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The influence of *GSTP*1 *A*313*G* polymorphism on susceptibility, chemotherapy-related toxicity and prognosis of Hodgkin's lymphoma in Ukrainian patients

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*The **aim** of this research was to study the influence of GSTP1 A313G polymorphism on susceptibility, chemotherapy-related toxicity and prognosis of Hodgkin's lymphoma in Ukrainian patients. **Methods**. The polymorphic variants of GSTP1 gene were analyzed using Allelic Discrimination Real-Time PCR. **Results**. The GSTP1 polymorphism is not directly involved in the development of Hodgkin's lymphoma and chemotherapy-related toxicity, but homozygous wild genotype of this gene is associated with a worse clinical response to the therapy and a higher risk of relapse. **Conclusions**. The investigation of GSTP1 polymorphism is very promising, since it might provide a possible application of this genetic marker as an independent prognostic factor of Hodgkin's lymphoma.*

Keywords: Hodgkin's lymphoma, polymorphism, GSTP1 gene.

Introduction. Hodgkin's lymphoma (HL) represents about 0.5–1 % of all malignancies and about 30 % of all lymphomas in the residents of developing countries. The morbidity of HL has two age-specific peaks: the first comprises the age between 15 and 35 years, while the second one is observed after the age of 50–60 years. In the age category of 15–24 years every sixth oncological diagnosis is HL [1, 2]. In Ukraine there is a steady upward trend in the incidence of malignant lymphomas, the rate of growth ranges from 4.2 % to 8.1 % in different regions of Ukraine [3].

Over more than 150-year history since the description of the first cases of HL till today scientists have faced many difficulties and contradictories from the recognition of absolute fatality of the disease to its potential curability. Even the first authors, who described HL, paid much attention to the diversity of clinical course

of the disease – from quick generalization, which led to the patient's death in several months, to the torpid period that could last for 20 years without treatment. The advancement of HL treatment is actually determined by the differential approach to the treatment in different risk groups distinguished by unfavorable prognostic factors. Modern risk-adapted treatment regimens for HL lead to good control of the disease and high cure rate for the most patients [4].

However, among the patients with different prognostic scores the treatment of about 10–20 % still has low efficiency. In addition, new aggressive treatment programs are highly toxic. Hence, identification of new biological and clinical markers that could help to select patients with a high risk of the treatment failure, remains a crucial challenge. The pharmacogenetics study indicates that even a tiny diversity in sequence of genome significantly influences the individual treatment response, toxicity, and survival of the HL patients. Germline poly-

morphisms in genes that code for the enzymes, involved in the pharmacodynamics of anticancer agents, are common and may result in altered drug pharmacokinetics [5, 6]. Current treatment regimens for HL include nitrogen mustards, anthracyclines, vinca alkaloids, and epipodophylotoxins, which are metabolized by enzymes of the glutathione S-transferase (GST) system. Glutathione-S-transferase P1 (GSTP1) is a member of the GST enzyme superfamily. *GSTP*1 gene is polymorphic that is characterized by adenine (A) to guanine (G) transition of nucleotide 313 in exon 5 (A313G), which causes isoleucine (Ile) to valine (Val) substitution at position 105 of the GSTP1 enzyme (Ile105Val), resulting in three possible genotypes: 313 *A*/*A*, 313 *A*/*G* or 313 *G*/*G* [7]. The proteins encoded by different alleles of the gene, show different abilities to metabolize carcinogens and anticancer agents. Watson et al. have shown that the individuals with two *GSTP*1 313 *G* alleles have lower catalytic activity when compared with the individuals possessing two *GSTP*1 313 *A* alleles, whereas an intermediate activity was reported for the heterozygotes [8].

Therefore, genetic polymorphism of *GSTP*1 could be essential in the determination of susceptibility to the toxic effects of chemicals and might also be involved in the tumor response to anticancer drugs and influence the clinical outcome [9].

In this report we present the results of the study on the influence of *GSTP*1 *A*313*G* polymorphism on the susceptibility, chemotherapy-related toxicity and prognosis of HL in Ukrainian patients.

Materials and methods. *Patients*. The case group comprised 97 patients with HL (median age: 31 years, range: 17–62; males: 34, females: 63) treated in the Oncohematological Department of National Cancer Institute from September 2008 to May 2011. HL was diagnosed according to the World Health Organization (WHO) classification, 2008. The patients were categorized by the Ann Arbor staging system and the International Prognostic Score. Anthracycline-based chemotherapy: ABVD (adriamycin/doxorubicin, bleomycin, vinblastine, dacarbazine) or BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, oncovin/vincristin, procarbazine, prednisone) were administered as a first-line therapy for all patients enrolled in this study. According to the randomization, the patients with stages IIB and III–IV received BEACOPP-14 or BEACOPP-esc. Radiotherapy was used to treat residual masses of bulky disease for stages III–IV and all injured areas for stages IIA–IIB. The treatment efficacy was estimated according to the Cheson criteria. Toxicity rate was evaluated with NCI-CTC, V4.0.

A control group included 158 healthy individuals (median age: 38 years, range: 19–71; 70 males, 88 females) with a medical history negative to any type of cancer and was not related to the patients.

An informed consent was obtained from both groups according to the requirements of the Ethical Commission of the National Cancer Institute.

DNA isolation. The blood samples of all individuals were collected in citric acid/EDTA anticoagulation tubes. Plasma was separated from whole blood by centrifugation at 350 *g* for 10 min and stored at –70 °C. Genomic DNA was isolated from the plasma samples using QIAamp® DNA Blood mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations and stored at –70 °C.

Genotype determination. GSTP1 genotypes (c. 313 *A* > > *G*, p. Ile105Val) were determined using Allelic Discrimination Real-Time PCR. Primers and TaqMan-MGB probes were designed using Assay-by-Design service («Applied Biosystems», USA): forward primer: 5'-CCTGGTGGACATGGTGAATG-3'; reverse primer: 5'-TGGTGCAGATGCTCACATAGTTG-3'; 313 G probe FAM-CTGCAAATACGTCTCC-MGB; 313 A probe VIC-TGCAAATACATCTCC-MGB. PCR amplification was performed in a volume of 25 μl containing 10 ng of genomic DNA, 12.5 μl 2X Taqman Universal PCR Master Mix («Applied Biosystems»), 200 nM probe, and 900 nM primer.

Cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles at 92 °C for 30 s and 60 °C for 1 min. The fluorescence emission was recorded using ABI Prism 7500 Sequence Detection System («Applied Biosystems»); end point plate read measurements allowed performing allelic discrimination using Sequence Detection System software («Applied Biosystems»).

Statistical analysis. The Hardy-Weinberg equilibrium was tested with chi-square statistic for the goodness-to-fit (one degree of freedom). Multivariate analysis was performed to assess the associations between

Patients characteristics

Variable	Patients	Variable	Patients
Age at diagnosis (years)		Ann Arbor stage	
< 45	72	IA–IIA	33
> 45	15	IIB–IV	64
Gender		B symptoms	
Male	34	No	47
Female	63	Yes	52
Histotype		Bulky disease	
cHL:		No	49
– nodular sclerosis	40	Yes	58
– mixed cellularity	31	Complete response after 1st line treatment	
– lymphocyte rich	7	Yes	75
– lymphocyte depletion	8	No	22
Nodular lymphocyte- predominant HL	2	Relapse from complete response	
Indefinite	9	Yes	18
–	–	No	57

*GSTP*1genotypes and HL. Odds ratios (OR) and 95 % confidence intervals (95 % CI) were used to estimate the risk of HL development and different grades of toxicity after chemotherapy. The clinical characteristics, treatment outcomes and toxicities in relation to the *GSTP*1 polymorphism were compared among the patients using chi-square, Fisher's exact tests, correlation analyses (Spearman and gamma coefficients). The value was considered as significant at $p < 0.05$. All computations were done using the STATISTICA 6.1. software (StatSoft, Inc., USA).

Results and discussion. *Characteristics of the patients.* The cohort consisted of 97 patients with HL. The clinical features of the patients are presented in Table.

For 97 patients the overall response rate was 91.7 % (89/97) with a complete response of 77 % (75/97) and a partial response – 13.5 % (14/97). Among the patients who achieved a complete response during the follow-up (median duration – 18 months; range 6–36 months), 18 had relapses with disease-free period 3–6 months (3 patients), 6–12 months (9 patients) and 12–24 months (6 patients).

During the anthracycline-based chemotherapy the patients developed some degree of hematologic (HT) and nonhematologic (NHT) toxicity (gastrointestinal, cardiovascular, hearing, nephro-, neurotoxicity). Among the patients who received ABVD, severe HT and NHT (grade 3–4) developed in 14 and 6 % of the cases respectively. The predominant HT types were anemia and neutropenia. For the patients who received BEACOPP-14 severe HT and NHT (grade 3–4) developed in 88 % and 24 % of the cases respectively, BEACOPP-esc – 87 % and 19 %. The special feature of BEACOPP-14 treatment was a significantly higher rate of anemia: 25 % compared with 12.5 % in the group of BEACOPP-esc. The most frequent NHT for all patients were nausea and vomiting.

*GSTP1 polymorphism. GSTP*1 genotype was assessed for 97 patients with Hodgkin's lymphoma, 46 (47.4 %) of which were homozygous of the *A/A GSTP*1 genotype, 38 (39.2 %) were heterozygous (*A/G*), 13 (13.4 %) were homozygous of the *G/G genotype*; and for 158 healthy blood donors, 76 (48.1 %) of which were homozygous of the *A/A GSTP1* genotype, 63
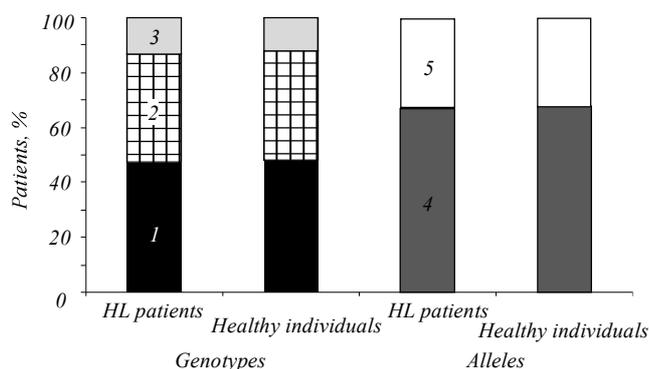
Fig. 1. *GSTP*1 genotypes and alleles distribution for the HL patients and healthy Ukrainian: *1 – G/G*; *2 – A/G*; *3 – A/A*; *4 – A*; *5 – G*
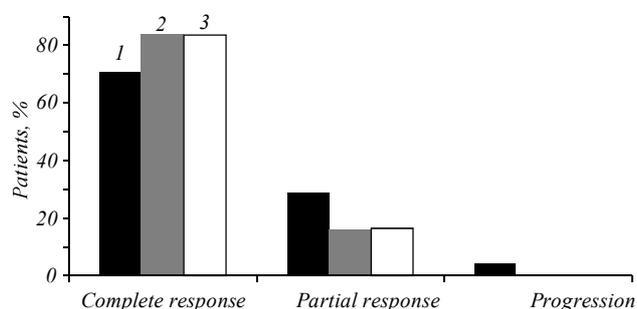

Fig. 2. *GSTP*1 genotype and clinical response to the therapy in Hodgkin's lymphoma patients: *1 – A/A*; *2 – A/G*; *3 – G/G*

(39.9 %) – heterozygous (*A/G*), 19 (12 %) – homozygous of the *G/G* genotype.

The distribution of the *GSTP*1 genotypes in both control and studied patient groups did not differ significantly from that predicted by the Hardy-Weinberg distribution. The observed G allele frequency was 0.32, similarly to the previous reports on allele frequencies for healthy Caucasians [10, 11]. The obtained results suggest that the *A*313*G* polymorphism of the *GSTP1* gene is not directly involved in the development of HL.

*GSTP*1 genotypes and alleles distribution for the HL patients and healthy Ukrainian are presented in Fig. 1.

We did not notice the association between demographic (age and gender), clinical and pathologic characteristics, including histotype, the stage of the disease, the presence of B symptoms, bulky disease characteristics of the patients with *GSTP*1 genotypes.

Significant relationship between chemotherapy-related toxicity for the patients with Hodgkin's lymphoma and *GSTP*1 genotype was not revealed, although for the patients with *G/G* genotype severe hematological toxicity was slightly more often.

We have observed a correlation between *GSTP*1 polymorphism and prognosis of HL: *A/A* genotype of *GSTP*1 gene can be considered as an independent unfavorable prognostic factor of HL. A complete response rate after the first-line therapy was better for the patients with the *A/G* or *G/G* genotype in comparison with the patients with homozygous wild genotype (84 % (43/51) versus 70 % (32/46), p = 0.066, correlation analyses, gamma coefficient). Progression of the disease during the therapy was observed for 4 % (2/46) of the patients with the *A/A* genotype of *GSTP*1 gene, while it was not noticed for the other genotype (Fig. 2).

We also analyzed how *GSTP*1 polymorphism influences the chemotherapy response and the disease prognosis for the patients grouped according to the stage (Fig. 3, 4).

As shown in Fig. 3, among the patients with the stages IA–IIA, who received ABVD as the first-line therapy, the complete response was achieved in 92.3 % (12/13) of the cases with *A/G* + *G/G* genotypes and only in 65 % (13/20) – with *A/A*. Moreover, we did not notice the Hodgkin's lymphoma progression during the therapy for the patients with *G/G* and *A/G* genotypes, while the patients with *A/A* have shown the progression in 10 % (2/20) of the cases. The frequency of the disease relapse was almost twice higher for the patients with homozygous wild genotype (30 % (6/20) *A/A* versus 16 % (2/13) *A/G* + *G/G*).

For the Hodgkin's lymphoma patients with stages IIB + (III–IV) (Fig. 4), who received BEACOPP (14/ esc) as the first-line therapy, the complete response was achieved in 82 % (31/38) of the cases with *A/G* + *G/G* genotypes and in 73 % (19/26) with *A/A*; the partial response – in 16 % (6/38) of the cases with *A/G* + *G/G* genotypes and in 23 % (6/26) with *A/A*. The frequency of the disease relapse was significantly higher for the patients with homozygous wild genotype (27 % (7/26) *A/A* versus 8 % (3/38) *A/G* + G/*G*, p = 0.003, correlation analyses, gamma coefficient).

The obtained results suggest that the *A*313*G* polymorphism of the *GSTP*1 gene is not directly involved in the development of HL and chemotherapy-related toxicity, but homozygous wild genotype of this gene (*A/A*) is associated with a worse clinical response to the therapy and a higher risk of the relapse for the HL patients independently from the stage of the disease.
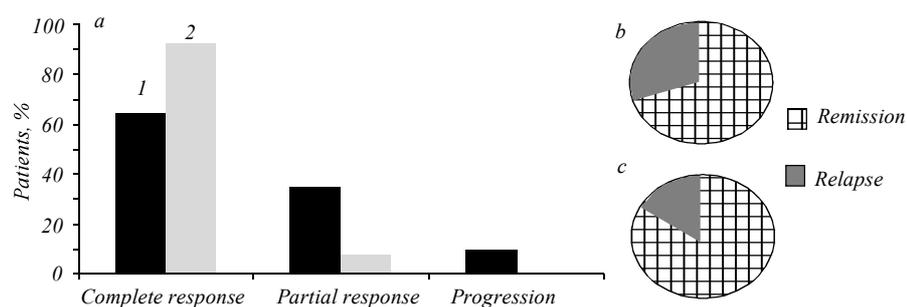
Fig. 3. Clinical response to the therapy (*a*: *1* – *A/A*; *2* – *A/G* + *G/G*) and prognosis of the disease (*b*, *c*) for the patients with Hodgkin's lymphoma stage IA–IIA according to *GSTP*1 genotype (*b* – *A/A*; *c* – *A/G* + *G/G*)
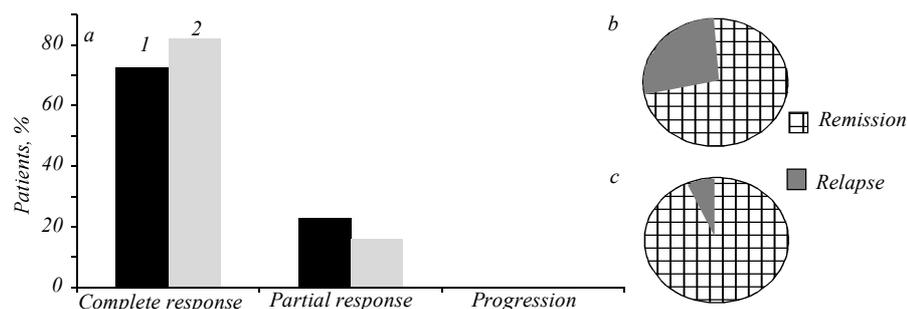
Fig. 4. Clinical response to the therapy (a: *1* – *A/A*; *2* – *A/G* + *G/G*) and prognosis of the disease (*b*, *c*) for the patients with Hodgkin's lymphoma stages IIB–IV according to *GSTP*1 genotype (*b* – *A/A*; *c* – *A/G* + *G/G*)

We assume that the association of *A/A* with unfavorable prognosis of HL may be attributed to a higher activity of the wild allele towards anticancer agents metabolism, as well as to its higher effect on the favouring tumor cell survival when compared with the variant allele, as previously reported [8]. Recently, Holley et al. have demonstrated that the wild allele protects the cells against apoptosis through a JNK-independent mechanism [12].

The prognostic impact of *GSTP*1 polymorphisms appears to vary according to the cancer type. Recently, the *GSTP*1 313 *G/G* genotype has been associated with a favorable prognosis following chemotherapy with the drugs known to be GSTP1 substrates in a variety of malignancies, such as pediatric acute lymphoblastic leukemia, myeloma, breast and colon cancer [6, 13–15]. The influence of *GSTP*1 polymorphism on the effectiveness of HL therapy has been previously analyzed by other authors with contradictory results [16–19]. The main difference has been associated with the different presentation of HL stages and clinical outcome of the patients.

Our results are in line with the previous reports of Italian researchers, which noticed the association of *GSTP*1 homozygous wild genotype with unfavorable outcome of HL patients [16, 17]. At the same time, some authors did not find any correlation between *GSTP*1 polymorphism and HL prognosis in Caucasian patients

[6]. The contradictive results might be due to the fact that small cohorts of patients have been analyzed along with the socioeconomic factors and late diagnosis in some countries.

Conclusions. Further research of the pharmacogenetic factors, such as *GSTP*1 polymorphism, including an analysis of larger cohorts of HL patients, may provide the understanding of molecular mechanisms of the development and progression of disease and help to estimate the individual detoxication potential that may influence the treatment response, susceptibility to the toxic effects of chemotherapy, and clinical outcome of the patients.

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Дослідження впливу поліморфізму гена *GSTP*1 *A*313*G* на виникнення лімфоми Ходжкіна, токсичність хіміотерапії та перебіг захворювання

Резюме

***Мета**. Дослідити поліморфізм гена GSTP1 A313G і визначити його вплив на виникнення лімфоми Ходжкіна, токсичність хіміотерапії, а також на перебіг захворювання. **Методи**. Поліморфні варіанти гена GSTP1 аналізували методом алель-специфічної ПЛР з детекцією результатів у режимі реального часу. **Результати**. Встановлено, що гомозиготний тип успадкування алеля дикого типу – генотип A/A – пов'язаний з несприятливим прогнозом перебігу лімфоми Ходжкіна: гіршою відповіддю на терапію і вірогідністю появи рецидивів. **Висновки**. Перспективним напрямком пошуку чинників впливу на перебіг хвороби є вивчення*

поліморфізму гена GSTP1, який у майбутньому може бути використаний як «фактор ризику» при формуванні прогностичних груп хворих та виборі тактики лікування.

Ключові слова: лімфома Ходжкіна, поліморфізм, ген GSTP1.

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Исследование влияния полиморфизма гена *GSTP*1 A313G на возникновение лимфомы Ходжкина, токсичность химиотерапии и течение заболевания

Резюме

Цель. *Исследовать полиморфизм гена GSTP1 A313G и определить его влияние на возникновение лимфомы Ходжкина, токсичность химиотерапии, а также на течение заболевания.* **Методы**. *Полиморфные варианты гена GSTP1 анализировали методом аллель-специфической ПЦР с детекцией результатов в режиме реального времени.* **Результаты**. *Установлено, что гомозиготный тип наследования аллеля дикого типа – генотип А/А – ассоциирован с неблагоприятным прогнозом течения лимфомы Ходжкина: худшим ответом на лечение и вероятностью появления рецидивов.* **Выводы**. *Перспективным направлением поиска причин, обусловливающих течение болезни, является изучение полиморфизма гена GSTP1, который в будущем, возможно, будет использован как «фактор риска» при формировании прогностических групп больных и выборе тактики лечения.*

Ключевые слова: лимфома Ходжкина, полиморфизм, ген GSTP1.

REFERENCES

1. *Roman E., Smith A. G.* Epidemiology of lymphomas // Histopathology.–2011.–**58**, N 1.–P. 4–14.

2. *Nakatsuka S., Aozasa K.* Epidemiology and pathologic features of Hodgkin lymphoma // Int. J. Hematol.–2006.–**83**, N 5.–P. 391–397.

3. *Fedorenko Z. P., Gajsenko A. V., Goulak L. O., Gorokh Ye. L., Ryzhov A. Yu., Soumkina O. V., Koutsenko L. B., Romanov D. V., Pushkar L. O.* Cancer in Ukraine 2008–2009 // Bulletin of National Cancer Registry of Ukraine / Ed. I. B. Shchepotin.– Kyiv, 2010.–N 11.–P. 68–72.

4. *Richardson S. E., McNamara C.* The management of classical Hodgkin's Lymphoma: past, present, and future // Adv. Hematol.–2011.–**2011**, ID 865870.–P. 1–17.

5. *Relling M. V., Dervieux T.* Pharmacogenetics and cancer therapy // Nat. Rev. Cancer.–2001.–**1**, N 2.–P. 99–108.

6. *Ribrag V., Koscielny S., Casasnovas O., Cazeneuve C., Brice P., Morschhauser F., Gabarre J., Stamatoullas A., Lenoir G., Salles G.* Pharmacogenetic study in Hodgkin lymphomas reveals the impact of UGT1A1 polymorphisms on patient prognosis // Blood.–2009.–**113**, N 14.–P. 3307–3313.

7. *Hayes J. D., Flanagan J. U., Jowsey I. R.* Glutathione transferases // Annu. Rev. Pharmacol. Toxicol.–2005.–**45**.–P. 51–88.

8. *Watson M. A., Stewart R. K., Smith G. B, Massey T. E., Bell D. A.* Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution // Carcinogenesis.–1998.–**19**, N 2.–P. 275–280.

9. *McIlwain C. C., Townsend D. M., Tew K. D.* Glutathione S-transferase polymorphisms: cancer incidence and therapy // Oncogene.–2006.–**25**, N 11.–P. 1639–1648.

10. *Harries L. W., Stubbins M. J., Forman D., Howard G. C., Wolf C. R.* Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer // Carcinogenesis.–1997.–**18**, N 4.–P. 641–644.

11. *Landi S., Norppa H., Frenzilli G., Cipollini G., Ponzanelli I., Barale R., Hirvonen A.* Individual sensitivity to cytogenetic effects of 1,2:3,4-diepoxybutane in cultured human lymphocytes: influence of glutathione S-transferase M1, P1 and T1 genotypes // Pharmacogenetics.–1998.–**8**, N 6.–P. 461–471.

12. *Holley S. L., Fryer A. A., Haycock J. W., Grubb S. E., Strange R. C., Hoban P. R.* Differential effects of glutathione S-transferase pi (GSTP1) haplotypes on cell proliferation and apoptosis // Carcinogenesis.–2007.–**28**, N 11.–P. 2268–2273.

13. *Dasgupta R. K., Adamson P. J., Davies F. E., Rollinson S., Roddam P. L., Ashcroft A. J., Dring A. M., Fenton J. A., Child J. A., Allan J. M., Morgan G. J.* Polymorphic variation in GSTP1 modulates outcome following therapy for multiple myeloma // Blood.–2003.–**102**, N 7.–P. 2345–2350.

14. *Sweeney C., McClure G. Y., Fares M. Y., Stone A., Coles B. F., Thompson P. A., Korourian S., Hutchins L. F., Kadlubar F. F., Ambrosone C. B.* Association between survival after treatment for breast cancer and glutathione S-transferase P1 Ile105Val polymorphism // Cancer Res.–2000.–**60**, N 20.–P. 5621–5624.

15. *Stoehlmacher J., Park D. J., Zhang W., Groshen S., Tsao-Wei D. D., Yu M. C., Lenz H. J.* Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer // J. Natl Cancer Inst.–2002.–**94**, N 12.–P. 936–942.

16. *Hohaus S., Massini G., D'Alo' F., Guidi F., Putzulu R., Scardocci A., Rabi A., Di Febo A. L., Voso M. T., Leone G.* Association between glutathione S-transferase genotypes and Hodgkin's lymphoma risk and prognosis // Clin. Cancer Res.–2003.–**9**, N 9.–P. 3435–3440.

17. *Hohaus S., Di Ruscio A., Di Febo A., Massini G., D'Alo' F., Guidi F., Mansueto G., Voso M. T., Leone G.* Glutathione S-transferase P1 genotype and prognosis in Hodgkin's lymphoma // Clin. Cancer Res.–2005.–**11**, N 6.–P. 2175–2179.

18. *Han X., Zheng T., Foss F. M., Lan Q., Holford T. R., Rothman N., Ma S., Zhang Y.* Genetic polymorphisms in the metabolic pathway and non-Hodgkin lymphoma survival // Am. J. Hematol.–2010.–**85**, N 1.–P. 51–56.

19. *Sarmanova J., Benesova K., Gut I., Nedelcheva-Kristensen V., Tynkova L., Soucek P.* Genetic polymorphisms of biotransformation enzymes in patients with Hodgkin's and non-Hodgkin's lymphomas // Hum. Mol. Gen.–2001.–**10**, N 12.–P. 1265–1273.