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Molecular diagnostics of cancers – practical approach

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Introduction of targeted therapies based on monoclonal antibodies or small-cell kinase inhibitors in cancer treatment led to major improvements of treatment outcomes in selected patients. However, achievement of prolonged progression-free survival or overall survival involves necessity to test a range of molecular markers at the diagnostic stage. Their number is determined by provisions of drug programmes and leads to serious problems with the right selection of individual markers. It is also an important challenge in the process of financial settlement of the performed tests. The present paper summarises the major aspects of molecular cancer diagnosis recommended and available in clinical practice in Poland.

Key words: targeted therapies, genetic diagnostics in cancers, settlement of genetic tests

The dynamic development of molecular biology led to exploration of a range of phenomena underlying the process of neoplastic transformation and contributing to fast development of therapies based on monoclonal antibodies and small-cell kinase inhibitors. However, multiple analyses have shown that these drugs are effective only in selected patients, and therefore it is necessary to test multiple molecular markers at the diagnostic stage to allow identification of those patients who can achieve the greatest benefits with the applied treatment. The number of tests imposed by provisions of drug programmes leads to multiple questions concerning selection of the testing method, quality standards to be met by diagnostic laboratories, and the major ones – concerning the possibility to settle the funding of individual tests. The present paper summarises the major aspects of molecular cancer diagnosis recommended and available in clinical practice in Poland.

Genetic testing at medical diagnostic laboratories

Genetic Diagnostics Departments/Labs at referential oncology centres should employ a staff of experienced lab diagnosticians and specialists in laboratory medical genetics. The basic role of these units is to perform diagnostic genetic tests designed to identify germinal mutations (constitutive mutations) and

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somatic mutations (genetic testing in acquired cancers). Genetic cancer diagnostics allows, above all, differential diagnosis, qualifying patients for targeted therapies, and also it enables monitoring of treatment course [1]. Within the diagnostic process, molecular analyses are also applied for assessing the development risk for the given cancer and as a basis for providing genetic counselling and prophylaxis for high-risk family members [2].

Genetic tests performed within a single medical centre allow introduction of integrated, interdisciplinary oncological diagnostics. The organisational structure and close multi-specialisation cooperation of lab diagnosticians, clinical physicians, pathomorphologists and geneticists enables specialist and comprehensive diagnostics in a single centre, without a need to send material to external cooperating units. Thus, the testing time is reduced to minimum, there is a possibility of consultation of the case with specialists in various medical specialties, and at the same time the risks involved in sample transport (e.g. sample loss or damage) are eliminated by application of consistent procedures for sample protection. Importantly, the material remains within the centre and if needed, it is available for re-analysis applying another technology. Further, if the result cannot be obtained, due to degradation of the genetic material or for other reasons, quick reaction is possible by re-harvesting a sample or using material harvested at another procedure or biopsy, if the archival material is representative [3].

Peripheral blood, drawn for assessment of germinal mutations or assessment of somatic mutations (referred to as liquid biopsy) on the level of extracellular nucleic acids – ctDNA (circulating tumour DNA) can be used material for genetic testing, if drawn upon prior written consent by the patient to diagnostic genetic testing. It should be referred directly to the genetics unit. Genetic testing of histopathology material (archival material fixed as paraffin blocks) performed upon acquisition of the patient's consent to diagnostic genetic testing, must be assessed by pathomorphologists to evaluate applicability of the material for molecular testing and to select the right sample (see: section about the role of pathomorphology in molecular diagnostics) [4].

A report of the completed genetic diagnostic test should include the test result, precise interpretation understandable for a clinical oncologist, clinical geneticist, pathomorphologist and the patient, as well as the scope and description of methods applied [5].

Genetic testing requires equipment with full technical documentation concerning repairs, validations and confirmation of annual inspections (Regulation of the Minister of Health of 21 March 2006 (Journal of Laws of 2006, no. 59, item 422, as amended). A genetic lab should hold experience of at least five years in working with peripheral blood material, tissue material, cytology material, extracellular nucleic acids; and it should have developed and implemented procedures, lab instructions, as well as internal quality control systems. It must be managed by a specialist in medical genetic diagnostics. The experience in testing germinal and somatic mutations should be documented by certificates of international quality control. The employed staff should be experienced and skilled in interpretation of the identified genetic variants based on medical databases, medical literature and bioinformatics analytical software *in silico*. All requirements set for diagnostic labs were described in the Regulation of the Minister of Health concerning quality standards for diagnostic labs and microbiology labs (Journal of Laws of 2019, item 1923).

A lab providing genetic testing for oncology diagnostics should ensure that the following tests are available at all times with no exceptions:

- **Sanger direct sequencing** – germinal and somatic mutations – testing of selected fragments of the DNA in genes where pathogenic variants can be located; targeted testing of selected genetic variants; verification of variants obtained by large-scale NGS methods. In assessment of histopathology material it is recommended that the tested preparations should contain no less than of 20% cancer tissue. Performance of macro- or micro-dissections is recommended to obtain the highest proportion of cancer tissue.
- **Next-generation sequencing (NGS)** – technology dedicated to comprehensive molecular diagnostics allowing parallel detection of multiple molecular markers and many classes of genetic mutations (point mutations, small deletions/insertions, big deletions, amplification, gene fusions), including genome signatures such as MSI (microsatellite instability), TMB (tumour mutational burden), HRD (homologous recombination deficiency). In testing germinal, as well as somatic mutations, panel (or targeted) next-generation sequencing can be applied, involving assessment of a selected pool of genes. In assessment of histopathology material it is recommended that the tested preparations should contain no less than of 20% cancer tissue (no less than 30% in the case of HRD testing). Performance of macro- or micro-dissections is recommended to obtain the highest share of cancer tissue.
- **qPCR** (modification of the PCR method referred to as quantitative real time PCR) –method dedicated to identification of only known genetic variants; quick method of high sensitivity of 1% to 0.2%. It allows identification of genetic mutations in material of scarce cancer tissue (5–15%) and ctDNA. Performance of macro- or micro-dissections is recommended to obtain the highest proportion of cancer tissue.
- **FISH** (fluorescent *in situ* hybridisation); **CISH** (chromogenic *in situ* hybridisation) – routine diagnosis of gene rearrangements, including gene fusions and gene amplifications.
- **MLPA** (multiplex ligation-dependent probe amplification) – method dedicated to assessment of large genetic rearrangements including deletions and duplications.

Dedicated mainly to assessment of germinal mutations. Frequently applied to verify mutations identified by large-scale techniques, such as NGS.

- **Other techniques: ddPCR** (droplet digital PCR) – one of the most sensitive techniques in molecular biology applied in testing selected genetic variants, especially on the ctDNA level. **Pyrosequencing** – method allowing assessment of methylation of selected DNA sequences. **aCGH** (array comparative genomic hybridisation) – cytogenetic method which involves detection of loss or amplification of chromosome regions or gene(s) characterised by very high resolution – for **SNP** assessment (single-nucleotide polymorphism) and evaluation of gene expression profile.

Role of pathomorphology in molecular diagnostics

Tissue and cytology material is used for molecular biology testing mainly in order to determine the right pathomorphological diagnosis of the cancer according to the currently binding classifications of the World Health Organisation (WHO) and to identify patients who may benefit the most from personalised therapies. Such tests require involvement of a diagnostic team including physicians specialising in pathomorphology, lab diagnosticians specialising in medical genetic diagnosis, biologists, biotechnologists and lab technicians. Labs/departments of pathomorphology (units specialising in pathomorphology diagnostics) within the highly-specialist healthcare institutions should have guaranteed access to the listed types of tests performed either in their own specialist labs or within a close cooperation with diagnostic labs specialising in analyses associated with medical genetic lab diagnostics [6, 7].

The quality of the genetic material (least possible degree of DNA/RNA degradation) is determined by observance of the right procedures at particular stages of processing of the biological material. The most important factors allowing maintenance of high quality of the tissue material include:

- delivery of the harvested material to the pathomorphology lab as fast as possible;
- fixing in 10% buffered formalin (4% solution of formaldehyde, pH 7.2–7.4, ambient temperature at most),
- adaptation of the fixing time to the size of the material (small histology material: up to 24/48 h, big histology material: up to 48/72 h).

Further processing of the tissue material must be standardised according to norms/requirements approved by the Ministry of Health and procedures recommended by the Polish Pathology Society and their latest updates. Each sample (paraffin block and corresponding microscopic preparation stained with hemotoxylin and eosin) – originating from the selected material for pathomorphology testing and designed for molecular testing – must be assessed by a physician specialising in pathomorphology to confirm the diagnosis, determine presence of cancer tissue and describe the proportion of cancer cells in the preparation. A physician specialising in pathomorphology chooses the best sample (procedure of qualifying material for molecular testing) in the context of molecular testing, considering also the sequence of planned diagnostic stages. In the case of materials sent from other centres, it is reasonable to provide all paraffin blocks to ensure the right qualification for the molecular testing considering the necessity to choose the material of the highest quality. If there is no adequate material for molecular testing (e.g. the material is too scarce, the proportion of cancer cells is too low or the material is technically damaged), a physician specialising in pathomorphology may recommend re-harvesting of material from the patient. The technical requirements concerning harvesting material from a paraffin block for isolation of nucleic acids (cutting blocks, their storage and delivery for molecular testing) are described in detail in the quoted guidelines.

Cytology smears (material for exfoliative and aspiration cytology, in the form of smear on basic glassware, fixed with alcohol 95–96%) and cyto-blocks (material for exfoliative and aspiration cytology fixed and submerged in a paraffin block) may also serve as valuable material for molecular testing. The binding rules for qualification of samples by a physician specialising in pathomorphology are the same as described above with respect to tissue material. In the case of smears, digital archiving of materials is recommended before their delivery for molecular analysis, because the biological material is entirely and irreversibly used.

The result of molecular assessment, necessary either for pathomorphological diagnosis, or for personalised treatment, should be included in the final/comprehensive pathomorphology report (including a summary or so-called synoptic report) in the case if the medical diagnostic lab is a part of the pathomorphology diagnostic unit or it may be attached to the report. Regardless of the organisational relations, provision of material for molecular testing requires cooperation and efficient communication to ensure fluent and optimal process of diagnostics. In order to ensure the right pathomorphology diagnostics, introduction of a separate model of funding of these tests is expected, based on the JGPato model, currently in development.

Funding of genetic diagnostic tests by the public payer

The right organisation of genetic diagnostics in oncology applying modern methods of molecular biology translates to improvement of the achieved outcomes of patient treatment, however, it requires additional funding [8]. Costs of genetic testing for oncologic patients vary depending on the applied testing technique and the number/type of procedures necessary to obtain an unequivocal, clinically useful result. There are several way of financial settlement of genetic tests within cancer diagnostics.

Considering the variable costs of genetic tests in oncology patients, the public payer introduced in 2017 a possibility to fund them within a hospitalisation agreement depending on ICD10 diagnosis, used diagnostic technology, number and type of the markets and moment of harvesting material for testing:

- archival material – provided from another centre or harvested at the given healthcare institution at a diagnostic procedure during earlier hospitalisation (fixed tissue and cytology material/paraffin blocks and preparations), or
- freshly harvested material sampled during hospitalisation (peripheral blood or material harvested during a surgical procedure and fixed as paraffin blocks or cytology material).

According to the Ordinance of the President of the National Health Fund concerning determination of terms of conclusion and implementation of agreements in the hospitalisation category (as amended), the possibility to settle diagnostic genetic testing in cancers was assigned to 15 areas both in conservative and surgical procedures (according to Attachment 1c to calculation): paediatric surgery, chest surgery, oncologic surgery, pulmonary disease / pulmonary disease in children, endocrinology, gastroenterology, oncologic gynaecology, haematology, neonatology, neurosurgery, paediatric oncology and haematology, clinical oncology, obstetrics and gynaecology, urology. It is not possible to settle genetic testing within general surgery.

For settlement of genetic testing in cancers within hospitalisation contracts, there are dedicated settlement products in catalogue 1c (for calculation) which allow funding of diagnostic genetic testing of material harvested during hospitalisation or archival material:

- basic genetic testing in cancers (code 5.53.01.0005001) – refund of **649 points**,
- complex genetic testing in cancers (code 5.53.01.0005002) – refund of **1,298 points**,
- advanced genetic testing in cancers (code 5.53.01.0005003) **–** refund of **2,434 points**.

Currently, this is the most favourable variant of settlement of genetic testing in cancers.

The basic condition of settlement of genetic testing in oncology within the hospitalisation agreement involves holding a contract with the National Health Fund concerning provision of healthcare services of the type "hospitalisation" in at least one area listed in catalogue 1c of the ordinance. Hospitalisation involving harvesting of material for genetic testing should be justified by medical considerations and correctly documented. Upon obtaining a result of a genetic test, the JGP group in catalogue 1a should be expanded by the correct settlement product as indicated by the genetics lab: simple, complex or advanced genetic testing in cancers.

Originally, reporting of genetic tests involved a necessity of hospitalisation of the patient, as harvesting of the material

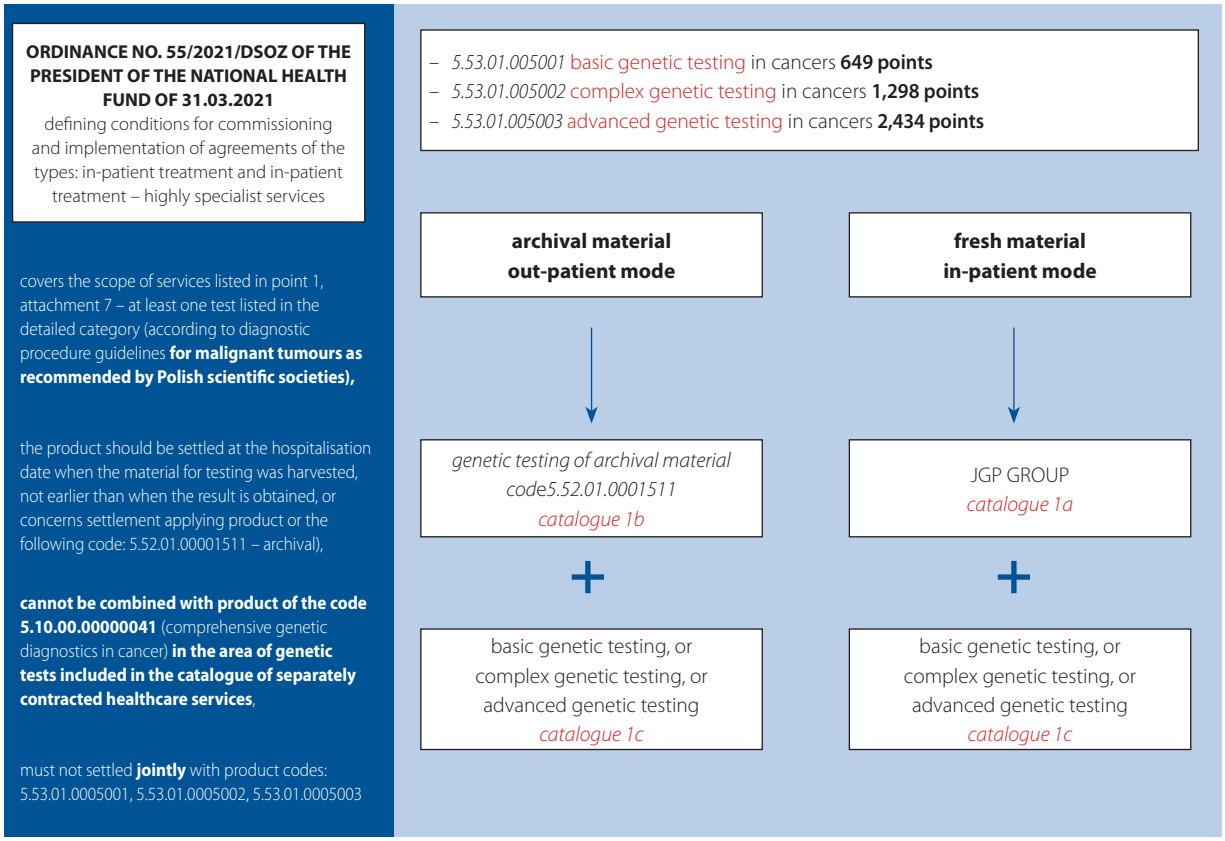

Figure 1. Settlement products in catalogue 1c (for calculation) which allow funding of diagnostic genetic testing of material harvested during hospitalisation or archival material

Table I. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

No. [1]	Name [2]	ICD 10 [3]	Objective of genetic testing [4]	Genetic testing basic profile minimum requirements [5]	Testing methods [6]	Material [7]	Funding method / settlement product [8]	Recommended extended profile (including genes of the basic profile and additional recommended genes), including markers significant for clinical trials [9]	Testing method [10]	Funding method [11]	Drug programme drugs as on 01.03.2023 [12]	Ministry of Health attachment no. [13]
1.	treatment of gastrointestinal Stromal Tumours (GIST)	C15, C16, C17, C18, C20, C48	qualification for targeted therapies[1] differential diagnosis[2]	*(KIT, PDGFRA)*[1,2]	sanger sequencing or NGS panel	• tissue – paraffin block • peripheral blood – in rare selected cases for assessment of germinal mutations	• 5.53.01.0005001 simple or 5.53.01.0005002 complex genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	*(KIT, PDGFRA)*[1,2] *(KRAS, NRAS, PIK3CA)2, BRAF*[1,2]*, SDHA/B/C/D*[2]*, NTRK3 (fusions)*[1,2]*, FGFR1 (fusions)*[1,2]*, BRAF (fusions)*[1,2]	• NGS panel • FISH (fluorescent in situ hybridisation), • MLPA, • aCGH micromatrices	• 5.53.01.0005003 advanced genetic tests in cancers on archival material (out-patient mode) – in-patient agreement	imatinib sunitinib sorafenib	B.3
2.	treatment of soft-tissue sarcomas	C48, C49	qualification for targeted therapies[1] differential diagnosis[2]	basic panel: *EWSR1, SS18, FOXO1, FUS, PDGFB, MDM2* (amplification), USP6, DDIT3	FISH (fluorescence *in situ* hybridisation), NGS panel recommended methods: • typical cases are covered by FISH method testing (individual rearrangements), • NGS panel – in the case of complex differential diagnostics	• tissue – paraffin block • peripheral blood – in rare selected cases for assessment of germinal mutations	• 5.53.01.0005001 simple or 5.53.01.0005002 complex genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	diagnostics: *(BCOR; CAMTA1; CIC; CSF1; CTNNB1; EPC1; ERG; ESR1; EWSR1; FOS; FOSB; FOXO1; FUS; GLI1; HMGA2; JAZF1)*[2] *(MDM2)*[1,2]*; (MEAF6; MET; MGEA5; MKL2; MYOD1; NCOA1; NCOA2; NR4A3; NUTM1; PAX3)*[2]*; (PDGFB)*[1,2]*; (PHF1; PLAG1; PRKCA; PRKCB; PRKCD; RAF1; SS18; STAT6; TAF15; TCF12; TFE3; TFG; USP6; VGLL2; YAP1; YWHAE, and others)*[2] targeted therapy: *(ALK; BRAF)*[1,2]*; EGFR2; (FGFR1, FGFR2, FGFR3)*[1]*, (NTRK1; NTRK2)*[2]*; NTRK3*[1,2]*; (RET; ROS1 and others)*[1]	• NGS panel (gene fusions), or • in selected cases comprehensive genome profiling (CGP): SNP, CNV, gene fusions, amplifications, gene signatures – MSI, TMB	no refund by the National Health Fund	trabectedin pazopanib sunitinib	B.8
3.	treatment of non-small-cell lung cancer and mesothelioma of the pleura	C34 C45	qualification for targeted therapies[1] differential diagnosis[2]	*(EGFR, KRAS* (p.Gly12Cys), *ALK, ROS1)*[1] immunohistochemistry testing	qPCR, FISH (fluorescence *in situ* hybridization), sanger sequencing, NGS panel recommended method: NGS panel	• tissue – paraffin block • peripheral blood – in rare selected cases for assessment of germinal mutations	• 5.53.01.0005001 simple or 5.53.01.0005002 complex or 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient	*(EGFR, KRAS , BRAF, HER2, ALK, ROS1, RET, NTRK1-3, MET,* and other, gene signatures TMB)[1]	• NGS panel (gene fusions), or • in selected cases comprehensive genome	NGS panel of tissue or cytology preparation: • 5.53.01.0005003 advanced genetic tests in cancers on	crizotinib osimertinib nivolumab pembrolizumab	B.6

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[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
				(e.g. PD-1 or PD-L1 expression degree)		• cytology preparations (cyto-blocks or smears on glass) • ctDNA: 1. *EGFR* testing selected mutations in exons 18,19,20,21 – in the case of nondiagnostic material or no material, 2. treatment monitoring – testing mutations of p.Thr790Met in *EGFR*, 3. complex genetic profiling with ctDNA (extended profile)	material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services		profiling (CGP): SNP, CNV, gene fusions, amplification, gene signa tures – MSI, TMB	archival material (out-patient mode) – in-patient agreement, • comprehensive genome profiling (CGP) – no refund	dacomitinib lorlatinib entrectinib cemiplimab ipilimumab durvalumab brigatinib ceritinib nintedanib alectinib atezolizumab afatinib	
4.	treatment of bone cancers	C48-C49	qualification for targeted therapies[1] differential diagnosis[2]	*TP53*[2], *CDK4*[2], *(MDM2)*[1,2], *RB1*[2], *IDH1/2*[2], *GNAS*[2], *(H3.3A)*[1,2], *H3.3B*[2], *BCOR*[2], *NR4A3*[2]	FISH (fluorescence *in situ* hybridization), NGS panel <u>recommended methods</u>: • FISH technique – typical cases, individual tests, • NGS panel – complex differential diagnosis	• tissue – paraffin block • peripheral blood – in rare selected cases for assessment of germinal mutations	• 5.53.01.0005001 simple or 5.53.01.0005002 complex genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	(*PTEN, FOS, FOSB, TF3, CAMTA1, NCOA2, PHF1, CSF1*)[2], TMB[1]	• extended NGS panel (gene fusions), or • in selected cases comprehensive genome profiling (CGP): SNP, CNV, gene fusions, amplification, gene signatures – MSI, TMB	no refund by the National Health Fund		
5.	treatment of melanoma of the skin or mucosa	C43	qualification for targeted therapies[1] differential diagnosis[2]	*BRAF*[1] 600 codon mutations, *NRAS*[2], *KIT*[1,2], (*GNAQ, GNA11*)[2], *TERT2* promoter gene	sanger sequencing, NGS panel <u>recommended methods:</u> • qPCR for quick diagnosis of mutations in 600 codon of the BRAF gene in the tissue and ctDNA;	• tissue – paraffin block • peripheral blood – in rare selected cases for assessment of germinal mutations • cytology preparations (cyto-blocks)	• 5.53.01.0005001 simple tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, • in the case of testing multiple genes possible application of 5.53.01.0005002 complex genetic tests in	*BRAF*[1], *NRAS*, *KIT*[1,2], *(GNAQ, GNA11, CTNNB1, MAP2K1, NF1, PIK3CA, PTEN, TP53)*[2], *NTRK1-3*[1], genome signature TMB[1]	• NGS panel (gene fusions), or • in selected cases comprehensive genome profiling (CGP): SNP, CNV, gene fusions, amplification,	• 5.53.01.0005003 advanced genetic tests in cancers on archival material (out-patient mode) – in-patient agreement,	ipilimumab nivolumab pembrolizumab vemurafenib cobimetinib dabrafenib trametinib	B.59

Table I. cd. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
					• Val600 variant verification by Sanger sequencing	• ctDNA liquid biopsy	cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services		gene signatures – MSI, TMB	• comprehensive genome profiling (CGP) – no refund	encorafenib binimetinib	
6.	treatment of patients with ovarian cancer, fallopian tube cancer or peritoneal cancer	C56, C57, C48	qualification for targeted therapies[1] differential diagnosis[2] prophylaxis[3]	*BRCA*[1] *BRCA2*[1] *HRD*[1]	*BRCA1, BRCA2* – NGS panel results verification by Sanger sequencing. **Note!** When a pathogenic variant is identified in tissue material of neoplastic origin, the genetic testing result should be delivered to the genetics office for verification in peripheral blood, if the variant is somatic or germinal. This is especially significant for prophylaxis for the patient's family. Genetic testing for a drug programme is requested by a clinical oncologist. If the patient has already had a genetic test result from a genetics office, it may be used for verifying eligibility for the drug programme	• tissue – paraffin block • peripheral blood – verification or no tissue	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • in the case of HRD: comprehensive genetic profiling (CGP) – no refund	(*BRCA1, BRCA2*)[1] HRD[1], (*BRAF, KRAS, PDGFRA, FOXL2,TP53*)[2]	• NGS panel, or • in selected cases comprehensive genome profiling (CGP): SNP, CNV, gene fusions, amplification, gene signatures – HRD, TMB	• 5.53.01.0005003 advanced genetic tests in cancers on archival material (out-patient mode) – in-patient agreement, • in the case of HRD: comprehensive genome profiling (CGP) – no refund	olaparib niraparib	B.50

Table I. cd. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
7.	treatment of the renal cancer	C64	qualification for targeted therapies[1] differential diagnosis[2]	• somatic: (*VHL, TSC1, TFE3* (fusions), *TFEB* (fusions), *ELOC*)[2], *ALK* (fusions)[1,2], *SMARCB1*[2], • germinal: *VHL,FH , TSC1/TSC2, SDHB/C/D, PTEN, BAP1, MET, FLCN*	NGS panel, small targeted panels to assess mutations and fusions NGS panel of peripheral blood, <u>recommended method</u>: NGS panel	• tissue – paraffin block • peripheral blood in selected cases of suspicion of genetic form	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract	(*PRBM1, BAP1, SET2D, KDMC5, TP53, PTEN, TET, ARID1A, TERT* promoter, *FOXI1, RHCG, MET*)[2]	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers on archival material – in-patient agreement	sunitinib everolimus sorafenib pazopanib axitinib nivolumab ipilimumab temsirolimus cabozantinib	B.10
8.	treatment of castrate-resistant prostate cancer	C61	qualification for targeted therapies[1] differential diagnosis[2] prophylaxis[3]	*BRCA1*[1] *BRCA2*[1]	<u>recommended methods</u>: • NGS panel to assess the status of genes BRCA1, BRCA2BRCA1, BRCA2 – verification of results by Sanger sequencing **Note!** For the purposes of targeted therapy in the drug programme, *BRCA1, BRCA2* testing is recommended by a clinical oncologist and based on archival material. If the tissue material is unavailable or non-diagnostic, *BRCA1, BRCA2* testing by liquid biopsy should be	• tissue – paraffin block • ctDNA – no tissue • peripheral blood - verification	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	*BRCA1*[1] , *BRCA2*[1] *PTEN*[2], *AR*[1]	• NGS panel of cancer tissue, or • in the case of no material available or non-diagnostic material, the NGS panel should be used applying ctDNA	ctDNA – no refund	olaparib enzalutamide radium dichloride Ra 223 apalutamide cabazitaxel daratumumab	B.56

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[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
					recommended (ctDNA). Currently liquid biopsy tested is not funded by the National Health Fund							
9.	treatment of patient with urothelial cancer (urinary bladder)	C67	qualification for targeted therapies[1] differential diagnosis[2]	*FGFR1/2/3*[1]	recommended methods: NGS panel to assess nucleotide level mutations, gene fusions, amplifications	• tissue – paraffin block	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract	(*RB1, CDKN2A, TP53, KDM6A, ELF3, ERCC2, CDKN2B, PIK3CA, EGFR, ERBB2/3/4*)[2], TMB[1]	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers (out-patient or in-patient harvesting of material) – in-patient agreement	avelumab	B.141
10.	treatment of the breast cancer	C50	qualification for targeted therapies[1] prophylaxis[3]	*BRCA1*[1;2] *BRCA2*[1;2] *PIK3CA*[1] *HER2*[1]	*BRCA1, BRCA2* – NGS panel result verification by Sanger sequencing *PIK3CA* – recommended qPCR panel *HER2* – IHC method (in selected cases verification by FISH) **Note!** Genetic testing of *BRCA1; BRCA2* in peripheral blood (germinal mutations) for a drug programme is requested by a clinical oncologist. If a pathogenic variant is identified, the patient should be referred to a genetics office so that the patient's family is covered	• peripheral blood – *BRCA1, BRCA2, PALB2, CHEK2* • tissue or ctDNA – PIK3CA • tissue – NTRK1-3 – paraffin block • tissue – gene signature TMB	• 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services, • 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisationl) – in-patient treatment contract, or • testing mutations in *BRCA1, BRCA2, PALB2, CHEK2* genes with NGS technique – separately contracted services	(*BRCA1, BRCA2, HER2, PIK3CA, ESR1, PALB2, CHEK2, NTRK,*)[1,3]	• NGS panel	• 5.10.00.000041 comprehensive genetic diagnostics in cancers – separately contracted services • no refund of advanced panels or out-patient material harvesting	trastuzumab emtansine lapatinib pertuzumab palbociclib ribociclib abemaciclib alpelisib talazoparib sacituzumab govitecan	B.9

Table I. cd. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
					by prophylactic care. If the patient already has a genetic test result from a genetics office, it may be used for verifying eligibility for the drug programme							
11.	treatment of the advanced colon cancer	C18, C19, C20	qualification for targeted therapies[1] differential diagnosis[2]	(*KRAS, NRAS, BRAF, MSI* – microsatellite instability)[1]	sanger sequencing, qPCR, recommended method: qPCR	• tissue – paraffin block • in rare selected cases peripheral blood can be applied for assessment of germinal mutations • ctDNA	• 5.53.01.0005002 complex genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services, or • in the case of peripheral blood material – ctDNA: *KRAS, NRAS, BRAF*	gene status assessment: (*ALK, BRAF*)[1], *BRCA1/2, EGFR, ERBB2 (HER2)*[1], *FGFR1, MET, MLH1, MSH2, MSH6, NRG1, PIK3CA, PMS2, POLE, PTEN, RET, ROS1, KRAS, NRAS*	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers (out-patient archival material) – in-patient agreement, • comprehensive genome profiling (CGP) – no refund	cetuximab panitumumab aflibercept trifluridine + tipiracil ipilimumab nivolumab pembrolizumab	B.4
13.	treatment of the pancreatic cancer	C25.4	qualification for targeted therapies[1] differential diagnosis[2] prophylaxis[3]	*BRCA1*[1,3] *BRCA2*[1,3]	recommended methods: NGS panel to assess the status of *BRCA1, BRCA2* genes **Note!** Genetic testing of *BRCA1; BRCA2* in peripheral blood (germinal mutations) for a drug programme is requested by a clinical oncologist. If a pathogenic variant is identified, the patient should be referred to a genetics office so	• peripheral blood – *BRCA1, BRCA2*	• 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	(*KRAS, SMAD4, FGFR1/2/3*)[2] (*GNAS, CDKN2A*)[2]	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or out-patient archival material) – in-patient agreement	everolimus sunitinib	B.53

Table I. cd. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
					that the patient's family is covered by prophylactic care							
14.	treatment of the advanced oesophageal and gastric cancer	C15, C16, C17, C18, C20, C48	qualification for targeted therapies[1] differential diagnosis[2]	*HER2* assessment[1]	FISH (fluorescence *in situ* hybridization)	• tissue – paraffin block	• 5.53.01.0005001 simple genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	*BRAF, EGFR, HER2*[1], *FGFR2, KIT, KRAS, MET, NRG1, PIK3CA, PDGFR, TP53*	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or out-patient archival material) – in-patient agreement	nvolumab pembrolizumab ramucirumab	B.58
15.	central nervous system	C71	qualification for targeted therapies[1] differential diagnosis[2]	(*IDH1, IDH2*)[2], *MGMT* promoter methylation[1], 1p/19q2 co-deletion	sanger sequencing, qPCR, FISH (fluorescence *in situ* hybridization), pyrosequencing	• tissue – paraffin block	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	(*IDH1, IDH2*)[2], promoter methylation MGMT[1], co-deletion 1p/19q[2], EGFR (amplification)[1,2], (*CDKN2A/B* (homozygotic deletion), mutation in the TERT gene promoter, *H3.3* (mutation), cytogenetic assessment of chromosome +7/–10)[2], fusions of BRAF, EGFR, (ROS1, ALK, NTRK1/2/3)[1, 2], and other fusions associated with neoplasms of the central nervous system	• NGS panel • FISH (fluorescent in situ hybridisation), • MLPA, • aCGH micromatrices	• 5.53.01.0005001 simple, 5.53.01.0005002 complex, or 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or out-patient archival material) – in-patient agreement, • comprehensive genome profiling (CGP) – no refund		
16.	thyroid cancers	C73	qualification for targeted therapies[1] differential diagnosis[2] *prophylaxis*[3]	*BRAF*[1,2], (*KRAS, NRAS, PIK3CA, TERT*)[2], (*RET, NTRK3*)[1] *RET*[3] DNA level mutations	qPCR, NGS panel <u>recommended methods</u>: • NGS panels	• tissue – paraffin block • peripheral blood – germinal	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or	none				

Table I. cd. List of genetic tests in selected cancers (solid tumours) conisdering the type of material, testing technology and treatment method

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]
					• qPCR recommended for fast *BRAF* diagnostics	mutations (in some cases)	• 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services					
17.	neoplasms of unknown primary origin	C80	qualification for targeted therapies[1] differential diagnosis[2]	(*EGFR, KRAS, BRAF, NTRK1/2/3, ALK, ROS1*)[1,2]	NGS panel	• tissue – paraffin block • ctDNA	• 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services • the test is not funded within the hospitalisation agreement due to lack of C80 diagnosis in attachment no. 7			• comprehensive genome profiling (CGP) – no refund		
18.	treatment of the endometrial cancer	C54	qualification of molecular subtype associated with prognosis and treatment[2]	*POLE*[2], *MSI*[2]	sanger sequencing, capillary electrophoresis	• tissue – paraffin block	• 5.53.01.0005001 simple genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.53.01.0005002 ccomplex genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract	(*POLE, TP53, MSH2, MSH6, MLH1, PMS2, BRCA1, BRCA2, CTNNB1, MSI* signature)[2]	• NGS panel	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or out-patient archival material) – in-patient agreement	dostarlimab not included in any drug programme	none
19.	treatment of patients with solid tumours with neurotrophic receptor tyrosine kinase (NTRK) fusions	ICD10 in solid tumours with *NTRK* fusion, tested upon qualification by a Coordination Team for Treatment of Solid Tumour Patients	qualification for targeted therapies[1]	(*NTRK1, NTRK2, NTRK3* – gene fusions)[1]	recommended method: NGS (RNA-seq)	• tissue – paraffin block	• 5.53.01.0005003 advanced genetic tests in cancers (material harvested during hospitalisation or archival out-patient material) – in-patient treatment contract, or • 5.10.00.000041 comprehensive genetic diagnostics of cancers – separately contracted services	*NTRK1, NTRK2, NTRK3* – gene fusions on the ctDNA level	• NGS panel with ctDNA	• comprehensive genome profiling (CGP) with ctDNA – no refund	larotrectinib	B.144

for diagnostic testing was an in-patient procedure. A change was introduced early in 2018 with introduction of a possibility to settle out-patient genetic diagnostic tests in cancers performed on archival material which could have been harvested by other providers. In this case, we apply product 5.52.01.0001511: genetic testing of archival material. The product's value is 0, but it allows reporting and settlement of genetic tests: simple, complex and advanced ones, if the treatment plan has to be modified. The service concerning genetic testing of archival material (code 5.52.01.0001511) is meant for out-patient procedures, but it is settled within an in-patient agreement. It is also obligatory to report the original date of harvesting of the material for testing.

Further, reimbursement of costs of genetic testing in cancers can be based on other agreements concluded between service providers and the National Health Fund:

1. Agreements concerning separately contracted services (SOK), which may fund tests on material harvested during an out-patient or in-patient diagnostic procedure as product (5.10.00.000041) – complex genetic diagnostics of cancers – 534 points.
2. The least favourable financial settlement involves an agreement concerning out-patient specialist care with settlement product (5.03.00.000021) – RNA/DNA detection with molecular tests (PCR/PFGE) – 300 points.
3. In the case of haemato-oncological drug programmes, it is admissible to settle genetic testing during qualification for drug programmes with so-called diagnostic lump amount.
4. Additionally, since September 2022 some service providers may perform specific genetic tests within a programme of care for families with high risk of hereditary breast cancer or ovarian cancer, as well as colon cancer or endometrial cancer.

Table I presents discussion of genetic diagnostics for particular cancers along with methods and type of funding.

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