating therapy as outpatients, this risk seems to be lower.

The potential relation between exposure to fungal sources prior to hospitalization and the development of invasive fungal infections (IFI) in adult patients newly diagnosed with AML after their first cycle of chemotherapy was evaluated in a prospective multicentre study involving 33 Italian hematology centres (107). In this study, in a multivariate analysis the following pre-treatment variables were identified as high risk factors for invasive fungal infections: *performance status* of 2 or more; chronic obstructive pulmonary disease; recent renovation of home or work environments with high exposure, such as construction works, agriculture and gardening.

In general, on the basis of literature, the risk factors for IFIs in AML can be classified in four main categories: leukemia-related factors (advanced phase of the disease, resistance to treatment/ failure to achieve complete remission), factors related to the patient (PS, comorbidities, advanced age, organ dysfunction, unfavourable karyotype), factors related to the treatment (severe and prolonged neutropenia, severe mucositis associated with chemotherapy) and factors related to the exposure of the patient to potential sources of fungi (rooms without HEPA filters, previous invasive fungal infection).

Risk of bacterial infection - prophylaxis and treatment

The possibility that the treatment with hypomethylating agents could worsen the risk of bacterial infections in patients affected by acute myeloid leukemia or myelodisplasia was considered for the purposes of phase III studies.

Rate of bacterial infections in phase III trials

In the AZA 001 trial, which evaluated the efficacy of azacitidine in MDS patients with an intermediate or high IPSS score, the percentage of infections per patient/year of exposure did not differ to a statistically significant degree between the azacitidine group and

the *best supportive care* group. Similarly, the percentages of pneumonia and sepsis were not different between the two groups (42,108). Similar results were found in the CALGB study (71). Even only considering patients affected by AML with a percentage of blasts between 20 and 30% extrapolated from the study AZA 001, the risk of experiencing a febrile event requiring the intravenous administration of antibiotics was not statistically significant, if the patients pre-selected for intensive chemotherapy were excluded from the analysis. The only statistically significant difference in the use of intravenous antibiotics was between the azacitidine group vs cytarabine at low doses (0.2 vs 0.8 events per patient/year). With regard to the patients affected by AML with blasts over 30% treated with azacitidine, there are no data from the AZA AML 001 study (46) on the use of intravenous antibiotics or on sepsis, but an incidence of febrile neutropenia grade 3 or 4 can be observed, similar in the study group and in the control groups (28%, 27,5%, 30,1% and 31% in the azacitidine, *best supportive care*, low-dose cytarabine and intensive chemotherapy groups, respectively).

With regard to decitabine, the phase III study on its use in AML (49) reports an incidence that is superimposable in the study group and the low-dose cytarabine group (21% vs 15%).

Therefore, on the basis of the results of the phase III studies, the risk of infection with hypomethylating therapy does not seem different from that existing with standard therapy.

Infection complications and risk factors: retrospective studies

The publications considered so far do not contain indications on antibacterial prophylaxis which, in the phase III trials, was left to the common clinical practice of the centre. There is, however, a series of retrospective studies that took into consideration antibacterial prophylaxis and risk factors for infection (Table 8).

In the Merkel study (109) 184 patients affected by high-risk MDS or AML were enrolled and treated with azacitidine. In a multivariate analysis, unfavourable cytogenetics, platelets counts of less than 20,000/mm3 or neutrophils below 500/mm3 at *baseline* were predictive of an increased risk of infection during the first two cycles of therapy, while no parameters emerged at *baseline* predictive of a risk of infection in later cycles. In the evaluation prior to each individual cycle of azacitidine, the presence of high-risk cytogenetics and platelet counts of less than 20,000/mm3 identified with a sensitivity of 53% and a specificity of 66% the cycles at risk of an infectious event. The infectious events decreased markedly as the cycles continued (26% in the first cycle, 7% after the fifth cycle).

In Falantes' retrospective study (110) 64 patients affected by MDS or AML were enrolled and treated with azacitidine. 48% of the patients manifested an infectious event in the course of three years. The most frequent type of infection was pneumonia, following by urinary tract infections. Also in this case the neutrophil count (greater or less than 500/mm3) had no effect on the probability of infectious complications. 86% of the episodes took place during the first three cycles.

In Schuck's retrospective study (111) 77 patients affected by MDS were enrolled and treated with azacitidine. 55 patients manifested at least one infectious event grade 3-4 during the treatment. 88% of infectious complications were attributed to bacterial infections. The percentage of infections was higher in the first 3 cycles, with a statistically significant difference. The response to azacitidine, both in terms of *hematological improvement* and in terms of remission, was associated with a lower percentage of infectious events.

In Lorenzana's retrospective study (112) 76 patients affected by MDS or AML were analysed during the first 4 cycles of azacitidine. 43% of the patients experienced infectious events: 34% of these were fevers of unknown origin (FUO), while the majority were diagnosed as pneumonia or urinary tract infections. In a univariate analysis, the IPSS, number of neutrophils and duration of the neutropenia during the cycle were found to be factors related to the number of infectious events. Prophylaxis with quinoline was administered in 41% of the cycles and the cycles during which it was used showed lower neutrophil counts, characteristic of the more severe form of the disease. Overall, the prophylaxis did not reduce the incidence of infectious events, but considering only the cycles with neutrophils less than 500/mm3, the incidence of infections was significantly lower (16 vs 51%, $p<0.001$).

Table 8. Summary of the evidence currently available on bacterial infections in patients affected by AML or MDS under hypomethylating therapy: retrospective studies.

References	Therapy	Patients (no.) and Diagnosis	Rate of bacterial infection events	Bacterial infection risk factors
Merkel, 2013 (108)	Azacitidine	184 pts High risk MDS/AML	16.5% of cycles; 59% of pathogens identified were bacteria	Unfavourable cytogenetics, platelests<20.000/mm3, neutrophils <500/mm3 at baseline (first 2 cycles of therapy).
Falantes, 2014 (109)	Azacitidine	64 pts MDS/AML	48% of pts; 86% of the events in first 3 cycles	-
Schuck, 2017 (110)	Azacitidine	77 pts MDS	71% of pts; 88% of bacterial infections; majority of events in first 3 cycles	No response to azacitidine
Lorenzana, 2017 (111)	Azacitidine	76 pts MDS/AML	43% of pts; 87% of episodes of bacterial origin	IPSS at diagnosis, no. of neutrophils, duration of neutropenia, no prophylaxis with quinoline in cycles with neutrophils <500/mm3.
Voso, 2016 (43)	Azacitidine	MDS/AML with low blast count	146 events/1528 cycles; 1st event after approx. 4 cycles	-
Ali, 2017 (77)	Decitabine 10 days	85 pts AML/MDS	77.5% of pts with AML; 96.3% of pts with MDS; 95% of episodes of bacterial origin.	Prophylaxis with quinoline is not related to multi-resistant bacterial infections nor does it reduce mortality.
Candoni, 2017 (114)	Decitabine	150 pts AML	14% of cycles; events more frequent in first 3 cycles	-

Bainschab's retrospective study (113) analysed 40 patients affected by AML treated with low-intensity regimens: azacitidine in 69% of the cycles, decitabina in 25% of the cycles and low-dose cytarabine in 6% of the cycles. 73% of the patients manifested at least one infectious complication during the administration of the chemotherapy. The most frequent manifestation was, again, pneumonia, followed by gastroenteritis and urinary tract infections. Antibiotic prophylaxis, mostly with quinoline, was administered in 37% of the cycles and appears to correspond with a statistically significant reduction in infections.

Ali's retrospective study (78) takes into consideration 85 patients affected by AML or MDS, treated for 10 days with decitabine. In the patients affected by AML 77.5% of 3-4 grade infections were documented, while a higher, statistically significant, incidence of infections was documented in patients with myelodysplasia (96.3%). These percentages are decidedly higher compared with the data of the 5-day schedule (114). The microorganisms most frequently isolated were Gram-positive cocci, perhaps due to the use of central venous catheters. The patients who were being administered prophylaxis with quinoline at the time of the infectious episode tended to have fewer positive cultures compared to those who were not receiving prophylaxis, but this difference was not statistically significant. It is important to point out that the

presence of prophylaxis with quinoline at the time of the infectious episode was not correlated to a higher incidence of infections microbiologically documented as multi-resistant bacterial infections, nor to a lower mortality rate.

In an Italian retrospective study that enrolled 150 patients in 17 Italian centres (115), the incidence of infectious events in patients affected by AML and treated with decitabine was found to be equal to 14% of the cycles. Infectious events were significantly more common during the first 3 cycles than in those that followed. Pneumonia accounted for 41% of the events, and sepsis for 21% (115).

In a retrospective study by Voso et al. (43), which enrolled patients affected by myelodysplasia or low-blast-count AML treated with azacitidine, 146 infectious events were reported during 1528 cycles of azacitidine. On average the first infectious episode took place after approx. 4 cycles of azacitidine. The number of neutrophils in relation to the infectious events did not appear statistically significant. The number of platelets on diagnosis appeared significantly higher in patients who developed an infectious complication. The most frequent were pneumonia, skin infections, genitourinary tract infections and upper airways infections.

Antibacterial prophylaxis in patients in treatment with hypomethylating agents

With regard to antibacterial prophylaxis in this patient *setting*, wishing to adhere strictly to current guidelines, the treatment indicated would be quinoline, since the neutropenia expected is prolonged. It should, however, be considered that not all of these patients recover a neutrophil value of over 500/mm3 and that the efficacy of the prolonged use of quinoline in reducing sepsis and, above all, death, in these times of multi-resistant microorganisms is, at the very least, doubtful. There are no prospective studies on the use of antibacterial prophylaxis in this patient *setting*; some suggestions come from the retrospective studies considered, but the level of evidence is really very poor. Similarly, also the evidence on the risk factors associated with infectious events (such as, for example, unfavourable cytogenetics, platelet counts of less than 20,000/mm3, neutrophils less than 500/mm3, failure to respond to the therapy, first 2-3-cycles of therapy) all derives from retrospective studies. For this reason, while awaiting prospective trials, our suggestion is that the use, if any, of prophylaxis with quinoline should only be considered during the first two cycles of therapy and only if indicated on the basis of the risk factors of the individual patient and local epidemiology (as regards multi-resistant microorganisms).

Antibiotic therapy in patients in treatment with hypomethylating agents

An extended discussion of the problems connected with antibiotic treatment for febrile neutropenia in elderly patients under hypomethylating therapy lies outside the scope of this publication, so only a few general principles are provided. In the case of febrile neutropenia in patients under chemotherapy for AML, empirical antibiotic treatment must be initiated rapidly. When choosing the antibiotic it is necessary to consider the possibility of using wide-spectrum drugs with an anti-Pseudomonas action, and to evaluate the possible presence of previous infections or colonizations by multi-resistant germs, local epidemiology (especially in terms of resistance), the patient's organ functions, potential sites of infection, allergies and recent exposure to antibiotics, including prophylaxis. The empirical antibiotic therapy can be modified on the basis of the results of the culture tests or if the patient becomes unstable. On the basis of the culture tests, it may be necessary to consider a possible *de-escalation* of the therapy (116,117).

Risk of fungal infection - prophylaxis and treatment

IFI in hematological patients are major complications to be feared, since their onset could trigger the terminal event of the hematological disease or, more often, complicate it at the beginning or during the various phases of treatment, significantly and negatively affecting the whole treatment plan (118). IFI in hematological patients most often take the form of filamentous fungi with a clear prevalence of aspergillosis (approx. 80 % of all IFI), followed by zygomycosis

and fusariosis; less frequent (with an incidence of less than10%) are yeast-related IFI (with a prevalence of non-albicans *Candida*, often resistant to fluconazole) (119).

The hematological patients at the highest risk for IFI today are still those with AML and those subjected to allogeneic bone marrow transplantation; however the introduction of new targeted molecular or antigene therapies are bringing about changes in the epidemiological scenario, with the emergence of new risk categories (such as patients with myeloma and chronic lymphoproliferative disorders treated chronically and/ or with repeated therapeutic lines, also including new agents) (119,120).

Risk factors for fungal infections

The risk factors for IFI in AML and high-risk MDS (MDS-HR) have already been dealt with in an earlier chapter. It should nonetheless be underlined that if they are correctly identified at an early stage, as recently proposed by the group SEIFEM (Sorveglianza Epidemiologica InFezioni nelle Emopatie, Epidemiological Surveillance of Fungal Infections in Hematological Malignancies), the patient can be appropriately placed in a high, intermediate or low IFI risk bracket, on the basis of their need for monitoring, prophylaxis and antifungal treatment (120). In particular, in patients with AML, advanced age, active leukemia (onset/relapsed/ refractory), severe (less than 100/mm3) and prolonged neutropenia, a previous IFI, mucositis or intensive polychemotherapy have high risk factors for IFI. In patients with MDS-HR, in addition to the risk factors for IFI already mentioned, iron overload consequent to an intensive transfusion regimen should also be included (60). In both cases, (AML and MDS-HR) the presence of comorbidities is also very important, especially diabetes, chronic obstructive pulmonary disease (COPD), the co-existence of other neoplasms or of autoimmune diseases treated with immunosuppressive therapy (60,121).

In particular, the degree of *fitness* of the elderly patient with AML and MDS-HR is an independent prognostic factor that is correlated with survival in patients treated with hypomethylating agents and which can affect the onset of intervening complications, also of an infectious nature (122,123). Recent studies have observed that PS (assessed with the aid of the ECOG Karnosky scales) is a subjective and rather imprecise evaluation of the *fitness* of the elderly hematological patient. Scales that include cumulative comorbidity indices are more objective. Such is the case of the CIRS scale (*Cumulative Illness Rating Scale,*) which makes it possible to calculate a comorbidity *score* by providing a cumulative numerical index which defines, objectively and precisely, the severity of the concomitant illnesses (123). The CIRS method for evaluating elderly patients' state of health has recently been made available also on the portal of the Italian Hematology Society (SIE) with the possibility to make the calculations *online*. Moreover, in 2013, GITMO-SIES and SIE jointly published the *consensus-based* definitions of "*unfitness to intensive and non-intensive chemotherapy in AML*" (124). The evaluation of the *fitness* of the elderly patient with AML is of fundamental importance for deciding objectively whether the patient is: 1) fit for intensive chemotherapy; 2) only fit for non-intensive chemotherapy (hypomethylating agents); 3) only fit for supportive care (124).

IFI incidence in patients treated with hypomethylating agents

With regard to the incidence of IFI in patients with AML and MDS, treated with hypomethylating agents (azacitidine and decitabine), no targeted prospective studies have been published yet. The data available on infectious complications derive partly from phase III studies on azacitidine and decitabine, in which the primary or secondary *endpoint* was not, in any case, the incidence of infections, or from retrospective studies with rather heterogeneous study populations, *endpoints* considered, treatments received, definition and detail of infectious events, and so they are not easily comparable (60,121,125). Overall, from the data available it can be seen that the infectious complications are common in patients treated with hypomethylating agents for both MDS-HR and AML and affect, in the studies published, not less than 30-60% of the patients treated (60,125). Table 9 reports the observational studies published over the last 4 years which had as their primary and secondary *endpoints*

Table 9. Retrospective observational studies published in the period 2014-2017 with primary and secondary endpoints, the incidence of infections in patients with AML or MDS-HR in treatment with hypomethylating agents (decitabine or azacitidine).

Reference	Therapy	Patients (no.) and Diagnosis	Prophylaxis *active moulds*	Rate of fungal infections	Risk factors for fungal infections
Ali, 2017 (77)	Decitabine 10 days (282 cycles)	85 pts AML (68%)/MDS	No primary *active mould* prophylaxis	Incidence of Infections: 96.3% in MDS and 77.5% in AML; infections microbiologically documented in 45%; **fungal infections: 6.6% of documented infections**.	-
Borlenghi, 2017 (126)	Decitabine 5 days (761 cycles)	150 pts AML	Not specified	Incidence of Infections: 73%; higher incidence in the first 3 cycles; pneumonia: 37% of documented infections; **fungal pneumonia: 9% of documented infections**.	-
Schuck, 2017 (110)	Azacitidine (614) cycles	77 pts MDS	Not specified	Incidence of Infections: 71%; higher incidence in the first 3 cycles; **fungal infections: 7% of documented infections**.	IFI incidence higher in non *responders* vs *responders* (p=0.002);
Trubiano, 2017 (125)	Azacitidine (884 cycles)	68 pts AML/MDS	In 29% of cycles (256/884) posaconazole or voriconazole	**Fungal infections: 4.5% of documented nfections;** higher incidence **i**in first 2 cycles	MDS *very high* IPSS-R; no *active mould* prophylaxis.
Pomares, 2016 (127)	Azacitidine (948 cycles)	121 pts AML (29%)/ MDS	Not specified	**Fungal infections: 1.6% of documented infections;** (4.1% in pts with severe neutropenia).	Severe neutropenia.
Falantes, 2014 (109)	Azacitidine (523 cycles)	64 pts AML (33%)/ MDS	No primary *active mould* prophylaxis	**Invasive aspergillosis: 8.3% of pts receiving azacitidine as salvage therapy; 2.3% with azacitidine as *front line***; incidence higher in first 3 cycles.	Salvage therapy compared with front line

the evaluation of the infectious complications arising during therapy with hypomethylating agents, also listing the data relevant to IFI incidence. From these studies it emerges that IFIs were found in a percentage varying from 1.6% to 9% of the infections recorded, with a higher incidence - according to some of these studies - in patients who had not been subjected to prophylaxis with *anti-mould* agents (126).

IFIs, like bacterial infections, were noted prevalently within the first 3 cycles of treatment with hypomethylating agents and in patients not responsive to the therapy or in those treated with hypomethylating salvage therapy (where the risk of the active hematological disease combines with the cytopenia caused by the treatment) (Table 9). The most frequent site of infection is the lung and the most frequent etiological agent is Aspergillus (Table 9).

Prophylaxis and treatment of fungal infections

There are no *evidence-based* guidelines available on antifungal prophylaxis and therapy in the *setting*

of AML and MDS-HR patients treated with hypomethylating agents, so it is necessary to integrate the epidemiological knowledge we have gleaned from the retrospective/observational studies available with the guidelines proposed for patients with AML and high-risk blood disorders, irrespective of the type of therapy received (polychemotherapy or hypomethylating agents). Starting out from this assumption, we can suggest the following indications:

a) **Primary antifungal prophylaxis**: this is justified in patients with MDS-HR and AML who are receiving hypomethylating therapy, in the first 2-3 cycles of the therapy, especially if the patients started out with severe neutropenia (neutrophil granulocyte count < 500/mm3), a high comorbidity index, concomitant diseases predisposing them to infection such as: diabetes, COPD, concomitant second neoplasm, autoimmune disorder under chronic immunosuppressive treatment, chronic liver disease, recent admission to hospital.
The prophylactic drugs should cover filamentous fungi and so, also taking into account their prescription status the drugs to be considered for prophylactic purposes are itraconazole and posaconazole. The prophylaxis can be reasonably suspended after the 3rd cycle of hypomethylating therapy, particularly if the patient is responsive and/or the neutrophil granulocytes reach stable values over 500/mm3.

b) **Secondary antifungal prophylaxis**: this may be considered in specific cases of MDS-HR or AML in hypomethylating therapy, with documented evidence of a previous IFI.
The drug to be considered in this case is voriconazole; with posaconazole as an alternative. The duration of the secondary prophylaxis should be evaluated on a case by case basis but should preferably be limited in time, taking into account the characteristics of the patient, the clinical situation, the response of the hematological disease (AML or MDS-HR) to the hypomethylating treatment in course, the characteristics of the past fungal infection and the possible adverse effects of prolonged prophylaxis. In particular, for voriconazole, it is important to point out that in the case of long-term exposure (in therapy or prophylaxis), i.e. over 180 days (6 months), the benefit/risk relationship must be very carefully assessed and the need to limit the period of exposure to the drug must be considered due to the possible emergence of skin lesions (also neoplastic) linked to phototoxicity.

d) **Antifungal Therapy**: the antifungal therapeutic approach must be guided as far as possible by the specific diagnostics, which must be carried out in all cases in which there is a clinical suspicion of an IFI. It is fundamental, in these cases, to take X-rays and perform a CT scan of the chest (or of other sites on the basis of the patient's clinical presentation), a Galactomannan (GM) assay and a serum beta-D-glucan assay and blood culture tests for fungi in patients with FUOs non responsive to wide-spectrum antibacterial therapy.

The drugs indicated for the empirical antifungal therapy are, as per the guidelines for hematological patients, caspofungin or liposomal amphotericin. For *pre-emptive* and targeted antifungal treatment (IFI possible, proven or probable) the indicated drugs are: liposomal amphotericin or amphotericin B lipid complex, voriconazole, isavuconazole (the choice will depend on the suspected/documented fungal agent, the site of the fungal infection, the comorbidities of the patient and on any possible pharmacological interactions).

Risk of viral infection - prophylaxis and treatment

Myeloid neoplasms are historically considered as being diseases with a low risk of viral infection, being characterised, from the pathophysiological standpoint, by an altered number and functionality of the cells deriving from the myeloid precursors (e.g. neutrophils), but by substantial conservation of the functionality of the lymphoid compartment. Although some studies in the past have specifically investigated the incidence of viral reaction in patients affected by AML (128,129), the viral complications in these patients are not responsible for major morbidity and mortality; in

consideration of this, specific antiviral prophylaxis has only been reserved for selected categories of patient, such as for example patients treated with chemotherapy containing fludarabine. The aim of this chapter is to understand whether the introduction of the new hypomethylating drugs for the treatment of elderly patients affected by high-risk MDS and AML have changed this scenario and whether patients treated with this molecule could benefit from specific antiviral prophylaxis (Table 10). The viral infections considered here are: Citomegalovirus (CMV) infections, the Herpes virus (including the Varicella-Zoster virus - VZV) and Hepatitis B (HBV) virus infections. Some therapeutical suggestions for the influenza virus will be discussed later in the chapter.

Diagnostic picture

Prior to initiating treatment with hypomethylating agents in a patient with AML and MDS-HR, the underlying virological picture must be studied through first level examinations that include: HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HCVAb and HIVAb. An evaluation of CMV IgG and IgM is also useful.

CMV, Herpes virus and VZV

CMV and other viruses from the Herpesviridae family are the viruses responsible for the childhood infections that then remain latent in the human body. Despite the vast number of studies that have sought

Table 10. Viral infections in patients with AML/MDS-HR subjected to treatment with hypomethylating agents: review of the studies published in recent years

References	Therapy	Patients (no.) and Diagnosis	Study type	Rate of viral infections	Type of viral infections
Ali, 2017 (77)	Decitabine	85 pts AML/MDS	Prospective	Incidence of viral infections: 3.7%.	Prevalence of influenza and parainfluenza virus No cases of CMV.
Radsak, 2017 (124)	Azacitidine	918 pts MDS	Review	Review of literature on almost 1000 patients treated with azacitidine. Viral infections not precisely quantified by the authors but reported as rare infectious complications.	-
Ofran, 2015 (130)	Azacitidine AML/MDS	216 pts	Retrospective	Incidence of viral infections: 4.3%	Prevalently infections of the high respiratory airways and pneumonia.
Sullivan, 2013 (131)	Azacitidine vs 103 pts	103 pts MDS	Control case .	Incidence of viral infections, 8%	Almost all of the episodes (7/8) were due to influenza viruses. One case of CMV reactivation.
Khan, 2011 (132)	Azacitidine	1 pt MDS	Case report	-	The authors report a case of hemorrhagic colitis due to CMV in an MDS patient treated with azacitidine.
Zhou, 2009 (133)	Azacitidine	1 pt MDS	Case report	-	Case of severe disseminated reactivation of VZV in an MDS patient treated with azacitidine.

to explain the latency mechanisms of these viruses, we are still a long way from understanding the exact physiological bases of their persistence. Many studies highlight that the site of persistence of these viruses could be the granulocyte-monocyte-macrophage system (134) and that T cell-mediated immunity plays a key role in controlling viral replication in immunocompetent patients (135). In particular, the main players in the immune regulation of viral infections are: the generation of cytotoxic CD8+ T lymphocytes and CD4+ virus-specific helper cells, the production of virus-specific antibodies by B lymphocytes, the production of cytochine/chemochine and the proliferation of NK cells in response to the virus. These pathophysiological considerations suggest that the reactivation of these viruses is an event typical of lymphoproliferative syndromes and patients subjected to hematopoietic stem cell transplantation, but less probable in myeloid disorders in which the function of the lymphoid compartment is substantially maintained. The introduction of the new hypomethylating drugs in the treatment of AML and high-risk MDS in elderly patients, has not yet significantly changed the epidemiological scenario in relation to the incidence of viral reactivation.

The studies conducted on patients treated with azacitidine are scarce and not specifically focused on the evaluation of viral infections. With the exception of some *case reports* concerning cases of reactivation of CMV or VZV (132, 133), a low incidence of viral infection, of between 0 and 4.3%, was observed in all of the studies conducted (125, 130, 131). Specific studies relating to the incidence of viral infections have not been carried out, not even for patients affected with AML/MDS-HR treated with decitabine. In a recent study published in 2017 evaluating the incidence of the infectious complications, the authors reported a global incidence of infectious viruses of around 3% and only one of these episodes was sustained by a virus from the Herpesviridae family (78). All the other studies in which the incidence of infectious complications was evaluated in these patients did not contain data relating to herpes or CMV viruses.

The epidemiological data, which do not seem to demonstrate a significant correlation between therapy with hypomethylating agents and viral infections, are corroborated by preclinical studies concerning the immunological changes brought about by the administration of these drugs. In fact, recent data suggest a possible role of hypomethylating drugs in the treatment of *Graft-Versus-Host-Disease* (GVHD) based on their capacity, through gene expression control mechanisms, to induce the expansion of regulatory T lymphocytes, cells considered as key in virus replication suppression in human beings.

Prophylaxis and treatment

On the basis of the data available today, and the low risk of CMV, Herpes virus and VZV reactivation in patients affected by AML/MDS treated with hypomethylating agents, the administration of antiviral prophylaxis is not recommended for these patients, apart from certain specific cases.

With regard to the therapy, the general indications are:

- HSV infections: aciclovir 400 mg 5 times a day orally (or 5 mg/Kg every 8 hours i.v.) or valaciclovir 1 g twice a day orally for 5 days.
- VZV infections: aciclovir 800 mg 5 times a day orally (or 10 mg/Kg every 8 hours i.v.) or valaciclovir 1 g 3 times a day orally for 7 days.
- CMV infections: ganciclovir 5 mg/Kg every 12 hours for 14 days, then 5 mg/Kg per day for 7 days (and/or depending on the *clearance* of the viremia).

Influenza virus

The seasonal influenza virus can be a major cause of morbidity and even mortality in patients affected by AML and high risk MDS under therapy with hypomethylating agents in a study conducted in 2013, an incidence of influenza episodes equal to 7% was reported in patients affected by MDS and treated with azacitidine (131).

Prophylaxis and treatment

At the moment there are no useful drugs that can be administered as prophylaxis for this infection and the most effective strategy is still the influenza vaccine (see the Vaccines chapter).

In influenza-like cases in patients affected by AML and high-risk MDS under therapy with hypomethylating agents (whether seasonally vaccinated against the virus or otherwise), it is useful to administer oseltamivir 75 mg twice a day, orally for 5 days.

HBV

Myeloid neoplasms are diseases that risk reactivating HBV both due to factors connected with the disease (e.g. immunodepression) and the treatment, and due to the patients' considerable exposure to potentially contaminated blood products and blood derivatives (138,139). Many cytostatic drugs, immunotherapy and tyrosine kinase inhibitors have been described as promoting factors of HBV reactivation and among these, the drugs used to treat high-risk AML/MDS are no exception (139). Specific data on the association between the reactivation of HBV and hypomethylating therapy are not available in literature and to our knowledge there are no descriptions of cases of acute hepatitis B in these patients. In the absence of specific data, we suggest following the currently published recommendations for patients with hematological malignancies (140).

Prophylaxis and treatment

On the basis of these recommendations, we maintain that the administration of prophylaxis with lamivudine should be suggested in the case of seropositivity for anti-core antibodies (HBcAb+), irrespective of the presence of HBV DNA, in patients with high-risk AML/MDS under treatment with hypomethylating drugs. We also believe it advisable to suggest antiviral treatment for all patients seropositive for the s antigene (HBsAg+) irrespective of the presence of HBV DNA with third generation antiviral drugs (entecavir or tenofovir).

In the case of active viral replication of HBV, hepatological tests should be carried out in order to be able to select the best antiviral therapy.

HCV

In the case of active viral replication of HCV, hepatological tests should be carried out in order to be able to evaluate and possibly select a suitable antiviral therapy.

Vaccinations

The effects on the immune system of hypomethylating drugs such as azacitidine and decitabine, used for the treatment of MDS and AML in the elderly, are far from clear. In addition to neutropenia, which is frequently encountered if rarely severe or in any case long-lasting, hypomethylating agents inhibit the activation and proliferation of T cells, decrease Th1 cells and increase the number of Treg cells (141).

This poor knowledge on the immunological effects mediated by azacitidine and decitabine, mean that the recommendations on vaccine protocols for patients under treatment with these drugs are largely based on the immunological deficit connected with the underlying disease and on the age of the patient.

Vaccinations in the hematological patient: general principles

Vaccinations have the power to reduce the mortality rate correlated with the infection, but the cover guaranteed by the vaccine and the duration of the antibody response in an immunocompromised patient is lower than that observed in healthy people.

Patients with a hematological malignancy generally have a time-window that precedes the start of the immunosuppressive treatment, during which they can be administered the vaccines since they are still immunocompetent or at least still have a certain degree of immunocompetence. It is important to underline, however, that the chemo/immunotherapy treatment must not under any circumstances be deferred to allow space for a vaccine protocol.

As a general rule, a live attenuated vaccine should not be administered during chemotherapy. After the administration of a live vaccine, the period for viral replication and the development of an antibody response generally takes less than 3 weeks; for this reason vaccination with live attenuated vaccines ≥4 weeks prior to immunosuppressive treatment (2 weeks in the case of vaccination with attenuated vaccines) may be considered safe enough (142).

Pneumococcal vaccine

The pneumococcal vaccine is universally recommended in immunocompromised individuals (142-145). Also in the general population, it is important to underline that the national vaccine prevention plan recommends the pneumococcal vaccine for all individuals over 64 years of age. In an observational multicentre study on approx. 800 cases of invasive pneumococcal infection, the mortality rate totalled to 9% among the general population but 24% among immunocompromised patients (146).

There are two types of vaccine available:

- The polysaccharide vaccine PPSV23 (Pneumovax) that gives protection against 23 different serotypes of pneumococcus bacteria that all cause pneumococcal disease known for being potentially responsible for an invasive form: 11 of these serotypes are not contained in any other type of vaccine available (PCV13).
- The conjugate vaccine PCV13 (Prevenar). This vaccine is more immunogenic than PPSV23, inasmuch as it evokes a more long-lasting memory T cell response with a duration of 3 to 5 years.

The main guidelines recommend the administration of a dose of PCV13 vaccine two weeks before starting chemotherapy followed by a dose of PPSV23 8 weeks later (142-145) (Table 11).

Influenza vaccination

The incidence of influenza in immunocompromised patients is approx. double that observed in the general population and hospitalization and mortality in this population reaches 20% and 50%, respectively (147).

The main guidelines recommend administration of the inactivated influenza vaccine once a year (142-145). Some guidelines recommend the administration of 2 doses of vaccine 4 weeks apart, while others recommend one dose annually (145). Also as regards the influenza vaccination, the national vaccine prevention plan recommends its administration to all members of the population over 64 years of age.

Recommendations for cohabitants

Cohabitants of immunocompromised patients must be vaccinated annually with the inactivated anti-influenza vaccine (142). It is important to remember, moreover, that immunocompromised patients must avoid contact with cohabitants who develop skin lesions after the vaccination against varicella/zoster.

Diagnostic approach to febrile neutropenia

One of the most unwelcome events in patients affected by MDS and AML is the onset of fever, as it places the clinician in the difficult position of having to carefully manage an event that has to be considered infectious until proven otherwise. If on one hand the onset of fever can be caused by a variety of factors (e.g. transfusion of blood products), on the other, patients with AML or MDS can rapidly degenerate into a state of sepsis and multiple organ failure. For this reason, it is of the utmost importance to carefully consider each single event of each single patient, in order to correctly interpret the clinical picture that is developing.

The correct management of a febrile event is of fundamental importance in the care strategy of the pa-

Table 11. Vaccine plan recommended for elderly patients with AML and high-risk MDS under treatment with hypomethylating agents.

Vaccination	Type of vaccine available	Recommendation
Pneumococcal	PPSV23 inactivated polysaccharide vaccine PCV13 inactivated conjugate vaccine	One dose of PCV13: 2 weeks prior to the start of the treatment, followed by one dose of PPSV23 8 weeks later.
Anti-influenza	Inactivated injectable	One annual dose

tient: a patient with MDS or AML who develops a severe infection might not be able to complete his/her treatment plan, having to defer or even suspend the treatment, thereby reducing the his/her probabilities of improvement. An epidemiological study, focused on evaluating the characteristics of patients affected by acute and chronic leukemia who developed documented sepsis, demonstrated that mortality at 21 days was higher in patients with long-lasting neutropenia than in patients with neutropenia lasting less than 10 days. In particular, in patients with AML, mortality at 21 days was higher in neutropenic patients than in non neutropenic patients (148).

Diagnostic picture of the febrile patient

A patient affected by MDS or AML who develops a fever while suffering from neutropenia must be subjected to an urgent medical evaluation: a careful objective examination must include an inspection of the mouth, skin, the insertion site of any venous catheters and the perianal region, pulmonary auscultation and palpation of the abdomen and the lymph node stations. Also of fundamental importance is a scrupulous evaluation of hemodynamic parameters, the presence of any associated symptoms such as expectorate, diarrhea, burning on passing urine and the patient's mental state: often the symptoms of severe sepsis can be very vague, linked to the condition of anergy which is typical of patients with MDS and particularly AML.

First level examinations include:

- complete blood count with formula,
- kidney and liver function,
- cholestasis and hemolysis indices,
- blood coagulation tests.

The discovery of abnormalities in the blood chemistry and/or blood coagulation tests can provide indirect indications on the origin of the febrile event, even if they are not sufficient to determine a severe event like an infection. Additionally, it is important to keep in mind the age and possible comorbidities of the patient, in addition to carrying out a logistic evaluation, considering for example the constant presence of a *caregiver* and the possibility of reaching a hospital quickly should the patient's clinical conditions worsen.

Predictive criteria of severity and prognostic scores

Clinical judgement is undoubtedly the first and most important evaluation tool for these patients, but it is not always sufficient. In order to identify the predictive criteria of severity and recognize which patients are most at risk for systemic complications and require antibiotic therapy during hospitalization, various cooperative groups have published and subsequently validated clinical *scores*. Already in 1992 three conditions were identified, otherwise known as "Talcott's Rules" to assess patients at risk of developing potentially fatal complications during febrile neutropenia: a hospitalization condition; a serious comorbidity, even if independent; uncontrolled cancer (149).

More recently, in 2000 the "*Multinational Association for Supportive Care in Cancer*" identified seven characteristics to which a score was attributed, making up the MASCC *score*: importance of the symptoms of febrile neutropenia, hypotension, COPD, previous fungal infection, dehydration, age <60 years, outpatient status (150). Numerous authors have validated this *score* as predictive of the severity of an ongoing state of infection; despite this, up to 11% of the patients classified by the MASCC *score* as being low risk patients developed a serious complication. Moreover, one of the major limitations of these *scores* resides in the variability of the patients being considered, also in the evaluation studies, which include both solid tumours and patients with AML as well as patients subjected to stem cell transplantation.

One of the most recent *scores* entitled CISNE succeeded in improving the predictive accuracy of severe complications in patients who were apparently low-risk, by attributing greater importance to pre-existing comorbidities rather than to the symptoms presenting at the infectious event; patients with AML or subjected to stem cell transplants were not included in the original study (151).

Radiological and microbiological investigations and antibiotic therapy

Most of the studies on neutropenic patients include a much larger number of patients with solid tumours than patients with MDS and AML: for this

reason, the indications listed and the value of the prognostic *scores* are only applicable to this population up to a certain point. A neutropenic patient with febrile MDS or AML, who does not present with any previous organ dysfunction or any new changes in his/her blood chemistry tests, does not report any associated symptoms and is able to reach the hospital quickly if his/her conditions worsen, can reasonably be started on antibiotic treatment *orally*. In any case, a clinical re-evaluation must be scheduled within 48-72 hours of the onset of the fever, or earlier than this, should new symptoms occur. If, during the evaluation of the patient, characteristics indicating a high risk of severe infection are identified (hemodynamic instability, respiratory symptoms, new onset of worsening of renal function, mucositis that prevents the administration of oral medication, a total indicating a severe infection in the above-mentioned scores), the patient will be hospitalized and commence antibiotic therapy, with intensive microbiological surveillance (116).

Blood cultures should be taken before antibiotic therapy is started (152), preferably by taking a couple of samples of peripheral blood and venous catheter blood, if any, in order to assess the differential time to positivity between the two samples (153). Even if carried out ubiquitously, the measuring of procalcitonin does not seem to be as effective in identifying an infectious event in neutropenic patients as it is in non neutropenic patients (154). Similarly, the measuring of the C-reactive protein has proved unequivocally to be a predictive marker for infection (155-157). Also useful is the determination of galactomannan and beta-D-glucan on peripheral blood (158,159), to be carried out twice a week, and the obtaining of urine culture and the conducting urine antigen tests for Legionella and Streptococcus before starting treatment with antibiotics. Perianal and pharyngeal swab tests, or fecal culture tests are also important. They make it possible to identify the contamination, if any, in order to be able to target the antibiotic therapy. Should respiratory symptoms emerge, an arterial blood gas test is indicated, since it also provides information on lactacidemia, an early marker of sepsis.

X-rays of the chest is the level 1 radiological investigation even although it does not identify abnormalities ascribable to an infection in a high number of cases. A thin slice CT scan of the chest is indicated in all cases of fever that persists notwithstanding the commencement of antibiotic therapy: the identification of pleural thickening indicates that a bronchoalveolar lavage should be performed in order to adjust the antibiotic therapy accordingly.

Depending on the clinical manifestations, other radiological and microbiological tests may be indicated. In the case of common cold symptoms during the period of seasonal viral infections, it is important to perform nasal and pharyngeal swab tests in order to ascertain whether a influenza virus infection is present In the case of nasal congestion, pain in the regions of the jaw and/or forehead, or other symptoms reminiscent of sinusitis, a CT scan of the facial bones can be useful in order to exclude the possibility of an invasive fungal infection. In the event of persistent diarrhea, a stool test is required to search for the main enterocolic pathogens, including the toxin *Clostridium difficile*, and to perform a CT scan of the abdomen in order to exclude typhoid. If the chest X-rays suggest a tubercular infection, it is useful to perform the Mantoux intradermal skin test, the Quantiferon diagnostic test, and carry out a microbiological examination of the bronchoalveolar lavage fluid.

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