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Organization of Clinical Trials of Photosensitizer based on 5-Aminolevulinic Acid Hexyl Ester

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Abstract

Background: This paper deals with the statistical and methodological study conducted in order to assess the possibility of combining in one study, Phase 2 and 3 clinical trials of the Russian drug Hexasense based on 5-aminolevulinic acid hexyl ester. **Methods:** Hexasense drug is intended for fluorescence diagnosis of bladder cancer (the route of administration - intravesical instillation of solution). The clinical trial involved a comparative study of the effectiveness of diagnostic fluorescence depending on the exposure time of the drug solution in the bladder (1 and 2 hours). This is due to the fact that the time interval of 2 hours checked on the research Phase 1 used to cause inconvenience to patients. **Results:** After selecting the optimal diagnostic mode (a task of Phase 2) it is necessary to evaluate the effectiveness of the selected mode as compared to standard white-light cystoscopy (a task of Phase 3). Comparison of the effectiveness of two diagnostic fluorescence diagnostic modes with different exposure time of solution of 1 and 2 hours has been planned. At the second stage, the selected fluorescence diagnosis mode will be compared with the results of a standard white-light cystoscopy obtained in the same group of patients. A difference of 10% will be taken for significant differences in values of sensitivity and specificity. The statistical analysis showed that the required number of patients at a significance level of 2.5%, power of 80%, and for minimum difference in proportions of 10% subject to the correction of 10% for the dropout and unassessed patients would be 134 people (two groups of 67 patients). In this case, the same sample of patients allows comparing reliably the effectiveness of two diagnoses with the correction for multiplicity of comparison. **Application:** This will help to minimize the number of patients involved in the study and not reduce the accuracy of the results at the same time. The Ministry of Health of the Russian Federation authorized the clinical trial of the drug Hexasense with Phases 2 and 3 combined.

Keywords: 5-Aminolevulinic Acid Hexyl Ester, Bladder Cancer, Clinical Trials, Organization of Clinical Trials

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1. Introduction

Methods of Fluorescence Diagnosis (FD) are based on the ability of the photosensitizing drug to fluoresce under the local exposure to light of a certain wavelength corresponding to its absorption peak[1–3]. FD is especially effective for detection of small-size tumors localizing in the surface layers, since the sensitivity of this method is significantly higher than that of other modern methods of early diagnosis[4,5].

One of the ways of creating the effective concentrations of the photosensitizer in the tumor tissue is the stimulation of the body to the endogenous photoactive compounds - porphyrins and, in particular, Protoporphyrin IX (PPIX). One of the compounds effectively inducing the synthesis of endogenous PPIX is 5-aminolevulinic acid (5-ALA) and its ester derivatives (5-ALA methyl and hexyl esters). 5-ALA is an intermediate metabolite of heme synthesis in human cells[6,7]. It is known that the tumor cells are prone to the increased accumulation of photoactive PPIX in the presence of exogenous 5-ALA due to increased activity of enzymes of initial heme synthesis in tumor cells, and to deficiency in ferrochelatase- an enzyme that disposes PPIX by its conversion into a heme. PPIX accumulation in tumor cells occurs within a few hours, and its high level is maintained for up to 6 hours, while PPIX in normal cells is disposed faster by its conversion into a non-photoactive heme[8,9]. The result is a high fluorescence contrast between the tumor and the surrounding tissue, which manifests itself in this period of time and reaches 10-15-fold size of different tumors, which is an important factor for the visualization of tumor boundaries during FD[10,11].

Possibilities of FD application with 5-ALA-based drugs in the diagnosis of cancer and precancerous diseases have been actively investigated both in Russia and abroad. By now, a number of 5-ALA-based drugs have been registered and approved for medical use in the world such as Levulan (Norway); Dusan (Canada) and Alasense (Russia), which showed a very high efficiency in diagnosing the malignant tumors of a number of locations, and the FD-method with their application has been used in leading clinics of the world as one of the most sensitive to clarify the prevalence of tumor process[11–13]. For example, after many years of research of the effectiveness of the Alasense-based FD in patients with tumors of the upper respiratory tract, upper digestive tract, colon, bladder, endometrial, pleura and peritoneum conducted in P. A. Herzen Moscow Scientific and Research Oncological Institute the following data on the effectiveness of diagnosis were obtained: The sensitivity ranged from 87.5% to 100%, and specificity - from 76% to 100%. At the same time, the FD allows effectively diagnosing hidden pre-cancer lesions, early cancer and superficial tumor recurrences[6].

In recent years, the FD has been performed with the use of drugs Metvix (Switzerland) and Hexvix (Norway) on the basis of 5-ALA-methyl and hexyl, respectively. Being more hydrophobic than the 5-ALA, the esters better overcome biological membranes, which contributes to their more rapid and greater accumulation in cells and incorporation into biosynthesis as a PPIX precursor. In the body, 5-ALA esters undergo metabolic transformation to 5-ALA[14–16].

In Russia, Federal State Unitary Enterprise, State Scientific Center "Scientific and Research Institute of Organic Intermediates and Dyes" together with P. A. Herzen Moscow Scientific and Research Oncological Institute developed the drug Hexasense based on 5-ALA hexyl ester synthesized by the original technology. The department of modifiers and anticancer therapy protectors of P. A. Herzen Moscow Scientific and Research Oncological Institute conducted pre-clinical studies of the developed drug[17–19]. In 2014, P. A. Herzen Moscow Scientific and Research Oncological Institute completed Phase 1 of the clinical trials of the drug organized by Federal State Unitary Enterprise, State Scientific Center "Scientific and Research Institute of Organic Intermediates and Dyes" under the protocol "Controlled open-label study of tolerability and diagnostic efficacy of Hexasense - photosensitizer for Fluorescence Diagnosis (FD) of bladder cancer". These studies revealed the absence in all studied doses (60-100 mg) of any general toxic reactions, hemato-, hepato- and nephro-toxicity, as well as the absence of local irritating action of the preparation solution on the mucous membrane of the bladder at a concentration of 0.12-0.2%.

The next stage of Hexasense development was the organization of the combined Phase 2-3 of clinical trials of the drug.

2. Materials and Methods

To calculate the required sample size of patients and evaluate the possibility of obtaining reliable results on this sample with the developed design of clinical study the following equation was used:

$$N = [A+B]^{2*}[p_1*(1-p_1)+p_2*(1-p_2)]/[p_1-p_2]^2 \quad (1)$$

where p_1 and p_2 – expectable proportions (sensitivity and specificity), A and B – tabular critical values of the normal distribution, corresponding to the predetermined levels of type 1 errors and the selected level of significance, (p_1-p_2) - the minimum clinically important difference, which can be detected.

For correct calculation, the expected values of sensitivity and specificity were evaluated and assigned, the minimum clinically significant difference was determined[20], and the correction for multiple comparisons by the Bonferroni method was made.

Upon calculation of the necessary sample of patients the statistical methods, which would be used in the future in the evaluation of the results of the clinical study, were considered. The estimated parameters of diagnostic efficacy (sensitivity and specificity) represent the frequencies measured in a percentage, and the testing of the statistical hypothesis about the same efficacy of two different diagnostic modes is supposed.

3. Results and Discussions

The main criterion for inclusion of patients in the study during the development of the clinical study design was morphologically verified diagnosis transitional cell bladder cancer at $T_{a\text{-}is}N_0M_0$ stage (T_a - noninvasive papillary carcinoma, T_{is} - preinvasive carcinoma (carcinoma in situ), N_0 - no metastases to regional lymph nodes, M_0 - no distant metastases) and at T_1N_0 stage (T_1 - tumor extends to the subepithelial connective tissue, N_0 - no metastases to regional lymph nodes, M_0 - no distant metastases), a primary or recurrent form of the disease. It should be noted that tumor incidence stage is important in calculating the required number of patients for the studies because it allows predicting the average number of tumor lesions in each patient.

Also, the main inclusion criteria were formulated:

- Age - 18 to 70 years old (inclusively).
- Decay- or necrosis-free tumors.
- Objectively assessable and measurable tumor lesions.
- Life expectancy - no less than 6 months.
- Blood pressure not more than 140/90 mmHg, creatinine level in the blood plasma not more than 97 mol/L, ALT, AST not more than 40 IU/L, total bilirubin not more than 20 mmol/L.
- Hematological parameters: white blood cell count > 4000/mm^3, platelets > 150,000/mm^3, hemoglobin 12 g/dl.
- Satisfactory condition of the patients (0-2 according to the WHO scale).
- Absence of intractable therapeutic control of co morbidities.
- Ability of the patient to perform the investigation procedure and provide written informed consent in accordance with GCP and local legislation.

Further, we have developed a method of diagnosis and its results evaluation, which was recorded in the Protocol No. 02-(FD-GE)-2014 of the Phase II-III of clinical trials of Hexasense.

Each patient must undergo morphological examination of the following lesions:

- Lesions defined as tumor in the white light (both fluorescent and non-fluorescent).
- Lesions of additional fluorescence (if any).
- Control lesions (non-fluorescent and defined in the white light as the unaltered mucous - for control "blind" biopsy, one lesion in each patient).

Minimum number of studied lesions - 2 lesions in a patient. The average predicted number (subject to the stage of the disease) - 4 lesions in a patient.

Evaluation of the diagnosis effectiveness (both FD and standard cystoscopy) must be carried out based on the parameters of sensitivity and specificity. The results shall be presented as the total number of true positive, true negative, false positive and false negative results (Table 1 and 2). The values of sensitivity and specificity parameters shall be calculated according to the Equation (2) and (3):

Table 1. Morphological study and FD comparability assessment

Results of FD	Results of morphological study	Assessment of results
Fluorescence available	Tumor cells available	True positive (TP)
Fluorescence available	No tumor cells	False positive (FP)
No fluorescence	Tumor cells available	False negative (FN)
No fluorescence	No tumor cells	True negative (TN)

Table 2. Morphological study and standard cystoscopy comparability assessment

Results of white-light cystoscopy	Results of morphological study	Assessment of results
Tumor lesion defined in the white-light	Tumor cells available	True positive (TP)
Tumor lesion defined in the white-light	No tumor cells	False positive (FP)
No tumor lesion defined in the white-light	Tumor cells available	False negative (FN)
No tumor lesion defined in the white-light	No tumor cells	True negative (TN)

It was decided to conduct within the framework of clinical study a comparative assessment of the FD effectiveness, subject to the exposure time of the preparation solution in the bladder of 1 and 2 hours (trial Phase 2, two groups of patients). Phase 1 exposure time of 2 hours caused inconvenience to the patients, therefore it is important to determine the optimal time of exposure of the solution, and then evaluate the FD effectiveness with the selected exposure time as compared to standard white-light cystoscopy (trial Phase 3). All patients included in the study can already undergo standard cystoscopy in the 2 Phase of the study (in addition to the FD), therefore we assumed that it is possible to use the results of the PD and the white-light cystoscopy, obtained at the beginning of the study, for the comparative analysis (retrospective analysis). This will allow solving the problems of the trial Phase 3 with no recruitment of new patients.

The maximum possible value that can be taken by $p^*(1\text{-}p)$ from Equation (1) is 0.25 at $p = 0.5$. If we set the minimum clinically important difference of 5%, then, at the total level of significance of 5% (corresponding to the significance level of 2.5% for each of the two comparisons), A = 2.24 and power of 80% (B = 0.84), each group must include:

$$N = [2.24 + 0.84]^{2*}[0.25 + 0.25]/[0.05]^2 = 1898 \text{ lesions.}$$

Given the prevalence of Ta-isN0M0 and T1N0M0 tumor process in patients included in the study we have assumed that the average number of such lesions in patients will be 4 (3 tumor lesions and 1 control lesion). Subject to the above-stated, each group shall include 475 patients. Furthermore, it is necessary to make a correction of at least 10% for that some patients may drop out of the study for various reasons (failure to comply with study procedures, development of severe adverse reactions, the patient's desire to terminate the study, the patient's pregnancy). Then, the total number of patients will be 1044.

In the given Equation (1), the main factor, which determines the sample size, is the value of the minimum clinically significant difference. The dependence of N value on this difference is given below (Table 3). To conduct the study, a minimum clinically significant difference between the sensitivity and specificity of 10% was taken. In this case, the total number of patients, according to the Table 3, will be 262 people.

The required number of patients also depends on the estimated values of the proportions (sensitivity and specificity) (Table 4).

The results of the clinical trials of Hexvix have shown that the sensitivity and specificity of Hexvix-based FD is not less than 85%. In this case, the required number of patients at a significance level of 2.5%, power of 80%, and

Table 3. The dependence of the required sample size on the minimum clinically significant difference in the effectiveness parameters of two diagnostic options. The calculation was performed with the significance level of 2.5% and power of 80%

$p_1 - p_2$, %	Number of lesions in a group	Patients in a group	Total patients in both groups	Total patients with 10% correction
2	11858	2965	5930	6522
5	1898	475	950	1044
10	**474**	**119**	**238**	**262**
15	211	53	106	116
20	119	30	60	66

Table 4. Dependence of the required sample size on the true values of p_1 and p_2 proportions. The calculation was performed with the significance level of 2.5%, power of 10% and for minimum difference in proportions of 10%

$p_1 = p_2$, %	Number of lesions in a group	Patients in a group	Total patients in both groups	Total patients with 10% correction
50	474	119	238	262
60	455	114	228	252
70	398	100	200	220
75	356	89	178	196
80	304	76	152	168
85	**242**	**61**	**122**	**134**
90	171	43	86	94
95	90	23	46	50
99	19	5	10	12

for minimum difference in proportions of 10% subject to the correction of 10% would be 134 people in two groups:

$N = [2.24 + 0.84]^2*[0.85*0.15 + 0.85*0.15]/[0.1]^2 = 242$ lesions.

In case of absence of significant differences in the effectiveness of the two modes after conducting the Phase 2 of the clinical trial (selection of the optimal solution time exposure), exposure time of 1 hour will be recommended for further evaluation as the time that causes the least inconvenience to the patient.

During Phase 3, the selected fluorescence diagnosis mode with the optimum exposure time will be compared with the results of a standard white-light cystoscopy obtained in the same group of patients. The difference of 10% will be taken for significant difference in the sensitivity and specificity values. In this case, the same sample of patients (67 patients in a group, each of which has undergone both types of diagnosis) allows comparing reliably the effectiveness of two diagnoses with the correction for multiplicity of comparison (the significance level of 2.5%). Documents proving the feasibility of combining Phase 2 and 3 of clinical trials of Hexasense drug were submitted for review to the Ministry of Health and approved. A permission was granted to conduct a clinical trials under the Protocol No. 02-(FD-GE)-2014 (version 2 of 04.24.2015) "Controlled open-label study of tolerability and diagnostic efficacy of Hexasense - photosensitizer for Fluorescence Diagnosis (FD) of bladder cancer" No. 304 of June 9, 2015. In June 2015, three research centers started clinical trials under the Protocol:

- P. A. Herzen Moscow Scientific and Research Oncological Institute (Branch of FSBI "NMRRC" of the Ministry of Health of the Russian Federation), Moscow.
- Moscow State Budgetary Healthcare Institution "Clinical Oncological Dispensary No. 1 of Moscow Health Department", Moscow.
- State Budgetary Healthcare Institution "City Clinical Hospital No. 40 of Moscow Health Department", Moscow.

4. Summary

- The possibility of combining Phase 2 and Phase 3 in the same trial has been shown.
- During Phases 2 and 3 of the trials it is necessary to perform a two-step assessment with sequential comparison of efficiency: The first stage - comparison of two fluorescent diagnostics mode, and the second stage - of the selected mode of fluorescent diagnostics with standard cystoscopy.
- The basic criteria for inclusion of patients in the trials under the Protocol No. 02-(FD-GE)-2014 of the Phase 2-3 of clinical study of Hexasense have been developed.
- The method of diagnosis and its results evaluation, which was recorded in the Protocol No. 02-(FD-GE)-2014 of the Phase 2-3 of clinical trials of Hexasense, have been developed.
- The number of patients required for inclusion in the trials has been calculated - 134 patients.

6. Conclusion

We have developed a design of the protocol of Phase 2-3 of clinical trials of Hexasense and statistically substantiated the possibility of combining Phase 2 and Phase 3 of clinical trials into one protocol. Statistical analysis has shown that the planned number of patients (67 patients in one group, total 134 patients) is sufficient to obtain statistically significant results for a given significance level of differences in parameters of sensitivity and specificity of 10%. The relevant documents have been submitted for examination to the Ministry of Health of the Russian Federation. The Ministry of Health of the Russian Federation approved the combined use of Phase 2 and Phase 3 of clinical trials of Hexasense under the same protocol and granted permission to conduct a clinical trials under the Protocol No. 02-(FD-GE)-2014 (version 2 of 04.24.2015) "Controlled open-label study of tolerability and diagnostic efficacy of Hexasense - photosensitizer for Fluorescence Diagnosis (FD) of bladder cancer" No. 304 of June 9, 2015. In June 2015, three research centers started clinical studies under the Protocol:

The study was performed as part of works under the state contract with the Ministry of Industry of the Russian Federation No. 13411.1008799.13.124 of 25.06.2013, in connection with the event "The organization and conduct of clinical trials of innovative drugs" of the Federal Target Program "Development of the Pharmaceutical and Medical Industry of the Russian Federation for the period until 2020 and for long term" (Government Resolution of February 17, 2011 No. 91).

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