Resistance to Hypomethylating Agents (HMA) in High-Risk Myelodysplastic Syndrome (HR-MDS): The role of the Adenosine Deaminase Acting on RNA 1 (ADAR1) enzyme.

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Background and Aims

The HMAs azacytidine (AZA) and decitabine (DAC) are the mainstay of treatment for HR-MDS, however their exact mechanism of action remains largely unknown. Though HMA induce a viral mimicry response on cancer lines, the latter was not associated with clinical responses when measured in primary leukemia cells. The differential expression of the A-to-I RNA editing enzyme, ADAR1, which depletes the immunogenic dsRNAs may be responsible for the disconnection between the induction of viral mimicry and clinical response to HMA. We sought to investigate the role of ADAR1 in the HMA resistance in HR-MDS patients utilizing bone marrow samples from well-annotated patient cohorts.

Materials and Methods

We measured ADAR1 levels and its two isoforms, ADAR1p150 and ADAR1p110, by quantitative real-time PCR in purified primary CD34+ cell subsets. We also performed functional immunophenotyping of STAT3 phosphorylation in primary CD34+ cells.

Results

Non-responders to HMAs (N=25, AZA=15, DAC=10) demonstrated significantly higher pretreatment ADAR1 levels compared to responders (N=22, AZA=18, DAC=4, p=0.0047), but the difference was significant only in AZA-treated patients (p=0.0006, **Figure 1a**). We further investigated the expression of the interferon-inducible ADAR1p150 isoform and the constitutively expressed ADAR1p110 one; both isoforms were upregulated in non-responders compared to responders to AZA ($p_{p150AZA}$=0.0080, $p_{p110AZA}$=0.0066, **Figures 1b-c**).

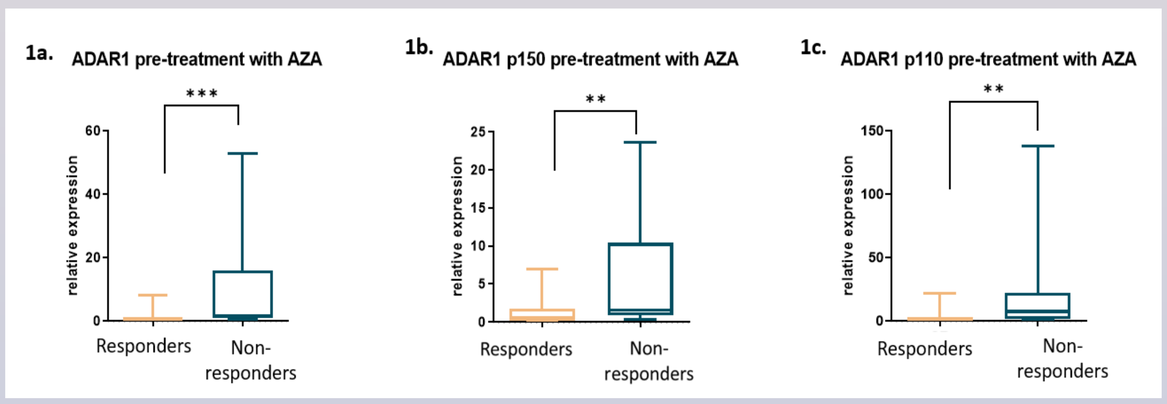

Figure 1a-1c. ADAR1, ADAR1p150, ADAR1p110 pre-treatment levels in primary CD34+ cells of non-responders compared to responders *p<0,05, **p<0,01, ***p<0,001.

The level of cellular differentiation where AZA acts by inducing the viral mimicry state is still unidentified; therefore, we measured ADAR1 levels in FACS-sorted hematopoietic stem and progenitor cell subpopulations. We observed significantly higher ADAR1p150 levels in the more primitive Lin-/CD34+/CD38- compartment of non-responders (N=17) compared to responders (N=11) to AZA (p=0.0060, **Figure 1d**), but not in the Lin-/CD34+/CD38+ committed progenitors (p=0.9879, **Figure 1e**), erythroblasts (p=0.4079, **Figure 1f**) or the mature Lin+/CD34+ subpopulation (p>0.99, **Figure 1g**).

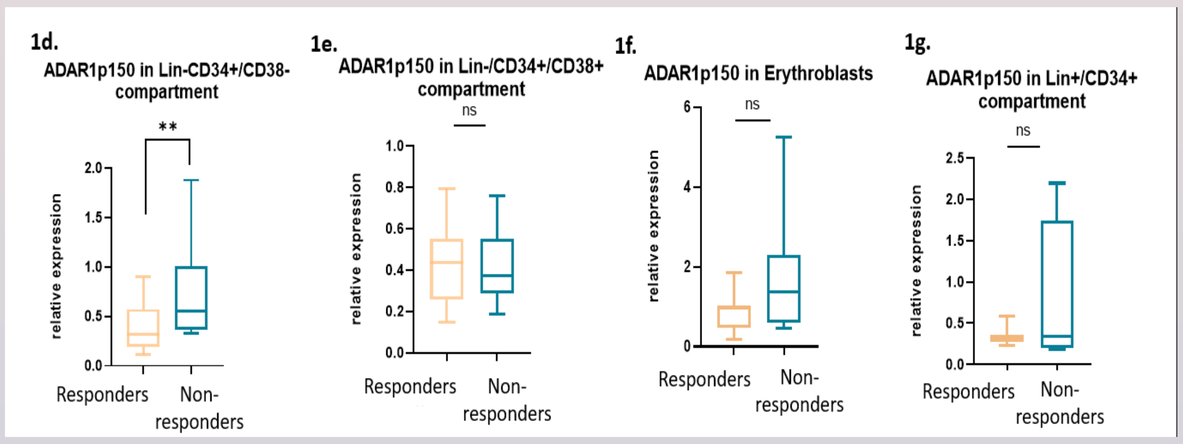

Figure 1d-1g. ADAR1p150 pre-treatment levels in primary FACS-sorted blast compartments of non-responders compared to responders *p<0,05, **p<0,01, ***p<0,001.

Recent data support the existence of a positive feedback regulatory loop between STAT3b and ADAR1, in which STAT3b promotes ADAR1 expression while, in turn, ADAR1 contributes to the stabilization of STAT3b.

We studied the expression of STAT3a and STAT3b in purified total CD34+ cells from HMA-treated HR-MDS patients, but we observed no significant associations with the response to HMAs (p_{STAT3a}=0.3277, p_{STAT3b}=0.2203, **Figures 2a-b**). However, we found a positive correlation between the relative expression of STAT3b and ADAR1p150 isoform only (**Figure 2c**), as well as between both total ADAR1 and the p150 isoform with the G-CSF-inducible STAT3 levels (p_{ADAR1}= 0.011, p_{p150} = 0.025), in non-responders (N=9) to AZA patients (responders N=6).

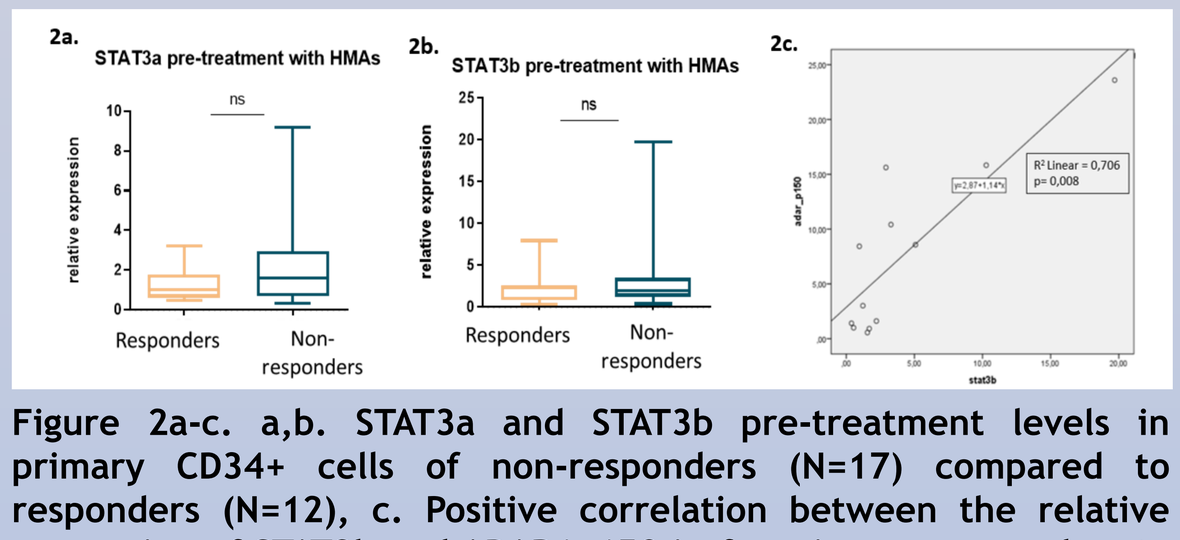

Figure 2a-c. a,b. STAT3a and STAT3b pre-treatment levels in primary CD34+ cells of non-responders (N=17) compared to responders (N=12), c. Positive correlation between the relative expression of STAT3b and ADAR1p150 isoform in non-responders to HMAs (N=12).

Summary and Conclusions

Remarkably, more than 50 years after their discovery and after 18 years of clinical experience, the mode of action of HMAs remains obscure, posing an obvious obstacle in developing novel therapeutic strategies that will overcome resistance and/or restore the response to HMA. In addition, there is lack of clinical and molecular predictors of outcome after HMA therapy. Our findings implicate the ADAR1 pathway in the resistance to AZA and further corroborate the potential existence of a vicious cycle among STAT3 and ADAR1 in the leukemic progenitors of HR-MDS patients. If confirmed in larger cohorts, our data argue for the utilization of ADAR1 as both a therapeutic target and a predictor of AZA response.

References

1.Roulois, D. *et al*. DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. *Cell* **162**, 961–973 (2015).

2.Chiappinelli, K. B. *et al*. Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. *Cell* **162**, 974–986 (2015).

3.Mehdipour, P. *et al*. Epigenetic therapy induces transcription of inverted SINEs and ADAR1 dependency. *Nature* **588**, 169–173 (2020).

4.Kordella, C., Lamprianidou, E. & Kotsianidis, I. Mechanisms of Action of Hypomethylating Agents: Endogenous Retroelements at the Epicenter. *Frontiers in Oncology* **11**, (2021).