


Prognostic value of indoleamine 2,3 dioxygenase in patients with higher-risk myelodysplastic syndromes treated with azacytidine

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Abstract

Hypomethylating agents (HMAs) are widely used in patients with higher-risk myelodysplastic syndromes (MDS) not eligible for stem cell transplantation; however, the response rate is <50%. Reliable predictors of response are still missing, and it is a major challenge to develop new treatment strategies. One current approach is the combination of azacytidine (AZA) with checkpoint inhibitors; however, the potential benefit of targeting the immunomodulator indoleamine-2,3-dioxygenase (IDO-1) has not yet been evaluated. We observed moderate to strong IDO-1 expression in 37% of patients with high-risk MDS. IDO-1 positivity was predictive of treatment failure and shorter overall survival. Moreover, IDO-1 positivity correlated inversely with the number of infiltrating CD8⁺ T cells, and IDO-1+ patients failed to show an increase in CD8⁺ T cells under AZA treatment. *In vitro* experiments confirmed tryptophan catabolism and depletion of CD8⁺ T cells in IDO-1+ MDS, suggesting that IDO-1 expression induces an immunosuppressive microenvironment in MDS, thereby leading to treatment failure under AZA treatment. In conclusion, IDO-1 is expressed in more than one-third of patients with higher-risk MDS, and is predictive of treatment failure and shorter overall survival. Therefore, IDO-1 is emerging as a promising predictor and therapeutic target, especially for combination therapies with HMAs or checkpoint inhibitors.

Keywords: myelodysplastic syndromes, immunomodulation, indoleamine-2,3-dioxygenase, azacytidine, prognosis.

Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell disorders characterised by dysplasia, bone marrow failure and an increased risk of developing secondary acute myeloid leukaemia (sAML). The prognosis of higher-risk patients is generally poor, with a high rate of early mortality, a low complete response (CR) and high relapse rates even after intensive chemotherapy or allogeneic haematopoietic stem cell transplantation (HSCT).¹

Hypomethylating agents (HMAs), such as azacytidine (AZA), have improved overall survival (OS) compared to

conventional care options, and are approved for patients with higher-risk MDS not eligible for allogeneic HSCT.^{2–4} However, resistance to HMAs occurs in more than 50% of patients. Furthermore, higher-risk MDS frequently progresses to sAML even under ongoing therapy with AZA, and virtually all responders eventually relapse.⁵ While somatic mutations in *TP53*^{6–8} have been associated with poor OS in some patient cohorts, the mechanisms of resistance to HMAs are still not well understood. Therefore, it is essential to identify reliable predictive markers and alternative treatment options.

In recent years, several immune regulatory factors have been discovered to play a role in tumour-mediated immune escape causing failure of standard therapy. Across different categories of MDS, alterations in natural killer cells, regulatory and cytotoxic T cells, as well as in myeloid-derived suppressor cells, have been reported.^{9–13} Moreover, therapy can induce immune reactions and lead to the upregulation of inhibitory cofactors, such as CTLA-4, PD-L1 and PD-1 in haematopoietic cells.¹⁴ Therefore, the combination of AZA with checkpoint inhibitors is an emerging new concept to overcome immune tolerance.

Mechanistic studies of immune escape have linked metabolic alterations and microenvironmental catabolism to immune tolerance. Indoleamine 2,3 dioxygenase (IDO-1) catalyses the degradation of tryptophan to kynurenine. Tryptophan is essential for proliferation of cytotoxic T cells and their immune response.^{15,16} In addition, kynurenine and downstream catabolites lead to the upregulation and activation of regulatory T cells (Tregs), inducing immune suppression.^{17,18} In AML, high IDO-1 protein expression, assessed by immunohistochemistry, is a predictor of poor outcome in patients who do not undergo HSCT.¹⁹ However, to the best of our knowledge, IDO-1 has not been studied in patients with MDS treated with HMAs. Therefore, we assessed IDO-1 expression in the bone marrow and its immunoregulatory capacity in patients with higher-risk MDS treated with HMAs and evaluated the prognostic value of IDO-1 in these patients.

Materials and methods

Patients

The study population included 95 patients with higher-risk MDS, sAML or MDS/MPN treated at the Technische Universität München and Heinrich-Heine Universität Düsseldorf (2004–2013). Informed consent in accordance with the declaration of Helsinki was obtained from all patients. Diagnosis was made according to the WHO-classification 2016.²⁰ MDS risk assessment was based on the IPSS-R.²¹ Karyotypes were classified according to the new comprehensive cytogenetic scoring system.²²

Treatment consisted of at least three complete cycles of AZA monotherapy. Patients who had undergone allogeneic HSCT before AZA treatment were excluded from the study. Nine patients underwent allogeneic HSCT after AZA treatment.

Definition of response and survival

Response to AZA treatment was scored according to the modified international working group criteria for MDS.²³ Reaching stable disease (SD) with haematological improvement (HI) appears to be associated with improved survival,² therefore it was also considered a response. The definition of

overall response rate (ORR) included complete remission (CR), partial remission (PR), marrow CR (mCR) and SD with HI. Survival was defined from the start of treatment until death. Patients were censored at the time of last observation, when still alive.

Immunohistochemistry and immunofluorescence

The following reagents were used: IDO-1 (Cell Signaling, Beverly, MA, USA) and CD3, CD8, CD11c, CD34, CD47 and CD68 (KP-1) (DAKO GmbH, Hamburg, Germany). Immunodetection was performed with the REAL detection kit (DAKO GmbH) and with Cy3-conjugated-goat anti-mouse and FITC-conjugated-goat anti-rabbit antibodies (Jackson ImmunoResearch, Cambridge, UK). IDO-1 immunohistochemistry was quantified according to the immunoreactive score (IRS),²⁴ with staining intensity (score 1–3) multiplied by percent of positive cells (IRS = 0–300). For further analysis, IRS score was calculated by grading percent of positive cells (0 = 0%; 1 = < 10%; 2 = 10–50%; 3 = 51–80%; 4 = > 80%). Results were divided into low expression (IRS = 0–3) and high expression (IRS = 6–12); no samples had a score of IRS = 5/6. Slides were scanned and analysed with Vectra Polaris (Perkin Elmer, Waltham, MA, USA).

Cell culture

The human MDS cell lines SKK-1 and MDS-L were cultured in RPMI-1640 with 10% heat-inactivated fetal bovine serum (Gibco, Thermo fisher scientific, Waltham, MA, USA). The medium for MDS-L additionally contained 50 µmol/l β-mercaptoethanol, 2 mmol/l L-glutamine, 10 mmol/l HEPES and 10 ng/ml IL-3 (R&D systems, Minneapolis, ME, USA). Human CD8⁺ T cells were purchased from Stem Cell Technologies (Cologne, Germany). For mitogen stimulation, 100 ng/ml anti-CD3 (OK3; Ortho Biotech, Rariton, NJ, USA) and 1 µg/ml CD28 (Pharming, BD Bioscience, San Jose, CA, USA) were used. When indicated, tryptophan (10 µmol/l; Sigma Aldrich, St. Louis, MI, USA) or IDO-1 inhibitor BMS-986205 (5 to 50 nmol/l; Selleck, Houston, TX, USA) were added. Tryptophan and kynurenine were quantified by ELISA (ImmuSmol, Pessac, France).

Statistical analysis

Clinical characteristics were compared with the χ^2 -test for categorical and Student's *t*-test for continuous variables. The association between clinical and histopathological variables was investigated using logistic regression. Cox proportional hazard models and Kaplan–Meier curves were used to assess the association of variables with OS. The significance level was set at 0.05, and analysis was conducted with SPSS version 25 (IBM, Chicago, IL, USA).

Results

Main patient characteristics

The demographic and clinical characteristics of the 95 patients are listed in Table I. The median age was 71 years. Of 61 patients with MDS, 28 patients with sAML and six with MDS/MPN, none belonged to the very low-risk cytogenetic risk group; 29 patients had a good-risk karyotype; 15 patients had an intermediate-risk karyotype; 20 patients had a poor-risk karyotype; and 31 patients had a very poor-risk karyotype.

Patients survived for a median of 10.2 months (median OS of MDS patients was 12.6 months and median OS of AML patients was 7.5 months). ORR of patients was 39% (MDS patients' ORR was 42% and AML patients' ORR was 37%); four patients achieved CR, one patient achieved partial remission (PR), 11 patients achieved marrow CR (mCR) and 21 achieved stable disease (SD) with haematological improvement (HI).

Macrophages and myelomonocytic cells of patients with MDS express IDO-1

We observed cytoplasmic IDO-1 positivity in mature macrophages (CD68⁺) and in a subset of mature to immature cells,

characterised by a round or bean-shaped nucleus with coarse chromatin (Fig 1A). Double immunofluorescence with IDO-1 and CD11c (myelomonocytic cells) showed clear colocalisation of IDO-1 and CD11c. Immunofluorescence with IDO-1, CD68 and CD34 (marrow blasts) showed that few mature macrophages were positive for IDO-1 and bone marrow blasts were negative (not shown). Hence, we could identify IDO-1 positivity not in CD34⁺ blasts but in mature macrophages, as well as in myelomonocytic cells of the bone marrow.

Thirty-five patients (37%) showed moderate to high cytoplasmic expression of IDO-1 (IRS 6-12), and 60 patients (63%) were negative or showed only weak expression of IDO-1 (IRS 0-3) (Fig 1D).

IDO-1 expression does not correlate with most well-established clinicopathological parameters, but correlates with CD8⁺ T cells

No significant differences in age, gender, diagnosis, number of marrow blasts, IPSS-R or p53 positivity were observed, as detailed in Table I. Only the cytogenetic risk classification showed a significance ($P = 0.014$), with more patients in the

Table I. Patient characteristics in the overall cohort by IDO-1 positivity.

	IDO-1+ ($n = 35$)	IDO-1 negative ($n = 60$)	P-value
Age			
Mean	70.9	69.9	n.s.
Median	72	70	
Range	47-84	40-87	
Gender (%)			
Male	69 (24)	55 (33)	n.s.
Female	31 (11)	45 (27)	
MDS/WHO 2016 (%)			
MLD	3 (1)	5 (3)	n.s.
EB1	3 (1)	12 (7)	
EB2	60 (21)	47 (28)	
Saml	23 (8)	33 (20)	
MDS/MPN	11 (4)	3 (2)	
Marrow blasts (%)			
0-4	9 (3)	8 (5)	n.s.
5-9	6 (2)	12 (7)	
10-19	63 (22)	47 (28)	
≥20	23 (8)	33 (20)	
IPSS-R			
Very good	0 (0)	0 (0)	0.014
Good	29 (10)	32 (19)	
Intermediate	31 (11)	7 (4)	
Poor	14 (5)	25 (15)	
Very poor	26 (9)	37 (22)	
p53 (IHC)			
Positive	26 (9)	35 (21)	n.s.
Negative	74 (26)	65 (39)	

IDO-1, Indoleamine 2,3 dioxygenase; MDS, myelodysplastic syndromes; WHO, World Health Organization; MLD, multilineage dysplasia; EB, excess blasts; sAML, secondary acute myeloid leukaemia; MPN, myeloproliferative neoplasms; IPSS-R, revised international prognostic scoring system; IHC, immunohistochemistry; n.s., not significant.

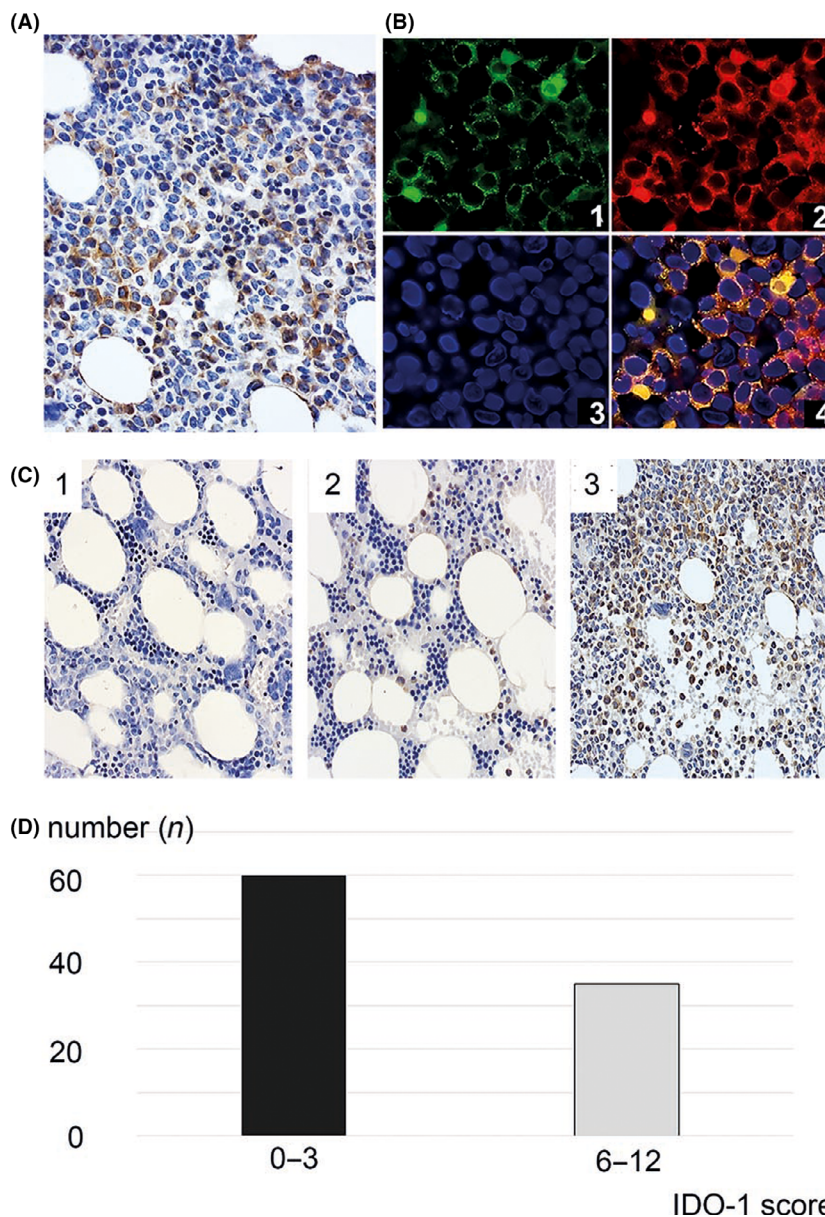


Fig 1. IDO-1 expression in bone marrow samples. IHC analysis was performed with IDO-1, and a moderate to high cytoplasmic IDO-1 expression can be observed in a subset of bone marrow samples (A). Double immunofluorescence analysis (B) with IDO-1 (green, FITC-conjugated secondary antibody (1)) and with CD11c (red, Cy3-conjugated secondary antibody (2)) shows co-expression of IDO-1 and CD11c (yellow, overlay of red and green (4)). IHC analysis of bone marrow samples showed IDO-1 negativity (C1), low expression of IDO-1 (C2) and strong expression of IDO-1 (C3). IDO-1 expression was semi-quantified by IRS; 63% of bone marrow samples were negative or showed weak expression of IDO-1 and 37% of bone marrow samples showed moderate to high expression of IDO-1 (D).

poor (25% vs. 14%) or very poor (37% vs. 26%) risk group and fewer patients in the intermediate (7% vs. 31%) risk group in the IDO-1-negative subgroup.

Because IDO-1 can directly upregulate Tregs (FOXP3⁺) and suppresses proliferation of cytotoxic T-lymphocytes (CD8⁺) (20, 21), we studied the immunoregulatory function of IDO-1 in our samples. We quantified T cells (CD3), CD8⁺ T cells, FOXP3⁺ T cells, PD1⁺ T cells, PDL1⁺ cells, TIM3⁺ cells and CD47⁺ leukocytes and performed regression analysis. PDL1 was expressed by bone marrow blasts and

macrophages (Fig S1). Baseline CD8/CD3 ratio was 0.46 for MDS patients and 0.37 for AML patients. Interestingly, we observed a strong negative correlation between the CD8/CD3 ratio to IDO-1 expression ($R^2 = 0.71$, $P < 0.0001$), whereas the correlation between IDO-1 and FOXP3 ($R^2 = 0.037$, $P = 0.060$), PD1 ($R^2 = 0.0263$, $P = 0.1163$), PDL1 ($R^2 = 0.0175$, $P = 0.2012$), TIM3 ($R^2 = 0.0387$, $P = 0.056$) or CD47 ($R^2 = 4.23 \times 10^{-3}$, $P = 0.95$) expression did not reach statistical significance (Fig 2), with a trend towards significance for FOXP3 and TIM3.

IDO-1 expression predicts response to AZA treatment

While it has been shown that AZA treatment can induce immune reactions, IDO-1 can inhibit immune reactions. As IDO-1 expression was correlated negatively with the CD8/CD3 ratio, suggesting an immunosuppressive role of IDO-1 in our samples, we next analysed whether IDO-1 expression has any impact on response to AZA treatment.

In the IDO-1⁻ group, the ORR was 52%, with an AZA treatment failure rate of 48%. In contrast, in the IDO-1⁺ group, the majority of patients showed treatment failure (83%), with an ORR of 17% ($P < 0.001$; Table II and Fig 3). Other factors (age, MDS type, p53- status) did not reach statistical significance (Table S1).

IDO-1 expression is a predictive factor of survival in patients treated with AZA

Moreover, IDO-1 expression did not only predict response to therapy but was also associated with OS. Univariate analysis showed significantly-improved survival in IDO-1⁻ patients (21.4 months) compared to IDO-1⁺ patients (10.8 months) (HR 0.615; $P = 0.034$) (Fig 4). No other markers significantly influenced OS, including the IPSS-R (HR 1.49; $P = 0.127$), sex (HR 1.008; $P = 0.971$), age ≤ 60 versus > 60 (HR 2.403; $P = 0.06$), sAML versus MDS (HR 1.153; $P = 0.550$), cytogenetic risk group (HR 1.07; $P = 0.426$) and p53 status (HR 0.983; $P = 0.884$) (Fig 3).

Table II. Response to azacytidine according to IDO-1 expression.

	IDO-1 negative ($n = 35$)	IDO-1+ ($n = 60$)
CR	3.30% (2)	2.90% (1)
PR	3.30% (2)	0% (0)
BM-CR	16.70% (10)	0% (0)
SD + HI	25% (15)	14.20% (5)
Failure	48.30% (29)	82.90% (29)
ORR	51.70% (31)	17.10% (6)

CR, complete remission; PR, partial remission; BM-CR, bone marrow complete remission; SD + HI, stable disease with haematological improvement; ORR, overall response rate.

To examine, if IDO-1 expression itself adds prognostic information to the IPSS-R multivariable analysis for survival was conducted. Multivariable analysis for survival was conducted to examine, if IDO-1 expression itself adds prognostic information to the IPSS-R (Table III).

IDO-1 expression correlates with CD8⁺ T cell response failure after AZA treatment

We identified 15 patients (5 IDO-1⁺; 10 IDO-1⁻) with follow-up bone marrow biopsies under AZA treatment which allowed us to obtain their status before and after/under AZA treatment. Remarkably, we observed a high and long-lasting increase in CD8⁺ T cells in all IDO-1⁻ patients; however, in IDO-1⁺ patients, the number of CD8⁺ T cells did not

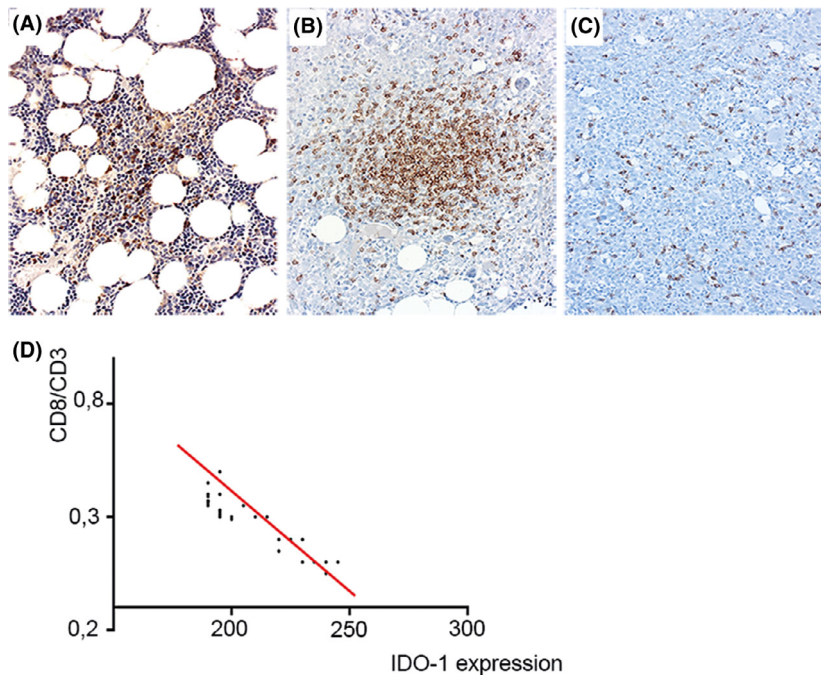


Fig 2. Correlation of IDO-1 expression with CD8/CD3 ratio in bone marrow samples. IHC analysis was performed with IDO-1 (A), CD3 (B) and CD8 (C). Correlation studies showed that IDO-1 expression inversely correlated significantly with the ratio of infiltrating CD8/CD3 positive T cells (D) with $R^2 = 0.71$ (Pearson correlation; total of 95 patients).

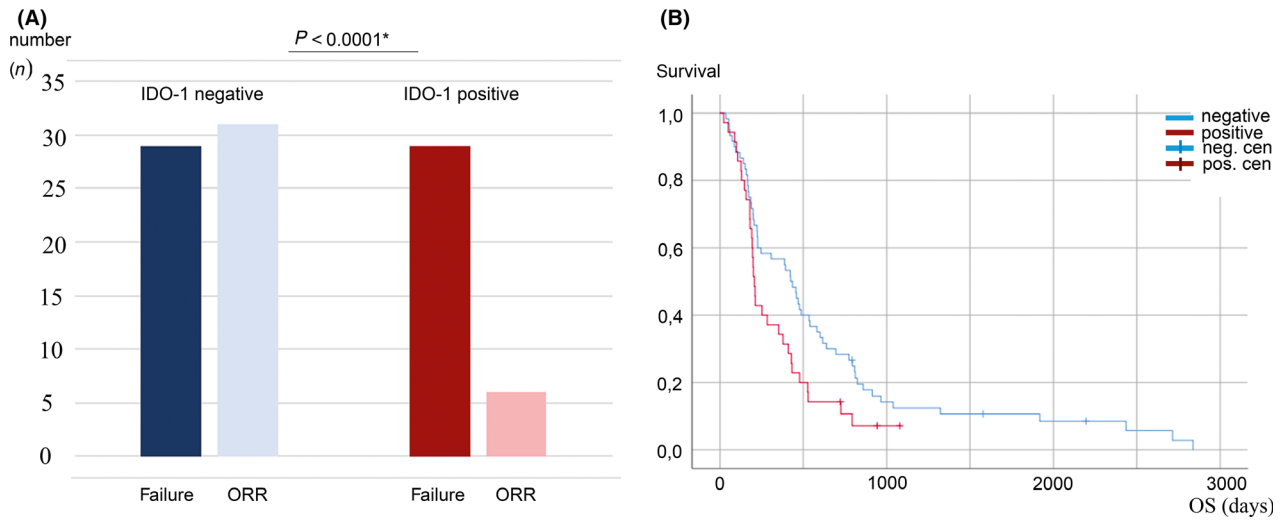


Fig 3. Correlation of IDO-1 expression and response to treatment as well as overall survival depending on IDO-1 expression. Correlation of IDO-1 expression and response to therapy was analysed by χ^2 test (A). 83% of the IDO-1+ patients failed to therapy, with 17% of ORR; in contrast, the overall response rate was 52% in the IDO-1 negative group ($P < 0.0001$). Kaplan-Meier curves for overall survival (B), with IDO-1+ and IDO-1 negative patients showing significantly shorter OS for IDO-1+ patients.

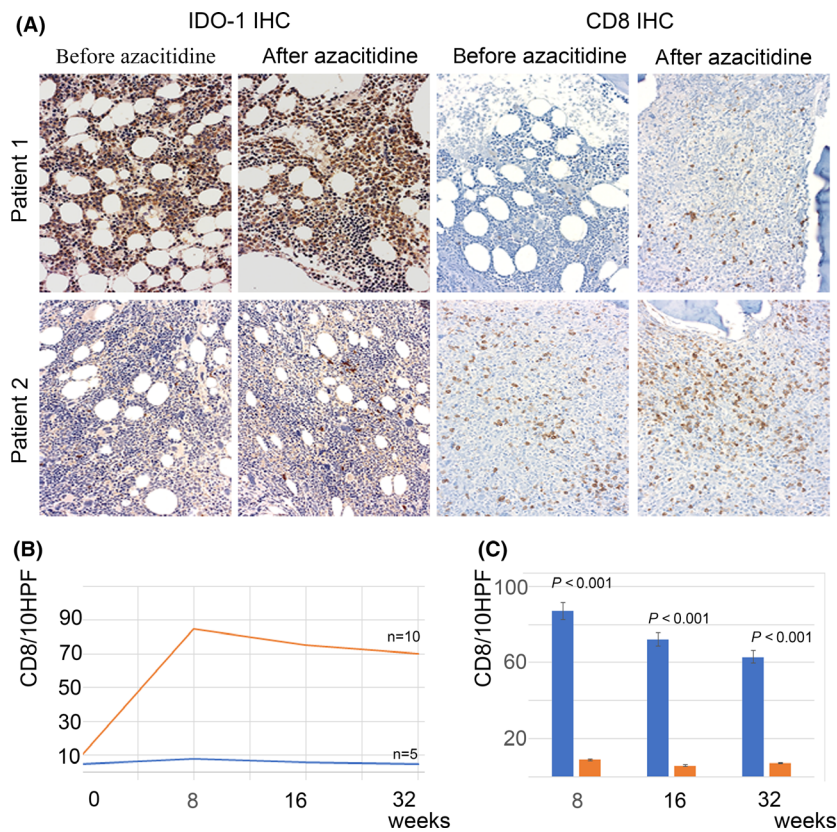


Fig 4. Increase of infiltrating CD8⁺ T cells in patients treated with AZA. Representative IHC analysis (A) of an IDO-1+ (patient 1) and of an IDO-1 negative patient (patient 2); displayed is the IDO-1 expression and the CD8-IHC before and after AZA treatment. Under AZA treatment a clear increase in the number of CD8⁺ T cells in IDO-1 negative patients with nearly no increase in IDO-1+ patients can be observed (B; Y-axis displays median gain of CD8⁺ T cells). This difference was statistically significant at all time points (C).

increase over time (Fig 4A,B). The difference in increase of CD8⁺ T cells was statistically significant ($P < 0.001$) (Fig 4C). We could also observe a decrease of Tregs, which was higher in IDO-1- MDS ($P = 0.061$).

IDO-1 in MDS cell lines leads to catabolism of tryptophan and depletion of CD8⁺ T-lymphocytes

It is well documented that cytotoxic (CD8⁺) T cells stop proliferating under tryptophan starvation. Therefore, we wanted to test if tryptophan in the cell culture medium can be metabolised by IDO-1⁺ MDS cell lines, and if proliferation of CD8⁺ T cells is indeed dependent on tryptophan under these conditions.

We cultured two MDS cell lines, SKK1 and MDS-L, with high expression of IDO-1 (Fig 5A) for 72 h. ELISA of the conditioned medium showed a nearly complete depletion of tryptophan to $< 0.5 \mu\text{mol/l}$ ($0.2 \mu\text{mol/l} \pm 0.2$), with subsequent increase in kynurenine to $20.5 \pm 1 \mu\text{mol/l}$ in both cell lines, which could be prevented by IDO-1 inhibition (Fig 5B).

IDO-1 inhibition had no direct effect on proliferation of the MDS cell lines, nor on the proliferation of CD8⁺ T cells (data not shown). We next investigated if tryptophan depletion in conditioned medium could reduce T cell proliferation. Activated CD8⁺ T cells stimulated with anti-CD3 (100 ng/ml) and CD28 (1 $\mu\text{g/ml}$) were cultured in conditioned medium and normal growth medium for 48 h, and proliferation was measured by MTT-assay. T cells grown in conditioned medium showed a significant reduction of relative proliferation to 20–25%, which could be nearly rescued by adding tryptophan to the medium (Fig 5C).

Discussion

In recent years, IDO-1 has emerged as a key target in cancer immunotherapy because it can induce tumour immune escape in various cancers.²⁵ Thus far, evidence supporting a role for this pathway in MDS is lacking, and the effect of IDO-1 expression under AZA treatment, a treatment that induces certain immune reactions, has not yet been studied.

In this study, we first demonstrated that IDO-1 is expressed in the bone marrow samples of patients with

higher-risk MDS. We performed a comprehensive analysis of patients ($n = 95$) treated homogeneously with AZA, while most previous studies have examined combined AZA or decitabine treatments.^{26–28} Our cohort patients with higher-risk MDS is in line with the approval of AZA.^{29,30} We observed cytoplasmic expression of IDO-1 in macrophages and myelomonocytic cells; however, we did not observe IDO-1 expression in bone marrow blasts. In a recent study of 29 AML patients,¹⁹ IDO-1 expression in leukemic blasts was found to be predictive of early mortality, though double immunofluorescence staining was not performed to truly clarify which cells were expressing IDO-1. Our observations are in line with numerous publications on solid tumours, lymphomas and melanomas,^{31–33} in which IDO-1 expression has been observed in the tumour cells themselves, as well as in by-standing antigen-presenting cells. Recently, it was also demonstrated that the levels of tryptophan metabolites are elevated in the sera of patients with MDS, and that higher tryptophan metabolite levels correlated with degree of cytopenia.³⁴

The main focus of our study was the immunomodulatory role of IDO-1 in MDS; therefore, we quantified infiltrating macrophages, T cells and specific T cell subsets. We observed only a small number of Tregs, and IDO-1 expression did not correlate with the number of Tregs. This is in contrast to previous studies showing that IDO-1 can activate Tregs and block their conversion to Th17 cells.³⁵ However, Tregs are suppressed by multiple cytokines (such as TGF- β , IL-10 and IL-35),³⁶ and, particularly, overactivation of the TGF- β pathway (which could eventually prevent IDO-1-induced Treg activation and proliferation) is well documented in MDS.³⁷

The bone marrow samples displayed a moderate to high number of infiltrating macrophages. Only a few macrophages were IDO-1+, and IDO-1 expression did not correlate with the number of infiltrating macrophages. This is not surprising, as it is well documented that in some (but not all) tumours, tumour cells can lead to the upregulation of IDO-1 in their microenvironment, yet an induction of macrophage proliferation has not been observed thus far. An important and common mechanism of IDO-1 in promoting tumour immune escape is the catabolism of tryptophan, which leads to the cell cycle arrest and apoptosis of cytotoxic T cells.¹⁶ Indeed, IDO-1 expression correlated inversely with the CD8/CD3 ratio in the bone marrow in our cohort. This is an exciting observation, which suggests that IDO-1 might be a key factor in the induction of an immunosuppressive bone marrow environment in MDS patients.

IDO-1 expression could not only predict the response to therapy, it was also the only prognostic factor for survival, and had an independent prognostic impact in multivariate analysis. In contrast, univariate analysis showed that IPSS-R, gender and p53 status could not predict response to AZA or survival. This is consistent with several publications in which TP53 mutations conferred a poor prognosis but were not predictive of the response to therapy,^{6,38,39} while IPSS-R,

Table III. Multivariate analysis of prognostic scores and IDO-1 expression.

	HR	P
IDO-1 expression	0.626	0.042
Medullary blasts according to IPSS-R	1.141	0.371
Cytopenia according to IPSS-R	0.607	0.194
Cytopenetics according to IPSS-R	1.138	0.177

IDO-1, Indoleamine 2,3 dioxygenase; IPSS-R, revised international prognostic scoring system.

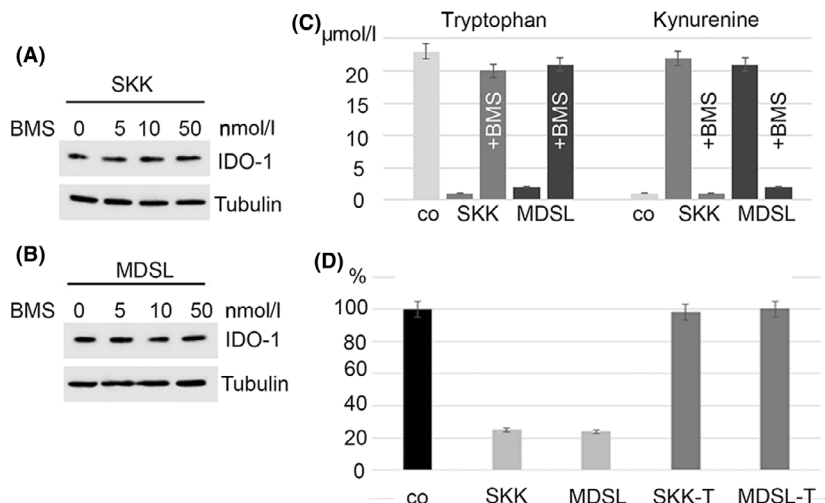


Fig 5. Tryptophan metabolism by MDS cell lines and inhibition of T cell proliferation. Western blot analysis of SKK (A) and MDS-L (B) cell lines display constant high expression of IDO-1 under BMS-986205 treatment in various concentrations (0–50 nmol/l). Tryptophan is catabolised in conditioned medium of SKK and MDS-L; simultaneously, kynurenine levels are increasing as measured by ELISA (C). MTT assays (D) show a clear reduction of T cell proliferation in conditioned medium (SKK, MDS-L), which can be rescued by addition of tryptophan (SKK-T, MDS-L-T).

gender and age did not qualify as predictors of response to therapy or survival. HMAs can potentiate antitumour immunity by inducing the expression of critical molecules involved in cognate recognition by cytotoxic T cells, such as MHC I and ICAM-1. Consequently, the T cell-mediated elimination of tumour cells is enhanced due to T cell recruitment and proliferation after reactivation.^{40,41} IDO-1 expression correlated inversely with the CD8/CD3 ratio in our bone marrow samples; therefore, it might well be that IDO-1 counteracts the immune cell activation of AZA and thereby promotes resistance to therapy.

The level of CD8⁺ T cells clearly increased after/under AZA treatment and remained constantly high. As stated above, HMA can stimulate the antitumour immune response, including activation and upregulation of antigen-specific cytotoxic T-lymphocytes.^{42,43} Excitingly, in all our IDO-1⁻ patients, we observed an increase in CD8⁺ T-lymphocytes; in contrast, all IDO-1⁺ patients showed constant low numbers of CD8⁺ T-lymphocytes, with no or only a slight transient increase (Fig 5). This effect was seen early during AZA treatment and drove our decision to include patients being treated with < 6 cycles AZA as well.

Functionally, IDO-1⁺ cell lines were able to catabolise tryptophan with subsequent increase of kynurenine, which led to depletion of T cells (Fig 5). Interestingly, the proliferation arrest could be rescued by addition of tryptophan. Previously,⁴⁴ it had been shown that human bone marrow stromal cells are capable of inhibiting allogeneic T cell responses; however, an induction of an immunosuppressive environment by MDS cells has not been demonstrated so far.

Our findings and functional experiments strengthen our hypothesis that IDO-1⁺ MDS can evade the antitumour immune response, thereby leading to AZA treatment failure.

This has clear implications for the development of new therapeutic strategies in the treatment of MDS. There are multiple IDO inhibitors in clinical trials.⁴⁵ Although a phase II trial of IDO-1 inhibitor⁴⁶ in 15 MDS patients has been disappointing, combinations of IDO-1 inhibitors with other chemotherapies or checkpoint inhibitors have resulted in promising ORRs in solid tumours^{47–49} and warrant further investigation in MDS on the basis of our data.

In conclusion, IDO-1 is expressed in macrophages and myelomonocytic cells of the bone marrow in patients with MDS, and is a predictor of both treatment failure and shorter OS under AZA treatment. Interestingly, IDO-1 expression was correlated inversely with the ratio of infiltrating CD8⁺ T cells, and in IDO-1⁺ patients, an increase in CD8⁺ T cells under AZA treatment was not observed. Therefore, IDO-1 positivity in the bone marrow might be useful for identifying patients with a low probability of achieving clinical benefit from HMA. In addition, our data further distinguish IDO-1 as an emerging promising target, especially for combination therapies with HMA in patients with higher-risk MDS.

Author contributions

C.M-T. and M.R. designed the research. C.M-T., M.R., K.G. and U.G. analysed and interpreted the data. C.M-T., M.R., K.G. and U.G. collected data. G.P., M.H. and M.S. performed experiments. C.M-T. and M.R. wrote the manuscript.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. PDL1 expression in MDS bone marrow.

Table S1. Univariate analysis of overall response rate (ORR)

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