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Triacetyl-5-Azacytidine Suppresses Experimental Allergic Encephalomyelitis (EAE) in Mice

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Abstract: Background/Objectives: The epigenetic drug 5-azacytidine (AzaC) is being used for the treatment of myeloproliferative diseases. It has multiple immunomodulating activities: it enhances the activity of Treg cells and suppresses effector T cell proliferation and function. Our aim was to repurpose AzaC for the treatment of multiple sclerosis (MS). AzaC treatment of myelodysplastic syndrome often improves the autoimmune disorders accompanying it. Another epigenetic drug, decytabin, was effective in EAE, suggesting that AzaC might behave similarly. Earlier, we found that AzaC improves aggrecan-induced arthritis in mice, further supporting our hypothesis. **Methods:** AzaC was tested in an animal model of MS: $MOG_{35–55}$-induced experimental allergic encephalomyelitis (EAE) in B6 mice. In addition to AzaC, its ester, prodrug triacetyl-5-azacytidine (TAC), reported earlier to exhibit improved stability and oral bioavailability, was also tested. **Results:** In our proof-of-concept experiment, i.p. administered AzaC ameliorated EAE. Then, we demonstrated that oral TAC is as effective as the positive comparator fingolimod. Next, we demonstrated that sub-optimal doses of oral TAC and fingolimod positively synergize. Importantly, the myelosuppression induced by TAC was not worse than that of the gold-standard fingolimod. **Conclusions:** Ours is the first study reporting the therapeutic activity of oral TAC. Both AzaC and TAC were effective in EAE; therefore, they can be proposed for the treatment of remitting–relapsing MS and possibly other autoimmune diseases. In addition, combination treatment with TAC and fingolimod might allow for lower individual drug doses, thus offering an alternative when side effects limit the use of current multiple sclerosis drugs.

Keywords: multiple sclerosis; epigenetics; experimental allergic encephalomyelitis; azacytidine

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1. Introduction

Multiple sclerosis (MS) is a chronic, progressive, inflammatory disease of the central nervous system. The most frequent form of the disease is relapsing–remitting MS. In a minority of patients, the disease manifests as a monophasic, primary progressive MS. In some cases, the relapsing–remitting form evolves into secondary progressive disease [1].

Relapsing–remitting MS has multiple treatment options; however, none of them are optimal. Three main types of agents are in use: type I interferons; monoclonal antibodies; and small-molecule drugs. The main targets of monoclonal antibodies are immune cells; most frequently, they are B cells, which are important contributors to pathology, both as antigen-presenting cells and sources of pathogenic autoantibodies [2]. The rather complex

mode of action of type I interferons is not definitely clarified yet, but apparently, they act on multiple processes of the immune response [3]. The currently used small-molecule drugs have four main types of molecular targets: sphingosine-1-phosphate receptor (fingolimod); Nrf2, nuclear factor (erythroid-derived 2)-like (dimethyl fumarate); the dihydroorotate dehydrogenase enzyme (teriflunomide); or DNA repair and synthesis (cladribine). The effectiveness of the available therapies varies, but each treatment modality is associated with specific, non-negligible side effects [4]. Our aim was to explore the possibilities of repurposing the epigenetic drug 5-azacytidine (AzaC) for the treatment of autoimmunity.

Notably, 5-azacytidine is a pyrimidine nucleoside analogue used in the treatment of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), primarily in elderly patients not eligible for haematopoietic stem cell transplant [5–7]. AzaC is thought to have two distinct mechanisms of action: a) it incorporates into ribonucleic acid (RNA) with a consequent disruption of messenger RNA and protein synthesis and b) inhibits deoxyribonucleic acid (DNA) methylation [5,7–9]. However, tentatively, additional modes of action are also postulated, including nonsense-mediated RNA decay inhibition [10], as well as the inhibition of T-cell proliferation and activation and decreasing the production of proinflammatory cytokines [11].

Three different lines of research have suggested that AzaC might be effective in multiple sclerosis. Myelodysplasia is often accompanied by various autoimmune and inflammatory disorders, and evidence is mounting about AzaC being effective in treating those as well [12–14]. Among others, whole-body erythroderma and Sweet's syndrome cases were resolved with AzaC treatment without concomitant steroid use [15]. In another case study, AzaC was effective (and thought to act with a different mechanism) in treating Behçet disease after the failure of several other drugs [11]. In four out of five Sweet's syndrome patients, a complete response was observed to AzaC [16]; autoimmune haemolytic anemia (AIHA) became Coombs-negative [17], and another warm AIHA patient also responded [18]. Major improvement was seen in systemic lupus erythematosus [19], and finally, neutrophilic dermatitis [20] and autoinflammatory lymphedema [21] were successfully treated.

Besides these observational data, we have experimental results that suggest that AzaC might be effective in treating MS. Earlier, we found that AzaC prevented the development of disease in aggrecan-induced arthritis, in a mouse model of rheumatoid arthritis. In this setup, the protective effect seemed to be based on blocking lymphoid follicle formation [22]. Based on our results in the autoimmune arthritis model, we have chosen AzaC over other epigenetic drugs for testing in EAE.

Corroborating our hypothesis, decytabin, another epigenetic drug, was shown earlier to be effective in mouse EAE models [23,24].

Based on these results, we tested the effectiveness of AzaC in the most widely used animal model of relapsing–remitting MS: $MOG_{35–55}$-induced experimental allergic encephalomyelitis (EAE) in B6 mice. Our positive comparator was fingolimod, a small-molecule disease-modifying drug used extensively in remitting–relapsing MS, equally active in EAE [25].

As was expected, AzaC applied intraperitoneally was found to suppress $MOG_{35–55}$-induced EAE. In clinical practice, AzaC is used subcutaneously, intravenously (Vidaza®) or orally (Onureg®). Triacetyl-5-azacytidine (TAC) is a triacetyl ester prodrug of AzaC. The structures of the two compounds are shown in Figure 1. TAC was reported to exhibit improved stability and oral bioavailability compared to the parent prodrug in rodent studies [26]. Based on this publication, we switched to this compound for further studies.

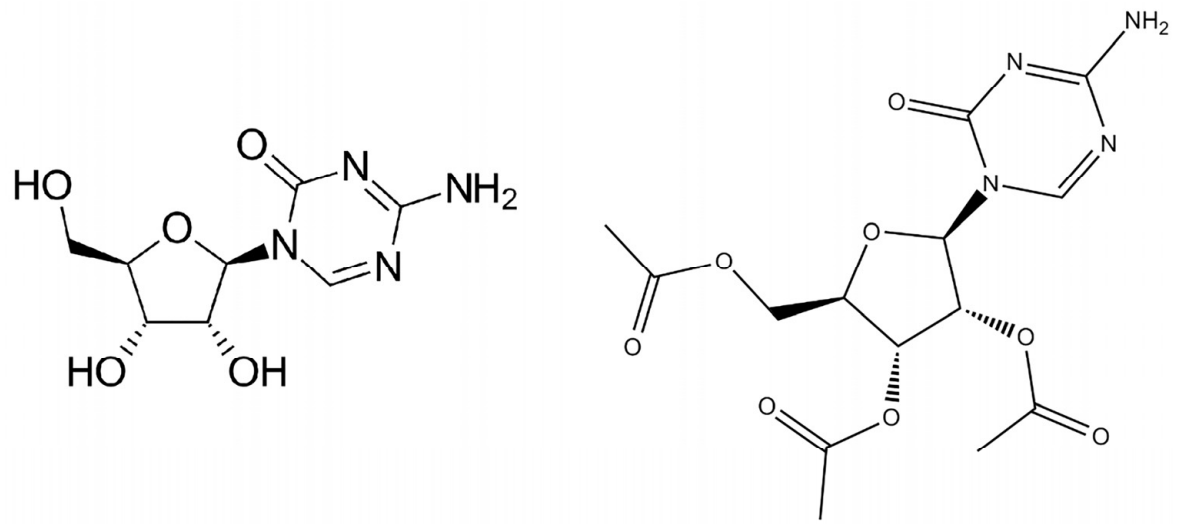

Figure 1. The chemical structures of azacytidine (AzaC) and its prodrug form triacetyl-5-azacytidine (TAC).

In the last step, we explored combination treatment with sub-optimal doses of TAC and fingolimod in the $MOG_{35–55}$ EAE model.

The main side effect of AzaC and TAC, as well as most immunosuppressive drugs, is myelosuppression, i.e., a decreased number of white blood cells. We checked this side effect by analyzing the blood cell composition of the treated mice.

2. Materials and Methods

Reagents: Synthetic $MOG_{35–55}$ peptide was kindly provided by our collaborator, Prof. G. Tóth, Institute of Medical Chemistry of University of Szeged. The purity of the peptide batch was checked by HPLC-MS and was found to be 97.7% (chromatograms available upon request). Incomplete Freund's adjuvant was purchased from SIGMA-Aldrich (Merck, St. Louis, MI, USA), Burlington, MA, USA, and heat-killed H37RA strain mycobacterium tuberculosis was obtained from BD (Franklin Lakes, NJ, USA). Pertussis toxin was purchased from SIGMA-Aldrich (Merck) and List Biological Laboratories, Campbell, CA, USA. AzaC (98+% purity) was obtained from Angene International Limited (London, England), while TAC (99.3% purity) was purchased from Xenoah Pharmaceuticals Inc. (Jiangsu, China). The active comparator fingolimod hydrochloride was purchased from Angene International Limited (London, UK).

Animal experiments: Remitting–relapsing EAE was induced in 6–8-week-old C57Bl/6 mice. Since, just as in the case of the human disease, females are more sensitive to central-nervous-system autoimmunity, the experiments were performed on female mice. The animals were obtained from Charles Rivers Laboratories (Wilmigton, MA, USA); they were kept in the specific-pathogen-free (SPF) animal facility of the Biological Research Center (Szeged, Hungary). The experiments started after a one-week acclimatization period. For the induction of EAE, the mice were immunized with synthetic MOG_{35-55} peptide. Briefly, the mice were injected subcutaneously in the flank with 2 mg MOG_{35-55} in incomplete Freund's adjuvant (SIGMA-Aldrich, Merck) substituted with 2 mg heat-killed H37RA strain mycobacterium tuberculosis. On day 0 and day 2, mice received, via the tail vein, 400 ng and 200 ng pertussis toxin, respectively [27]; in our previous projects, this protocol worked better than the more widespread standard protocol that applies 2 × 200 ng pertussis toxin. Mice were monitored at least once daily, and clinical grades of the disease (grade 1: limp tail, grade 2: weakness of hind leg, grade 3: paralysis of hind leg, grade 4: paralysis of hind legs plus a front leg, grade 5: moribund, 6: dead) were recorded. The

grading and the treatments were performed in random order in a blinded fashion, i.e., the person performing the interventions was not aware of the treatment groups. The drug treatment started when the first sign of the disease—tail limpness (grade 1 EAE)—appeared in the first mice. At that point, the animals were randomly assigned to treatment groups, consisting of 10 mice each in all of the experiments; this sample size is regarded as standard in EAE experiments on mice. The control groups received the vehicle per os. In each in vivo experiment, when mice showed signs of severe EAE, food and water were supplied at the floor level of the cage. Since severe paralysis was accompanied by incontinence, abundant, absorbent bedding was provided and changed daily. Moribund mice were euthanized and registered as grade 6 EAE. AzaC was administered by i.p. injection at 2 mg/kg of body weight, while TAC was administered by gavage with a syringe and a curved feeding needle (Harvad Apparatus, Holliston, MA, USA) at 5, 10 and 25 mg/kg, respectively, in a volume of 200 µL. Since TAC is unstable in gastric acid, based on our preliminary stability experiments in simulated gastric acid, it was administered in 100 mM, pH 7 phosphate buffer (VWR, Radnor, PA, USA). Fingolimod was administered by gavage at 1 mg/kg dissolved in dH_2O, at a volume of 200 µL. At the end of the experiments, the mice were over-anesthetized by pentobarbital injection, and blood samples were collected by heart puncture.

Hematology: In some of the experiments, blood samples were collected from the retroorbital plexus in isoflurane anesthesia in heparinized tubes, and then standard hematology analysis was performed in a routine diagnostic laboratory (HR Pharma, Szeged, Hungary) using the mouse preset of their standard hematology instrument (Advia 12, Siemens Healthineers AG, Forchheim, Germany).

Statistics: The choice of 10 mice/per group, regarded as standard in the EAE literature, was supported by our power analysis: at the expected clinical grade of 3.0, at a sample size of 10, a clinically meaningful improvement of 1.5 clinical grades is safely detectable (alpha = 0.05; power = 0.8 in the case of n = 9). The EAE grades were compared with the GraphPad Prism 5.01 statistical software with one-way ANOVA followed by Dunnet's post-test. The survival ratios were compared with Fisher's exact test.

Ethics: All animal experiments were performed according to institutional and EU ethical guidelines, equivalent to the Guide for the Care and Use of Laboratory Animals, with ethical clearance obtained from the institutional review board of the BRC and the National Animal Experimentation and Ethics Board (XVI./719/2020, issued at 8 May 2020).

3. Results

3.1. AzaC Administered Intraperitoneally Ameliorates MOG_{35-55}-Induced EAE

In the first proof-of-concept experiment, the AzaC treatment started at the onset of symptoms. Because of its known disadvantageous pharmacokinetic properties, the agent was administered i.p. three times a week. As expected, the AzaC treatment improved the clinical symptoms of EAE by two clinical grades, from the mean of grade 4 (paralysis of hind and front limbs) to grade 2 (not more than hind leg weakness). The AzaC treatment also decreased EAE-induced mortality (Figure 2 and Table 1).

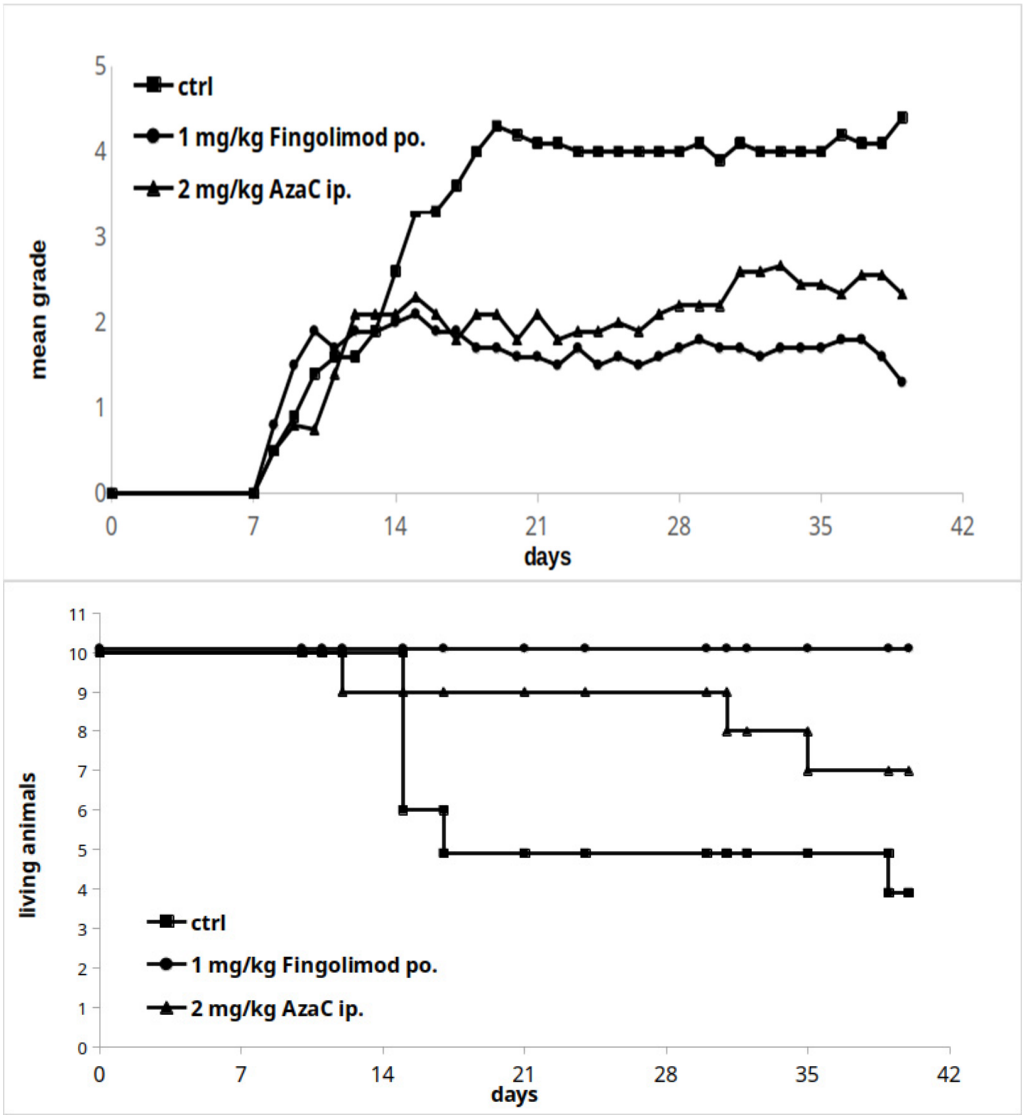

Figure 2. Proof-of-concept experiment showing the efficacy of AzaC. After the first signs of EAE symptoms, mice were randomized and AzaC was administered 3×/week. Because of its low oral bioavailability, the mice received AzaC i.p. The positve comparator was fingolimod applied po. 3×/week. Upper panel: mean EAE clinical grades. Ten mice per group were used. Lower panel: urvival of the mice from the same experiment.

3.2. Oral TAC Is as Effective as Fingolimod in Suppressing MOG$_{35-55}$-Induced EAE

After we ascertained that AzaC has the potential to suppress EAE, we tested its triacetyl prodrug TAC, which was reported to exhibit improved oral bioavailability. We compared different oral administration protocols with the i.p. AzaC treatment (Figure 3). TAC was indeed found to be effective in this setup; the optimal dose was 25 mg/kg three times a week (Table 1). At the highest dose tested (100 mg/kg), 3/10 mice died without severe EAE symptoms. Unlike the deaths preceded by severe EAE symptoms, deaths occurring in absence of EAE symptoms were attributed to toxicity of the highest dose of TAC. At the optimal TAC dose, 25 mg/kg, the most severe symptom was tail and rear leg weakness; no paralysis or incontinence developed in this group, and no death occurred. In contrast, deaths at the lower TAC doses were preceded by EAE symptoms, so they were attributable to EAE. At the plateau phase of the disease at day 28, the untreated mice showed severe EAE (mean grade ± SD = 4.0 ± 2.1), while in the case of fingolimod and 25 mg/kg TAC, it attenuated to grade 1.7 ± 0.7 and 1.7 ± 0.7, respectively. The optimal

TAC dose and schedule was confirmed in another independent experiment that produced similar results (Table 1).

Table 1. Summary of the EAE experiments.

	Clinical Grade at Plateau at Day 28					Survival at Day 40		
	N	**Mean**	**SD**	***p*, *t*-Test, Ctrl vs. Untreated [1]**	***p*, *t* Test, Ctrl vs. Fingolimod**	**N, Living Animals**	***p*, Fisher's Exact Test, Ctrl vs. Untreated**	***p*, Fisher's Exact Test, Ctrl vs. Fingolimod**
EAE ctrl, experiment 1.	10	4.0	2.1	-	*	4	-	*
AzaC i.p., 3×/week	10	2.2	1.5	ns	ns	7	ns	ns
TAC p.o., 5 mg/kg, 3×/week	10	2.9	1.7	ns	ns	4	ns	*
TAC p.o., 10 mg/kg, 3×/week	10	2.4	1.3	ns	ns	9	ns	ns
TAC p.o., 25 mg/kg, 3×/week	10	1.7	0.7	*	ns	10	*	ns
TAC p.o, 100 mg/kg, 3×/week	10	1.1	1.9	***	ns	7	ns	ns
Fingolimod, po., 1 mg/kg, 3×/week	10	1.7	0.7	*	-	10	*	-
EAE ctrl, experiment 2.	10	2.3	1.5	-	**	9	-	ns
25 mg/kg TAC 1×	10	1.8	0.6	ns	*	10	ns	ns
25 mg/kg TAC, 1×/biweekly	10	1.8	0.6	ns	*	10	ns	ns
25 mg/kg TAC, 1×/week	10	1.0	1.2	*	ns	10	ns	ns
TAC 25 mg/kg, 3×/week	10	0.9	1.0	*	ns	10	ns	ns
Fingolimod, 1/mg/kg, 3×/week	10	0.6	1.0	**	-	10	ns	-
EAE ctrl, experiment 3.	10	1.9	0.3	-	ns	10	-	ns
Fingolimod, 0.1 mg/kg 3×/week	10	3.0	1.7	ns	**	10	ns	ns
Fingolimod 1 mg/kg/, 3×/week	10	1.3	1.1	ns	-	10	ns	ns
TAC 10 mg, 3×/week	10	1.9	0.6	ns	ns	10	ns	ns
0.1 mg fingolimod + 10 mg TAC, 3×/week	10	0.8	0.9	ns	ns	10	ns	-

[1] One-way ANOVA followed by Dunnett's post-test. ns: non significant, $p > 0.05$, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. The statistical analysis was performed sperately in the case of the three independent experiements.

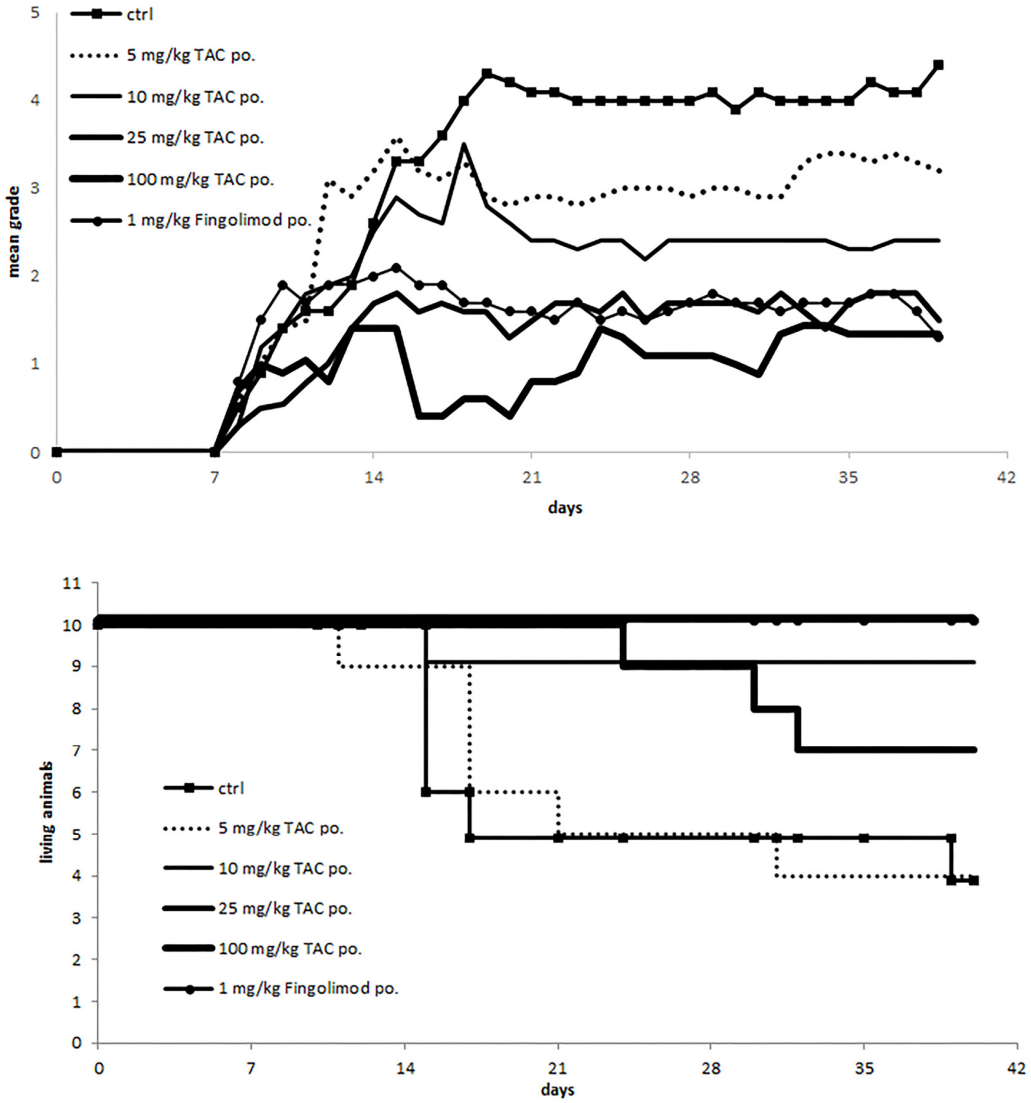

Figure 3. Therapeutic effect of graded doses of oral TAC. After the first signs of EAE symptoms, mice were randomized and AzaC, TAC or fingolimod were administered 3×/week. AzaC was administered i.p., while TAC and fingolimod orally by gavage. Upper panel: mean EAE clinical grades. Groups of 10 mice were used. Lower panel: survival data from the same experiment.

3.3. Low-Dose TAC Positively Synergizes with Low-Dose Fingolimod

We explored combination treatment with a suboptimal dose of TAC and a suboptimal dose of fingolimod (Figure 4 and Table 1). We found that the combination of the suboptimal doses, i.e., 0.1 mg/kg fingolimod plus 10 mg/kg TAC, was as effective as fingolimod at its optimal dose in suppressing the EAE symptoms. The suboptimal fingolimod dose aggravated the disease. Due to natural variability typical for this autoimmunity model, in this specific in vivo experiment, no deaths occurred. In this experiment, the suboptimal dose of fingolimod, 0.1 mg/kg, did not ameliorate the disease but aggravated it. The clinical grades at the plateau phase at day 25 were equally moderate in the case of the optimal dose of fingolimod (1 mg/kg) and the low-dose combination treatment (mean ± SD: 1.3 ± 1.1 vs. 0.8 ± 0.9).

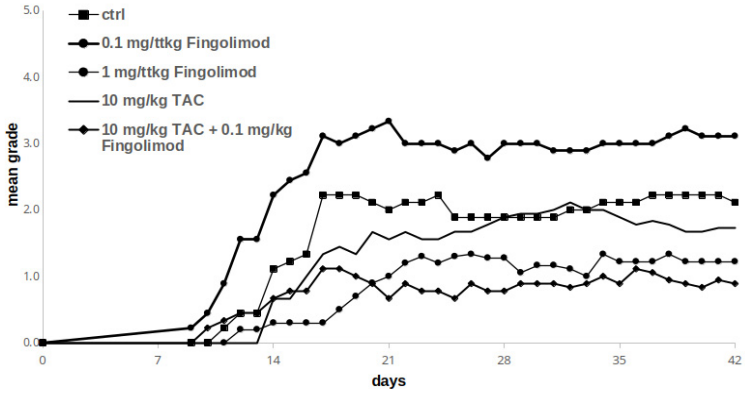

Figure 4. Positive synergy between TAC and fingolimod. After the first signs of EAE symptoms, mice were randomized and TAC was administered 3×/week, either alone, or in combination with fingolimod. Mean grades are plotted. In this specific experiment, no mortality occurred. Groups of 10 mice were used.

3.4. The Myelosuppression Induced by the Optimal TAC Dose Is Comparable with That of Fingolimod-Induced Myelosuppression

Based on previous clinical data summarized in review papers of the field, the main side effect of both agents—fingolimod [4] and AzaC [28]—is immunosuppression. In our experiments analyzing blood samples obtained from drug-treated mice suffering from EAE, we demonstrated that the myelosuppression caused by the optimal oral TAC dose was not significantly different from the myelosuppression caused by the optimal fingolimod dose, and the decrease in total white blood cell number was not significantly different in the case of the two drugs: mean WBC number ± SD: 1.43 ± 0.97 (fingolimod) vs. 2.12 ± 0.55 (25 mg/kg TAC). The combination treatment with suboptimal doses of TAC and fingolimod resulted in myelosuppression comparable with that caused by the optimal dose of fingolimod: WBC number ± SD: 1.48 ± 0.46 (fingolimod) vs. 1.29 ± 0.51 (combination treatment) (Figure 5 and Table 2).

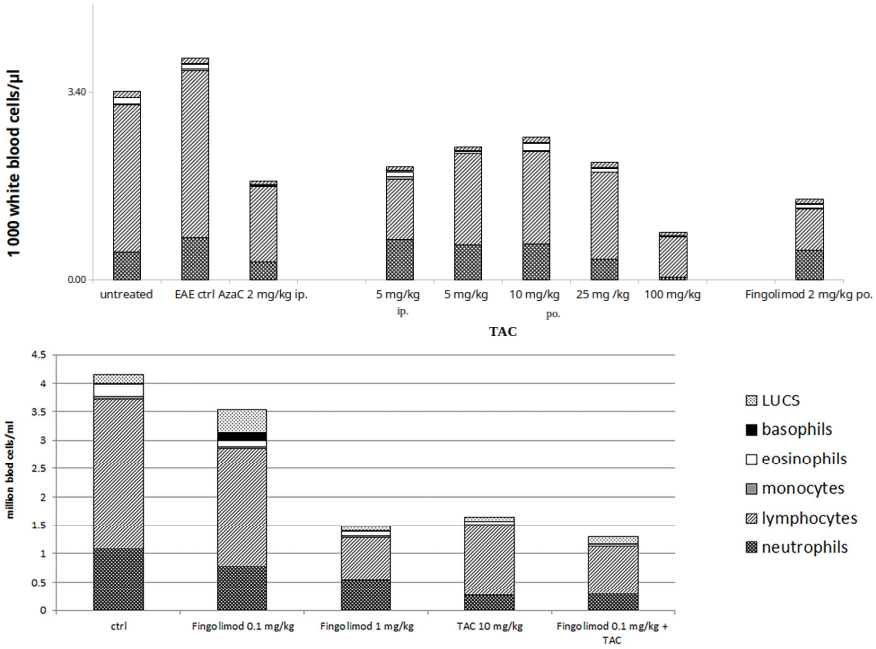

Figure 5. Hematological analysis of the treated mice. The analysis was performed at day 43, 24 h after the last treatment. Mean blood cell numbers of the surviving animals (4–10 per group) are plotted. LUC: large unstained cells. Upper panel: comparison of AzaC, different doses of TAC, and fingolimod. Lower panel: combination treatment with suboptimal dose of TAC and suboptimal dose of fingolimod.

Table 2. Hematology analysis performed at day 40.

Treatment	N	WBC × 10^6/mL Mean	WBC × 10^6/mL SD	*p*, *t*-Test, Ctrl vs. Untreated [1]	*p*, *t*-Test, vs. Ctrl vs. Fingolimod	Neutrophil × 10^6/mL Mean	Neutrophil/× 10^6/mL, sd	*p*, *t*-Test, vs. Untreated	*p*, *t*-Test, vs. Fingolimod
EAE ctrl, experiment 2.	6	3.42	1.32	-	***	0.50	0.26	-	ns
Untreated ctrl	4	4.25	0.60	ns	***	0.77	0.24	ns	ns
AzaC ip, 3x/week	7	1.80	0.40	**	ns	0.33	0.17	ns	ns
TAC p.o., 5 mg, 3x/week	7	2.37	0.52	ns	ns	0.61	0.22	ns	ns
TAC p.o., 10 mg, 3x/week	9	2.58	0.86	ns	*	0.66	0.24	ns	ns
TAC p.o., 25 mg, 3×/week	10	2.12	0.55	*	ns	0.38	0.13	ns	ns
TAC 100 mg, 3×/week	7	0.84	0.65	***	ns	0.03	0.05	*	*
Fingolimod po., 3×/week	10	1.43	0.97	***	-	0.53	0.29	ns	-
EAE ctrl, experiment 3.	9	4.16	1.93	-	**	1.08	0.95	-	ns
0.1 mg fingolimod	7	3.40	3.05	ns	ns	0,78	0.56	ns	ns
Fingolimod 1 mg, 3×/week	9	1.48	0.46	**	-	0.54	0.33	ns	-
TAC 10 mg, 3×/week	9	1.64	0.30	**	ns	0.27	0.08	**	ns
0.1 mg fingolimod + 10 mg TAC, 3×/week	9	1.29	0.51	**	ns	0.28	0.14	**	ns

[1] ANOVA followed by Dunnett's post-test. ns: non significant, $p > 0.05$, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. The statistical analysis was performed separately in the case of the two independent experiments.

4. Discussion

We have demonstrated that AzaC and its prodrug TAC are promising drug candidates for the treatment of MS. Ours is the first demonstration of the in vivo therapeutic effect of AzaC and TAC in EAE; in addition, it is also the first demonstration of the in vivo biological activity of TAC. Our results are in accordance with (1) recent clinical data accumulated on the immunomodulating "collateral effects" of epigenetic drugs in myelodisplastic syndrome [29], (2) the effectiveness of another epigenetic drug, decitabin, in mouse EAE models [23,24], and (3) our own data on AzaC in a mouse model of another autoimmune disease, rheumatoid arthritis, i.e., proteoglycan-induced arthritis [22].

Since we hypothesized that the pharmacokinetic properties of AzaC might be improved using a prodrug, after the proof-of-concept experiments with intraperitoneal AzaC, we explored the therapeutic effect of an orally administrable derivative of AzaC: TAC. We have demonstrated that oral TAC is as effective as the gold-standard fingolimod in suppressing EAE. Importantly, fingolimod is regarded the most effective small-molecule drug in in MS. It is equally active in EAE in mice—in fact, the discovery and preclinical development of fingolimod was conducted in rodents. To the best of our knowledge, these are the first published results on the biological activity of TAC; in earlier studies, only its pharmacokinetics were

studied [26]. In this next step, we have determined the optimal dosage and schedule of oral TAC. Importantly, the major side effect at the optimal TAC dose—myelosuppression—was not worse than that of the gold-standard small-molecule drug fingolimod.

The therapeutic effects of TAC likely result from multiple complementary mechanisms. One key mechanism involves the hypomethylating activity of its active metabolite, AzaC, which has been shown to enhance Treg function by reducing promoter methylation in Treg-associated genes [30]. Given that Treg deficiency is a well-established factor in autoimmunity [31], this mechanism aligns with the broader immunomodulatory effects observed with other hypomethylating agents. Additionally, we have previously demonstrated that AzaC disrupts lymphoid follicle formation, thereby impairing B-cell activation [22]. This is particularly relevant in MS, where B cells contribute to disease pathogenesis through antigen presentation, cytokine secretion, and autoantibody production. Furthermore, the leukopenia observed in our models suggests that TAC exerts a direct antiproliferative effect on pathogenic immune cells, potentially limiting the expansion of autoreactive lymphocytes. Beyond these known effects, epigenetic drugs such as AzaC and TAC may influence additional immune pathways, including cytokine signaling and innate immune activation. These mechanisms require further exploration, particularly in the context of MS, where immune dysregulation is multifaceted. Given the emerging interest in combination therapies, it would also be valuable to investigate whether TAC can enhance the efficacy of existing MS treatments, such as fingolimod, by modulating distinct immunological pathways.

While our study provides compelling evidence for the therapeutic efficacy of TAC in an EAE model of multiple sclerosis (MS), several limitations should be acknowledged. First, MS is a chronic, lifelong disease requiring prolonged treatment, whereas EAE models typically reflect an acute or relapsing–remitting phase of the disease. Thus, our study cannot fully address the long-term safety and efficacy of TAC in a chronic setting. In particular, prolonged exposure to hypomethylating agents like AzaC raises concerns about cumulative toxicities, including potential myelosuppression, off-target epigenetic modifications, and secondary malignancies. These risks require further investigation in long-term preclinical and clinical studies. Second, while our data indicate that TAC is as effective as fingolimod in suppressing EAE, it remains to be determined how TAC compares in a broader range of MS models, including those that incorporate progressive disease features. Moreover, our study does not address potential drug–drug interactions—an important consideration for MS treatment, where polypharmacy is common. Finally, while TAC's improved oral bioavailability compared to AzaC is a significant advantage, its precise pharmacokinetics and biodistribution in MS patients remain to be elucidated. Future studies should aim to establish optimal dosing regimens, ensuring a balance between efficacy and toxicity.

In conclusion, AzaC can be considered a new therapeutic agent of remitting–relapsing MS and possibly also other autoimmune diseases. Its considerable advantage is that its ADMETox properties are well known due to its clinical use for other indications. Alternatively, because of its better oral bioavailability, TAC might also be worth consideration. An important factor may be the relatively low manufacturing price of both compounds, especially compared with recombinant monoclonal antibodies that are generally among the most expensive routinely used drugs. In addition, combination treatment with the hypomethylating agents and fingolimod might also be considered in a clinical setup, promising lower individual drug doses, hopefully accompanied by attenuated side effects, thus offering an alternative when side effects limit the use of current multiple sclerosis drugs.

5. Conclusions

Multiple sclerosis is currently treated by different drug classes, including small molecules, interferons, and monoclonal antibodies, all associated with specific side ef-

fects that limit their use. Another limiting factor is the prohibiting price of these agents, especially monoclonal antibodies. Adding a new class of drugs, like the epigenetic drugs proposed by us, might widen the repertoire of available treatment modalities, hopefully avoiding the dose-limiting side effects. Based on our in vivo results, we propose these two options for testing in multiple sclerosis: azacytidin, with its ADMETox properties known due to its routine clinical use for other indications, and its derivative TAC, with proven oral activity. Further knowledge concerning these new agents might also lead to their use in new therapeutic areas, especially in other autoimmune diseases.

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Conflicts of Interest: T.A. Rauch, is among the inventors of a patents describing the potential clinical use of azacytdidine in rheumatoid arthritis and other autoimmune diseases. The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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