ALTO-300 as Adjunctive Treatment for Major Depressive Disorder Supported by Mechanistic Validation of Patient Selection Biomarker and Well-Established Safety and Tolerability Profile

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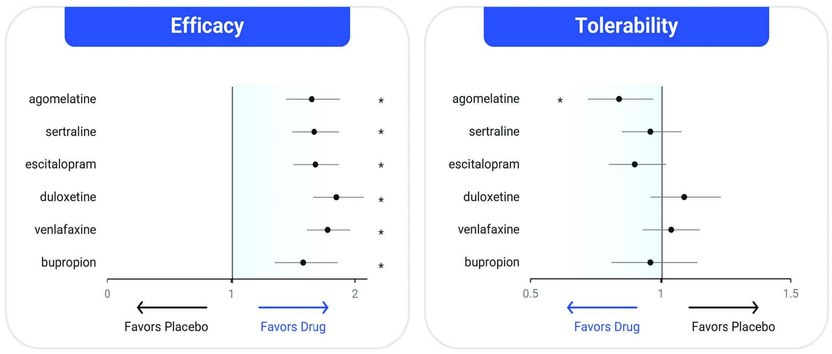
1. Introduction

- Agomelatine is an approved antidepressant medication in Europe and Australia, at both 25mg and 50mg, but has not been approved in the United States.
- **Alto is developing ALTO-300 (25mg agomelatine) as an adjunctive treatment in the United States for patients with major depressive disorder (MDD) characterized by a predictive EEG biomarker,** used to identify patients most likely to respond to treatment.
- Previously, agomelatine has been studied as monotherapy in thousands of patients globally, showing superior tolerability and similar efficacy as other antidepressants:



Cipriani, Lancet, 2018

Agomelatine at 25mg maintains efficacy and avoids liver function test (LFT) elevation observed with the 50mg dose:

- Meta-analyses have shown **equivalent agomelatine efficacy at 25mg and 50mg**.
- LFT elevations are dose-dependent: 0.5, 1.3 and 2.5% in placebo, 25, and 50 mg, respectively (Servier-run trials that did not consistently monitor baseline LFTs).
- In Novartis-run US Phase 3 trials, the **25mg dose led to similar rates of LFT elevation as placebo** (these studies incorporated baseline LFT screening).
- **Low rates of LFT elevation and no safety issues in data from tens of thousands of patients in long-term monitoring trials and real-world data demonstrate**:
- LFT elevations occur early in treatment, are non-cumulative, and resolve quickly (*Perlemuter et al., CNS Drugs, 2016*).
- LFT elevations do not result in liver failure following long-term use *(Pladevall-Vila et al., CNS drugs, 2019).*
- **The 25mg dose of agomelatine potentially balances efficacy and avoids LFT monitoring requirement.**

No unexpected adverse events were observed in Alto's completed Phase 2a trial of ALTO-300

- The most common adverse event was headache.
- Treatment emergent adverse events (TEAEs) were consistent with prior agomelatine studies.

Overall TEAEs	
Safety Analysis Set	**N (%)**
Total Participants	239
At least one TEAE	172 (72.0)
No TEAE	67 (28.0)
SAEs (none related)	6 (2.5)
AEs leading to discontinuation	12 (5.0)
	% of TEAEs
Related TEAEs	35.7

TEAEs for ≥5% of the Population	
Safety Analysis Set	**N (%)**
Headache	35 (14.6)
Nausea	18 (7.5)
Dyspepsia	15 (6.3)
Insomnia	15 (6.3)
COVID-19 Infection	14 (5.9)
Rash (10 from wearable)	12 (5.0)
	35 (14.6)

Note: participants may have had more than one AE

- Alto's Phase 2a and Phase 2b ALTO-300 trials have involved monitoring for elevated liver enzymes (≥ 3 times the upper limit of normal), with the Phase 2b trial including a stopping rule for elevated liver enzymes.
- **No LFT elevations ≥ 3 times the upper limit of normal were observed in the Company's completed 239-patient Phase 2a trial**.
- In our ongoing blinded Phase 2b trial no patient has been stopped due to an LFT elevation ≥ 3x ULN

Mechanistic link between ALTO-300 and the electroencephalography (EEG) biomarker:

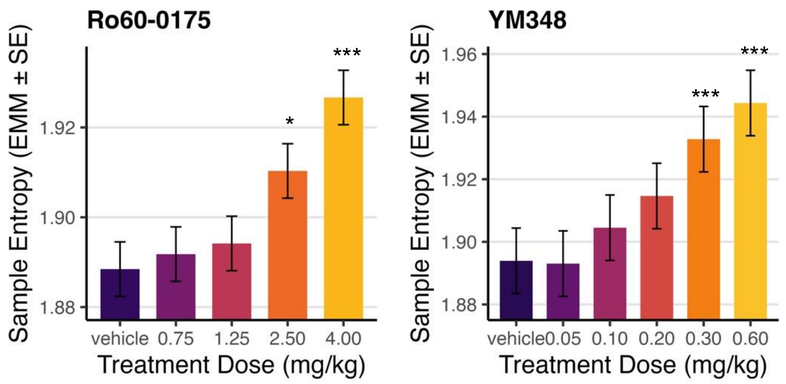
- ALTO-300, a melatonin MT1/MT2 agonist and 5-HT2C antagonist, increases dopamine and noradrenaline release via its 5-HT2C antagonism.
- The ALTO-300 Phase 2a trial in MDD identified a **reproducible, readily scalable and easily administered machine learning-derived EEG biomarker**, which is a measure of gamma band **sample entropy (SE) representing neural noise**. This biomarker is being used to **predict clinical response** and identify patients who are most likely to benefit from ALTO-300.
- We hypothesized that increasing 5-HT2C activity or directly depleting dopamine (both the opposite mechanistic effect of ALTO-300) would increase SE, creating a more biomarker positive-like EEG pattern.

2. Study Design – 5-HT2C Agonists in Mice

- Two 5-HT2C agonists were tested in two separate cohorts of mice: RO60-0175 and YM348.
- Each animal received all doses of the relevant drug, including a vehicle control, in a randomized sequence, with a wash-out period at least 72 hours.
- EEG recordings were taken from frontal and parietal screws.

3. Dose-dependent SE Increases in Mice

- Gamma band frequency range was used to calculate SE.
- SE features were computed for every 10-second epoch and then averaged between 0.5 and 1.5 hours post-dosing.
- Estimated values from a mixed model accounting for dosing sequence and individual variances are shown below for both drugs.

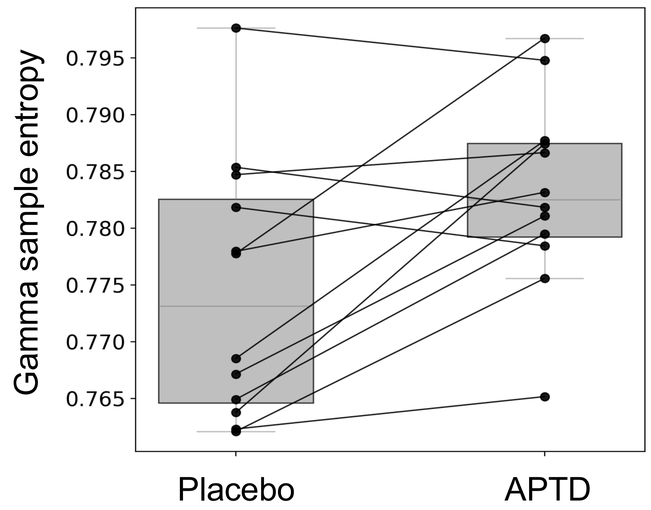


4. Study Design – APTD Human Study

- A previously conducted study enrolled twelve healthy male participants aged 18–30 years in a double-blind, placebo-controlled, crossover dietary dopamine depletion study.
- In a counterbalanced design, participants consumed both a nutritionally balanced amino acid mixture (placebo) and a mixture deficient in tyrosine and phenylalanine (APTD) separated by 7 days.
- EEG data were recorded during a cognitive task, and gamma band sample entropy was calculated from the continuous recordings.

5. APTD Increases SE in Humans

- APTD significantly increased gamma-band sample entropy compared to placebo (Cohen's d=0.94, p=0.006).



6. Conclusions

- ALTO-300 has continued to be well tolerated, with no evidence of increased rates of LFT elevation **(supporting the selection of the 25mg dose as an adjunctive treatment).**
- The ALTO-300 predictive biomarker signal likely reflects increased neural noise due to elevated 5-HT2C tone and reduced dopaminergic activity.
- The mechanism of action of ALTO-300 involves an increase in dopamine in part through 5-HT2C antagonism.
- These findings provide a direct link between ALTO-300 and the EEG biomarker used to identify MDD patients who are more likely to be responders to treatment.

7. Acknowledgments

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