The role of Immunomodulatory drugs in Acute Myeloid Leukemia

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# Abstract

Immunomodulatory drugs (IMiDs)  are analogues of thalidomide. They present immunomodulatory, antiangiogenic and pro-apoptotic properties and exert a role in regulating tumor microenvironment.

Recently IMiDs have been investigated for their pleiotropic properties and their therapeuthic applications in both solid tumors (melanoma, prostate carcinoma and differentiated thyroid cancer) and haematological malignancies. Nowadays, they are applied in de novo and relapsed/refractory (R/R) multiple myeloma (MM), in myelodisplastic syndrome (MDS), with a specific use of lenalidomide in del5q syndrome and B-cell lymphoma.

Several studies have been conducted in the last few years to explore IMiDs’ possible use in the acute myeloid leukemia (AML) treatment.

Here we report on the mechanisms of action of immunomodulatory drugs in AML and their possible possible future therapeutic application in this disease.

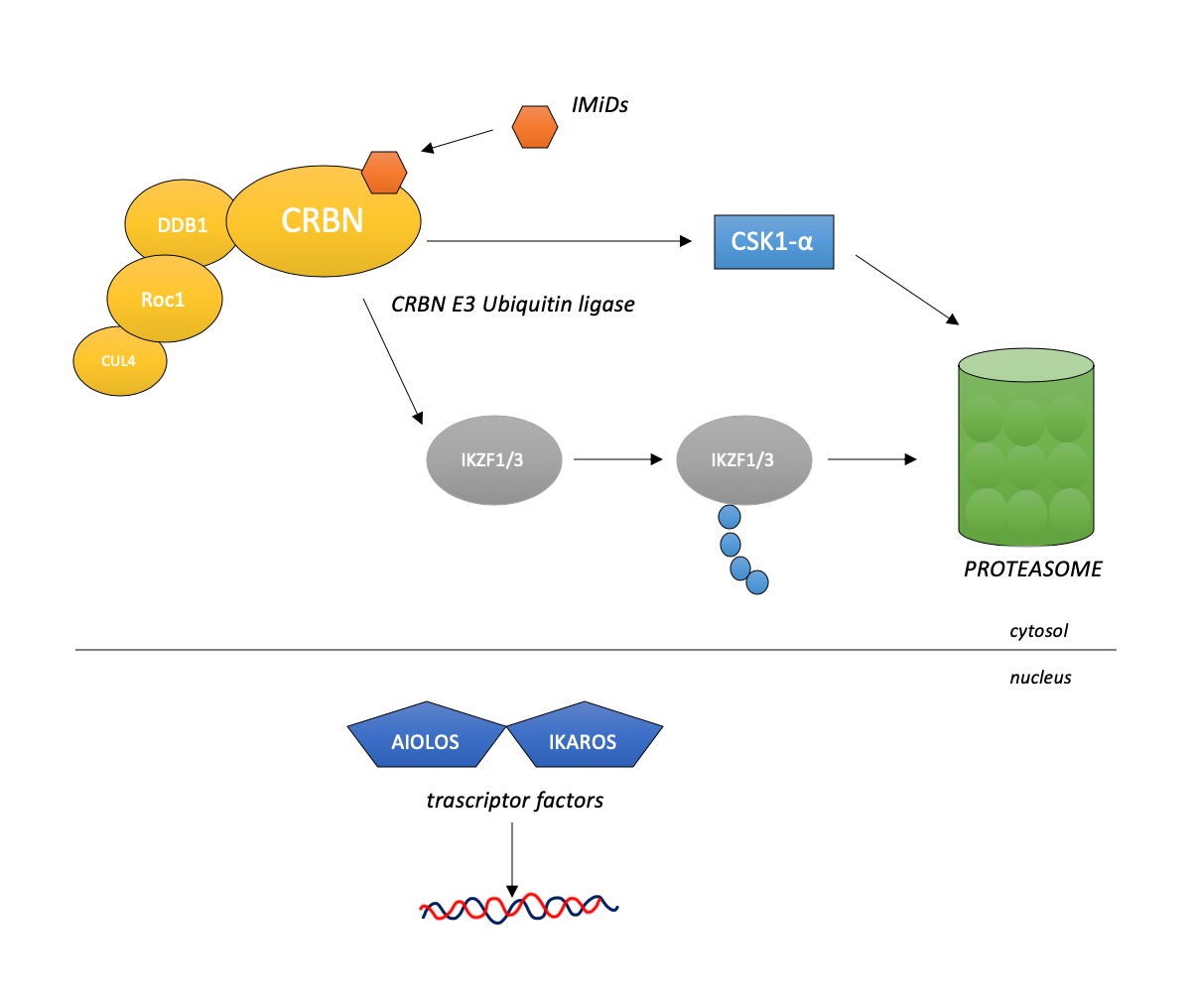
# 1. Introduction

Immunomodulatory drugs (IMiDs) are a class of molecules composed by thalidomide and its anologues: lenalidomide, pomalidomide and the new molecules CC-122, CC-220, CC-885, CC-9009. They have immunomodulatory and anti-proliferative properties and are applied in the treatment of several haematologic and solid malignancies as well as in autoimmune diseases . 1

IMiDs are the backbone of multiple myeloma therapies and recently their use has been extended to B-cell lymphomas and myelodisplastic syndromes.  2

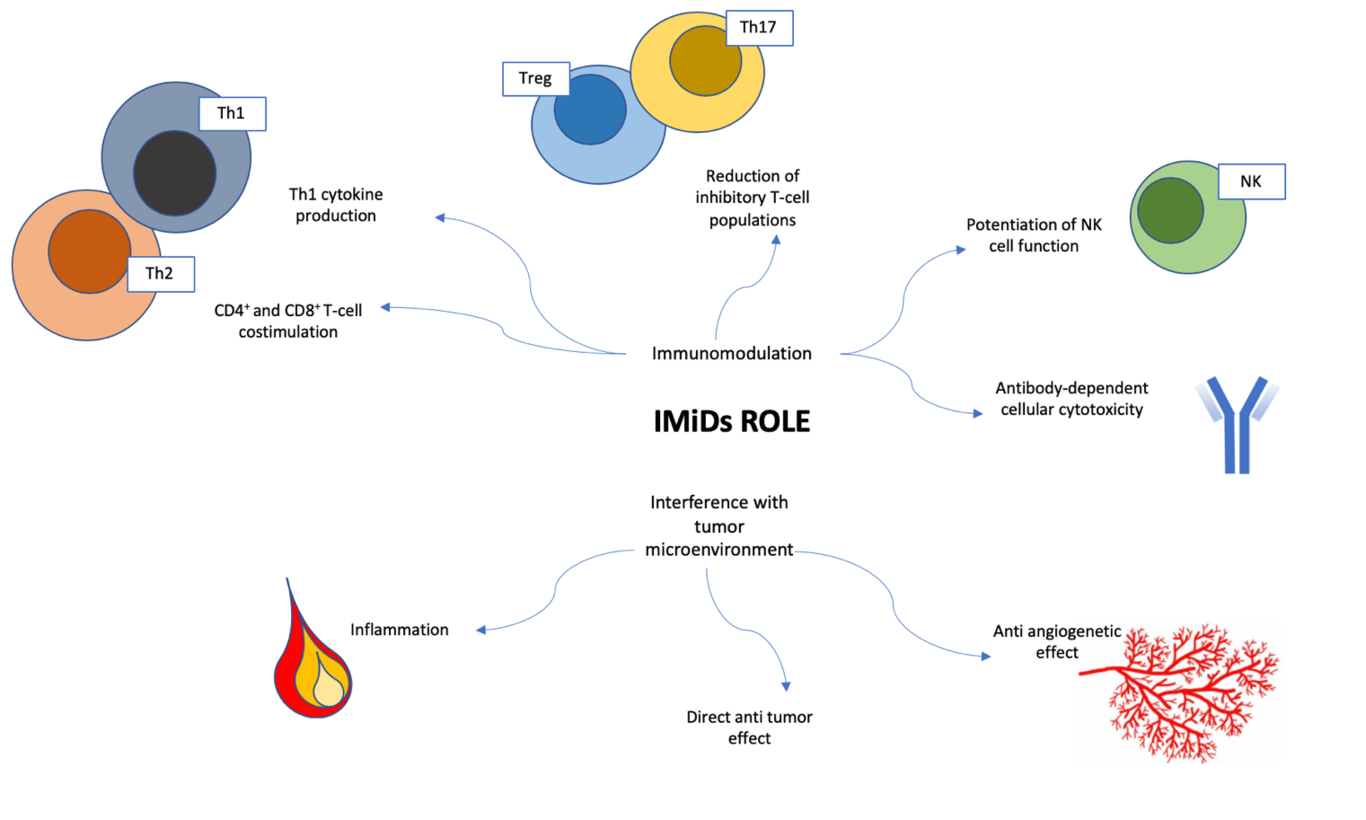
Recent studies have clarified the IMiDs mechanism of action. In fact, they exert their pleiotropic effect by binding a single primary target, cereblon (CRBN), rather than affecting multiple molecular substrates.3

CRBN is part of CLR4 E3 ubiquitin ligase complex with damage-binding protein-1 (DDB1), cullin 4 (Cul4A or Cul4B) and regulator of cullins (Roc1). CRBN acts as a substrate receptor and binds some proteins facilitating their ubiquitination and the proteasome-dependent proteolysis. 4 The alteration of CRBN molecular downstream is involved in the antiangiogenetic and  immunomodulatory effects of IMiDs.  3



CRBN, a single primary target of IMiDs.

The increasing knowledge and interest about IMiDs’ therapeuthic action promoted several studies on their use in AML treatment. In this scenario, IMiDs seem to have a direct impact on AML blasts and also on the bone marrow microenvironment. There are some interactions between leukemic cells and the immune system which are involved in AML pathogenesis; therefore, the tumor microenvironment makes difficult the immune system to act against cancer cells. 5



The role of IMiDs.

IMiDs affect the immune surveillance against leukemia: on one hand they induce the activation of effector cells (NKT, NK cells, γδ T cells, macrophage, cytotoxic T cells), on the other hand they reduce inhibitory T-cell populations (regulatory Th cells (Treg) and IL-17-producing Th cells (TH17)). 6 Moreover, these drugs have a costimulatory effect on T and NK cells, by increasing interleukine 2 (IL-2) and interferon-γ (IFN-*γ*).7

NK cells can enhance the immune response against cancer cells by means of creating different molecular bindings. The first theory proposed to explain this mechanism was defined as “missing-self” hypothesis: NK cells were able to recognize malignant cells that lost HLA class I ligands.  8

Today, it is well-known that the anti-tumor activity of NK cells depends on different signaling molecules: Killer Immunoglobulin-like Receptor (KIR), that are inhibitory receptors who recognize HLA class I ligands, Natural Killer Group 2A (NKG2A, inhibitory as well) and Natural Cytotoxic Receptors (NCRs, an activator family). 9

In the AML setting, activating NK cells receptors (DNAM 1, NKG2D) are down-regulated reducing NK killing. Moreover, the bindings between AML blasts and NKs are ineffective because of up-regulation of inhibitory signals; such changes have an impact on NK activity and AML prognosis.10 Chretien et. al described three NK maturation stages in AML patients (hypermaturation, intermediate maturation and hypomaturation); NKs with hypomaturation feature are associated with a poor prognosis. 11

IMiDs downregulate the expression of HLA class I molecules on AML cells, in order to increase  their disruption by NK cells, and increase the expression of key ligands on AML cells (particularly ligands of DNAM-1 and NKG2D)  involved in blasts recognition.  9  12 Furthermore, these drugs promote NK cell phenotypic modifications, improving the immunological synapse between NK cells and leukemic cells (upregulation of CD56 and downregulation of KIR2D, NKp30, NKp46 on NK cells). 9  Finally, they enhance the NK cell degranulation and production of cytokines  (IFN- and TNF-) and  promote NK-cell mediated Antibody Dependent Cell-Mediated Cytotoxicity against tumor cells (ADCC).  9

It is well known that angiogenesis plays a relevant role in the progression of cancer; IMiDs have also an anti-angiogenetic effect by reducing tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), fibroblast growth factor-β (FGF-β) and interleukine-6 (IL-6). 13 In the AML context, as blasts and endothelial cells depend on each other for survival and proliferation, therapy directed against several pro-angiogenic factors might help to enhance the AML outcome.

Several investigations have been carried out to find new therapeutic agents for AML treatment.  AML has been proved to be typical of elderly patients. In this particular subset of patients, the response to chemotherapy is inferior because of the biological characteristics of the disease and the toxicity of standard therapies. 14 Moreover, the outcome for patients with refractory or relapsed (R/R) AML is even worse and, currently, there is not any standard therapy. IMIDs in high risk, older and R/R AML patients can be effective as shown in several studies.

# 2. Thalidomide

The first molecule in IMiDs family is thalidomide. Thalidomide has different mechanisms of action and has been tested both in untreated and R/R AML patients.

This drug has a great antiangiogenic activity in AML, due to its direct inhibition on endothelial cell proliferation, reduction of fibroblast growth factor (FGF) and downregulation of a VEGF receptor, neuropilin-1, which is overexpressed in AML and whose high levels are correlated with lower survival.  15  VEGF levels are higher in AML bone marrow;  Aguayo et al. demonstrated that VEGF levels are higher in AML bone marrow and high levels of VEGF in AML are linked to a worst prognosis.  16This suggests that thalidomide may play a role in treating patients with AML.

Besides, thalidomide has important immunomodulatory effects by decreasing TNF-α synthesis  and selectively modulating T cell subsets; in fact this molecule shifts the T cell population towards T helpers.

In a phase I/II trial Steins et al. investigated the use of thalidomide as a single agent in AML patients who were poorly candidates for intensive cytotoxic chemotherapy; such AML patients were refractory towards at least 2 standard induction chemotherapies and were not eligible for allogeneic stem cell transplantation. In 20 evaluated AML patients, there were 4 and 4 patients with hematologic improvement (HI) and with partial response (defined as reduction of leukemic blast infiltration of 50% in bone marrow, PR), respectively. 17

A phase II trial was conducted on 16 patients with R/R AML, previously treated with a cytarabine-containing regimen. They were treated daily with thalidomide 200–800 mg orally for an average of 27 days. At the end of the study, only one patient (6%) achieved complete remission (CR) lasting for 36 months; noteworthy, this patient had chromosomal aberration del(5)(q22q35). Toxicities were a limiting factor for the study: common side effects included fatigue, sedation, neurotoxicity. This study suggests that thalidomide used as a single agent is not a good strategy for poor prognosis de novo, and R/R  AML.  18

Combination strategies with thalidomide and chemotherapy have been tested; thalidomide has been associated with several agents, such as 5-azacitidine, amifostine, topotecan, and arsenic trioxide biblio?. A randomized study of liposomal daunorubicin and ara-C with or without thalidomide (400 mg daily escalated to 600 mg daily) in poor risk karyotype untreated AML did not show any extention in the duration of CR with the addition of thalidomide. 19

Another phase II study assessed the efficacy of a quadruple regimen of thalidomide, fludarabine, carboplatin, and topotecan (FCTT) in poor prognosis patients with R/R, previously treated with conventional chemotherapy, or secondary AML. Five and 5 patients achieved CR and an incomplete platelet recovery (CRp), respectively, for an overall CR rate of 24% (10/42). The karyotype didn’t seem to correlate with response. However, FCTT regimen compared to a phase I evaluation of fludarabine, carboplatin, topotecan regimen (FCT) alone led to similar results: this suggests that the addition of thalidomide does not improve the effect of chemotherapy.  20

A study by Chen et al. included two different arms. The first one, the control arm, was treated with a non-intensive regimen composed of cytarabine, aclarubicin and G-CSF in order to induce remission; in the second arm, the investigational arm, patients were given thalidomide in addition at a maximum dose of 200 mg/day. 70 elderly patients were enrolled. A trend towards better overall survival (OS) and event free survival (EFS) was observed in the investigational arm, although no statistical relevant survival benefit was seen between the 2 treatment arms.   21

The most promising data went from the combination of thalidomide and 5-azacitidine, an hypomethylating agent. Thalidomide and 5-azacitidine have different mechanisms of action and toxicity profiles, so they could be combined with good results. In Raza’s phase II study, the use of low-dose thalidomide in association with 5-azacitidine was effective in patients with AML post MDS. The study population was made up of 40 patients with AML (de novo or post MDS) or MDS with an average age of 72 years. Eight out of 14 (57%) AML patients responded to treatment, among them, 4 achieved CR. 22  In a phase I/II trial, 80 patients with clinically advanced MDS, CMML and low blast AML were treated with thalidomide and 5-azacitidine. The elegibility criteria were: no treatment with thalidomide or its analogs in the 30 days prior to the study, and no prior treatment with azacitidine or other demethylating agent. Patients received azacitidine 75mg/mq daily for seven days every 28 days and thalidomide from 50 mg up to 100 mg daily with a median of 9 cycles. The combination of thalidomide and azacitidine appeared to be more effective than azacitidine as a single agent; CR was observed in 26% patients, PR in 5% and HI in 14%; the overall response rate (ORR) according to intention to treat (ITT) was 63%. The drugs were tolerated well; most common side effects were infections, while the 27% and 35% patients had grade 3+ neutropenia and grade 3+ thrombocytopenia, respectively.  23 These two studies are the springboard to further studies with the association of 5-Azacitidine with other IMiDs like lenalidomide, which is more effective than thalidomide and has a more favorable toxicity profile.

The inhibitory effects of thalidomide on angiogenesis and VEGF expression in leukemic cells are emphasized in an in vitro study of combination of thalidomide with arsenic trioxide. Moreover, both ATO and thalidomide facilitate an interruption in AML cell cycle at G1 phase. 24

# 3. Lenalidomide

In the last few years,  lenalidomide has been employed as an important anti-cancer drug. It was approved by the FDA in the treatment  of multiple myeloma, del5q myelodysplastic syndrome and mantle cell lymphoma.

It has several mechanisms of action such as a direct anti-tumor effect, inhibition of angiogenesis, anti-inflammatory activity, and immunomodulation (NK cell activation, T cell co-stimulation, Treg suppression). In this way, lenalidomide promotes tumor cell apoptosis in a direct and indirect way, by means of anti-angiogenic and anti-osteoclastogenic effects, immunomodulatory activity and inhibition of bone marrow stromal cell support. 25

In the last years, literature produced evidences about the potential therapeutic role of lenalidomidein AML.

## 3.1 Lenalidomide as a single agent

The use of lenalidomide in studies  reported in literature generally concerns a particular subset of AML patients: untreated elderly patients, with poor cytogenetic risk, or R/R patients.

A meta-analysis and systematic review of Chun-Hong Xie et al. reported that in AML patients treated with lenalidomide as monotherapy the CR rate was relatively low (14%) and the ORR was 22%. 26

In a phase II study by Fehniger et al., untreated AML patients aged 60 years or more (median age, 71 years) received high dose (HD) lenalidomide (50 mg/day) for up to two 28-day-cycles. Thirty-three patients with intermediate (55%), unfavorable (39%) and unknown (6%) cytogenetic risk were enrolled. 27

The overall CR/complete remission with incomplete blood count recovery (CRi) rate was 30%. Moreover, in another report 16% of R/RAML patients treated with HD lenalidomide achieved CR/CRi.28

Fehniger et al. reported two elderly AML patients (one untreated and one treated previously) treated with HD lenalidomide. They had trisomy 13 as the only cytogenetic abnormality and they both showed a CR of 9 months.  29 Moreover, Lancet et al. described a 55-year-old AML patient with chromosome 5q deletion, treated with lenalidomide as single agent at dose of 10 mg/day), who achieved a CR. Currently, the precise pathogenetic role of chromosome 5q deletion, wich is associated with a poor prognosis, in AML is not well-known but this case provides a basis for further investigations on the use of lenalidomide in myeloid pathologies.  30

Some phase-II studies showed that lenalidomide as single agent at  standard dose (5 to 25 mg daily) has a limited activity in R/R AML and MDS patients with chromosome 5q deletion in the context of a complex karyotype; on the other hand, lenalidomide may represent a treatment option for AML and MDS patients with this kind of chromosomal aberration in non-complex karyotype, in particular for unfit patients.  31

Relevant data about safety showed that the most common side effects regarding lenalidomide monotherapy were myelosuppression, fatigue and electrolyte disturbance. Infection and neutropenic fever are the most frequent complications.   26

**3.2 Lenalidomide and chemotherapy**

The most important lenalidomide associations described concern its employment with cytarabine, clofarabine and in therapeutic regimens: MEC (Mitoxantrone, Etoposide, Cytarabine) and cytarabine plus daunorubicin.

De Angelo. et al. enrolled 35 R/R AML patients aged 18-70 years in order to evaluate the safety and tolerability of lenalidomide with MEC. The dose of lenalidomide was escalated starting from 5 mg to 50 mg. The maximum tolerated dose (MTD) of lenalidomide combined with MEC was 50 mg/day in days 1-10. The median OS for all patients was 11.5 months; the CR rate was 34% while, historically, the CR in MEC reinduction therapy was approximately 18-26%  32 33. The safety profile, in this study, was similar to events reported in other lenalidomide and MEC trials.  34

Jain et al. described the association of lenalidomide and clofarabine in 4 patients with high risk MDS and R/R AML, unsuitable for intensive chemotherapy or allogenic stem cell transplantation. Two subjects achieved stable disease and one achieved a partial response. However, all patients were taken out of study because of disease progression (3 subjects) and liver toxicity (1 subject). Despite the limited sample size of this study, it led to an interesting finding underlined by the authors: pre-treatment samples showed elevated expression of exhaustion markers (PD1, LAG3 and TIM3) in T cells, markers which are known to produce impairment of T-cells.  35 The Authors also found that NK cells had an higher expression of LIR1, an inhibitory molecule known to induce impairment of leukemia killing. The hypothesis is that in responders and stable disease subjects, clofarabine may produce lympho-depletion and decreased the number of exhausted T CD4 and NK cells with inhibitory markers. This lympho-depleting effect could create a favorable microenvironment for subsequent lenalidomide therapy stimulating NK and T cell reconstitution.

In the study by Ades  et al. was described the association of classical “3+7” with an escalating dose of lenalidomide. The authors enrolled 82 patients with MDS or AML with 5q deletion (62 with AML). Sixty-two 78% patients had a complex karyotype.  The 46% of patients achieved CR  and the overall response rate (ORR) was 58.5%; the CR rate in AML was a little lower  (40%). Among the patients with a complex karyotype, 27 (44%) achieved CR. The 1-year cumulative incidence of relapse was 64.6% and the median overall survival was 8.2 months. By comparison with conventional intensive chemotherapy, the treatment protocol used by the authors produced higher haematologic CR  rates in patients with very poor cytogenetics risk, but the response duration was short. 36

The association lenalidomide/cytarabine was investigated in different AML setting, giving controversial results.

Griffiths et colleagues enrolled 32 patients with R/R AML.  The MTD for this association was 10 mg/daily administered on days 6–26 in a 28 day cycle. The CR/CRi rate was about 30% and the median OS was 5.8 months. This trial was associated with marked skin toxicity and other toxicities such as nausea, vomiting, mucositis, electrolyte disturbance.  37 In a second study, Visani et. al described 66 elderly AML patients (median age 76 years) unsuitable for intensive chemotherapy or allotransplantation, who were consecutively treated with low dose lenalidomide (10 mg/day, days 1-21) plus low dose cytarabine (10 mg/m2, twice a day, days 1-15, every six weeks, up to 6 cycles) . The CR rate was 36%; the achievement of CR was not predicted by bone marrow blast count, molecular markers, cytogenetics, white blood cell count or prior MDS. The toxicities of this association were represented by trombocytopenia, anemia and neutropenic fever, while non-haematological toxicities were less relevant.  38

Another study by Visani et al. described 31 elderly AML patients with unfavourable (52%) and intermediate (48%) karyotype treated with low dose lenalidomide (10 mg/day, days 1-21) plus low dose cytarabine (20 mg/m2, twice daily, days 1-15). The CR rate was 33%. In this study non-hematological toxicities were mild.  39

**3.3  Lenalidomide and Azacitidine**

Pollyea et al. evaluated the efficacy and safety of sequential azacitidine plus lenalidomide for elderly patients with untreated AML. They enrolled 42 AML patients aged more than 60 years; they were given azacitidine (75 mg/m2 for 7 days) followed by an escalating dose of lenalidomide daily for 21 days, starting on day 8 of each cycle, for 6 weeks. The ORR was 40%, the median duration of response was 28 weeks and the OS for responders was 20 weeks. The lenalidomide MTD was 50 mg/daily. Patients with adverse risk in terms of genomic abnormalities had responses similar to those with lower risk. These findings suggest that unknown gene alterations could cause treatment sensitivity in such patients. The results suggest that sequential administration of lenalidomide and azacitidine in untreated elderly AML patients is a treatment option preferable to a single-drug treatment because there is a higher ORR and it is relatively well tolerated.

Ramsingh et al., in a phase I-study, partly confirmed the findings of Pollyea et al 40. In this study, the authors enrolled 19 newly diagnosed or R/R AML elderly patients (median age 72 years) and treated with HD lenalidomide and azacitidine simultaneously. Thirteen patients out of 19 completed at least one induction cycle. The 30.8% of the 13 evaluable patients obtained a CR/CRi.   41

In a systematic review and meta-analysis by Chun-Hong Xie et al. some studies on lenalidomide and azacitidine in AML patients were considered. The analysis revealed that the CR rate was 22% and the ORR was 31%. In this meta-analysis the authors found out that the cytogenetic risk had a minimal impact on the outcomes of patients who received a combination of lenalidomide and azacitidine. Myelosuppression was the most common toxicity, while infection and neutropenic fever were the most frequent complications. 26

Another important study by Kunacheewa et al. biblio? evaluated the efficacy and the adverse effects of azacitidine plus lenalidomide in patients with AML, MDS and chronic myelomonocytic leukemia. In this meta-analysis the authors considered 10 studies with a total of 406 patients treated with lenalidomide-plus-azacitidine regimen. The pooled CR rate was 33% while the pooled ORR was 49%. In addition, the analysis of the subgroups revealed that patients with 5q deletion had a higher CR rate (43.8%). The most common adverse events included: grade 3-4 neutropenia (48.8%), platelet toxicity (54.7%) and febrile neutropenia (36.7%), while the most frequent non haematological toxicities were the acute renal failure (9.2%) and thrombotic events (5.3%).  42

Therefore, positive results following the association of lenalidomide and azacitidine in AML have great evidence in literature. It should be better defined which specific AML population could benefit from the association of these drugs.

## 3.4 Lenalidomide and allogenic stem cell transplantation

The use of a lenalidomide-based maintenance post allotransplantation has been evaluated in the phase II trial LENAMAINT 43. Ten patients were recruited: 1 patient with high risk MDS and 9 patients with AML, with a median age of 65 years, in CR after Hematopoietic stem cell transplantation (HSCT). In this trial, lenalidomide maintenance (10 mg/day for 21 days of a 28-day cycle) has been suspected of inducing acute graft-versus-host disease (aGVHD) in 6 out of 10 patients (60%). Therefore, the use of a lenalidomide based maintenance therapy after allogenic stem cell transplantation does not seem to be a good choice.

However, more promising results derive from the association with azacitidine. In VIOLA phase I trial, 29 patients with AML or MDS relapsed after allotransplantation were treated with azacitidine and lenalidomide with a median follow up of 23 months. The major clinical response was achieved in 7 out of 15 patients (46,6%). In this trial, GVHD appears less frequently as an adverse effect, probably because of the association with azacitidine that favours T-regulatory cell expansion, as observed in mouse models  44.

# 4. Pomalidomide

Pomalidomide is a third-generation immunomodulatory drug approved for the treatment of R/R multiple myeloma.

As the other molecules previously described, pomalidomide has anti-cancer, anti-angiogenic and immunomodulatory properties. It seems to enhance anti-tumor immune response costimulating T cells, increasing both the activity and the proliferation of NK and NKT cells. In contrast to thalidomide, it reduces the level of Treg and Th17 cells.  6

Recent studies have investigated pomalidomide’s role in AML treatment providing limited evidence but underlyning its action on NK and T cells.

In “in vitro” and murine models, pomalidomide has a direct and indirect activity against AML blasts, reducing their vitality and sensitizing leukemic cells to NK.  As explained above, IMiDs and pomalidomide particularly, reduce expression of HLA-I class molecule on AML blasts, increase expression of CD56 and dowregulate the expression of inhibitory receptor (KIR2D) on NK cells. Similarly to lenalidomide, this drug enhances NK degranulation, improving the polarization of this immunological synapse. 9

The binding between CRBN and pomalidomide seems to be involved in immunomodulatory effects. In fact, pomalidomide enhances ubiquitination and degradation of ikaros and aiolos, two transcription factors expressed in several haematological diseases and crucial for the development of lymphoid lineage and lymphocytes functions. Aiolos is also expressed in AML and its role is not completely understood. Gandhi et al. biblio? reported that aiolos is an IL-2 promoter repressor; consequently, its degradation, induced by pomalidomide, facilitates IL-2 costimulation of CD4+ Th1 lymphocytes. It will be necessary to clarify the role of aiolos in AML and its action on T cells populations and interleukins production. 45

In a phase I trial (NCT02029950), Zeidner et al. enrolled 51 AML patients (46 de novo non favorable risk AML, 4 high risk MDS and 1 chronic myelomonocytic leukemia-2) who received timed sequential therapy (TST) as induction treatment (AcDVP-16 regimen, cytarabine, etoposide, daunorubicin), pomalidomide escalating doses at early lymphocyte recovery (ELR) and as manteinance therapy. The first objective was to establish the pomalidomide MTD: 4 mg/day for 21 days of a 28-day cycle; additionally, the trial demonstrated an improvement in OS and EFS in pomalidomide-treated high risk AML.   46

Furthermore, this study shows preliminary results regarding pomalidomide immunomodulatory activity. During the immunomodulant administration, aiolos concentration was reduced in CD4+ and CD8+ T-lymphocytes from both bone marrow (BM) and peripheral blood (PB) samples. IL-2 and TNF-α production increased in BM CD8+ and PB  CD4+; similarly there was a growth in frequency of subpopulation of effector memory lymphocytes T. Gene expression analysis reveals an upregulation in genes involved in metabolic and immune processes in CD8+ and CD4+ associated with a downregulation of exhaustion genes as well as Treg genes.  46

Further studies are necessary to define the effect of pomalidomide on ELR, T lymphocytes and NK subpopulations and to clarify the impact of immunological modifications on both AML immune surveillance and disease outcome.

# 5. New Molecules

New thalidomide analogues have been recently developed. They are involved in different clinical trials in order to define their mechanisms of action.

The effects of the new molecules depend on their binding to CRBN. CC-122 (iberdomide) and CC-220 (avodamide) enhance aiolos and ikaros degradation and they are under investigation for their possible role in lymphoproliferative and rheumatologic disorders. 47

CC-885 inhibits cancer growth, and it has been tested against different cells lines and AML leukemic blasts isolated from patients’ samples. Particularly, CC-885 facilitates the binding of GSPT1 (G1 to S phase transition 1, a translation termination factor) to CRBN, enhancing the degradation of this protein. GSPT1 binds to eukaryotic translation termination factor 1(eRF1) and the deriving complex recognizes proteins’ stop codons, allowing their release from ribosome. The inhibition of this process reduces cellular fitness and seems to have an anti-proliferative effect.  48

CC-9009 enhances the binding of ikaros and aiolos to CRBN. 7 In preclinical studies on AML cell lines, CC-9009 binds to GSPT1 inducing apoptosis of AML blasts. Nowadays, CC-9009 has been applied in a phase 1 clinical trial in vivo to evaluate its pharmacodynamics, pharmacokinetics and tolerability in heavily pretreated R/R AML patients. In this setting, the new drug seems to improve the outcome and to reduce GSPT1 concentration in leukemic blasts as well as T cells from patients’ PB samples. 49

The involvement of CLR4 E3 ubiquitin ligase complex in IMiDs’ action highlights their role in protein ubiquitination. Proteolysis of targeting chimeras (PROTACs) are composed of two different molecules binding a target protein with an E3 ubiquitine ligase, promoting target intracellular ubiquitination. dBET1 is a PROTAC, composed of thalidomide and JQ1; the latter binds bromodomain and extra-terminal motif transcription factor (BET, specifically BRD4) and allows its degradation. 50

BRD4 is a chromatin reader protein that acts as a transcriptional coactivator involved in physiological hematopoiesis and recruited in cancer development. In AML this protein promotes chromatin remodeling and transcription of genes pivotal for the tumor survival, remarkably BRD4 inhibition seems to affect specifically the transcription of cancer-promoting genes. 51

ARV-285 is composed of pomalidomide and OTX015, a BRD4 inhibitor. A preliminary study conducted on AML cell lines and cells derived from secondary AML patients (post Myeloproliferative Neoplasms) indicates that PROTAC performs a profound disruption of s-AML oncogenes through BRD4 degradation. 52

These small molecules promote the CRBN-mediated ubiquitination of BRD4, being thus a possible candidate for AML treatment.

# 6. Conclusions

In this review we reported about the application of IMiDs’ pleiotropic action in the AML scenario. The majority of clinical trials are focused on thalidomide and lenalidomide applied as single agents or in combination therapy. The results are interesting but not completely conclusive.

Thalidomide exerts its immunomodulatory effect by means of co-stimulation of T cells (partially activated by their T-cell receptor) and enhancement of NK cells cytotoxicity. On the other hand, thalidomide is less active against T-reg proliferation. 6 T-regs level is high in AML patients at diagnosis and at ELR after TST induction therapy; they constitute an independent prognostic factor and facilitate immunesurveillance escape. 53

Moreover, thalidomide binds directly CRBN and indirectly DDB1 (two compounds of CLR4 E3 ubiquitin ligase complex), but this binding is less strong than those produced by lenalidomide and pomalidomide and even ikaros and aiolos degradation is less active.  3 As a consequence, thalidomide can be less effective than its more recently developed analogues in activating the immune system against leukemic blasts.

Thalidomide’s clinical trials often involve cohorts composed of heavily pretreated AML patients, probably affecting clinical results and drug impact on immune system. In the studies examined, this drug used as a single agent did not prove to be really effective; toxicities were often significant, especially at high dose, and precluded dose escalation and study continuance.

The association of thalidomide with hypometylating drugs like azacitidine in trials is attractive and it is the starting point for new further studies with its derivative drug, lenalidomide.

Lenalidomide is the most investigated molecule in clinical trials about the role of IMiDs in AML. This drug has a better toxicity profile and efficacy than thalidomide.

Previous studies provided some evidence about the effects of lenalidomide in the AML treatment. Probably, the most significant results occur following two combination therapies: lenalidomide plus cytarabine and lenalidomide plus azacitidine, suggesting a possible synergic action of these molecules.  The most important studies reported a response rate of approximately 30% for these two combinations  26 It was shown that the cytogenetic risk had exerted only a minimal impact on the outcomes of lenalidomide-based regimens. Finally, it is possible to speculate that there is a particular subset of AML patients who could benefit from these treatments: elderly patients as well as R/R who are not elegible for intensive treatment. Surely, further studies and evidences are required to define better the target population to treat with this kind of drugs association. As regards safety, lenalidomide-based regimens were well tolerated but the association with cytarabine or azacitidine can increase toxicities.

It is noteworthy that lenalidomide has a remarkable impact on the treatment of MDS associated with del(5q) as single chromosomal alteration, because it induces casein kinase 1 (Ck1) degradation. This protein is encoded by a gene mapped on 5q chromosome and lenalidomide enhances its reduction allowing p53 activation.  3.

Järås et al. showed that, in AML Ck1 partial inhibition promotes p53 activation and leukemic blast elimination, highlighting its crucial role for tumor survival and the potential importance as therapeuthical target. 54

Hypometilating agents downregulate NKG2D inhibitory ligands production by AML cells, improving NK activity. 12 This evidence might at least partially provide en explanation for the potential synergical activity with lenalidomide.

Another consideration concerns the impact of the combination of cytarabine with lenalidomide. The mentioned studies showed an improvement in the outcome: cytarabine cytotoxicity seems to act synergically with lenalidomide’s impact on microenvironment to disrupt leukemic blasts.

As far as the use of pomalidomide and the new molecules in AML are concerned, studies are still few but promising.

Several studies have highlighted the relevance of NK and T cells in AML development, but it is necessary to further clarify the role of these cells as therapeutic targets. The effect of IMiDs on AML immunosurveillance seems to be the clue to understand their clinical application in this setting. Of interest is also the discovery that interactions with the cells of the microenvironment play a relevant role in activating processes of proliferation of leukemic cells. The central role of CRBN binding in the wide range of mechanisms and targets of action of IMiDs have made their application in AML patients noteworthy; certainly, further studies will be required in order to establish the therapeutic use of these drugs in AML.

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