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RESEARCH LETTER

Anti-thymocyte globulin in immune-mediated cytopenias: A monocentric experience and literature review

To the Editor,

Diagnostic work-up of isolated cytopenias in adults includes a careful personal and familiar anamnesis, blood count history and investigations of possible culprits such as infection, systemic diseases and/or metabolic deficiencies, drug reactions, bone marrow failure syndromes (e.g. aplastic anaemia [AA]) and signs of haematological neoplasia.[1,2] Following exclusion of secondary and destructive causes, that is, likely antibody-mediated, the residual immune-mediated cytopenias are mostly related to T cell-mediated pathogenesis. Admittedly, such a classification has drawbacks as in some cases antibody and T-cell autoimmunity may be present, while others may occur in association with or be triggered by, often subclinical, either B- or T-cell dyscrasia. The latter are chiefly represented by large granular lymphocyte leukaemia (LGLL) either of T-cell or less commonly NK-cell type.

The treatment of presumed T-cell-mediated (immune-mediated) cytopenias includes various immunosuppressive regimens applied serially, each with mixed success rates. In addition to corticosteroids, the currently most applied therapies include cyclosporine, tacrolimus, sirolimus, methotrexate and mycophenolate.[3,4] We have also reported the application of new subcutaneous low intensity alemtuzumab regimens which showed considerable efficacy without the historically high complication rate of the intense IV regimens.[5] However, a significant minority of cases remains refractory, and in severe cases, we have used intense immunosuppression with anti-thymocyte globulin (ATG) as a salvage regimen. For this analysis, antilymphocyte globulin (ALG) and ATG were included and combined. While ATG is widely used in transplant conditioning, GvHD prophylaxis and AA, no systematic studies of ATG in refractory immune-mediated cytopenias were conducted, and only case reports or small case series can be found in the literature.[6–8] Due to the rarity of immune-mediated cytopenias, the otherwise consistent response with standard immunosuppressive therapies and the lack of commercial interest, prospective clinical trials of potential salvage therapies, including ATG, are unlikely. However, it would be important to assess whether this agent may have a role in the current armamentarium of salvage agents available for these conditions. As a high-volume, quaternary referral centre, over the years, we have encountered a significant number of multi-drug refractory immune-mediated cytopenias. To better guide future salvage therapy, we reviewed treatment results of ATG in these cases.

When we searched health records of patients with immune 'malproductive' cytopenias ($N = 312$), which included 74 pure red cell aplasia (PRCA) and 238 idiopathic neutropenia (IN) with or without the associated LGLL, we identified a total of 20 patients who received ATG in the multirefractory/relapsing setting and 14 of these with detailed treatment and follow-up information were included in the analysis (Figure 1A). Of these, five patients received ATG in combination with ciclosporin (CSA). Response criteria are reported in Table S1. We also performed a literature search for corresponding cases, treated with ATG or ALG from 2000 to 2024 and selected 33 studies with a total of 42 patients. After excluding patients with secondary aetiology of cytopenia, incomplete information or unclear clinical history, progression to AA, lack of a clear response assessment and treated with drug schedules different from the ones used nowadays (Supporting Information and Table S2), we were able to select only three informative reports, describing six patients, treated with ATG-CSA combination, who were used to supplement our case series.[9–11] Of note is that all the patients we added had acquired amegakaryocytic thrombocytopenia (AMT) who received salvage ATG (Figure S1) in analogy to the isolated IN and PRCA in our series.

While the characteristics of our cohort, response criteria and the gene panel of our deep targeted sequencing are separately presented in Tables S3 and S4, for the purpose of drawing more qualified conclusions, curated meta-analytic cases and our series were merged and analysed together. The characteristics of the combined cohort ($n = 20$; median age of 56 years, interquartile range: 51–67 and equal gender distribution) are summarized in Table 1. LGLL was found in 11 patients (55%; 8 presenting with anaemia, 3 with neutropenia) and monoclonal gammopathy of undetermined significance (MGUS) was present in 43% (6 of 14 tested; all patients presented with anaemia; 4 patients had both LGL and MGUS). No patient presented with chronic lymphocyte leukaemia. The presence of paroxysmal nocturnal haemoglobinuria (PNH) clones is strongly suggestive of immune-mediated aplastic anaemia. However, no patient with immune cytopenia had a PNH clone. Bone marrow cellularity was heterogeneous: 26% presented normal cellularity, 32% were hypocellular for

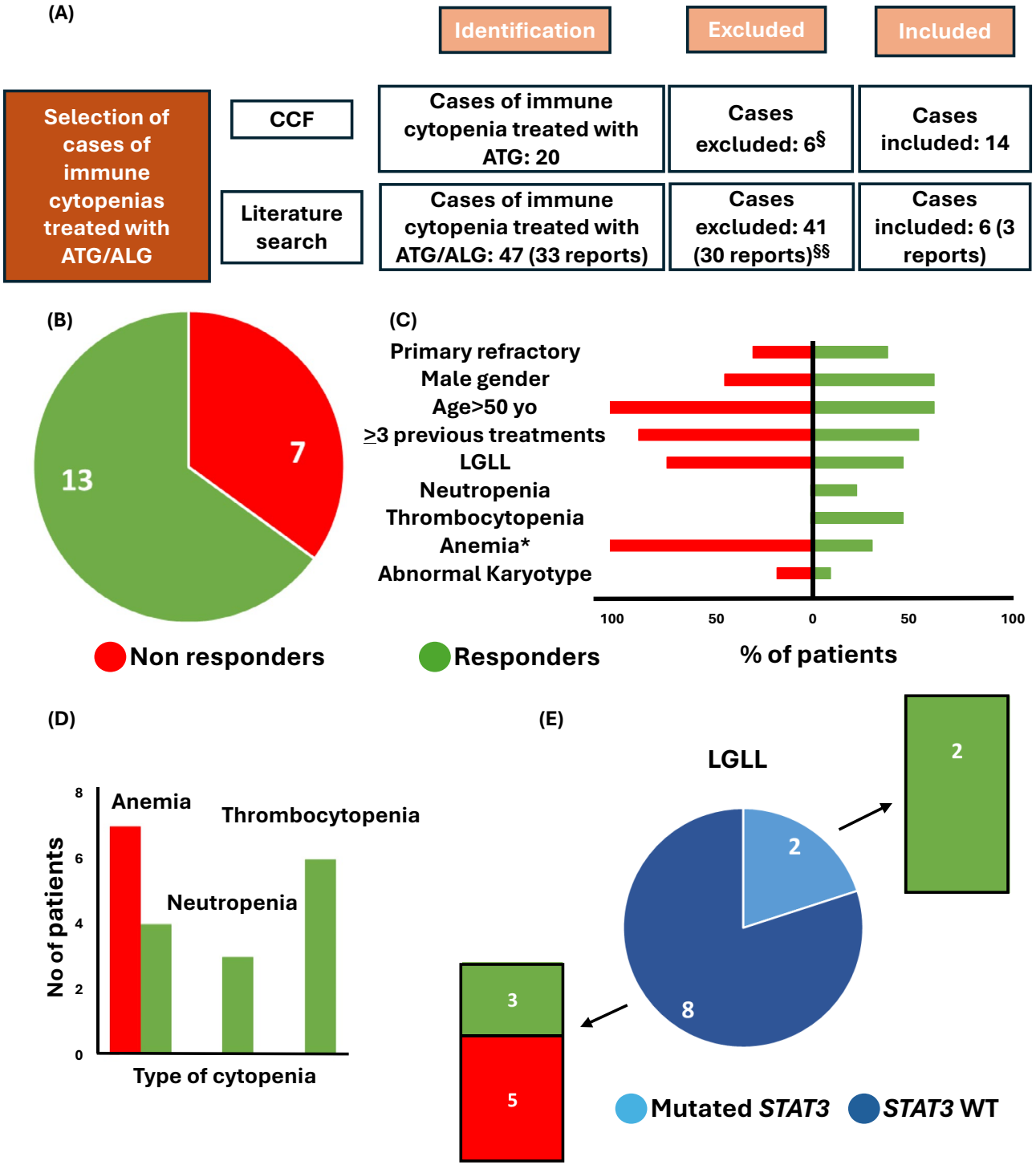

FIGURE 1 Flow chart of the study and outcome predictors. A, Schematic representation of patient enrolment from The Cleveland Clinic Foundation and literature review. Exclusion criteria were: missing demographic data, unclear clinical history and/or type of cytopenia, lack of response assessment, unusual or unknown drug schedule and cases reported before 2000 (See Supporting Information for further details). B, Pie chart showing the outcome of the overall cohort of patients. C, Comparison of features between responders and non-responders. D, Histogram showing the outcome of the patients divided by type of cytopenias. E, Analysis of LGLL patients with available *STAT3* mutational status. §Four cases were excluded because of the unavailability of response assessment; two cases were excluded because they received ATG at another institution. §§Two cases were excluded because of premature discontinuation of the drug. One case was excluded due to a possible secondary aetiology of immune cytopenia. In two cases, the clinical response was unclear and/or without sufficient follow-up to assess a proper, stable clinical effect of the drug. Three reports described treatment in cases suspected to be progressed, or overtly progressed, to aplastic anaemia. Twenty-seven patients underwent drug schedules not used nowadays and thus were not considered for subsequent analysis. Six cases were excluded because they were reported before 2000. Asterisk in panels C refers to the level of significance: $*p < 0.05$. ALG, anti-lymphocyte globulin; ATG, anti-thymocyte globulin; CCF, Cleveland Clinic Foundation; LGLL, large granular lymphocyte leukaemia; wt, wild type.

TABLE 1 Characteristics of study population.

Features		Study population ($n = 20$)
Age	Median (range)	55.8 (38–82.4)
Gender	M, *n* (%)	11 (55)
LGLL	*n* (%)	11 (55)
	STAT3 mutation, *n* (%)	2 (20)
	NA, *n*	1
MGUS	*n* (%)	6 (43)
	NA, *n*	6
Karyotype	Abnormal, *n* (%)	2 (12.5)
	NA, *n*	4
Mutational screening	*DNMT3A*, *n* (%)	1 (8.3)
	No mutations, *n* (%)	11 (75)
	NA, *n*	8
Type of cytopenia	Anaemia, *n* (%)	11 (55)
	Neutropenia, *n* (%)	3 (15)
	Thrombocytopenia, *n* (%)	6 (30)
Bone marrow cellularity	Normal, *n* (%)	5 (26.3)
	Hypocellular, *n* (%)	6 (31.6)
	Hypercellular, *n* (%)	8 (42.1)
	NA, *n*	1
Primary refractory	*n* (%)	7 (35)
No of previous treatment	Median (range)	4 (1–8)
Responders	*n* (%)	13 (65)

Abbreviations: LGLL, large granular lymphocyte leukaemia; M, male; MGUS, monoclonal gammopathy of undetermined significance; NA, not available.

age but with lesser depletion of haematopoiesis (no severe aplasia was observed as pathognomonic for AA hypocellularity) and 42% hypercellularity. Cytogenetics and molecular screening revealed abnormal karyotype in two patients (of 16 available [12.5%]; one patient with del(Y) and one patient with dup(2)) and a mutation in *DNMT3A* (p.L373V, variant allele frequency [VAF]: 10.3%) in one patient (of 12 available [8.3%]) consistent with the presence of clonal haematopoiesis of indeterminate significance (CHIP) or clonal cytopenia of undeterminate significance. Among patients with LGLL ($n = 11$), two patients had *STAT3* mutations (out of 10 available [20%]; the mutations detected were p.S614R, VAF: 21.6% and p.Y640F, VAF: 7.6%).

The cohort included 55% of cases with are generative anaemia, followed by thrombocytopenia (30%) and neutropenia (15%). Overall, seven patients (35%) had a primary refractory disease, while the rest displayed a response to previous immunosuppressive treatment, reaffirming the immune-mediated pathophysiology of the cytopenia; in 5/7 refractory cases, responses with ATG were observed, indicating that likely more powerful immunosuppression may salvage some patients. Fifteen patients (75%) received three or more previous treatments (median of 4 previous therapies, range 1–8).

In total, 13 patients responded to ATG (65%, Figure 1B). When comparing the features of responders versus non-responders, we observed, in the second group, a higher median age (range): 66.3 (53–82) vs. 52 (38–77) years ($p = 0.056$), anaemia presentation (100% vs. 30.8%, $p = 0.005$) and a lower percentage of patients presenting with thrombocytopenia (0% vs. 46.1%, $p = 0.051$; Figure 1C). No differences were observed in patients with MGUS or in patients with different levels of bone marrow cellularity. Response rate in patients presenting with anaemia, neutropenia and thrombocytopenia was 36.4%, 100% and 100% respectively (Figure 1D). The group of non-responders was enriched in LGLL patients (71.4% vs. 46.1, $p = 0.374$; Table S5).

Of note is that the two *STAT3* mutant LGL patients (presenting both with neutropenia) achieved remission, whereas among the remaining eight *STAT3* wild-type cases, all presenting with anaemia, the response rate was about 37% ($p = 0.444$; Figure 1E). Patients who failed to respond also presented a higher pre-ATG treatment burden [median (range): 5 (3–7) vs. 4 (1–8) previous therapies, $p = 0.296$; Table S5]. For consistency, the analysis of our cohort is provided separately in Table S6 and the treatment history with clinical responses of each patient included in the analysis is summarized in Figure S1.

To the best of our knowledge, this is the first report analysing systematically ATG efficacy in a population of T-cell-based immune cytopenia patients. Surprisingly, our literature search produced only three usable reports (clear diagnosis, refractory setting, contemporary dosing) in the last 25 years, for a total of six patients, highlighting the novelty of our report which aims to fill an important gap in the management of patients with PRCA and IN.

We acknowledge a possible 'publication' bias derived from literature cohort which is more likely to select for studies in which positive responses were observed. Indeed, aware of this possibility, for consistency, we decided to report, in all our analysis, the outcomes of our in-house cohort and overall cohort separately.

Despite the low number of patients included in our analysis, because of the precise and stringent criteria used in the selection of the patients, we were able to derive informative conclusions helpful in managing such refractory cases: (i) It appears that ATG leads to a successful response in up to 65% of cases (50% in our case series) of heavily pretreated patients with immune-mediated cytopenia; (ii) as expected, younger, less pretreated patients are more likely to achieve a response; (iii) PRCA patients (in particular the ones with LGL) may be less responsive to ATG, but the sample size does not allow for assertive conclusions.

Thus, cytopenic patients in particular those presenting with anaemia and with concomitant LGLL confirmed to constitute a clinical challenge and a pathophysiological conundrum. In fact, the boundaries between LGLL and less polarized immune cytopenias are blurred and their refractoriness may be due to distinct/additional underlying causes, such as myelophthisis, bone marrow toxicity and

concomitant conditions, such as autoimmune haemolytic anaemia (5% of the cases with anaemia), PRCA (30% of the cases with isolated anaemia) and immune thrombocytopenia.[12,13] In this context, the nature of the strong relation between LGLL and PRCA has not been comprehensively explored. LGLL with PRCA seems to share many clinical features with other cytopenias, but PRCA cases appear to be less responsive to standard immunosuppressive therapies.[4,12] It is possible that PRCA is associated with a more profound immune dysfunction, responsible not only for a more pervasive clonal lymphocyte expansion but also for a T-cell-mediated precursor cells' clearance.

In contrast to PRCA, neutropenia associated with LGLL harbouring *STAT3* mutation was successfully treated. This observation is in line with previous studies which suggested, in these patients, a shorter time to treatment but no differences in terms of disease progression or overall survival.[14]

In conclusion, our findings provide a rationale for the use of ATG, which still appears to be a viable salvage approach for refractory, in particular, younger patients.

AUTHOR CONTRIBUTIONS

L.G. and A.A. supervised the study, collected, analysed, interpreted clinical and molecular data and wrote the manuscript. C.B-P., O.D.O., M.D., Z.B., S.U., C.H. and A.M. interpreted clinical and molecular data and edited the manuscript. A.D. analysed molecular data. C.G. analysed data and provided important feedback. V.V. analysed data and provided invaluable help to the manuscript preparation. J.P.M. provided invaluable help to the manuscript preparation, generated and conceived the study design, designed figures and tables and edited the manuscript. All authors participated in the critical review of the final paper and submission.

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KEYWORDS

amegakaryocytic thrombocytopenia, anti-thymocyte globulin, immune cytopenia, immune neutropenia, pure red cell aplasia

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

All data to ensure reproducibility are provided in the main text and supplemental material. Additional information can be requested by e-mailing the corresponding author: maciejj@ccf.org.

CONSENT FOR PUBLICATION

Informed consent was obtained according to the protocols approved by the Institutional Review Board of the Cleveland Clinic Foundation (IRB#5024) and in accordance with the ethical principles set forth by the Declaration of Helsinki.

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SUPPORTING INFORMATION

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