Clinical Study Report

An Open-labelled, Randomized Phase II Multicentre Study to Investigate Efficacy of Autologous Lymphoid Effector Cells Specific Against Tumour-Cells (ALECSAT) in Patients with Glioblastoma Multiforme Measured as Progression Free Survival Compared to Avastin/Irinotecan

ALECSAT

Title Page

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Development Phase	Phase II
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Indication	Glioblastoma Multiforme
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Study Initiated	22-Apr-2014
Study Completed	Study prematurely terminated on 12-Jun-2015
Sponsor	CytoVac A/S, SCION DTU Forskerparken, Venlighedsvej 6, 2970 Hørsholm, Denmark
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Report Date	31-Mar-2016

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Signature Page

I have prepared or read this report and confirm that to the best of my knowledge it accurately describes the changes in the study	
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Synopsis

TITLE OF STUDY
An Open-labelled, Randomized Phase II Multicentre Study to Investigate Efficacy of Autologous Lymphoid Effector Cells Specific Against Tumour-Cells (ALECSAT) in Patients with Glioblastoma Multiforme Measured as Progression Free Survival Compared to Avastin/Irinotecan

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PUBLICATIONS
None at the time of this report

STUDY PERIOD	**DEVELOPMENT PHASE**
22-Apr-2014 to 12-Jun-2015	Phase II

OBJECTIVES

Primary Objective

- To compare progression-free survival (PFS) in subjects with relapsed glioblastoma multiforme (GBM) when the subjects were either treated with ALECSAT immunotherapy or standard praxis therapy with Avastin/Irinotecan.

Secondary Efficacy Objectives

- To evaluate the overall survival (OS) during the study period in subjects treated with ALECSAT compared to subjects treated with Avastin/Irinotecan by Kaplan-Meier methodology.
- To evaluate time to progression in the two treatment groups.
- To compare PFS in the two treatment groups by Kaplan-Meier methodology upon study completion.
- To compare PFS in a landmark analysis in the two treatment groups after a duration of 6 and 12 months after initiation of treatment.
- To compare objective response rate (ORR).
- To investigate Quality of Life (QoL) and performance status during the study period for subjects treated with ALECSAT compared to subjects treated with Avastin/Irinotecan.
- To investigate any changes in leucocytes and lymphocytes during the study period for subjects receiving ALECSAT.
- To investigate radiological changes as measured by magnetic resonance imaging (MRI) during the study period for the ALECSAT group and compare the results with the Avastin/Irinotecan treated group.

Safety Objectives

- To characterise the safety and tolerability of ALECSAT treatment.

METHODOLOGY
This study was a prospective, open-label, randomised, parallel group study with ALECSAT compared to Avastin/Irinotecan in GBM patients with verified relapsed disease after or during treatment with standard regimen or another recognised first-line treatment.
The subjects in the two treatment groups were to be followed at 18 planned study visits for up to 62 weeks. After informed consent and check of inclusion and exclusion criteria, baseline evaluations were performed, including medical history, electrocardiogram (ECG) and baseline MRI scan of tumour.
Subjects in the ALECSAT group donated blood for production of the ALECSAT product 3 weeks prior to each

ALECSAT treatment (at weeks 4, 9, 14, 26 and 46). Subjects in the Avastin/Irinotecan group were treated with Avastin/Irinotecan on visit 1 (day 0) and every 2 weeks according to standard practice.
MRI scan, QoL and performance status was performed regularly for efficacy assessments. In addition to MRI scan, ^{18}F-fluoro-ethyl-tyrosine (18F-FET) Positron emission tomography (PET) scan was performed at Copenhagen University Hospital, Rigshospitalet.
Safety blood samples, vital signs, physical examination and adverse event monitoring were performed frequently during the study.

NUMBER OF SUBJECTS PLANNED AND ANALYSED

A total of 175 subjects were planned, distributed as 105 subjects in the ALECSAT group and 70 subjects in the Avastin/Irinotecan group.
Since the study was terminated early, 25 subjects were analysed, 15 subjects in the ALECSAT group and 10 subjects in the Avastin/Irinotecan group.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

The subjects enrolled in this clinical study were patients with advanced GBM with documented relapse during, or after completing, first line treatments.

Inclusion criteria:

1. Histologically confirmed GBM tumour with recurrence during or after completing the recognised first-line treatments, tumour recurrence documented by MRI
2. Minimum age of 18 years old capable of understanding the information and giving informed consent
3. Minimum height of 155 cm
4. Expected survival time (life expectancy) of over 3 months
5. Adequate performance status ≤ 2 (according to WHO/ECOG [Eastern Cooperative Oncology Group]performance status score)
6. Clinically normal erythrocyte volume fraction (EVF)
7. Women in fertile conditions could only be included with a negative pregnancy test at screening and had to use appropriate contraceptives during the study

Exclusion criteria:

1. Positive tests for anti-HIV-1/2; HBsAg, anti-HBc, Anti-HCV or being positive in a *Treponema pallidum* test (syphilis)
2. Subjects who may have been exposed to West Nile virus, Dengue or Ebola virus or HTLV-1 virus prior to donation should be excluded
3. Concurrent illness, e.g. uncontrolled epilepsy, cardiovascular-, cerebrovascular-, and/or respiratory disease which could worsen or cause complications in connection with blood donation
4. Clinically significant autoimmune disorders or conditions of immune suppression
5. Haemoglobin count ≤ 7.5mmol/L (men and women)
6. Lymphocyte-numbers below 0.5 x 10^9/L
7. Body weight below 40 kg (men) and 50 kg (women)
8. Clinically abnormal ECG as judged by the Investigator
9. Pregnant or breastfeeding women
10. Inclusion in other clinical studies 4 weeks prior to inclusion in the study
11. Any medical condition that will render participation in the study risky or, according to the Investigator will make the assessment of the study endpoints difficult
12. Treatment with any immunotherapy, cytotoxic therapy or, biologic therapy 4 weeks prior to enrolment in this study
13. Subjects that either may be put at risk due to the blood donation or where it is not expected that an ALECSAT product of good quality can be produced (judged by the Investigator)
14. Subjects with uncontrolled serious bacterial, viral, fungal or parasitic infection
15. Blood transfusions within 48 hours prior to donation of blood for ALECSAT production
16. Known or suspected intolerance to Avastin, Irinotecan or any of the excipients as well as intolerance to recombinant humanised antibodies
17. Performance status ≥ 3 (according to WHO/ECOG performance status score)

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

The ALECSAT treatment contained $1\times10^7 - 1\times10^9$ cytotoxic T-lymphocytes (CTL) and natural killer (NK) cells generated from autologous blood donated by the individual study subject prior to each treatment cycle. Each batch of ALECSAT was therefore a subject-specific single dose.

The first ALECSAT treatment was given on visit 2 (week 4). The following administrations were given in uneven intervals at weeks 9, 14, 26 and 46. Due to early termination of the study, no subjects received study product at weeks 26 and 46.

DURATION OF TREATMENT

Treatment duration was planned for up to 62 weeks.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Subjects allocated to the Avastin/Irinotecan treatment were treated in accordance with standard practice in Denmark for GBM patients. Treatment with Avastin/Irinotecan started at study visit 1 (day 0) and was given as up to 16 treatment cycles with 4 weeks duration. Each cycle consisted of 2 dosing days; day 1 and day 15 in the cycle.

CRITERIA FOR EVALUATION – EFFICACY

Since the study was terminated early, some endpoints deemed irrelevant or unfeasible to analyse as planned. Some endpoints were re-defined, and others (especially exploratory analyses) were deemed irrelevant and not performed.

Primary Endpoint

- PFS measured by MRI. Progression of disease was defined according to the response evaluation criteria for solid tumours (Response Assessment in Neuro-Oncology Criteria, RANO).

Secondary Efficacy Endpoints

- OS, the number of subjects still alive at the end of study.
- Time to progression.
- PFS time-to-event analysis.
- PFS landmark after 6 and 12 months – was changed to event rates after 3, 4 and 5 months due to early termination of the study.
- OS landmark proportions after 6 and 12 months – was changed to event rates after 3, 4 and 5 months due to early termination of the study.
- ORR defined as the response rate of subjects having a complete or partial response.
- EORTC QoL Questionnaires EORTC QLQ-C30 and QLQ-BN20 and performance status according to WHO/ECOG performance status score.
- Changes in leucocytes and lymphocytes in the ALECSAT group.
- Changes in tumour size measured by MRI – this was not performed since tumour diameters were not available.

CRITERIA FOR EVALUATION – PHARMACOKINETICS

Not applicable for this study.

CRITERIA FOR EVALUATION – SAFETY

The safety and tolerability of ALECSAT in this study was measured by:

- Presence of adverse events (AEs) and serious AEs during the study period.
- Presence of clinically significant changes in biochemistry and haematology parameters.
- Presence of medical events of special interest (MESI) – MESIs were not classified or reported due to early termination of the study.
- Changes in vital signs.
- Changes in ECG.

STATISTICAL METHODS

- Descriptive statistics of demographics and other baseline characteristics were presented by treatment group
- In the protocol, all time-to-event related endpoints were defined as time-from-randomisation-to-event endpoints, but due to the asymmetric duration from randomisation to first study product administration in the two treatment arms, some endpoints were supplemented by a corresponding time-from-first-study-product-administration-to-event endpoint.
- All statistical tests were performed using a two-sided test at a 5% significance level. Results from analyses were

presented with estimates, 95% confidence intervals and p-values. For log-transformed analyses, the anti-log transformation was applied before presentation.

- Numerical data were presented in summary tables by number of subjects, arithmetic mean (geometric mean and coefficient of variation [CV] where applicable), median, standard deviation, minimum and maximum (and CV in % where applicable). Categorical data were to be presented by the number and the percentage of subjects (and number of events where applicable).
- Subjects with neither disease progression nor death were censored at the last tumour assessment date where they were known not to have progressed. Subjects with no tumour assessments after baseline but who were still alive at the time of the clinical cut-off were censored at day 1 (worst case assumption). If several response evaluations for a subject were progressive disease, the date of the first of these measurements was used in the survival analysis of PFS.
- The primary efficacy hypothesis to be tested was that the median time to event for PFS within the ALECSAT treatment group would be superior to the median time to event for PFS in the comparator treatment group. This hypothesis was tested by using a two-sided log-rank test at a significance level of 5% to test the null hypothesis of the hazard ratio, HR, being equal to 1 with the alternative of the hazard ratio being different from 1.
- Estimated survival curves for OS and time to progression were tabulated and displayed in Kaplan-Meier plots, and the treatment groups were summarised similar to the primary efficacy analysis, including a comparison based on a log-rank test.
- The objective response rates (ORR) were tabulated for the two treatment arms and compared by a two-sided chi-square test along with the estimated odds ratio and the corresponding 95% confidence interval.
- QoL scales were as to be tabulated and displayed in figures according to treatment and visit, but no formal statistical evaluation had been planned.
- An interim analysis for futility was planned, but since the study was prematurely terminated after 25 subjects, the analysis was no longer relevant and therefore not performed.

DEMOGRAPHY OF STUDY POPULATION

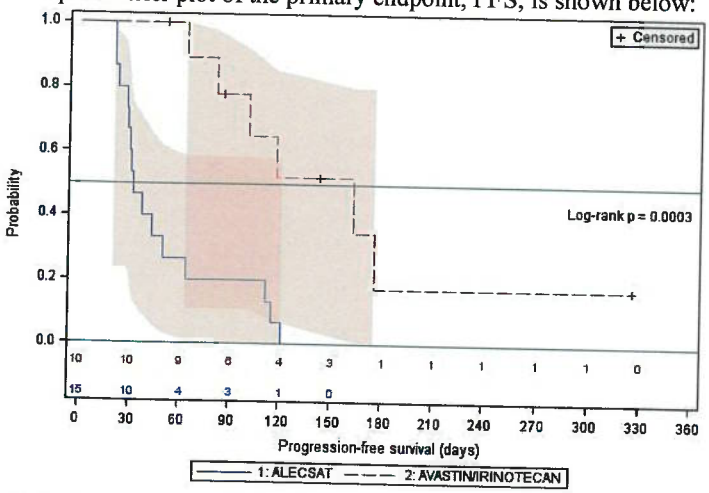
- A total of 27 subjects were screened and 25 subjects were enrolled and treated in the study, 15 in the ALECSAT group and 10 in the Avastin/Irinotecan group. All subjects were discontinued prior to completing the study; 18 subjects discontinued due to progression of disease, 2 subjects discontinued due to Investigator's recommendation, 3 due to other reasons, and 2 subjects were discontinued when the study was terminated by Sponsor.
- A total of 12 females and 13 males, representing ages from 46 to 73 years were included in the study. All subjects were white; the average height was 1.74 m and the average weight 78.1 kg. There was a slight imbalance in body weight at baseline; otherwise no apparent imbalances were found in the baseline characteristics.
- All subjects had a normal ECG or ECG with no significant findings at baseline.
- Physical examination of abdomen, chest, heart, lungs, skin, and peripheral pulse was normal for all subjects.
- The physical examination revealed abnormal clinically significant findings for the head in 2 subjects in the ALECSAT group, for ears/eyes/nose/throat in 1 subject in each treatment group, and for other physical conditions / undefined in 1 subject in each treatment group.
- All subjects had performance status score of 0 or 1 at baseline. More subjects in the ALECSAT group compared to the Avastin/Irinotecan group were asymptomatic at baseline (66.7% vs. 40.0% of subjects, respectively).
- All enrolled subjects had advanced cancer of the brain. Additionally, most subjects (72.0%) had other concurrent disease reported as ongoing medical history. The most frequently reported medical history terms were hypertension reported by 7 subjects (28.0%), epilepsy reported by 4 subjects, and depression reported by 3 subjects (12.0%).
- All subjects used concomitant medication during the study. Drug classes used by most subjects were alimentary tract and metabolism products (23 subjects), systemic hormonal preparations (23 subjects), and nervous system products (22 subjects).

EFFICACY RESULTS

- The average (SD) exposure was 2.2 (1.2) months in the ALECSAT group and 4.2 (2.9) months in the Avastin/Irinotecan group. Total patient-years of exposure (PYE) were 2.72 years in the ALECSAT group and

3.52 years in the Avastin/Irinotecan group.

- A Kaplan-Meier plot of the primary endpoint, PFS, is shown below:



- Median PFS was statistically significantly shorter in the ALECSAT group (32 days) compared to the Avastin/Irinotecan group (163 days) (p=0.0003), and the Hazard Ratio [95% CI] for PFS in the ALECSAT group was 0.16 [0.05; 0.49].
- Sensitivity analysis of PFS from first study product administration yielded similar results as the primary analysis.
- Median OS was not significantly different between the ALECSAT group (150 days) and the Avastin/Irinotecan group (203 days) (p=0.1907). The Hazard Ratio [95% CI] for OS in the ALECSAT group was 0.45 [0.13; 1.54].
- Median time to progression was statistically significantly shorter in the ALECSAT group (32 days) compared to the Avastin/Irinotecan group (163 days) (p=0.0001). The Hazard Ratio [95% CI] for time to progression in the ALECSAT group was 0.12 [0.03; 0.42].
- PFS event rates (i.e. for no progression or death) were statistically significantly lower in the ALECSAT group compared to the Avastin/Irinotecan group at all time points. The Hazard Ratio [95% CI] for PFS was 0.26 [0.10; 0.69] at month 3 and 0.16 [0.05; 0.49] at month 5.
- OS event rates (i.e. for no death) were slightly lower in the ALECSAT group compared to the Avastin/Irinotecan group, but were not statistically significantly different between the treatment groups at any time point.
- No subjects in the ALECSAT group had objective response to treatment compared to 6 subjects in the Avastin/Irinotecan group, resulting in an odds ratio [95% CI] for objective response rate in the ALECSAT group of 0.17 [0.001; 0.39].
- Overall, the scores for EORTC QLQ-C30 global health status and functional scales were similar between groups apart from a slightly worse cognitive and role functioning and better social functioning subjects in the ALECSAT group at some weeks.
- Overall, the EORTC QLQ-C30 symptom scores were similar between groups apart from worse gastrointestinal symptom scores (appetite loss, constipation and diarrhoea) in the Avastin/Irinotecan group around treatment, consistent with the expected gastrointestinal adverse reactions for this treatment.
- Overall, the BN20 global health status and domain scores were similar between groups. However, communication deficit and motor dysfunction domains were worse for subjects in the ALECSAT group at some weeks.
- Overall, BN20 single item scores were similar between groups. However, subjects in the Avastin/Irinotecan group had worse hair loss, drowsiness and weakness of legs, consistent with the expected adverse reactions for this treatment.
- Overall, the ECOG performance status deteriorated during the study. At baseline and week 4, a greater proportion of subjects were asymptomatic in the ALECSAT group compared to the Avastin/Irinotecan group. At weeks 6 and 14, the performance status was more similar between the treatment groups and most subjects in both groups were symptomatic but completely ambulatory.

- Overall, leukocytes increased slightly from baseline while lymphocytes decreased. Leukocyte and lymphocyte values outside normal ranges were reported as not clinically significant.

PHARMACOKINETIC RESULTS

Not applicable for this study.

SAFETY RESULTS

- A total of 129 AEs in 23 subjects occurred during the study; In the ALECAT group, a total of 62 events in 13 subjects were reported, corresponding to 2.36 subjects with AEs per PYE. In the Avastin/Irinotecan group a total of 67 events in 10 subjects were reported, corresponding to 3.52 subjects with AEs per PYE.
- The most frequently reported AEs were nausea (3 events in 2 subjects in the ALECSAT group and 11 events in 5 subjects in the Avastin/Irinotecan group), diarrhoea (1 event in 1 subject in the ALECSAT group and 12 events in 8 subjects in the Avastin/Irinotecan group), fatigue (6 events in 6 subjects in the ALECSAT group and 7 events in 4 subjects in the Avastin/Irinotecan group), and cystitis (4 events in 3 subjects in the ALECSAT group and 3 events in 2 subjects in the Avastin/Irinotecan group).
- Most of the AEs were of mild (67 of 129 AEs) or moderate (40 of 129 AEs) severity. A total of 22 events had intensity of CTCAE grade 3 or above.
- A total of 39 events in 11 subjects were assessed as related to study product. Only 2 of these events (diarrhoea and vomiting) were assessed as related to ALECSAT (0.36 subjects with adverse drug reactions [ADRs] per PYE) compared to 37events in 9 subjects in the Avastin/Irinotecan group (3.17 subjects with ADRs per PYE). The majority of ADRs in the Avastin/Irinotecan group were gastrointestinal disorders.
- Time to first onset of treatment-emergent AE from randomisation was longer in the ALECSAT group compared to the Avastin/Irinotecan group (p=0.0006). However, there was no statistically significant difference between the treatment groups in time to first onset of a treatment-emergent AE from first study product administration.
- No AEs or SAEs led to discontinuation of subjects.
- Medical events of special interest (MESIs) were not classified or reported due to early termination of the study.
- A total of 21 SAEs in 13 subjects were reported during the study. Of these, 2 events of embolism (1 moderate and 1 severe) in 2 subjects in the Avastin/Irinotecan group were assessed as related to study product.
- One subject died during the study due to progression of GBM.
- No clinically significant values were reported for any high or low out-of-range biochemical or haematology laboratory parameters, and no AEs were reported for any laboratory values.
- There were no obvious differences between groups in vital signs.
- Abnormal physical examination results were found for abdomen, ears/eyes/nose/throat, extremities and head. Overall, more abnormal results were found in the ALECSAT group compared to the Avastin/Irinotecan group.
- Overall, the ALECSAT treatment appeared well tolerated and no safety concerns were raised. There were fewer treatment-emergent AEs and treatment-emergent ADRs in the ALECSAT group compared to the Avastin/Irinotecan group, especially the incidence of gastrointestinal events were different between groups. The frequencies and preferred terms appearing were in line with expectations for a study in this indication and study population.

CONCLUSION

Data from the study showed that second line treatment of late stage GBM patients with ALECSAT could not extend PFS or OS when the treatment was given as monotherapy. This may due to the fact that ALECSAT was administered 28 days later than control treatment was initiated in the reference group, and the delay in treatment may have caused early discontinuation of subjects due to progression of disease.

The rapid disease progression of GBM together with the treatment regimen used in this study precluded the detection of any delayed effects of ALECSAT treatment. The ALECSAT treatment appeared well tolerated and no safety concerns were raised.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.