Supplementary Material

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Materials and Methods

Overall Study Design and Plan

This Phase 2a, single-center, 3-month randomized, double-blind, placebo-controlled, split-mouth design study was conducted in adults with existing chronic gingival inflammation (presented as gingivitis or periodontitis), as determined by mean full mouth MGI of ≥2.0 and percent BOP of ≥40% at screening/baseline. The study was conducted to determine whether the local complement inhibition resulted in reversal of clinical signs of gingival inflammation.

The total study duration was 16 months including 4 months of subject participation from the screening visit (within 30 days of baseline). There was a 2-month pause to the study enrollment due to State restrictions for coronavirus 2019 (COVID-19) pandemic.

Dose-Escalation Phase

The study design included a dose escalating phase to choose the safe and effective dose for the main study. Escalating doses of AMY-101 and placebo were injected into interproximal gingival tissues at buccal and lingual aspects in different halves of the mouth (split-mouth design) once a week on Days 0, 7, and 14. Initially 4 subjects received 0.025 mg dose/interdental papilla of AMY-101 and evaluated for safety and injection-site reactions (ISRs). With no significant events observed up to Day 28, another 4 subjects received 0.05 mg/interdental papilla of AMY-101 who were observed up to Day 28. With no serious TEAEs or significant ISRs reported, another 4 subjects received 0.10 mg dose/interdental papilla who were observed up to Day 28. For interim

analysis, the 3 cohorts (0.025 mg/interdental papilla, 0.05 mg/interdental papilla and 0.10 mg/interdental papilla) were assessed for safety and efficacy parameters, and based on this assessment, the PI and DSMB decided to treat the additional 28 subjects with 0.10 mg dose/interdental papilla.

Supplementary Table 1. Dose Escalation and Stopping Rules

If 0/4 subjects had DLT	Escalated to the next higher dose level
If 1/4 subjects had DLT	Repeated the current dose level or escalated to the next higher dose level
If ≥2/4 subjects had DLT	Lowered the dose or stopped further dosing
DLT = dose limiting toxicity	

Main Study Phase

The study consisted of a Screening, a Baseline Visit (Study Visit 1), and Study Visits 2 to 8.

Screening Visit

Subjects underwent evaluation of eligibility criteria, collection of medical, dental and medication history, urine pregnancy test (in women with childbearing potential), and complete oral examination, and periodontal clinical measurements after providing an informed consent to participate in the study.

Study Visits

Baseline Visit (Day 0, Study Visit 1)

Within 30 days after Screening, subjects returned for the Baseline Visit. At this visit, subjects underwent a medical and dental history review; complete oral examination; urine pregnancy test (in women with childbearing potential); measurements of height and weight; assessment of concomitant medications; evaluation for unanticipated problems (UPs); collection of baseline biological samples; collection of samples for anti-drug antibody assessment; assessment of vital signs; and baseline periodontal measurements. Baseline periodontal assessments were performed on 6 sites per tooth for all teeth (excluding third molars) and included the following: modified gingival index (MGI), probing depth (PD), bleeding on probing (BOP), measurement of distance from the cementoenamel junction (CEJ) to the free gingival margin (GM), and plaque index (PI). Clinical attachment level (CAL) was calculated by subtracting the distance between CEJ and GM from the PD.

The GCF samples were collected from two sites with highest gingival index in each quadrant at Baseline to detect the levels of matrix metalloproteinases in the GCF.

Safety and efficacy parameters were assessed before treatment at Baseline. Once biological samples were collected and clinical examination completed, randomly assigned halves of the mouth (split-mouth design) received injections of either AMY-101 (0.025 mg, 0.05 mg or 0.1 mg in 25 µl or 50 µl) or Placebo (25 µl or 50 µl) in every interproximal papilla at both buccal and palatal/lingual aspects with Gingival Index score of ≥1. The injections were administered once a week on Day 0 (Baseline), Day 7, and Day 14.

Study Visits 2 through 8 (Day 3 - Day 90)

- Subjects returned to clinic on Days 3, 7, 14, 21, 28, 60, and 90 after initial application of AMY-101 and placebo for safety evaluations, periodontal assessments, and biological sampling
- At each visit, the subject underwent a medical and dental history review, complete oral examination, assessment of concomitant medications, assessment of vital signs, and evaluation for AEs and UPs
- Safety parameters were assessed on Days 3, 7, 14, 21, 28, 60, and 90 after initial treatment
- Efficacy parameters were assessed on Days 21, 28, 60, and 90 after initial treatment
- After the 90 days follow-up visit, subjects received a complete oral debridement consisting of supra and sub gingival prophylaxis, and referred to further periodontal treatment, if needed.

The clinician who performed periodontal and oral examination assessments in each subject was different from the clinician who injected AMY-101 and placebo. A participant was considered to have completed the study if he or she had completed all phases of the study including the last visit or the last scheduled procedure.

Study Design Rationale

The split-mouth design was chosen to minimize subject level variability between groups, optimize recruitment efforts and allow for the use of more powerful paired statistical tests. Given that local

application of AMY-101 at the doses proposed had demonstrated no significant systemic effects on complement inhibition in a pre-clinical model of non-human primates, a split-mouth design helped to examine the local effects of direct application of the drug into gingival tissues by comparing results to the placebo-injected side of the mouth. In this split-mouth design, the interdental papilla between teeth 8 and 9 as well as 25 and 24, were not treated, to specifically apply the split mouth and separate two halves. Sites with gingival index of ≥1 were treated, to ensure well balanced groups regarding inflammation parameters at the site level. Subjects with generalized gingivitis (MGI ≥2.0 and 40% BOP) were included in the study, but more specifically, to address the severity between mouth halves, a randomized designation of treatments for each subject was applied.

Selection of Study Population

Inclusion Criteria

Each subject had to meet all of the following criteria to be eligible for the study:

1. Provide a signed and dated informed consent
2. State his/her willingness to comply with all study procedures and availability for the duration of the study
3. Was 18 to 65 years of age
4. Had ≥20 natural teeth (excluding third molars)
5. Had generalized plaque-induced gingival inflammation determined by MGI and percent BOP (MGI ≥2.0, BOP ≥40%). Subjects could have been diagnosed with stable (treated) Stage I-IV periodontal disease according to CAL
6. Had a good general health, as evidenced by medical history
7. Female subjects of reproductive potential used licensed hormonal contraception or practiced barrier methods or abstained for at least one month prior to Screening, and agreed to use such a method during study participation
8. Male subjects of reproductive potential agreed to use condoms or other methods which ensured effective contraception with partner.

Exclusion Criteria

A subject who met any of the following criteria was excluded from the study:

1. Presence of orthodontic appliances (including fixed lingual retainer)
2. Soft or hard tissue tumor of the oral cavity
3. Carious lesions requiring immediate treatment
4. Presence of gross plaque and calculus at the Investigator's discretion
5. Missing natural teeth on one side of the jaw (right or left upper and lower) only
6. Presence of more than six crowns in the mouth
7. Participated in any other clinical study within 30 days of screening or during the study
8. Received any antibiotic therapy within the last 30 days
9. History of chronic use (≥3 times/week) of anti-inflammatory medications (e.g., non-steroidal anti-inflammatory drugs [NSAIDs] steroids). Low dose (<325 mg) aspirin was allowed
10. Was immune compromised (e.g., subjects with Human Immunodeficiency Virus [HIV] infection, neutropenia, complement deficiency, etc.)
11. Medical history or any concomitant medication that could have affected the assessment of the study treatment or periodontal tissues, such as diabetes (irrespective of level of control), rheumatoid arthritis, Crohn’s disease, nifedipine, phenytoin (Dilantin), anticoagulant medications (e.g., warfarin [Coumadin] etc.), ongoing cancer treatment either with radiation or chemotherapy
12. Involvement in the planning or conduct of the study
13. History of any clinically significant disease or disorder which, in the opinion of the Investigator, could have either put the subject at risk because of participation in the study, or interfered with interpretation of the subject’s study results
14. Was pregnant or lactating
15. Uncontrolled chronic diseases (e.g., kidney disease, chronic obstructive pulmonary disease, pulmonary fibrosis, Hepatitis C)

16. Autoimmune disorders (Down's Syndrome, Sjogren's Disease, Psoriasis, Chediak-Higashi Syndrome)
17. Conditions requiring antibiotic prophylaxis
18. Underwent periodontal therapy within the past one year
19. Gross tooth decay, as determined by the Investigator
20. Periodontal or dental abscesses
21. Root fragments, pericoronitis, endo-perio lesions
22. Smoked cigarettes or other tobacco products (including e-cigarette or recreational drug use) within one year before the screening visit.

Stopping or Suspending the Study

Circumstances that warranted early termination of study included, but were not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient adherence to protocol requirements
- Data that were not sufficiently complete and/or evaluable
- Planned to modify, suspend, or discontinue the development of the IP
- Determination of futility. No formal futility analysis was planned. However, if serious safety concerns required the unblinding of all study subjects, a lack of efficacy could be considered along with the safety concerns as a reason for the premature termination of the study.

Results

Supplementary Table 2. Primary Efficacy Analysis—Changes in Mean Modified Gingival Index at Day 28

Statistics	AMY-101 [0.1 mg] N=31	Placebo N=31
LSM	-0.285	-0.104
SE	0.0259	0.0217
95% CI	-0.336, -0.234	-0.147, -0.062
LSM Difference TRT-Placebo	-0.181	-
SE	0.0340	-
95% CI	-0.248, -0.114	-
p-value	<0.001	-

CI = confidence interval, LSM = least square mean, SE = standard error, TRT = treatment.

Least square means (LSM) with 95 % Confidence Interval (CI), standard error (SE), LSM difference along with its standard error (SE), 95% CI, and p-value was obtained through a Generalized Estimating Equations (GEE) method with normal distribution and Identity link including treatment group, study visit (up-to Day 28) and interaction between treatment group and study visit (up-to Day 28) as fixed effects with baseline as covariate.

Supplementary Table 3. Secondary Efficacy Analysis: Changes in Mean Modified Gingival Index at Days 21, 60, and 90

Visit	Statistics	AMY-101 [0.1 mg] (N=31)	Placebo (N=31)
Day 21	n	31	31
	LSM	-0.238	-0.102
	SE	0.0163	0.0163
	95% CI	-0.270, -0.206	-0.134, -0.070
	LSM Difference TRT-Placebo	-0.136	
	SE	0.0231	

Visit	Statistics	AMY-101 [0.1 mg] (N=31)	Placebo (N=31)
	95% CI	-0.182, -0.091	
	p-value	<0.001	
Day 60	n	28	28
	LSM	-0.211	-0.062
	SE	0.0236	0.0147
	95% CI	-0.257, -0.164	-0.091, -0.033
	LSM Difference TRT-Placebo	-0.149	
	SE	0.0275	
	95% CI	-0.203, -0.095	
	p-value	<0.001	
Day 90	n	30	30
	LSM	-0.169	-0.039
	SE	0.0227	0.0176
	95% CI	-0.214, -0.125	-0.073, -0.004
	LSM Difference TRT-Placebo	-0.131	
	SE	0.0192	
	95% CI	-0.168, -0.093	
	p-value	<0.001	

Supplementary Table 4. Secondary Efficacy Analysis: Changes in Mean Bleeding on Probing (BOP)

Visit	Statistics	AMY-101 [0.1 mg] (N=31)	Placebo (N=31)
Day 21	n	31	31
	LSM	-0.190	-0.061
	SE	0.0227	0.0111
	95% CI	-0.235, -0.146	-0.083, -0.039
	LSM Difference TRT-Placebo	-0.129	

Visit	Statistics	AMY-101 [0.1 mg] (N=31)	Placebo (N=31)
	SE	0.0228	
	95% CI	-0.174, -0.085	
	p-value	<0.001	
Day 28	n	31	31
	LSM	-0.250	-0.074
	SE	0.0233	0.0150
	95% CI	-0.296, -0.204	-0.103, -0.044
	LSM Difference TRT-Placebo	-0.177	
	SE	0.0282	
	95% CI	-0.232, -0.121	
	p-value	<0.001	
Day 60	n	28	28
	LSM	-0.182	-0.032
	SE	0.0329	0.0157
	95% CI	-0.246, -0.117	-0.062, -0.001
	LSM Difference TRT-Placebo	-0.150	
	SE	0.0309	
	95% CI	-0.211, -0.090	
	p-value	<0.001	
Day 90	n	30	30
	LSM	-0.187	-0.020
	SE	0.0244	0.0195
	95% CI	-0.235, -0.139	-0.058, 0.018
	LSM Difference TRT-Placebo	-0.168	
	SE	0.0328	
	95% CI	-0.232, -0.103	
	p-value	<0.001	

Supplementary Table 5: Summary of Treatment Emergent Injection Site Reactions by System Organ Class and Preferred Term, Safety Population

	AMY-101		
System Organ Class Preferred Term	**0.025 mg (N=4) n (%)**	**0.05 mg (N=4) n (%)**	**0.1 mg (N=32) n (%)**
Total Number of Treatment Emergent ISRs	0	0	3
Number of Subjects with at Least one ISR	0	0	2 (6.3)
Gastrointestinal disorders	0	0	1 (3.1)
Gingival erythema	0	0	1 (3.1)
Gingival swelling	0	0	1 (3.1)
Nervous system disorders	0	0	1 (3.1)
Ageusia	0	0	1 (3.1)

AE = adverse event, ISR = injection site reaction, N = number of subjects in safety population in each dose group; TEAE = treatment emergent adverse event. Percentages were based on N.