AM PHARMA

Title: Exploratory analyses following first interim analysis for the REVIVAL trial	**Document number:** SAP-CLIN-REVIVAL-001-v1.0-FINAL

Title: Exploratory analyses following first interim analysis for the REVIVAL trial

Study: AP-recAP-AKI-03-01

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Two-Arm Parallel-Group, Multi-Center Phase 3 Pivotal Trial to Investigate the Efficacy and Safety of Recombinant Human Alkaline Phosphatase for Treatment of Patients with Sepsis-Associated Acute Kidney Injury

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1. INTRODUCTION

Results from the first interim analysis of approximately 400 main trial population patients from the AP-recAP-AKI-03-01 (REVIVAL) trial were presented to and reviewed by the Data Monitoring Committee (DMC), as planned, during June 2022. Safety data were also presented to and reviewed by the DMC for the moderate CKD and COVID-19 populations. Following the review of the results from the first interim analysis, the recommendation from the DMC was to stop the REVIVAL trial based on futility according to the pre-defined thresholds for predictive probability of success. The DMC did not express any concerns with the safety data presented.

Blinded total group exploratory analyses were conducted by AM-Pharma and PHASTAR to determine if potential reasons why the criteria for futility had been met would emerge, but no firm conclusions could be drawn from the blinded analyses performed, mainly because treatment group allocation remained unknown. These blinded analyses are documented separately to the analyses that will be performed as part of this SAP.

This current prospective statistical analysis plan (SAP) details the exploratory analyses that will be conducted on unblinded data by AM-Pharma and PHASTAR in accordance with the objectives defined for these analyses.

Major challenge is the heterogeneity of the syndromes included (both AKI and sepsis), major aim is to sequester the various sources of variation **(in bold the most important)**;

1. For AKI
    a. **Pre-sepsis renal function**
        i. Known or assumed
        ii. CKD 1, 2, 3A, 3B, or 4
    b. Duration (time from diagnosis to treatment)
    c. Depth ergo max AKI stage (and when?)
2. For sepsis
    b. **Severity ergo SOFA/Lactate/CRP**
        i. At baseline
        ii. Recovery pattern
    b. Preexistent morbidity
    c. Medical/Surgical
    d. Causative organ
    e. Causative micro-organism
        i. Suspected
        ii. Proven
    f. APACHE (with the note that we have a time shift)
3. For disease severity: Based on APACHE/SOFA: therapeutic efficacy might be less pronounced/absent in patients that are likely to survive anyway (low severity score) or who are likely to die anyway (high severity score).

2. OBJECTIVES

The objectives for the analyses described in this SAP include:

- Identifying an effect of ilofotase alfa on short- and long-term renal function and mortality compared to controls

- Identifying sub-groups within patients with SA-AKI that have differential benefit of ilofotase alfa compared to control

3. DATA

Following further information being provided by the DMC and further discussion within AM-Pharma, the decision was taken to stop recruitment for the REVIVAL trial on 21JUL2022. Patients ongoing in the trial are to be followed up according to the protocol until 22 August 2022 where the data cut will occur. This will result in approximately 650 patients who have a chance for follow-up through day 28 and approximately 550 patients who have a chance for follow-up to 90 days. The data that will be used in these exploratory analyses will include patients from all 3 trial populations defined in the trial protocol:

- The main trial population
- The moderate CKD population
- The Corona Virus Disease 2019 (COVID-19) population

Analysis populations will be defined as follows, and analyzed in the order of:

1. One overall combined population consisting of the main trial population, the moderate CKD population and the COVID-19 population
2. The three individual trial populations
    - Main trial population
    - Moderate CKD population
    - COVID-19 population
3. One combined population consisting of the main trial population and the moderate CKD population
4. Other combinations to be defined

For the analyses by individual trial population, patients that were incorrectly randomized into the wrong trial population based on their pre-AKI reference eGFR, but then rerandomized in the correct trial population, will be analyzed in the trial population they were rerandomized to.

For the analyses described in this SAP, patients that have relevant data will be included in the analyses described; no data imputation will be performed. However, for patients that do not complete visits due to the trial being stopped, their data may be included in the mixed model repeated measures (MMRM) analysis, if appropriate, in order to include the data they have for the visits they have completed.

All patients will be analyzed according to the treatment they received regardless of what their planned treatment was.

4. UNBLINDING

An unblinded DMC support team at Labcorp was responsible for the production and presentation of the unblinded results to the DMC. The programming of the analysis datasets and outputs was initially performed and validated by the blinded programming team at Labcorp, and the validated programs passed to the unblinded team to apply the actual treatment codes and produce the unblinded results.

For the analyses described in this SAP, responsibilities for creating unblinded data are described in the following sections. Labcorp will take the responsibility for updating their programs to create the

unblinded SDTM and ADaM datasets from which PHASTAR will create additional analysis variables and datasets as required for the analyses described in this SAP.

5. SUBGROUP ANALYSES

The endpoints to be used in the subgroup analyses are given in Table 1.

Table 1: Endpoints to be used in the subgroup analyses

Priority	Variable	Definition	Responsibility
1	MAKE90a	- Death until Day 90 - > 25% drop in eGFR at Day 90 visit (compared to pre-AKI reference eGFR) - On RRT at Day 90 **OR** on RRT through Day 28 - Rehospitalization	PHASTAR
2	MAKE90b	- Death until Day 90 - > 25% drop in eGFR (compared to pre-AKI reference eGFR) at Day 28 **AND** Day 90 - On RRT at day 90	LABCORP
3	Mortality D28	All-cause Mortality up to and including Day 28	LABCORP
4	Mortality D90	All-cause Mortality up to and including Day 90	LABCORP
5	Mortality D180	All-cause Mortality up to and including Day 180	LABCORP
6	eGFR at each timepoint	Patients on RRT at each timepoint will have an imputed value of '0' for eGFR at that timepoint	LABCORP /PHASTAR
7	Relative change from baseline in eGFR at each timepoint	Patients on RRT at each timepoint will have an imputed value of '0' for eGFR at that timepoint	LABCORP /PHASTAR

For the eGFR analyses, the available value from each timepoint will be included in a MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline eGFR as the single continuous covariate. The MMRM will be applied overall and for each of the subgroups defined in Table 2.

The variables that will be categorized into subgroups are defined in Table 2. PHASTAR are responsible for programming all subgroups as defined in Table 2.

Table 2: Variables that will be categorized into subgroups and the corresponding subgroup definitions

Priority	Variable	Subgroup definitions
1	Baseline mSOFA	Define 3 subgroups based on tertiles: Baseline mSOFA <= 33.3 percentile Baseline mSOFA from 33.3 to <= 66.6 percentile Baseline mSOFA > 66.6 percentile Present actual cut-off values in dataset/output
2	Baseline eGFR	Define 3 subgroups based on tertiles: Baseline eGFR <= 33.3 percentile Baseline eGFR from 33.3 to <= 66.6 percentile Baseline eGFR > 66.6 percentile Present actual cut-off values in dataset/output
3	KDIGO AKI Stage at baseline	Define 3 subgroups as: KDIGO AKI stage at baseline was 0 or 1 KDIGO AKI stage at baseline was 2 KDIGO AKI stage at baseline was 3
4	AKI Diagnosis relative to VP start	Define 2 subgroups as: AKI diagnosis before or at the same time as VP start AKI diagnosis after VP start Compare minimum VP start date/time with AKI diagnosis date/time and define subgroups based on this
4	Pe-AKI Reference eGFR	Define 3 subgroups based on tertiles: Pe-AKI Reference eGFR <= 33.3 percentile Pe-AKI Reference eGFR from 33.3 to <= 66.6 percentile Pe-AKI Reference eGFR > 66.6 percentile Present actual cut-off values in dataset/output
4	APACHE II	Define 3 subgroups based on tertiles: APACHE II <= 33.3 percentile APACHE II from 33.3 to <= 66.6 percentile APACHE II > 66.6 percentile Present actual cut-off values in dataset/output

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Priority	Variable	Subgroup definitions
5	Improving AKI	Define 2 subgroups as: Improvement of AKI No improvement of AKI Improvement of AKI is defined as > 0.3 mg/dL decline in SRCR (SRCR on which diagnosis of AKI was made vs baseline SRCR). In case baseline SRCR is not available OR from same sample as the SRCR on which diagnosis of AKI was made, SRCR on Day 1 is used (if Day 1 not available, Day 2 is used) Subjects on RRT at baseline/Day 1/Day 2 will be regarded "No improvement of AKI"
6	Main cause of sepsis	Define 2 subgroups as: Cause of sepsis: medical Cause of sepsis: surgical or trauma
7	Baseline CRP	Define 3 subgroups based on tertiles: Baseline CRP <= 33.3 percentile Baseline CRP from 33.3 to <= 66.6 percentile Baseline CRP > 66.6 percentile Present actual cut-off values in dataset/output
8	Baseline lactate	Define 2 subgroups as: Baseline lactate <= 2 mmol/L Baseline lactate > 2 mmol/L
9	Time from AKI diagnosis to treatment start	Define 2 subgroups as: <= median time from AKI diagnosis to treatment start > median time from AKI diagnosis to treatment start Present actual cut-off values in dataset/output
10	Mechanical ventilation status at baseline	Define 2 subgroups as: On mechanical ventilation at baseline Not on mechanical ventilation at baseline Patients that started mechanical ventilation from their date of informed consent up to and including the date/time of trial drug administration will be considered on mechanical ventilation at baseline. Patients ongoing for mechanical ventilation at their date of informed consent and/or ongoing at the date/time of trial drug administration will also be considered on mechanical ventilation at baseline. All periods of mechanical ventilation will be considered regardless of modality (invasive or non-invasive)

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Priority	Variable	Subgroup definitions
11	Baseline P/F ratio	Define 3 subgroups based on tertiles: Baseline P/F ratio <= 33.3 percentile Baseline P/F ratio from 33.3 to <= 66.6 percentile Baseline P/F ratio > 66.6 percentile Present actual cut-off values in dataset/output
12	Charlson co-morbidity index	Define 3 subgroups based on tertiles: Charlson co-morbidity index <=33.3 percentile Charlson co-morbidity index 33.3 to <=66.6 percentile Charlson co-morbidity index >66.6 percentile Present actual cut-off values in dataset/output
13	Recruitment numbers at site	Define 3 subgroups as: patients from sites with <=2 patients recruited patients from sites with 3 to 14 patients recruited patients from sites >=15 patients recruited
14	Gram-bacterial infection	Define 3 subgroups as: Gram positive Gram Negative Mixed Data taken from the Sepsis Diagnosis Confirmation page, where 'If Bacterial' is recorded as Gram pos, Gram neg or Mixed

No data imputation will be performed. For all subgroup analyses, patients that could not be assigned to a specific category because of partial or completely missing information will be included in a missing category for that variable.

For the derivation of tertiles, all relevant values available for the analysis population of interest (as defined in Section 3) will be used to determine the cut-off values to be used in the subgroup definitions.

Tables displaying the number and percentage of patients by treatment group (ilofotase alfa or placebo) and total for each subgroup will be presented. For example:

	Treatment group					
	Placebo		ilofotase alfa		Total	
	N	Number (%) that died	N	Number (%) that died	N	Number (%) that died
Number (percentage) that died*	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
	Number in subgroup	Number (%) that died	Number in subgroup	Number (%) that died	Number in subgroup	Number (%) that died
Subgroup 1**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Subgroup 2**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Subgroup 3**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)
Missing**	nn	nn (xx%)	nn	nn (xx%)	nn	nn (xx%)

* Percentages are based on N

** Percentages are based on the number within each treatment group and subgroup

6. ANALYSIS OF BIOLOGICAL ENDPOINTS

The biological endpoints for further analysis are given in Table 3.

Table 3: Biological endpoints of interest for further analysis.

Priority	Variable	Method of analysis	Responsible for programming
1	mSOFA	MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline mSOFA as the single continuous covariate	PHASTAR
1	Relative change from baseline in mSOFA	MMRM with treatment group and Day as fixed effects, mSOFA score used to randomize the patient as an ordered categorical variable (i.e. obtained from IRT), site as a random effect, and baseline mSOFA as the single continuous covariate	PHASTAR
2	Relative change from baseline in CRP	Descriptive statistics (n, mean, standard deviation, minimum, median and maximum) for Day 3 and Day 28	PHASTAR
2	Absolute change from baseline in CRP	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR
3	Relative change from baseline in Lactate	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR

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Priority	Variable	Method of analysis	Responsible for programming
3	Absolute change from baseline in Lactate	Descriptive statistics (n, mean, standard deviation, min, median and max) for Day 3 and Day 28	PHASTAR
4	Days alive and free of organ support MV, RRT, vasopressor through Day 28	Defined as the number of days alive up to and including Day 28 with no organ support MV or RRT or vasopressors or inotropes. Death up to and including Day 28 is counted as zero days The method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
4	Days alive and free of organ support MV through Day 28	Defined as the number of days alive up to and including Day 28 with no organ support MV. Death up to and including Day 28 is counted as zero days The method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP

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Priority	Variable	Method of analysis	Responsible for programming
4	Days alive and free of, RRT through Day 28	Defined as the number of days alive up to and including Day 28 with no RRT. Death up to and including Day 28 is counted as zero days The method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
4	Days alive and free of vasopressor through Day 28	Defined as the number of days alive up to and including Day 28 with no vasopressors or inotropes. Death up to and including Day 28 is counted as zero days The method for assessing statistical significance will be a re-randomization test comparing the treatment median values for days alive and free of organ support, respecting randomization according to site and mSOFA score. Two-sided exact 95% CIs for the difference in the medians based on the Hodges-Lehmann location shift estimate will be constructed to aid interpretation. This method does not respect the randomization according to site and mSOFA score. More details are provided below.	LABCORP
5	Patients alive and free of AKI on Day 7/ICU discharge and at Day 28	A patient is defined as 'free of AKI' at Day 7/ICU discharge and at Day 28 if the derived AKI stage value on the KDIGO AKI staging page is equal to zero. The number and percentage of patients with this property will be calculated for each time point.	LABCORP

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Priority	Variable	Method of analysis	Responsible for programming
6	Patients alive and free of new onset CKD or worsening of CKD on Day 90	A patient is defined as free of new onset or worsening of CKD (defined as any increase in CKD Stage) at Day 90 if neither of the two conditions mentioned in this endpoint is present. Especially, • For patients with a pre-AKI reference CKD stage (s. Section 7.4.6) of 1 or 2: 'New onset of CKD' is defined as KDIGO CKD stage of at least 3a. • For patients with a pre-AKI reference CKD of stage 3a or higher: 'Worsening of CKD' is defined as an increase of KDIGO CKD stage (including a change from stage 3a to 3b) as compared to the pre-AKI reference CKD stage. The number and percentage of patients with this property will be calculated.	LABCORP

The p-value of the re-randomization test will be calculated as follows:

- Step 1: Calculate X0 = Median days alive and free of xxx in recAP group – median days alive and free of xxx in Placebo group.
- Step 2: Within each stratum (site and mSOFA score) permute the treatment group labels. Call this a permutation resample.
- Step 3: Calculate Xi = Median days alive and free of xxx in 'permuted recAP' group – median days alive and free of xxx in 'new Placebo' group.
- Step 4: Repeat steps 2 and 3, 1000 times to create a permutation distribution.
- Step 5: The p-value is the proportion of the 1000 resamples (X1 to X1000) that are greater than or equal to X0.

7. OTHER ANALYSES

Baseline Characteristics

Descriptive statistics will be presented for the following baseline characteristics by analysis population as defined in Section 3 and treatment group, overall and by subgroup (as defined in Table 2).

- Gender (Male, Female) (n %)
- Age (years) (median [IQR])
- Weight (kg) (median [IQR])
- Height (cm) (median [IQR])
- BMI (kg/m^2) (median [IQR])
- Race (n%)
- APACHE II score (n %)

- MV status at baseline (on/off) (n %)
- AKI stage (n %)
- AKI diagnosis eGFR (mL/min/1.73m^2) (median [IQR])
- Pre-AKI reference eGFR (mL/min/1.73m^2) (median [IQR])
- Baseline mSOFA score (median [IQR])
- Time (h) from AKI diagnosis to start of treatment (median [IQR])
- KDIGO Criteria (n %)
- Baseline CRP (mg/L) (median [IQR])
- Baseline lactate (mmol/L) (median [IQR])
- P/F ratio (median [IQR])
- Main cause of sepsis (n %)
- Charlson Comorbidity Index (median [IQR])
- Dose of norepinephrine and other vasopressors and inotropes (n %)
- Time (h) from start of vasopressor therapy to start of first IV antibiotic (median [IQR])
- Suspected vs proven infection at randomization (n %)
- Known or suspected infection site (by category: pulmonary, abdominal, urinary tract, skin or soft-tissue, CNS, unknown or other) and known or suspected pathogen (by category: viral, bacterial (Gram positive, Gram negative, mixed), other or unknown) (n %)

Further descriptive analyses

- Number of patients on RRT per day (Day 1 to Day 28) by treatment group
- For patients not on RRT: for each day (Day 1 to Day 28) and treatment group, present the number of patients with a > 0.3 mg/dL decline in baseline SRCR (SRCR on which diagnosis of AKI was made vs baseline SRCR). In case baseline SRCR is not available OR from same sample as the SRCR on which diagnosis of AKI was made, SRCR on Day 1 is used (if Day 1 not available, Day 2 is used)