IDO in MDS/AML disease progression and its role in resistance to azacitidine: a potential new drug target?

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In this issue, Müller-Thomas *et al.*[1] explored the predictive value of indoleamine 2,3 dioxygenase (IDO) expression in 96 patients with high-risk myelodysplastic syndromes (MDS) and secondary AML (sAML) treated with azacitidine.

MDS are clonal haematopoietic stem cell (HSC) disorders characterised by progressive bone marrow (BM) failure resulting in cytopenias, with approximately one-third of patients progressing to sAML,[2] a process during which the increased ratio of apoptosis to proliferation is inversed and replaced by a differentiation block. The pathophysiology of early-stage MDS is a much-debated topic, and it remains unclear whether (i) the MDS clone emerged first (e.g. via mutations),[3] followed by $CD8^+$ cytotoxic T cell (CTL) attack against aberrantly expressed tumour-associated antigens ('T against the clone scenario'); (ii) the immune defect came first, with expansion of autoreactive or cross-reactive polyclonal CTLs targeting normal HSCs ('autoimmune attack'); or (iii) microenvironmental defects came first, with (ii) and (iii) resulting in selection pressure for MDS clones.[4]

What is clear, however, is that both the microenvironment and the immune system are severely disturbed in MDS and AML (hereafter referred to as MDS/AML), and that disease progression is paralleled by a progression from immunosurveillance to immunoselection and ultimately immuno-subversion, resulting in tumour immune escape. MDS/AML are characterised by a plethora of numerical and functional changes in virtually all cellular components of the immune system and microenvironment. Much of the immune suppression and evasion results from intense cross-talk between the MDS/AML clone with mesenchymal stem and progenitor cells (MSPCs). Under inflammatory licensing conditions, which prevail in the BM of MDS/AML patients, tumour-educated Type-2 MSPCs exert their strongly immunosuppressive function, that is, via secretion of high levels of IDO. IDO is expressed by different cells throughout the body, including macrophages and dendritic cells, but is also found in tumour cells and the tumour microenvironment.[5] IDO has been linked to the development of several cancer types, including MDS/AML, via suppression of the immune system, propagation of cancer cell growth, migration and invasion.[5–9] IDO is an endocellular monomeric enzyme that degrades the essential amino acid L-tryptophan to L-kynurenine. Tryptophan starvation results in T-cell cycle arrest, and kynurenine and its metabolites are also directly toxic for many T- and natural killer cells (NKCs). IDO also induces a plethora of regulatory cells and the switch from M0- to M2-macrophages, which, together with the induction of T-cell anergy, results in a tolerogenic microenvironment, strong immunosuppression, tumour immune escape and disease progression (Fig 1). Furthermore, the enzyme inhibits the production of erythropoietin and may also be mutagenic, thus contributing to genetic instability.[10,11]

Thus far, only a few groups have studied IDO in the context of MDS/AML. Constitutive overexpression of the strongly-immunosuppressive enzyme has been detected in primary human AML blasts[12] and patient sera[13]. It has been correlated with increased levels of circulating T regulatory cells (T-regs) at initial diagnosis[14] and linked with decreased relapse-free and overall survival.[15] In MDS, elevated IDO metabolites were detected in patient sera and correlated with the degree of cytopenia.[16] Primary MSPCs from MDS patients have been shown to secrete IDO.[17] The fact that IDO has emerged as a key target in cancer immunotherapy, and the paucity of data regarding this critical switch towards immune suppression and evasion in MDS/AML, highlight the relevance and need of the current report by Müller-Thomas *et al.*[1] Immunohistochemistry staining for IDO in BM sections revealed that 37% of their cohort showed moderate to high expression of IDO, with cytoplasmic positivity being observed mainly in $CD11c^+$ myelomonocytic cells and in a few mature $CD68^+$ macrophages, while BM blasts remained negative. In line with the reported immunosuppressive function of IDO, the group observed a significantly lower CD8/CD3 ratio ($P < 0{\cdot}0001$) and a trend for lower FOXP3

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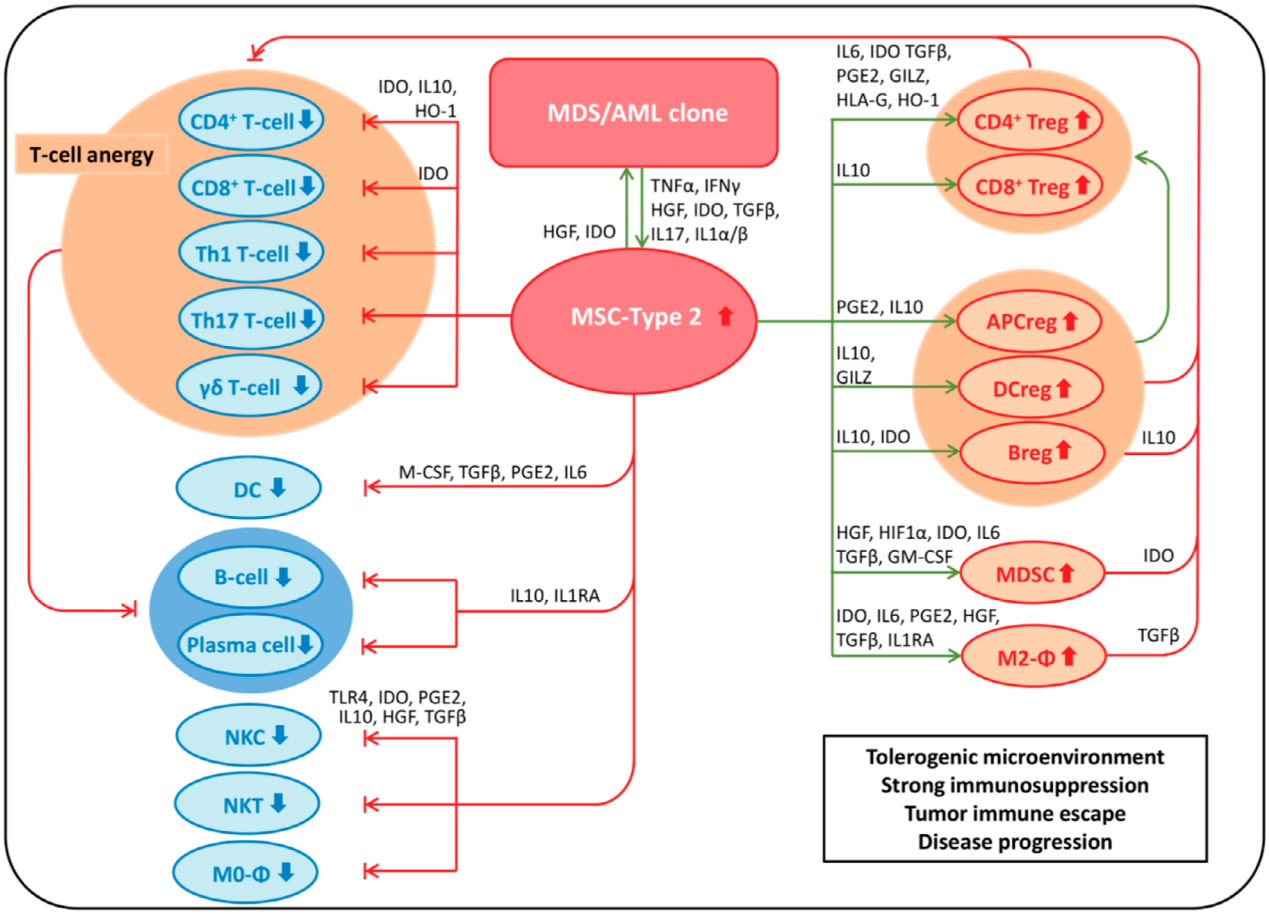

Fig 1. Mechanisms of immune evasion in MDS/AML. Reprinted with permission from Pleyer *et al.*[11] Early stage MDS/AML are ssociated with a stage of inflammation. The inflammatory BM microenvironment is believed to recruit proinflammatory Type-1 MSCs and license them to adopt a Type-2 immunosuppressing and tumour-promoting phenotype. Together with the leukemic clone, tumour-educated Type-2 MSCs recruit additional immunosuppresive cells, and suppress those cells capable of targeting the leukemic clone, resulting in a strongly immunosuppressive environment, enabling tuour immune escape and disease progression. [Colour figure can be viewed at wileyonlinelibrary.com]

expression ($P = 0{\cdot}060$), which serves as a lineage specification factor for T regulatory cells, in the BM of patients with high IDO expression.

Müller-Thomas *et al.*[1] are the first to analyse IDO in a patient cohort uniformly treated with the hypomethylating agent (HMA) azacitidine. According to current NCCN guidelines and numerous expert opinions,[18–20] HMA are the recommended front line treatment of choice in patients with MDS and AML who are unfit for intensive chemotherapy and/or allogeneic stem cell transplantation. Of note, azacitidine is approved for both MDS and AML in the EU and the US, whereas decitabine is approved for AML (but not MDS) in the EU and for MDS (but not AML) in the US. So far, azacitidine is the only treatment modality shown to prolong survival in MDS in a phase III randomised trial,[21] whereas both azacitidine and decitabine (albeit in a *post hoc* sensitivity analysis) demonstrated prolonged overall survival (OS) in patients with AML.[22,23] However, approximately half of patients do not respond to HMA, and all of the responders eventually experience progressive disease and die. Therefore, several groups have attempted to predict which patients will benefit from HMA treatment.[24–27]

In their report, Müller-Thomas *et al.*[1] explored the predictive value of IDO expression in 95 MDS/AML patients treated with azacitidine. Median OS was 12·6 and 7·5 months, and the overall response rate (ORR) was 42% and 37% for MDS and AML patients, respectively. Notably, both are lower than those reported by others, including ourselves. In an analysis of 339 MDS/AML patients treated with azacitidine within the Austrian Registry of Hypomethylating Agents, median OS was 23·7, 18·9, 13·5 and 13·1 months for patients classified as MDS-RAEB-I, MDS-RAEB-II, low blast count sAML and AML with >30% BM blasts, respectively. ORR ranged from 49·0 to 55·9%.[10] These discrepancies might be due to the high percentage of patients with poor and very poor-risk karyotype in Müller-Thomas's cohort (54%). Importantly, the group demonstrated that high expression of IDO in the BM predicts azacitidine treatment failure (83 vs. 48%, $P < 0{\cdot}001$) and significantly shorter OS (10·8 vs. 21·4 months, $P = 0{\cdot}034$) in IDO positive *versus* negative

patients respectively, despite the fact that the IDO positive group had significantly fewer patients with poor and very poor IPSS-R risk categories than the IDO negative group (40 vs. 62%, $P = 0{\cdot}014$). IDO expression remained prognostically significant for OS in multivariate analysis.

Azacitidine is known to increase $CD8^+$ CTLs[28] and has also been reported to induce T regulatory cells.[29] Müller-Thomas *et al.*[1] demonstrated that IDO positivity significantly correlated with a lack of increase of $CD8^+$ CTLs ($P < 0{\cdot}001$) in a small subgroup of patients ($n = 15$) with a follow-up BM sample. These results may be seminal if reproduced in a larger set of samples, as they show that IDO (i) is expressed in the BM of MDS/AML patients, (ii) induces an immunosuppressive microenvironment, thereby relevantly contributing to azacitidine treatment failure and poor survival associated therewith, and (iii) may thus represent an interesting drug target in MDS/AML, especially for combination therapies.

So how can the expression of an enzyme in mainly non-blast cells be related to the efficacy of an HMA on a mechanistic basis? It has been shown that HMAs exert pleiotropic effects on a plethora of cells relevant to MDS/AML development and progression.[30] Besides hypomethylation of silenced tumour-suppressor genes and direct cytotoxicity, HMAs modulate numbers and functions of various immune cells (i.e., T-cell subsets, NKCs, MSCs and myeloid-derived suppressor cells (MDSCs)) to reactivate dormant anti-tumour immune responses.[31–33] One could speculate that high IDO expression in the BM of MDS/AML patients might be a surrogate marker for advanced disease in terms of advanced tumour immune evasion and strong immunosuppression, without necessarily being strictly associated with BM blast count or other laboratory parameters currently included in prognostic scoring systems. In MDS/AML patients with high IDO levels and a severely dysfunctioning immune system, monotherapy with azacitidine might not suffice to reverse these changes. As such, the present study forms a basis for further exploring therapeutic inhibition of IDO in MDS/AML. Several IDO inhibitors exist, including indoximod, epacadostat and BMS-986205.[34] A recent phase II trial with epacadostat monotherapy in MDS demonstrated safety, but did not reveal relevant activity, with disease stabilisation in 80% of the patients being the best reported outcome.[35] From the mechanisms reported above, this does not seem too surprising, as one might expect IDO inhibition to be more effective as an add-on, rather than as a stand-alone therapy. IDO inhibitors are currently being tested in solid tumours, including combination strategies with checkpoint inhibitors (NCT04106414, NCT04047706, NCT03915405, NCT03695250, NCT03414229, NCT03347123, NCT03291054, NCT03085914, NCT02073123), but selected trials are also starting to look at this potentially promising target in AML (NCT02835729). As immune therapies (most excitingly the field of chimeric antigen receptor T-cell therapies) develop forwards in the field of MDS and AML, combinatorial targeting of IDO will be an interesting avenue to explore, with the aim to further increase therapeutic efficacy of existing treatments. Clinical trials with these combination strategies are therefore eagerly awaited.

Author Contribution

M.L. and L.P. contributed equally.

Conflict of Interest

L.M.: Honoraria from Bristol-Myers-Squibb, Pfizer, Takeda; travel support from Novartis, Roche. R.G.: Honoraria from Bristol-Myers-Squibb, Cephalon, Amgen, Eisai, Mundipharma, Merck, Janssen-Cilag, Genentech, Novartis, AstraZeneca, Boehringer Ingelheim, Pfizer, Roche, and Sanofi Aventis; research funding from Cephalon, Celgene, Amgen, Mundipharma, Genentech, Pfizer, GSK, and Ratiopharm; consulting work for Bristol-Myers-Squibb, Cephalon, and Celgene. L.P.: Honoraria from Abbvie, Agios, Bristol-Myers-Squibb, Celgene, Inflection Point Biomedical Advisors, Novartis; travel support from Celgene, Gilead, and Novartis.

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