Supplementary data

Complete Methodology

Study design and population

This was a phase 2, randomized, multicenter, double-blind, placebo-controlled trial to evaluate the safety and efficacy of allogeneic bone marrow-derived human MSCs (AC607) in patients undergoing cardiac surgery with laboratory evidence of postoperative AKI. We conducted the trial in 27 clinical sites in the United States and Canada in accordance with the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and registered on clinicaltrials.gov (NCT01602328). Research teams at each center obtained Institutional Review Board or Ethics Committee approval and signed informed consent from each patient or his or her surrogate decision-maker prior to participation. The first patient was randomized on January 10, 2013 and the study was terminated on March 31, 2014, following a recommendation from the Data Monitoring Committee that enrollment be ceased.

We included adults (>21 years of age) undergoing cardiac surgery utilizing cardiopulmonary bypass (CPB), who had a baseline serum creatinine measured within 30 days before surgery and who developed postoperative AKI. Based on an epidemiologic assessment, the trials design group recommended the AKI definition as a postoperative rise in serum creatinine greater than 0.5 mg/dL from baseline within 24 hours of removal from CPB. The group extended the time window to 48 hours from end of CPB in a protocol amendment dated July 17, 2013. Confirmation of a rise in serum creatinine more than 0.5 mg/dL from baseline was required with at least one additional serum creatinine measurement. We excluded patients with stage 5 chronic kidney disease (CKD) based on pre-surgical eGFR, active cancer or active treatment for cancer (with the exception of squamous cell or basal cell carcinoma of the skin), surgery for thoraco-abdominal aortic aneurysm, presence of a medical condition or

mechanical circulatory assist device (such as intra-aortic balloon pump, ventricular assist device or extracorporeal membrane oxygenation) that would preclude or compromise femoral artery catheter placement, or current or expected receipt of dialysis within 24 hours of enrollment or dosing. All inclusion and exclusion criteria are provided in **e-Table 1**.

We randomized enrolled subjects to receive AC607 (2×10^6 cells/kg body weight) or placebo in a 1:1 ratio using an interactive web-based response system. We stratified randomization by CKD status, defined as an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2.

We performed laboratory assessments at screening (either prior to or after completion of surgery), daily during the post-operative hospital stay from the day of randomization until discharge, and at 30 days and 90 days after study drug administration. We measured serum creatinine twice-weekly until day 30 post-dosing or return to baseline values, whichever came first, in patients whose serum creatinine failed to return to or below baseline during their hospital stay.

Procedures

We administered AC607 or placebo as a single dose within 48 hours of removal from CPB and 8 hours of AC607 reconstitution, via a femoral artery catheter, over a period of 1 to 3 minutes. The proceduralist positioned the catheter tip in the aorta at the level of T10-T12 with placement confirmed by imaging prior to dosing.

AC607 was manufactured, processed, packaged and labelled by Lonza Walkersville, Inc. (Walkersville, Maryland) from a starting bone marrow material from two donors. Each 20-mL vial of AC607 contained more than 100 million viable human MSC, 10% v/v dimethyl sulfoxide, 5% v/v human serum albumin in PlasmaLyte A, pH 7.4, and was stored frozen at a temperature below -130°C. After thawing and reconstitution, the final concentration of MSCs

was 2 x 10^6 cells/kg body weight in a total volume of 100 mL. Cell viability and stability data indicated that the cryopreserved product dose contained 120 million cells with 95% viability at 9 months. Furthermore, when tested through the 4F, 70 cm pigtail administration catheter, AC607 retained viability without cell aggregation. We performed all viability and stability studies according to standardized manufacturing practices for stem cells and submitted the results to the United States FDA and Health Canada for review. We ensured maximum viability by maintaining the product dose on refrigerated gel packs during the period between reconstitution and administration. The placebo also contained 10% v/v dimethyl sulfoxide, 5% v/v human serum albumin in PlasmaLyte A, pH 7.4. Clinical trials teams at each institution ensured blinding of investigators, clinical staff and patients by preparation of AC607 or placebo in identical transfer packs before delivery to the patient's location.

Study endpoints

The primary endpoint was the time to recovery of kidney function after post-operative AKI, defined by the time in days from dosing until the first of two post-dosing serum creatinine samples that returned to the patient's baseline level. We did not impute missing serum creatinine concentrations. We considered patients with missing data that was required to establish recovery of kidney function, including deaths and withdrawals, as not recovered during the study and the recovery time was censored at the last creatinine collection during hospitalization. Secondary efficacy endpoints included all-cause mortality or provision of dialysis at 30 and 90 days post study drug administration, all-cause mortality at 30 and 90 days post administration, and proportion of patients who required dialysis during the 30-day evaluation period. Exploratory efficacy endpoints included ICU and hospital lengths of stay, in-hospital mortality, rate of hospital readmissions within 30 days of end of CPB, time to kidney recovery from post-operative AKI using an alternative definition based on return to

the pre-operative serum creatinine level + 0.2 mg/dL with confirmatory measurement, area under the concentration-time curve (AUC) of serum creatinine concentrations above a pre-specified recovery threshold during hospitalization, peak rise in serum creatinine during hospitalization, and kidney function (eGFR) at 6 months and 1 year follow-up.

We performed safety analysis on all patients who underwent cardiac surgery, provided informed consent, and were treated with AC607/placebo. We predominantly assessed safety using treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), vital signs and laboratory values. Adverse events of special interest (AESIs) included injection reactions, *de novo* development of cancer or manifestations of ectopic MSC differentiation.

Statistical Analysis

We performed efficacy analysis following the intention-to-treat principle including all patients who underwent cardiac surgery, provided informed consent, had a documented acute rise in serum creatinine of ≥0.5 mg/dL from pre-surgical values within 48 hours of removal from CPB, and received AC607 or placebo. We constructed Kaplan-Meier product limit plots, and determined relative hazard (AC607 versus placebo) using proportional hazards ("Cox") regression with treatment group as a dependent variable. We calculated the difference between treatment groups in proportions using the exact Clopper-Pearson methodology.

The trials design group estimated the sample size based on the relative hazard for recovery of kidney function greater than 1.25. Assuming 12 days to recovery of kidney function in control-treated patients, 150 treated patients would provide 96%, 92% or 85% power if time to recovery of kidney function were reduced to 7.5 days, 8.0 days or 8.5 days, respectively, in AC607-treated patients.

We conducted all statistical analyses using SAS® software version 9.2 or higher (SAS Institute, Cary, NC).

Supplementary Table 1: Study inclusion and exclusion criteria

Inclusion Criteria:

- Age ≥ 21 years
- Had cardiovascular surgery utilizing cardiopulmonary bypass
- Have a pre-operative (baseline) serum creatinine value collected within 30 days of surgery (if multiple laboratory results are available within this time window, the most recent serum creatinine value prior to surgery to be used to establish the baseline)
- Willing and able to comply with visit schedule and study procedures including posthospitalization
  discharge follow-up
- Ability to give informed consent or have a legally acceptable representative do so for them
- Have AKI defined as ≥ 0.5 mg/dL rise in serum creatinine from baseline within 48 hours of removal from cardiopulmonary bypass

Exclusion Criteria:

- Active cancer and/or receiving active treatment for cancer, with the exception of squamous cell or basal cell carcinoma of the skin
- Surgery for thoraco-abdominal aortic aneurysm (TAAA)
- Current participation in another interventional drug or device clinical study
- Prisoner or other detainee
- Current medical condition that would preclude or compromise femoral artery catheter placement
- Intra-aortic balloon pump (IABP) in place within 2 hours prior to study catheter placement
- Ventricular assist device (VAD) or extracorporeal membrane oxygenation (ECMO) in place at the time of study catheter placement
- Prior history of solid organ or bone marrow transplant
- Stage 5 CKD (based on a pre-surgical eGFR) or currently on dialysis
- Expected to receive dialysis within 24 hours of enrollment or dosing
- Complication during surgery or post-operatively that, in the opinion of the principal investigator, significantly increased the risk of complications to the subject and therefore precluded dosing the subject
- Pregnant or lactating - women with childbearing potential may be tested for pregnancy at the discretion of the principal investigator.

Supplementary Table 2 – Mortality data

Age	Sex	Type of Operation	Postop Dialysis	AKI recovery	Date of Death (Study Day)	In hospital Death	SAE/Cause of Death	Relatedness to Study
AC607								
69	Male	Valve	Yes	Yes	143	No	Multi-organ failure	Unrelated
50	Male	Redo valve	No	No	10	Yes	Hemothorax	Unrelated
50	Female	Pulmonary thrombectomy	Yes	Yes	198	No	Unknown	Unrelated
74	Female	CABG, Valve	Yes	Yes	31	Yes	Multi-organ failure	Unrelated
62	Female	CABG, RV Repair	No	No	34	No	Stroke	Unrelated
81	Female	Valve	No	No	216	No	Respiratory failure, GI bleed	Unrelated
67	Male	CABG	Yes	Yes	17	Yes	Respiratory failure	Unlikely
63	Male	Valve	No	Yes	219	No	GI bleed	Unrelated
83	Male	CABG, Aortic dissection repair	No	No	0	Yes	Cardiogenic shock	Unrelated
62	Male	Valve	Yes	No	14	Yes	Cardiorespiratory arrest	Unrelated
84	Female	Valve, VSD/PFO closure, RV myomectomy	No	No	7	Yes	Ischemic bowel	Unrelated
55	Male	CABG	No	No	1	Yes	Cardiogenic shock	Unlikely
Placebo								
66	Male	Ascending Aorta replacement, coronary repair	No	Yes	17	Yes	Hypoxic brain injury	Unlikely
82	Male	CABG	No	No	9	No	Arrhythmia	Unrelated
66	Female	CABG	Yes	Yes	15	Yes	Sepsis	Unlikely
56	Male	CABG	No	No	85	No	Unknown	Unlikely
63	Female	Valve	Yes	Yes	20	Yes	Encephalopathy	Unknown
78	Male	Valve	No	No	9	Yes	GI bleed	Unrelated

Abbreviations: SAE – serious adverse event

Supplementary Table 3 – Treatment Emergent Serious Adverse Events (TESAE) up to 90 days post-treatment

		AC607 (N= 67)	Placebo (N= 68)
Number of Subjects with at least one TESAE		41 (61.2%)	34 (50.0%)
TESAEs Occurring in ≥ 5% of Subjects Within a Single Cohort			
Cardiac Disorders	Overall	18 (26.9%)	19 (27.9%)
	Cardiac failure congestive	3 (4.5%)	7 (10.3%)
	Atrial fibrillation	2 (3.0%)	4 (5.9%)
	Cardiogenic shock	4 (6.0%)	1 (1.5%)
Respiratory and Thoracic Disorders	Overall	13 (19.4%)	9 (13.2%)
	Pleural effusion	5 (7.5%)	3 (4.4%)
	Respiratory failure	6 (9.0%)	2 (2.9%)
Infections	Overall	12 (17.9%)	9 (13.2%)
	Pneumonia	4 (6.0%)	1 (1.5%)
Renal and Urinary Disorders	Overall	7 (10.4%)	6 (8.8%)
	Acute renal failure	3 (4.5%)	4 (5.9%)

Supplementary Table 4 – Vital Signs and Urine Output

Parameter	Group	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Discharge
Heart Rate (beats/min; mean±SD)	AC607	84.5 ± 14.2	81.5 ± 14.5	83.2 ± 13.0	82.7 ± 16.2	87.2 ± 15.2	85.3 ± 14.1	81.5 ± 12.0
	Placebo	82.5 ± 14.3	82.3 ± 15.2	83.5 ± 14.2	80.0 ± 15.0	81.0 ± 15.3	78.8 ± 11.4	79.6 ± 12.4
Systolic Blood Pressure (mmHg; mean±SD)	AC607	116.7 ± 15.8	125.8 ± 20.5	127.3 ± 20.4	127.7 ± 21.5	130.3 ± 23.1	128.7 ± 23.0	123.6 ± 18.5
	Placebo	120.1 ± 17.1	125.8 ± 19.6	122.9 ± 18.2	123.3 ± 21.0	125.8 ± 17.7	125.5 ± 16.8	125.2 ± 18.7
Diastolic Blood Pressure (mmHg; mean±SD)	AC607	56.0 ± 9.9	59.1 ± 10.4	62.6 ± 11.1	63.1 ± 12.3	64.5 ± 11.3	65.9 ± 11.3	67.8 ± 10.8
	Placebo	57.3 ± 9.6	60.5 ± 8.9	63.1 ± 9.4	64.2 ± 12.5	65.4 ± 12.9	64.1 ± 12.1	69.4 ± 10.3
Respiratory Rate (breaths/min; mean± SD)	AC607	18.6 ± 5.6	19.3 ± 4.8	19.2 ± 4.7	18.2 ± 3.6	18.6 ± 2.9	19.4 ± 3.7	18.3 ± 2.4
	Placebo	19.1 ± 5.4	19.2 ± 4.8	19.3 ± 4.5	19.0 ± 3.3	19.0 ± 2.8	19.4 ± 4.0	18.4 ± 2.1
Temperature (° Celsius; mean±SD)	AC607	37.0 ± 0.5	36.8 ± 0.6	36.9 ± 0.6	36.9 ± 0.6	36.9 ± 0.5	36.8 ± 0.7	36.6 ± 0.6
	Placebo	37.1 ± 0.6	37.0 ± 0.7	36.8 ± 0.6	36.8 ± 0.6	36.8 ± 0.5	36.9 ± 0.5	36.7 ± 0.4
Urine Output (ml/day; mean±SD)	AC607	1582 ± 1209	2073 ± 1485	2172 ± 1351	2235 ± 1531	2149 ± 1536	1779 ± 1234	-
	Placebo	1698 ± 1529	2237 ± 1669	2284 ± 1533	1964 ± 1185	1933 ± 1064	1449 ± 831	-

Supplementary Table 5 – Selected Clinical Laboratory Data

	AC607 (n=66)		Placebo (n=68)		
Parameter	**# Cases**	**Incidence Rate (Cases Per Person-Week)**	**# Cases**	**Incidence Rate (Cases Per Person-Week)**	**Crude Incidence Rate Ratio**
ALP ≥ 2 x ULN	28	0.165	55	0.307	0.54
ALT ≥ 3 x ULN	40	0.227	56	0.306	0.74
ALT ≥ 10 x ULN	9	0.051	15	0.082	0.62
AST ≥ 3 x ULN	58	0.326	72	0.392	0.83
AST ≥ 10 x ULN	18	0.101	22	0.120	0.84
GGT ≥ 3 x ULN	42	0.334	48	0.505	0.66
Serum Creatinine≥ 2.0 mg/dL	326	1.483	247	1.135	1.31
Uric Acid ≥ 8.5 mg/dL (females), ≥ 10.5 mg/dL (males)	106	0.646	87	0.536	1.20
LDH ≥ 3 x ULN	36	0.267	18	0.159	1.68
BUN > 30 mg/dL	400	2.347	360	1.980	1.19

Abbreviations: ALP – alkaline phosphatase; ULN – upper limit of normal; ALT - alanine transaminase; AST – aspartate aminotransferase; GGT - gamma-glutamyl transferase; LDH - lactate dehydrogenase; BUN - blood urea nitrogen