<u>**Supplementary 1. Protocol**</u>

Clinical study protocol

An open prospective non-randomized trial of the efficacy and safety of combination of vemurafenib and cytarabine/ 2-chloro-2'-deoxyadenosine in children with Langerhans cell histiocytosis with BRAF V600E mutation

Principal Investigator:	Prof. Michael Maschan, MD, Ph.D
Study coordinator:	Dmitry Evseev, MD
Co-investigators:	Irina Kalinina, MD, Ph.D
	Elena Raykina, MD, Ph.D

Local profile expert committee approval date 20.03.2017

Local ethical committee approval date 20.03.2018

Date of last revision 19.06.2018

List of abbreviations

LCH – Langerhans cell histiocytosis

PCR – polymerase chain reaction

MAPK – mitogen-activated protein kinase

BRAF – rapidly activating fibrosarcoma isoform B

ORR – overall response rate

DAS – disease activity score

CTCAE – common terminology criteria for adverse events

MRD – minimal residual disease

Ara-C – cytosine arabinoside, cytarabine

2-CdA – 2-chloro-2'-deoxyadenosine, cladribine

I.   Content

II. Justification

LCH is a disease characterized by pathological clonal expansion of cells phenotypically similar to Langerhans cells and their dissemination into various organs and tissues. The manifestations of this disease are multifaceted and range from a self-resolving skin rash or a single focus in the structure of bone tissue (the so-called eosinophilic granuloma) to severe multisystem processes affecting the bone marrow, spleen and liver.

The modern classification implies the division of LCH into mono- and multisystem. Mono or single-system (SS) forms include those that affect one organ or one system (most often skin or bones are affected), and the bones lesions are divided into uni- and multifocal. In multisystem (MS) LCH lesions can affect so-called risk organs, which include the liver, spleen and bone marrow[1].

The basis of the pathogenesis of LCH is the clonal proliferation of cells expressing markers CD1a and CD207[2] on their surface. The most likely source of these cells are myeloid progenitors, in which the MAPK pathway is overactivated due to different reasons[3]. The most common established reason for this is the substitution of glutamic acid for valine at position 600 of the *BRAF* gene. The influence of this gene and its product on the pathogenesis of LCH was established in 2010 by a group of researchers led by Gayane Badalian-Very[4]. According to various sources, from 38 to 64% of all LCH are caused by clonal proliferation of cells carrying the V600E mutation in the *BRAF* gene[5–8]. This mutation leads to the loss of the protein's ability to self-regulate and self-inhibit, which contributes to the uncontrolled division of pathological cells.

Upon further study, a theory of misguided myeloid precursor was elaborated. According to it the level of occurrence of the mutation determines the degree of dissemination of the lesion: when it appears at the level of cells migrating into the skin, an isolated skin form occurs, while a mutation at the level of CD34+cells leads to severe, multisystem forms[6].

To date, it is not fully known whether the presence of BRAF V600E mutation affects the prognosis in LCH. While some researchers have not noted a direct correlation [4, 6, 9], more recent studies have noted an increased risk of reactivation and resistance to first-line therapy[10].

Despite the fact that the five-year survival rate for SS and MS RO- patients is quite high (100 and 95%, respectively) [11, 12], the treatment of MS RO+ patients, especially those refractory to the first line of therapy, remains a significant problem as the survival rate drops to a depressing 40-50%[13]. The "salvage therapy" regimen with high-dose cytarabine and cladribine proposed for such patients turned out to be quite effective, but at the cost of high toxicity, which, together with a high initial premorbidity of this group of patients, limits its use[14].

Thus, the main problem in LCH is the choice of therapy for ultra-high-risk patients and those patients who, due to their infectious status or other somatic problems, cannot start high-dose therapy. In the presence of a V600E mutation in the BRAF gene, such patients are indicated for therapy with BRAF V600E inhibitors (vemurafenib or dabrafenib).

The first attempts of vemurafenib therapy were made in 2013 by the French group in adult patients with Erdheim-Chester disease and LCH[15]. According to the observations, the main features of the use of vemurafenib in LCH and other histiocytic pathologies are:

1) quick response to therapy;
2) achieving a "plateau" effect (keeping the signs of the disease at the same level) with long-term use of vemurafenib;
3) rapid reactivation of the disease upon discontinuation of vemurafenib;
4) absence of resistance phenomena when resuming therapy;
5) cardiac and skin toxicity (prolongation of the QTc interval, skin rash).

Further use of vemurafenib (including among children) confirmed these theses[16–18].

This protocol integrates the use of vemurafenib and low-dose cytarabine and cladribine, which have also proven themselves as therapy in high-risk patients, but are significantly less toxic than salavage regimen[19].

In Dmitry Rogachev National Research Center, vemurafenib was used in 5 patients with LCH. The M:F ratio was 4:1. The median onset of the disease was 3.2 months (1–8 months). All patients had liver, spleen, bone marrow, and skin involvement. Bones were affected in 60% of cases, lymph nodes - in 20%. The median of hepatomegaly was 4.8 cm (3–6 cm), the median of splenomegaly was 5.6 cm (3–8 cm). The median age at diagnosis was 10 months (5–22 months). Only 1 out of 5 patients was diagnosed at the age of over 1 year.

All patients received LCH-IV therapy. Three out of 5 patients received 1 or more high-dose therapy course. At the same time, 2 of them subsequently showed the progression of the underlying disease.

The mutation in the *BRAF* gene was determined by immunohistochemistry in a tumor biopsy (skin, bone lesion) and by PCR in a tumor biopsy and / or bone marrow aspirate by Sanger sequencing.

Vemurafenib was started because patients were refractory to salvage therapy or because of the presence of life-threatening infections as the risks of starting high-dose therapy were too high.

The starting dose of vemurafenib in all patients was 480 mg/day (2 pills a day). The median dose per kilogram was 43 mg/kg/day (37–50 mg/kg/day).

By + 30th day from the start of vemurafenib, all patients showed a response to therapy. All patients showed positive dynamics: in 4 out of 5 - restoration of hematopoiesis, reduction in the size of the liver (median reduction by 45% was 0–75%), spleen (median reduction by 48.4% was 0–80%), decrease in the size of soft-tissue bone lesions.

Side effects from the therapy were observed in 4 out of 5 patients. The main effects were cardiotoxicity (3 out of 4) in the form of prolongation of the QTc interval (up to a maximum of 0.5 s) and skin toxicity (2 out of 4). All side effects were mild and did not require pause or discontinuation of therapy.

III. Purpose of the study

To evaluate the safety and efficacy of combination of vemurafenib and cytarabine and cladribine in patients with LCH with BRAF V600E mutation.

IV. Endpoints and statistical analysis

The foreign data[18, 20], as well as our pilot study of the use of vemurafenib in the treatment of patients with LCH with BRAF V600E mutation showed that it is possible to achieve a faster response to therapy and a "plateau" effect in comparison with the standard regimens.

Primary endpoint

To evaluate ORR which is defined as the sum of the values of partial and complete responses. A complete response will be defined as a decrease in DAS 0–1 point after 16 weeks from the start of therapy. A partial response will be defined as a decrease in DAS after 16 weeks from the start of therapy.

Secondary endpoints:

1. Reactivation-free / progression-free survival is defined as the time from initiation of therapy to reactivation, progression, death from any cause or date of last examination for censored patients without events at the time of analysis. Survival will be calculated using the Kaplan – Mayer method with a 95% CI.

2. Evaluation of the proportions of patients with severe side effects. Severe side effects will include skin lesions and grade III – IV cardiac conduction abnormalities according to CTCAE (version 4.0). The toxicity assessment will be carried out once at the highest reported grade.

3. Overall survival is calculated from the date of initiation of therapy to the date of death from any cause or to the date of last contact. The calculation by the Kaplan-Mayer method is performed 2 years after the start of therapy with a 95% CI.

In accordance with the available experience, it is planned to include 10 patients with LCH with BRAF V600E per year.

Given the potentially small number of enrolled patients, the calculations are based on the adaptive two-step Simon method[21, 22]. An alternative hypothesis is formulated as follows: the number of patients with an overall response in this study will be higher compared with historical data, where the response rate to therapy is less than 30%[23, 24], with an expected ORR of 65–70%.

Thus, when $p_0 = 0.25$, $p_1 = 0.65$, $p_2 = 0.7$, $\alpha = 0.10$, $\beta_1 = 0.2$, $\beta_2 = 0.1$;

1. The required number of patients for the first stage of the study is 5.

2. If no patient responds to the therapy at that point, the study will prove the null hypothesis and will be stopped.

3. If the number of patients with ORR is from 1 to 4, the study will proceed to the second stage and up to 14 additional patients will be recruited to confirm the

alternative hypothesis. And when the ORR is reached in more than 6 patients, the alternative hypothesis will be considered confirmed.

4. If the number of patients with ORR at the first stage is 5, the study will proceed to the second stage and up to 7 additional patients will be recruited to confirm the alternative hypothesis. And when the ORR is reached in more than 5 patients, the alternative hypothesis will be considered confirmed.

Stage 1			Stage 2 (ORR $\leq r_1$)		Stage 2 (ORR $> r_1$)	
s_1	r_1	n_1	s	m	r	n
0	4	5	6	14	5	7

Thus:

1. The first interim analysis will be performed after 16 weeks after the enrollment of the 5th patient.

2. Conditions for premature termination of the study - failure to achieve a response to therapy in the first 5 patients.

Study start date: March 2018

Estimated end of the study date: March 2020.

V. Inclusion criteria

1. Age 0-18 years.

2. Histologically verified diagnosis of LCH (CD1a + / CD207 +).

3. Verified BRAF V600E mutation in the lesion and /or in the CD34 + isolate proven by RT-PCR or IHC.

4. QTc interval <0.5 s.

5. No documented heart disease.

6. Signed informed consent.

N.B! In life-threatening conditions, BRAFi therapy can be without verification of the mutation at all. In the absence of clinically significant efficacy during therapy with vemurafenib within 7 days, it is recommended to interrupt therapy. If a negative BRAF V600E result is obtained, therapy should also be interrupted.

VI. Exclusion criteria

1. Revocation of signed informed consent.

2. QTc> 0.5 s or the presence of long QT syndrome.

3. Intake of antiarrhythmic drugs.

4. Uncorrectable electrolyte disorders

Study design

The initial assessment of the patient's status occurs on the 0 day (before the therapy start, MRD point 0).

The therapy protocol begins with a 28-day vemurafenib induction without the use of other chemotherapeutic agents. All patients receive vemurafenib without randomization with a starting dose of approximately 20 mg / kg / day orally, rounded to the whole capsule (240 mg).

On the 28th day, the efficacy of vemurafenib therapy is assessed (MRD point 1) and the drug is discontinued simultaneously with the start of Ara-C + 2-CdA №1 course.

Vemurafenib therapy is resumed on day + 1 after Ara-C + 2-CdA course. The interval between courses is 28 days.

Before starting the mono 2-CdA courses, the efficacy of the therapy is assessed (MRD point 4) with bone marrow aspiration and detection of BRAF V600E allele load in FACS CD34+CD117+ cells using ddPCR. Vemurafenib therapy is stopped completely, further courses of 2-CdA monotherapy are given at intervals of 28 days.

Disease activity score (DAS) is used to assess the efficacy of therapy (see Appendix 1).

After the end of therapy, the assessment of disease status is carried out according to the recommendations (see Appendix 2).

Ara-C + 2-CdA course:

-   Cytarabine 100 mg/m^2/every 12 h with 1h i.v. infusion, days 1–5
-   2-chloro-2'-deoxyadenosine (cladribine) 6 mg/m^2/day with 1h i.v. infusion, days 1–5

2-CdA course:

-   2-chloro-2'-deoxyadenosine (cladribine) 6 mg/m^2/day with 1h i.v. infusion, days 1–5

Supportive therapy:

-   NaCl 0.9% and glucose 5% i.v. infusions 1500 ml/m^2/day;
-   antifungal prophylaxis: micafungin or caspofungin at an age-appropriate dosage for the period of aplasia. Prophylaxis with azole drugs is not recommended due to the high risk of hepatotoxicity;
-    Pneumocystic pneumonia prophylaxis: sulfamethoxazole/trimethoprim at a dose of 5 mg/kg for trimethoprim 3 times a week;
-   GSCF if granulocyte level is below 500/mcl.

VII. Monitoring

Pre-treatment studies:

1. Peripheral blood

• CBC + leukocyte count on blood smears;

• biochemical blood test: bilirubin, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, gamma-glutanyl transpeptidase, alkaline phosphatase, creatinine, urea, albumin, total protein;

• assessment of the levels of troponin I, BNP, creatine phosphokinase, MB-KFK;

• immunophenotypic study of peripheral blood with the relative and absolute amount of CD3 +, CD4 +, CD8 +, CD56 +;

• serum immunoglobulins (classes A, G and M).

2. Examination of the bone marrow

• bone marrow aspiration from 2 anatomically different points;

• morphological examination of the bone marrow;

• identification of BRAF V600E mutation in bone marrow aspirate by a method with a validated sensitivity of at least 5%;

• isolation of CD34 +CD117+ cells in bone marrow aspirate, identification of BRAF V600E mutation in this cell line with a validated sensitivity of at least 5%.

3. Specific studies

• biopsy of the affected lesion (bone, skin, lymph node, etc.) with subsequent morphological and immunohistochemical examination (including BRAF);

• molecular analysis of a biopsy specimen for BRAF V600E mutation.

4. **Virological study**

• PCR of peripheral blood for HBV, HCV, HIV, CMV, HHV-VI, EBV (according to indications);

• determination of circulating antibodies (IgM and IgG) to CMV, EBV.

5. **General urine test** (in case of a decrease in relative density – Zimnitsky urine test).

6. **Radiography** of all bones or PET combined with computed tomography, or total body MRI.

7. **Brain MRI** in T1, T2, FLAIR modes using gadolinium contrast (if there is a suspicion of central nervous system lesions).

8. **Computed tomography of the chest organs** - for all patients, abdominal organs with contrast enhancement - according to indications.

9. **Bronchoscopy** with bronchoalveolar lavage and its cytological and microbiological examination (bacteriological culture, determination of CMV, EBV, HHV6, Pneumocystis, Rhinovirus, Adenovirus by PCR method) - according to indications.

10. **Ultrasound** examination of the abdominal organs.

11. **Electrocardiography**, echocardiography, consultation with a cardiologist.

Laboratory and instrumental monitoring during the study and follow-up

1. **CBC**: automatic blood test - during the period of aplasia at least 1 time in 3 days, hemogram with the manual calculation of the leukocyte formula - 1 time per month.

2. **Biochemical analysis of blood**: the first 2 weeks of taking vemurafenib - at least 2 times a week, then at the discretion of the attending physician.

3. **Electrocardiography**: in the first month of vemurafenib therapy - at least 1 time per week, then at the discretion of the attending physician, but at least 1 time per 1 month.

4. **Echocardiography**: in the first month of vemurafenib therapy - at least 1 time in 2 weeks, then at the discretion of the attending physician, but not less than 1 time in 1 month.

5. **Evaluation of markers of myocardial injury**: creatine phosphokinase, troponin I, atrial natriuretic peptide - on vemurafenib therapy at least 1 time/month.

Criteria for stopping vemurafenib therapy:

• Intolerance/hypersensitivity to vemurafenib.

• Withdrawal of signed informed consent.

• Development of grade IV toxicity associated with the use of vemurafenib.

• Progression of the underlying disease during therapy with vemurafenib.

N.B! The main reason for stopping therapy is the cardiac toxicity of vemurafenib. The criteria for discontinuation of therapy are the prolongation of the QTc interval >0.5 s, uncontrolled arterial hypertension, and the development of cardiomyopathy.

Toxicity

To describe the frequency of adverse reactions, the following classification is used: very often ($\geq$10%), often ($\geq$1% and <10%), infrequently ($\geq$0.1% and <1%), rarely ($\geq$0.01% and < 0.1%), very rarely (<0.01%).

The most common adverse reactions (>30%) with vemurafenib were arthralgia, fatigue, rash, photosensitivity reaction, nausea, diarrhea, alopecia, pruritus, and skin papilloma. Very common cases of squamous cell carcinoma of the skin have been reported, and treatment has generally been surgical.

Benign, malignant, and unspecified neoplasms (including cysts and polyps): very often - squamous cell carcinoma of the skin, seborrheic keratosis, skin papilloma; often - basal cell carcinoma, new primary melanomas; infrequently - squamous cell carcinoma of non-cutaneous localization.

Metabolic disorders: very often - loss of appetite, weight loss.

Nervous system disorders: very often - headache, dysgeusia (distortion of taste perceptions), peripheral neuropathy; often - paralysis of the facial nerve, dizziness.

Organs of vision disorders: often - uveitis, including iritis; infrequently - occlusion of the retinal veins.

Vascular disorders: infrequently - vasculitis.

Respiratory, thoracic and mediastinal disorders: very common - cough.

GI tract disorders: very often - diarrhea, vomiting, nausea, constipation.

Skin and subcutaneous tissue disorders: very common - photosensitivity reaction, actinic keratosis, rash, maculopapular and papular rash, pruritus, hyperkeratosis, erythema, alopecia, dry skin, sunburn, palmoplantar erythrodysesthesia syndrome; often - panniculitis, including erythema nodosum, follicular keratosis, folliculitis; infrequently - toxic epidermal necrolysis, Stevens-Johnson syndrome.

Musculoskeletal and connective tissue disorders: very often - arthralgia, myalgia, pain in the extremities and back, musculoskeletal pain, arthritis.

Other: very often - fatigue, fever, peripheral edema, asthenia.

Laboratory and instrumental work-up: very often - an increase in the activity of gammaglutamyl transpeptidase (Grade III or IV severity), creatinine concentration increase (including 1.2% of cases of Grade III or IV severity); often - increased activity of alanine aminotransferase (Grade III), alkaline phosphatase (Grade III), bilirubin concentration (Grade III); infrequently - increased activity of aspartate aminotransferase (Grade III or IV severity).

MRD evaluation

An important goal of the protocol is to evaluate the MRD. Currently, there is no validated method of MRD measurement for LCH, however, the work of Marie-Louis Berres (2014) directly indicates a correlation between the activity of the underlying disease and the presence in the blood of cell-free BRAF V600E DNA[6]. In addition, during the treatment and a decrease in the clinical

signs of the disease, a decrease in the amount of BRAF V600E cfDNA was noted[25]. Thus, summarizing the data, it can be assumed that for patients with a proven BRAF V600E mutation, it is possible to measure the MRD to assess the effectiveness of therapy and predict relapses of the disease.

In this study, the days immediately before the polychemotherapy blocks were chosen as control points for the collection of blood. In these samples, the mutant DNA load will be measured using the digital droplet PCR technique, which has proven itself in the search for other mutations in cfDNA[26]. At points 0–6, it is planned to collect 5–9 ml of peripheral blood into test tubes. In addition, a bone marrow puncture is planned at point 4 in order to isolate a CD34+CD117+ cell line, in which it is planned to search for BRAF V600E mutation with a validated sensitivity of at least 5%. This study is designed to support the hypothesis that the progenitor cell in multisystem LCH with risk organs involvement is a CD34+CD117+ myeloid cell[6,27]. This is followed by PCR and direct Sanger sequencing for BRAF V600E in this cell line.

Control of vemurafenib concentration in peripheral blood in patients receiving protocol therapy

Currently, vemurafenib is a widely used agent in patients with metastatic melanoma. In the example of this cohort of patients, some pharmacokinetic and pharmacodynamic features were determined:

- Plasma concentration of vemurafenib greater than 40.4 mg/l on the 15th day of therapy is associated with better overall survival and progression-free survival;

- the same concentration is associated with more frequent side effects on the skin;

- no other correlation of vemurafenib plasma concentrations with side effects was found 28.

However, such studies have not been conducted for pediatric patients and those with LCH. In this regard, we intend to measure the concentration of vemurafenib in blood plasma on the 14th day of administration. The concentration will be measured 30 minutes, 1, 2, 4, and 8 hours after taking the drug.

VIII. References:

1.   Haupt R, Minkov M, Astigarraga I, et al. Langerhans cell histiocytosis (LCH): guidelines for diagnosis, clinical work-up, and treatment for patients till the age of 18 years. *Pediatr Blood Cancer* 2013; **60**(2): 175-84.
2.   Bechan GI, Egeler RM, Arceci RJ. Biology of Langerhans cells and Langerhans cell histiocytosis. *Int Rev Cytol* 2006; **254**: 1-43.
3.   Collin M, Bigley V, McClain KL, Allen CE. Cell(s) of Origin of Langerhans Cell Histiocytosis. *Hematol Oncol Clin North Am* 2015; **29**(5): 825-38.
4.   Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. *Blood* 2010; **116**(11): 1919-23.
5.   Satoh T, Smith A, Sarde A, et al. B-RAF mutant alleles associated with Langerhans cell histiocytosis, a granulomatous pediatric disease. *PLoS One* 2012; **7**(4): e33891.
6.   Berres ML, Lim KP, Peters T, et al. BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. *J Exp Med* 2014; **211**(4): 669-83.
7.   Haroche J, Charlotte F, Arnaud L, et al. High prevalence of BRAF V600E mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses. *Blood* 2012; **120**(13): 2700-3.
8.   Bubolz AM, Weissinger SE, Stenzinger A, et al. Potential clinical implications of BRAF mutations in histiocytic proliferations. *Oncotarget* 2014; **5**(12): 4060-70.
9.   Sahm F, Capper D, Preusser M, et al. BRAFV600E mutant protein is expressed in cells of variable maturation in Langerhans cell histiocytosis. *Blood* 2012; **120**(12): e28-34.
10.   Heritier S, Emile JF, Barkaoui MA, et al. BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy. *J Clin Oncol* 2016.
11.   Titgemeyer C, Grois N, Minkov M, Flucher-Wolfram B, Gatterer-Menz I, Gadner H. Pattern and course of single-system disease in Langerhans cell histiocytosis data from the DAL-HX 83- and 90-study. *Med Pediatr Oncol* 2001; **37**(2): 108-14.
12.   Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. *Blood* 2013; **121**(25): 5006-14.
13.   Hutter C, Minkov M. Insights into the pathogenesis of Langerhans cell histiocytosis: the development of targeted therapies. *Immunotargets Ther* 2016; **5**: 81-91.
14.   Bernard F, Thomas C, Bertrand Y, et al. Multi-centre pilot study of 2-chlorodeoxyadenosine and cytosine arabinoside combined chemotherapy in refractory Langerhans cell histiocytosis with haematological dysfunction. *Eur J Cancer* 2005; **41**(17): 2682-9.
15.   Haroche J, Cohen-Aubart F, Emile JF, et al. Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation. *Blood* 2013; **121**(9): 1495-500.
16.   Haroche J, Cohen-Aubart F, Emile JF, Donadieu J, Amoura Z. Vemurafenib as first line therapy in BRAF-mutated Langerhans cell histiocytosis. *J Am Acad Dermatol* 2015; **73**(1): e29-30.
17.   Haroche J, Cohen-Aubart F, Emile JF, et al. Reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAF(V600E)-mutated Erdheim-Chester disease. *J Clin Oncol* 2015; **33**(5): 411-8.
18.   Heritier S, Jehanne M, Leverger G, et al. Vemurafenib Use in an Infant for High-Risk Langerhans Cell Histiocytosis. *JAMA Oncol* 2015; **1**(6): 836-8.
19.   Rosso DA, Amaral D, Latella A, Chantada G, Braier JL. Reduced doses of cladribine and cytarabine regimen was effective and well tolerated in patients with refractory-risk multisystem Langerhans cell histiocytosis. *Br J Haematol* 2016; **172**(2): 287-90.
20.   Shan G, Wilding GE, Hutson AD, Gerstenberger S. Optimal adaptive two-stage designs for early phase II clinical trials. *Stat Med* 2016; **35**(8): 1257-66.
21.   Lin Y, Shih WJ. Adaptive two-stage designs for single-arm phase IIA cancer clinical trials. *Biometrics* 2004; **60**(2): 482-90.
22.   Gadner H, Grois N, Arico M, et al. A randomized trial of treatment for multisystem Langerhans' cell histiocytosis. *J Pediatr* 2001; **138**(5): 728-34.
23.   Minkov M, Grois N, Heitger A, et al. Response to initial treatment of multisystem Langerhans cell histiocytosis: an important prognostic indicator. *Med Pediatr Oncol* 2002; **39**(6): 581-5.
24.   Hyman DM, Diamond EL, Vibat CR, et al. Prospective blinded study of BRAFV600E mutation detection in cell-free DNA of patients with systemic histiocytic disorders. *Cancer Discov* 2015; **5**(1): 64-71.

25. Zhu G, Ye X, Dong Z, et al. Highly Sensitive Droplet Digital PCR Method for Detection of EGFR-Activating Mutations in Plasma Cell-Free DNA from Patients with Advanced Non-Small Cell Lung Cancer. *J Mol Diagn* 2015; **17**(3): 265-72.

26. Milne P, Bigley V, Bacon CM, et al. Hematopoietic origin of Langerhans cell histiocytosis and Erdheim-Chester disease in adults. *Blood* 2017; **130**(2): 167-75.

27. Kramkimel N, Thomas-Schoemann A, Sakji L, et al. Vemurafenib pharmacokinetics and its correlation with efficacy and safety in outpatients with advanced BRAF-mutated melanoma. *Target Oncol* 2016; **11**(1): 59-69.

Supplementary 2. The detailed description of methods.

Cell-free ddPCR

Minimal residual disease (MRD) was measured at specific predetermined points (initially, before the start of each course, and at the end of the therapy) using digital droplet PCR (ddPCR) on blood-derived cell-free DNA (cfDNA). Blood samples were collected in 10-mL PlasmaProtect tubes (Evrogen, Russia) designed to prevent cellular DNA from being released into the plasma. After centrifugation and plasma collection, cfDNA extraction was performed using the QIamp Circulating Nucleic Acid Kit (Qiagen, Germany). The PCR mixtures with two probes (wild-type BRAF exon 15 and V600E-specific) designed by Bio-Rad (USA) were processed using a QX200 droplet generator with AutoDG (Bio-Rad, USA) for droplet generation and a QX200 (Bio-Rad, USA) for droplet counting. The results were analyzed using QuantaSoft software (Bio-Rad, USA). At specific predetermined points, we performed bone marrow (BM) aspiration. Isolation of the CD34+CD117+ population was performed, and ddPCR was used to measure the BRAF V600E allelic load. Isolation of myeloid progenitor cells was performed on freshly collected BM samples. The antibody panel used for staining consisted of CD34-ECD, CD117-PC5.5, HLA-DR-PacificBlue, CD45-KromeOrange, and CD1a-PE antibodies (all from Beckman Coulter, BC, Indianapolis, IN, US). Before staining, all samples were treated with fixative-free erythrocyte lysis buffer (Pharm Lyse, BD). Then, optimized concentrations of appropriate antibodies were added to the cell suspensions. The lysing and staining procedures were performed per the technical protocol of BD Biosciences.

The sensitivity of ddPCR varied depending on the DNA input. The median input of cfDNA per reaction was 6.2ng (94 copies/µl (8.7-1013)). The sensitivity threshold of ddPCR was determined for each sample individually, according to the manufacturer's guideline, which reflects the correspondence between the amount of DNA input and the sensitivity of the method. The median

sensitivity threshold was 0.16% (0.05%-1.7%). All results that were lower than an individually calculated sensitivity threshold were considered below the level of detection.

Myeloid progenitors gating strategy and ddPCR output

In brief, the immunophenotype of myeloid progenitor cells was defined as CD45+CD34+CD117+HLA-DR+. Flow cell sorting was performed on a FACS Aria III or FACS Melody flow cell sorter (both from BD) in "Purity" sort mode. Cells were collected into Eppendorf tubes with CellWASH buffer (BD). At least 10000 myeloid progenitors were isolated in each case. After sorting, the cell suspensions were immediately frozen at -80 °C and stored for further analysis.

The median number of sorted CD34+CD117+ cells was 40000 (7000-223000), the median amount of isolated DNA that was used as an input was 88.6ng (1343 copies/µl (376-5120)). The sensitivity threshold was also determined for each sample individually and the median was estimated as 0,011% (0,003%-0,04%). All results that were lower than an individually calculated sensitivity threshold are considered as 'below level of detection'.

Vemurafenib serum concentration detection methods

The serum concentration of vemurafenib was quantified by high-performance liquid chromatography–mass spectrometry (HPLC–MS). A Shimadzu LCMS-8030 mass spectrometer with an LC-20AD pump, a SIL-20A autosampler, and CTO-20A column oven was used. The separation of components from biological samples (venous blood) was carried out on a C18(2) Phenomenex Luna® 250*4,6 mm column with an internal diameter of 5 µm and a sorbent pore diameter of 100 Å and with a Security Guard 4.0x3.0 C18 cartridge system at 45 °C. As a mobile phase, we used a gradient of 2 solutions: A – 94.9% Milli-Q water, 5% acetonitrile, and 0.1%

formic acid; and B – 100% acetonitrile. The mixture components were identified in positive ionization mode. The vemurafenib concentration was evaluated by external standards using calibration solutions ranging from 100 ng/ml to 30 µg/ml.