2 Synopsis

NAME OF COMPANY AGC Biologics S.p.A (formerly MolMed S.p.A)	Individual Study Table Referring to Part of the Dossier: Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)
NAME OF FINISHED PRODUCT Not applicable		
NAME OF ACTIVE INGREDIENT HSV-TK cells		
Title of the study: Randomized phase III trial of haploidentical HCT with or without an add-back strategy of HSV-TK donor lymphocytes in patients with high-risk acute leukemia		
Investigators: 24 Principal investigators (5 in Italy, 4 in Germany, 4 in France, 3 in Belgium, 3 in US, 2 in Spain, 1 in Greece, 1 in Israel and 1 in Lithuania)		
Study centers: 24 investigational study sites (5 in Italy, 4 in Germany, 4 in France, 3 in Belgium, 3 in US, 2 in Spain, 1 in Greece, 1 in Israel and 1 in Lithuania)		
Publication (reference): Not applicable		
Study period: First patient enrolled: 12/04/2010; Last patient completed: 30/11/2019; End of study: 30/11/2019		
Study phase: Phase III		
Objectives: Primary Objective: To compare disease-free survival (DFS) in patients with acute high-risk leukemia treated with Herpes Simplex Virus-1 Thymidine Kinase (HSV-TK) add-back strategy versus standard strategy following haploidentical Hematopoietic Cell Transplantation (HCT) Secondary objective: To compare the following endpoints in the two treatment arms: • Overall survival (OS) • Cumulative incidence of non-relapse mortality (NRM) • Cumulative incidence of relapse (CIR) • Time to T-cell immune reconstitution (IR) To evaluate the incidence of severe infections and to assess acute and chronic treatment-emergent graft versus host disease (GvHD) and drug-related treatment-emergent GvHD To evaluate the acute and long-term toxicity related to HSV-TK cells infusions		
Methodology: This was a prospective, randomized (3:1), 2-arm, open-label, multicenter, multinational phase III trial. As part of the Conditional Marketing Authorization (CMA) granted in August 2016 by the European Medicines Agency (EMA), this trial was classified as a Category 2 study. The EMA reviewed safety data based on Periodic Safety Update Reports (PSURs) every 6 months and the benefits and risks of the medicinal product annually. Approximately 170 patients were to be randomized in a 3:1 ratio to either group A (experimental) or Group B (control) using disease status at the time of transplantation (first or subsequent complete remission or relapse), Eastern Cooperative Oncology Group (ECOG) performance status (0 or 1) and country as stratification factors. In group A, patients received the infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg), followed by the infusion of HSV-TK genetically modified CD3+ cells.		

In group B, the physician chose whether the patient received the infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg) or an unmanipulated haploidentical bone marrow or peripheral blood transplant followed by high-dose cyclophosphamide.

The study consisted of a screening phase (at maximum 30 days before haploidentical HCT), the haploidentical HCT on day 0 followed by study treatment (see test and reference therapy for details) and a post-HCT phase.

At screening the following evaluations were performed: collection of medical history and complete physical examination, hematology and chemistry, serology, evaluation of neoplastic disease on bone marrow or peripheral blood, cytogenetics and/or molecular tests of bone marrow and/or peripheral blood for markers of disease according to local practice, left ventricular ejection fraction by MUGA scan or echocardiography, spirometry with diffusing capacity of the lungs for carbon monoxide (DLCO), serum or urine pregnancy test in women of childbearing potential, QTc interval by electrocardiogram (ECG), donor tests (as detailed in the study protocol).

In the post-HCT phase, patients underwent disease evaluation monthly up to month 6 (± 7 days), at month 9 and month 12 (± 7 days), and then yearly (± 1 month) until disease relapse or progression. Immune cell phenotyping, i.e. the quantification of circulating CD3+, CD3+CD4+ and CD3+CD8+ cells, was performed with flow cytometry after hematologic engraftment of haploidentical HCT weekly up to IR and, after IR, monthly up to month 6 (± 7 days) and at month 9 and month 12 (± 7 days). In the test arm, immune cell phenotyping and monitoring of HSV-TK cells were also to be performed in the presence of GvHD related to HSV-TK cells before ganciclovir (GCV)/valganciclovir (VCV) treatment, 4 days after the beginning of GCV/VCV treatment and 1 day after the discontinuation of GCV/VCV treatment. An assessment of the quality of life (QoL) by questionnaire was to be performed before HCT, at months 9 and 12, and yearly after HCT until disease relapse. Overall safety was assessed by recording adverse events, including infectious events, important identified and potential risks and GvHD events, as detailed below. Laboratory assessments were performed after HCT on day 15 of month 1 (± 2 days), monthly up to month 6 (± 7 days) and at month 9 and month 12 (± 7 days). A physical examination, including vital signs (blood pressure, heart rate), body weight, ECOG performance status and clinical signs of GvHD, was performed monthly up to month 6 and at month 9 and month 12. In the test arm, the collection of samples for replication-competent retrovirus (RCR) analysis in modified T-cells was to be performed at baseline (immediately before the first HSV-TK cells infusion), at month 3 and month 6 (± 7 days) and at year 1 (± 1 month) after the first HSV-TK cells infusion.

The study was also to include a long-term follow-up of 15 years after the first year from HCT or after disease relapse/progression. Patients were followed up for further antileukemia treatments and survival on an every 6-month basis, as well as for safety and, for patients treated with HSV-TK cells, also for RCR and long-term follow-up (treated patients only).

The study was early terminated on 30 November 2019 and the enrolment was stopped at the randomization of the 92nd patient.

Number of patients (total and in each arm):

	Randomized	STDP	ITT	PP
Total	92	80	92	64
Arm A	64	53	64	38
Arm B	28	27	28	26

Study population

This was a study of patients with high-risk acute leukemia in first or subsequent complete remission or in relapse and suitable for haploidentical HSCT.

Diagnosis and main criteria for inclusion:

- Any of the following conditions:
  - AML and ALL in first complete remission (CR)
  - AML and ALL in second or subsequent CR
  - Secondary AML in CR

- AML and ALL in first or second relapse or primary refractory
- Left ventricular ejection fraction > 45%
- Signed informed consent of patients and donors or independent witnesses

Criteria for exclusion:

- Life-threatening condition or complication other than study indication
- Contraindication to haploidentical HCT as defined by the investigator
- Active CNS disease
- Pregnancy or lactation. Both males and females of reproductive potential had to practice effective contraceptive measures throughout the study. Women of childbearing potential had to have a negative pregnancy test (serum or urine) within 14 days prior to registration

Test product, dose and mode of administration:

- Name: HSV-TK cells: allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and herpes simplex I virus thymidine kinase (HSV-TK Mut2) as add-back strategy to haploidentical T-depleted transplant.
- Dose: $1 \pm 0.2 \times 10^7$ cells/kg for each infusion, up to 4 infusions, with the first one administered between day 21 and day 49 after haploidentical HCT (day 0) and an interval between each infusion of 30 days, in the absence of spontaneous immune reconstitution (IR) documented by two consecutive findings of circulating CD3+ cells ≥ 100/µl and/or development of GvHD
- Mode of administration: iv infusion over a period of 20-60 minutes

Duration of treatment: see test and reference therapy

Reference therapy, dose and mode of administration:

Haploidentical HCT on day 0 (T-depleted transplant, i.e. infusion of CD34+ cells plus a dose of approximately 1×10^4/Kg of T cells, or an unmanipulated transplant, i.e. bone marrow or peripheral blood, followed by cyclophosphamide at 50 mg/kg iv on days +3 and +4) as standard strategy.

After unmanipulated transplant, administration also of filgrastim on day +4 (5 mg/kg/day by subcutaneous injection until recovery of neutrophils to > 1000/µL for 3 days) and, as GvHD prophylaxis, from day +5 tacrolimus or cyclosporine until day 180 and mycophenolate mofetil until day 35.

Criteria for evaluation:

Efficacy

Primary endpoint: DFS, i.e. the time in months from randomization to relapse, disease progression or death from any cause plus one day

Secondary endpoints:

- OS, i.e. the difference in months from the date of randomization to that of death from any cause plus one day
- Cumulative incidence of NRM (i.e. death from any cause without previous documented relapse (or progression)). NRM was measured from the date of randomization until death from any cause without previous documented relapse. Survival time was computed as the difference in months from the date of randomization to the date of the first event among death, relapse or progression
- CIR, defined based on morphologic evidence of leukemia in bone marrow or other sites. Survival time was computed as the difference in months from the date of randomization to the date of the first event among relapse (or progression) and death
- Time to T-cell IR, i.e. the time to reach a level of circulating CD3+ greater than or equal to 100/µL for two consecutive observations starting from the date of transplantation (study day 0). It was computed as the difference in months from the transplant date to the date of first event among IR, death and relapse (or progression)

Safety

Safety endpoints

1. **Adverse events**
2. **Severe infections**
3. **Important identified and potential risks**
4. **GvHD**
5. **Laboratory assessments:** hematology and blood chemistry
6. **Vital signs and physical examination:** heart rate and systolic and diastolic blood pressure, and body weight and ECOG performance status
7. **Others:** immunophenotype, engraftment, cardiac examination, childbearing potential, functional tests, follow up information, long-term follow-up questionnaire, quality of life questionnaire, donor demography and serology

Statistical methods:

Analysis populations:

- Intention to Treat Population (ITT): all randomized patients, regardless of their eligibility and compliance to the assigned treatment and to the follow-up protocol, who were analyzed according to the treatment assigned to at randomization.
- Per Protocol (PP) set: all patients who were administered at least one dose of study treatment and did not have significant protocol violations concerning inclusion/exclusion criteria or that could condition efficacy evaluations.
- Standard Population (STDP): all randomized patients who received at least one haploidentical HCT.

Efficacy analyses were performed on the ITT and PP populations, with those on the PP set considered supportive. Safety analyses were performed on the STDP.

Efficacy and safety analyses were repeated on the ITT and Standard population, respectively, excluding center 164.

Efficacy:

Primary efficacy

The log-rank and Wilcoxon tests were used to compare DFS curves estimated with Kaplan-Meier method in the two treatment arms. The analysis was repeated using day 21 after transplant date as start date. Of note, patients without an event were censored at the last time known to be alive and free of relapse (i.e. the last disease evaluation) and patients without any follow-up information were censored at the date of randomization.

Secondary efficacy

- OS
  The log-rank and Wilcoxon tests were used to compare OS Kaplan-Meier curves in the two treatment arms. The analysis was repeated using day 21 after transplant date as start date. Of note, living patients were censored at last contact.
- Cumulative incidence of NRM
  The cumulative incidence of events is reported graphically partitioned into its components: crude cumulative incidence of non-relapse mortality and crude cumulative incidence of relapse (or progression). Gray's test was used to compare the sub-distribution functions of the death without previous relapse (or progression) and relapse (or progression) events in the two treatment groups. The analysis was repeated using day 21 after transplant date as start date.
- CIR

  The cumulative incidence of events is reported graphically partitioned into its components: crude cumulative incidence of relapse (or progression) and crude cumulative incidence of non-relapse mortality. Gray's test was used to compare the sub-distribution functions of the relapse (or progression) and death without previous relapse (or progression) events in the two treatment groups

- Time to T-cell IR

  A competing risks analysis was performed considering death, relapse and disease progression as competing events to compare time to T-cell IR in the two treatment groups. The cumulative incidence of events is reported graphically partitioned into its components: crude cumulative immune reconstitution, crude cumulative incidence of relapse (or progression) and crude cumulative incidence of death without previous relapse (or progression) and immune reconstitution. Gray's test was used to compare the sub-distribution functions of the immune reconstitution, relapse (or progression) and death without previous relapse (or progression) or immune reconstitution events in the two treatment groups

Safety

Summaries of the number of treatment-emergent adverse events (TEAEs), number of patients with at least one AE, number of patients with at least one TEAE related to HSV-TK cells, number of patients with at least one TEAE related to HCT, number of patients with study adjustment, interruption, delay or discontinuation due to a TEAE and number of patients with fatal TEAE are provided. TEAEs, suspected related TEAEs and serious TEAEs are also summarized by SOC, PT and severity. Severe infectious AEs and important identified and potential risks are also summarized by severity. Summaries of patients with GvHD by grade and patients experiencing at least one case of GvHD are provided for acute and chronic treatment-emergent GvHD and drug-related treatment-emergent GvHD. The number of patients in the two groups who experienced at least one case of GvHD were compared for exploratory purposes using a Chi square test or a fisher's exact test when the number of observations or the expected number in at least one group was less than 5. Laboratory data including hematology and blood chemistry, vital signs and other safety information are listed.

Study population:

Ninety-two (92) patients were enrolled in this study: 64 in arm A and 28 in arm B. Out of 92 enrolled patients, 70 patients (76.09%) discontinued from the study (78.13% in group A and 71.43% in group B) and 22 (23.91%) early terminated the study (21.88% in group A and 28.57% in group B).

All enrolled patients were randomized and included in the ITT population. Sixty-four (64) out of 92 patients (69.57%) in the ITT were administered at least one dose of study treatment and did not have significant protocol violations concerning inclusion/exclusion criteria or that could condition efficacy evaluations, and thus were included in the PP set (38 patients, 59.38% in group A and 26, 92.86% in group B). Eighty (80) enrolled patients (86.96%) received at least one haploidentical HCT and thus were included in the STDP: i.e. 53 patients (82.81%) in group A and 27 (96.43%) in group B.

Most patients in the ITT had a diagnosis of AML and had a complete response at baseline. Overall, patients in both groups most frequently presented with acute leukemia in first complete remission. No major differences between the two groups were reported concerning demographic and other baseline characteristics.

Efficacy results:

Primary efficacy variable:

ITT population

Most patients had a relapse or disease progression or died from randomization up to the early termination of the study in both treatment groups: 45 patients (70.31%) in group A and 20 patients (71.43%) in group B. Median DFS was 6.70 months (95% CI: 5.36 to 10.02) and 5.55 months (95% CI: 3.71 to 12.58) in group A and group B, respectively. DFS probability at 1 year was 0.37 (95% CI: 0.24 to 0.49) for patients in the experimental arm and 0.35 (95% CI: 0.18 to 0.53) for those in the control arm. No significant difference in DFS was detected between the two groups (log-rank test p=0.8974; Wilcoxon test p=0.7612).

PP population

Supportive results on DFS for the PP population were consistent with those obtained for the ITT population. Most patients had a DFS event: 25 out of 38 patients (65.79%) in group A and 18 out of 26 patients (69.23%) in group B. Median DFS and DFS probability at 1 year were 8.74 months (95% CI: 6.47 to 16.26) and 0.48 (95% CI: 0.32 to 0.63), respectively, in the experimental arm and 5.80 months (95% CI: 4.11 to 13.67) and 0.38 (95% CI: 0.20 to 0.56) in the control arm. No

significant difference in DFS was observed between the experimental group and the control group (log-rank test p=0.3351; Wilcoxon test p=0.1233).

Similar results were obtained considering day 21 after transplant instead of randomization as start date for DFS computation for both the ITT and PP populations.

Secondary efficacy variables

- OS
  The majority of patients died during the study after being randomized: 42 patients (65.63%) in the experimental arm and 18 patients (64.29%) in the control arm. Median OS (computed as the difference in months from the date of randomization to that of death from any cause plus one day) was 9.07 months (95% CI: 6.44 to 14.75) and 13.93 months (95% CI: 5.75 to 16.46) among patients in group A and those in group B, respectively (ITT population). No significant difference in OS was observed between the experimental group and the control group (log-rank test p=0.7660; Wilcoxon test p=0.6706). Similar results were obtained in the PP set, as well as for the ITT population considering day 21 after transplant instead of randomization as start date for OS computation.
- Cumulative incidence of NRM
  NRM (defined as death from any cause without previous documented relapse or progression from the date of randomization) occurred in 27 patients (42.19%) in the experimental group and 9 patients (32.14%) in the control group (ITT population). No statistically significant difference in the cumulative incidence functions of NRM was detected between the experimental and the control group (Gray's test p=0.4035). Similar results were obtained in the PP set and considering day 21 after transplant instead of randomization as start date for NRM computation for both the ITT and PP populations.
- CIR
  CIR (defined based on morphologic evidence of leukemia in bone marrow or other sites) was of 18 (28.13%) and 11 patients (39.29%) in group A and group B, respectively (ITT population). No significant difference in the cumulative incidence functions of relapse (or progression) was detected between the two groups (Gray's test p=0.3526). Results for the PP population were consistent with those obtained for the ITT population.
- Time to T-cell IR
  Thirty-eight (38) patients (65.52%) in the experimental arm and 22 (78.57%) in the control arm showed T-cell IR after randomization without previous relapse or progression. Six (6) patients (10.34%) in the experimental group and 1 patient (3.57%) in the control group had a relapse or progression without previous IR, while 13 (22.41%) and 5 patients (17.86%) died with no previous IR nor relapse or progression in group A and group B, respectively (ITT population). No significant difference in the cumulative incidence functions of IR, relapse (or progression) and death with no previous relapse (or progression) nor IR events was detected between the experimental and the control group (Gray's test p=0.1735 for IR; p=0.5958 for death; p=0.2889 for relapse or progression). Similar results were obtained for the PP population.

Similar results were obtained with all subset primary and secondary efficacy analyses on the ITT population excluding center 164.

Safety results:

Adverse events

Forty-two (42) patients (79.25%) in the experimental group in the STDP and 27 (100%) in the control group had at least one TEAE. The incidence of mild, moderate, severe, life-threatening and fatal TEAEs in the STDP, evaluated by considering the worst severity for each patient throughout the study, was higher or slightly higher in the control group (81.48%, 81.48%, 62.96%, 40.74% and 59.26%, respectively vs. 58.49%, 66.04%, 56.60%, 24.53% and 39.62% in the experimental group). The most frequent TEAEs were "Infections and infestations" (62 patients – 77.50%), with a higher incidence in the control group (25 out of 27 patients, 92.59%) compared with the experimental group (37 out of 53 patients, 69.81%).

Seventeen (17) patients (21.25%) adjusted or interrupted or delayed or permanently discontinued treatment due to a TEAE: 14 patients (26.42%) in the experimental group and 3 (11.11%) in the control group.

HSCT-related TEAEs were reported in 59 patients (73.75%), 39 patients (73.58%) in the experimental group and 20 (74.07%) in the control group. Moreover, 22 patients (41.51%) in the experimental group had at least one TK-related TEAEs (defined as suspected related if the causal relationship to HSV-TK cells was missing and TEAE started after the first HSV-TK cells infusion or it was assessed as "related", "unknown" to "Rel. to HSV-TK" in the opinion of the Investigator). Most patients reported TK-related TEAEs with moderate severity (11, 20.75%) and severe (9, 16.98%), as worst severity. Only 1 patient had life-threatening TK-related TEAEs (causal relationship assessed as unknown), namely "Leukopenia", "Lymphopenia", "Neutropenia" and "Thrombocytopenia", while no TK-related TEAE was fatal. The most frequent TK-related TEAEs were "Immune system disorders", being reported by 17 patients (32.08%).

At least one serious TEAE was reported by 60 patients (75.00%), i.e. 35 patients (66.04%) and 25 patients (92.59%) in the experimental and control groups, respectively. Of note, 31 (38.75%) had at least one severe serious TEAE (22, 41.51% in group A and 9, 3.33% in group B), and 14 (17.50%) had at least one serious TEAE classified as life-threatening (5, 9.43% in group A and 9, 3.33% in group B), as worst severity. Thirty-seven (37) patients (46.25%) died due to at least one serious TEAE (21, 39.62% in group A and 16, 59.26% in group B). The most frequent serious TEAEs were "Infections and infestations", being reported by 37 patients (46.25%) overall, 22 patients (41.51%) in the experimental group and 15 (55.56%) in the control group.

Thirty-nine (39) patients (48.75%) reported at least one TEAE leading to death, with an incidence slightly higher in the control group (16 patients, 59.26%) compared with the experimental group (23 patients, 43.40%). Overall, 52 patients out of 80 (65%) in the STDP died during the study: 35 (66.04%) in the experimental group and 17 (62.96%) in the control group. The most frequent primary reason of death was relapse (9 patients, 16.98% in group A and 8, 29.63% in group B), followed by sepsis (6 patients, 11.32% in group A and 4, 14.81% in group B).

Severe infections

In randomized patients in the STDP who underwent haploidentical HCT for the control arm and who received also at least one HSV-TK cells infusion for the experimental arm the most frequent severe infections reported during the study were sepsis and lung infection. Severe sepsis occurred in 6 out of 43 patients (13.95%) in the experimental arm and 3 out of 27 (11.11%) in the control arm. Moreover, sepsis was life-threatening for 3 patients (11.11%) in group B and fatal for 7 patients (16.28%) in group A and 5 (18.52%) in group B. For 1 patient in group A the severity of sepsis was classified as missing. Seven (7) patients (16.28%) in group A presented with severe lung infection. A life-threatening lung infection occurred in 2 patients (7.41%) in group B, while 2 patients (4.65%) in group A and 2 (7.41%) in group B died for lung infection.

Important identified and potential risks

In randomized patients in the STDP who underwent haploidentical HCT for the control arm and who received also at least one HSV-TK cells infusion for the experimental arm the most frequent important identified and potential risks were a severe systemic infection (23 patients, 53.49% in group A and 6, 22.22% in group B) and CMV reactivation of moderate severity (12, 27.91% in group A and 11, 40.74% in group B). Of note, a severe systemic infection was life-threatening for 1 patient (2.33%) in group A and 5 (18.52%) in group B and fatal for 8 patients (18.60%) in group A and 6 (22.22%) in group B.

GvHD

At least one case of treatment-emergent chronic GvHD (cGvHD) occurred in 8 patients out of 53 (15.09%) in the experimental arm and 5 patients out of 27 (18.52%) in the control arm. All treatment-emergent cGvHD in the experimental arm were reported as TK-related. No significant difference in the number of patients who experienced at least one case of suspected-related treatment-emergent chronic GvHD was observed between the two groups (fisher's exact test p=0.7532). Patients experienced more frequently suspected related treatment-emergent cGvHD of grade II (3 patients, 5.66% in group A and 2 patients, 7.41% in group B) and grade III (4 patients, 7.55% in group A and 1, 3.70% in group B).

Twenty (20) patients (37.74%) in the experimental group and 8 (29.63%) in the control group had at least one treatment-emergent acute GvHD (aGvHD), with no significant difference between the two groups (fisher's exact test p=0.4723). The most frequent treatment-emergent aGvHD were of grade II (10 patients, 18.87% in group A and 4, 14.81% in group B) and grade I (6 patients, 11.32% in group A and 2, 7.41% in group B). At least one case of TK-related treatment-emergent aGvHD occurred in 14 patients out of 53 (26.42%) in the experimental arm: 4 patients (7.55%) had a grade I TK-related

treatment-emergent aGvHD, 8 (15.09%) a grade II and 2 (3.77%) a grade III. No significant difference in the number of patients who experienced at least one case of suspected related treatment-emergent aGvHD was observed between the two groups (fisher's exact test p=0.7608). Similar results were obtained with all subset safety analyses on the STDP population excluding center 164.
Conclusions: This study further supports the safety of TK gene therapy, but found no evidence of long-term beneficial effects of HSV-TK cells add-back strategy on disease-free survival, overall survival, cumulative incidence of non-relapse mortality and relapse, and time to immune reconstitution, as well as incidence of severe infections and control of GvHD in patients with high-risk acute leukemia.