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Molecular diagnostics in clinical oncology: an overview

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Abstract

Molecular diagnostics has become an integral part of modern clinical oncology. There are several dozen hereditary cancer syndromes; the detection of germline pathogenic variants in tumor-predisposing genes allows for the identification of subjects at-risk as well as guides the administration of cytotoxic and targeted drugs. The development of predictive tests for personalized drug-target matching is the best-known achievement of molecular oncology. For the time being, these assays are routinely utilized for the management of lung, breast, ovarian, colorectal, thyroid, biliary tract, endometrial, urothelial, and other malignancies. We are currently witnessing the emergence of practical applications of liquid biopsy. The detection of circulating tumor DNA (ctDNA) is a highly sensitive and specific procedure, which is currently used for the detection of secondary drug-resistant mutations, and holds great promise for the monitoring of malignant disease in oncological patients and early cancer detection in healthy individuals. While the utilization of molecular tests is currently limited to particular categories of cancer patients, their use is likely to become significantly more widespread in the near future. This trend will affect educational standards, requiring practicing physicians to become more familiar with molecular biology, and, *vice versa*, claiming some fluency in clinical oncology from laboratory specialists.

Keywords

Actionable mutation, molecular diagnostics, targeted therapy

Introduction

Molecular diagnostics is a complex of technologies aimed at detection of alterations in individual genes and gene-encoded molecules (RNA, proteins) for clinical purposes. This field emerged several decades ago, starting from the identification of selected tumor-specific protein markers [1]. A breakthrough in molecular genetics, i.e., the development of polymerase chain reaction (PCR), conventional and, particularly, next-generation sequencing (NGS), ultrasensitive methods of DNA analysis, etc., as well as the completion of the Human Genome Project shifted the focus of molecular diagnostics towards the profiling of alterations in genes and their transcripts. Advances in molecular medicine have changed the daily practice of clinical

oncologists. Timely identification of subjects with hereditary forms of cancer is a mandatory component of the examination of cancer patients. Many targeted therapies are tailored to particular genetic alterations in tumor cells; therefore, mutation-drug matching has become a part of clinical routine [2] (Table 1). We are now witnessing significant progress in liquid biopsy, i.e., DNA-based non-invasive monitoring of the course of cancer disease [3]. This article provides an overview of the applications of molecular diagnostics in various areas of clinical oncology.

Table 1. Druggable genetic alterations in different tumor types

Target	Frequency of actionable mutations	Examples of targeted drugs
Lung cancer		
EGFR ex19del, L858R	10–20% in Europeans; 40–70% in Asians; more common in females and non-smokers	EGFR inhibitors (erlotinib, gefitinib, afatinib, osimertinib, etc.)
ALK fusions	5%; more common in young patients, females, and non-smokers	ALK inhibitors (crizotinib, alectinib, lorlatinib, etc.)
RET fusions	4%; more common in young patients, females, and non-smokers	RET inhibitors (selpercatinib, pralsetinib)
ROS1 fusions	1.5–2%; more common in young patients, females, and non-smokers	ROS1 inhibitors (crizotinib, entrectinib, repotrectinib)
NTRK1-3 fusions	0.2%; more common in young patients, females, and non-smokers	NTRK inhibitors (entrectinib, larotrectinib)
HER2 amplification	1%	HER2 inhibitors (trastuzumab, pertuzumab)
HER2 exon 20 insertions	2–3%	HER2 inhibitors (trastuzumab deruxtecan, pyrotinib)
MET exon 14 skipping	2.5%; more common in elderly patients	MET inhibitors (capmatinib, tepotinib, crizotinib)
BRAF V600E, V600K, and other V600 mutations	2%	BRAF inhibitors (vemurafenib, dabrafenib, encorafenib) in combination with MEK inhibitors
KRAS G12C	10%; significantly more common in smokers	$KRAS^{G12C}$ inhibitors (sotorasib, adagrasib)
Breast cancer		
BRCA1/2 mutation	7–10%; more common in patients with clinical signs of hereditary disease	Platinum compounds, PARP inhibitors (olaparib, talazoparib, niraparib, rucaparib)
PTEN mutation	5%	AKT inhibitor (capivasertib)
PIK3CA mutation	40%	AKT inhibitor (capivasertib), PI3K inhibitor (alpelisib)
AKT1 mutation	4%	AKT inhibitor (capivasertib)
Ovarian cancer		
BRCA1/2 mutation	25–40%	Platinum compounds, mitomycin C, PARP inhibitors (olaparib, talazoparib, niraparib, rucaparib)
Colorectal cancer		
BRAF V600E	4–8%	BRAF inhibitor (encorafenib) plus EGFR inhibitor (cetuximab)
POLE mutation	< 1%	Immune checkpoint inhibitors (pembrolizumab, nivolumab, etc.)
HER2 amplification	1–2%	HER2 inhibitors (trastuzumab, pertuzumab, lapatinib, etc.)
Pancreatic cancer		
BRCA2 mutation	2%	PARP inhibitors (olaparib, talazoparib, niraparib, rucaparib)
PALB2 mutation	0.5%	PARP inhibitors (olaparib, talazoparib, niraparib, rucaparib)
KRAS G12C	2%	$KRAS^{G12C}$ inhibitors (sotorasib, adagrasib)
Biliary tract tumors		
FGFR2 fusion or activating mutation	20%	FGFR inhibitors (futibatinib, pemigatinib, infigratinib)
HER2 amplification or mutation	4%	HER2 inhibitors (trastuzumab, pertuzumab, trastuzumab deruxtecan, pyrotinib)
BRAF V600E	2%	BRAF inhibitors (vemurafenib, dabrafenib, encorafenib) in combination with MEK inhibitors

Table 1. Druggable genetic alterations in different tumor types (*continued*)

Target	Frequency of actionable mutations	Examples of targeted drugs
Melanoma		
BRAF V600E	60%	BRAF inhibitors (vemurafenib, dabrafenib, encorafenib) in combination with MEK inhibitors
KIT mutation	15% in acral and mucosal melanomas	KIT inhibitors (imatinib, nilotinib, dasatinib)
Thyroid cancer		
RET activating mutation	10–25% of medullary carcinomas	RET inhibitors (selpercatinib, pralsetinib)
BRAF V600E	Up to 50% of papillary carcinomas	BRAF inhibitors (vemurafenib, dabrafenib, encorafenib) in combination with MEK inhibitors
RET fusion	10% of papillary carcinomas	RET inhibitors (selpercatinib, pralsetinib)
Endometrial cancer		
POLE mutation	8%	Immune checkpoint inhibitors (pembrolizumab, nivolumab, etc.)
HER2 amplification	10%	HER2 inhibitors (trastuzumab, pertuzumab)
Urothelial cancer		
FGFR3 activating mutation	15–20%	FGFR inhibitors (erdafitinib, futibatinib, pemigatinib, infigratinib)
HER2 amplification or mutation	20–30%	HER2 inhibitors (trastuzumab, pertuzumab, trastuzumab deruxtecan, pyrotinib)

Molecular tests for particular tumor types

Lung cancer

Molecular analysis of lung cancer is currently limited to non-squamous non-small cell lung carcinomas, while there are virtually no advances in DNA-assisted guidance of the treatment of squamous and small-cell lung malignancies. Histological diagnosis of lung cancer is often complicated; therefore, it is recommended to test patients with high probability of actionable genetic findings, i.e., women and non-smokers, irrespective of tumor histology [4–6].

EGFR gene mutations are the most common druggable alterations in lung adenocarcinomas. They account for approximately 10–20% of patients of European race and 40–70% of subjects of Asian ancestry. Although these manifold differences in racial distribution of *EGFR* mutations were acknowledged immediately after their discovery, the causes of this phenomenon remain unknown. *EGFR* mutations are significantly more common in females and non-smokers [7]. *EGFR* exon 19 deletions (ex19del) are associated with higher tumor sensitivity to EGFR tyrosine kinase inhibitors (TKIs) and demonstrate similar frequencies in patients of various ages. *EGFR* L858R mutations are particularly common in elderly subjects [8].

Approximately 20% of *EGFR* genetic events are defined in scientific literature as “rare” or “uncommon” mutations, i.e., they are represented by other than ex19del or L858R alterations [9]. The majority of these uncommon lesions are druggable by conventional inhibitors, with the exception of *EGFR* exon 20 insertions which require a distinct category of drugs [9–11]. Early *EGFR* PCR kits focused mainly on the detection of hotspot ex19del and L858R mutations, while current standards of *EGFR* testing call for comprehensive analysis of exons 18–21.

The cumulative frequency of tyrosine kinase gene rearrangements is around 10%, being 5% for *ALK*, 4% for *RET*, 1.5–2% for *ROS1*, and around 0.2% for the *NTRK* receptor family (*NTRK1*, *NTRK2*, and *NTRK3*). These fusions are strongly associated with young patient age, female gender, and non-smoking status. Reliable and time-efficient detection of gene fusions still presents a challenge, despite significant advances in techniques for molecular analysis. RNA hybridization-based NGS is the most proficient technology in this respect; however, its routine utilization remains limited due to extraordinary complexity, high costs, and significant turn-around time. Amplicon-based RNA and DNA NGS are more accessible, but these techniques have a risk of missing some translocations. Immunohistochemistry (IHC) analysis is widely used for *ALK* testing, and, to a lesser extent, for *ROS1* and pan-*NTRK* screening, despite having significant drawbacks with

regard to specificity and sensitivity. There are several elegant techniques for rapid and cost-efficient detection of *ALK*, *ROS1*, *RET*, and *NTRK1-3* gene fusions that address the disadvantages described above, but their use is restricted to facilities that have managed to achieve scrupulous in-house adjustment and validation of these laboratory-developed protocols [6].

The medical significance of error-free *ALK*, *ROS1*, *RET*, and *NTRK1-3* testing is perhaps the highest among all known predictive markers. For example, earlier studies suggested that patients with metastatic ALK-driven lung cancer may gain up to 7 years in their life expectancy upon targeted therapy, and these estimates are likely to exceed 10 years with more modern drugs [12, 13]. In addition to the above genes, *NRG1* rearrangements appear to be more or less actionable; however, their testing has not been yet incorporated in minimal diagnostic standards due to the low occurrence of these fusions (around 0.2%) and moderate survival benefit from matched therapies [14, 15].

Activating *MET* mutations result in exon 14 skipping, thus significantly increasing the half-life of this tyrosine kinase. Their occurrence reaches approximately 2.5%. These events are confined to elderly patients, with almost all mutation-positive subjects being well above 70 years old [16, 17]. Surprisingly, IHC cannot be used for preliminary screening of these mutations. Exon 14 skipping *MET* alterations can be detected either by RNA-based NGS or specifically adjusted PCR allele-specific expression tests [18]. *MET* amplification coupled with gene overexpression is also associated with potential tumor sensitivity to MET inhibitors, although this variety of molecular testing and clinical attitudes towards *MET*-amplified tumors have not been standardized yet [19, 20].

BRAF activation occurs in approximately 4% of lung carcinomas. Only substitutions affecting codon 600 are druggable; they are observed in less than 2% of tumors. *BRAF* V600-mutated malignancies are treated with a combination of BRAF and MEK inhibitors [6, 21]. Other *BRAF* alterations are represented by exon 11 mutations, non-codon-600 substitutions located in exon 15, and gene rearrangements. None of the latter events are clearly actionable [22].

HER2 amplification accompanied by gene overexpression plays a driving role in approximately 1% of lung carcinomas. These tumors can be managed by various anti-HER2 therapeutic agents [23–25]. Some lung malignancies produce excessive amounts of this receptor tyrosine kinase without being HER2-dependent. The analysis of other mutated genes in MAPK pathway, particularly *RAS* mutations, may help to discriminate between driver and passenger *HER2* amplifications [25]. Activating *HER2* mutations, mainly exon 20 insertions, occur in approximately 2–3% of lung carcinomas. These tumors can be targeted by novel low-weight TKIs or HER2 antibody-drug conjugates [26, 27].

KRAS mutations are seen in 30% of lung carcinomas. *KRAS* G12C substitution is amenable to therapy by sotorasib or adagrasib [28]. While actionable alterations affecting *EGFR*, *BRAF*, *MET*, *HER2*, *ALK*, *RET*, *ROS1*, and *NTRK1-3* oncogenes are strongly associated with the lack of smoking history, *KRAS* G12C substitutions occur almost exclusively in smokers, with the frequency about 1 out of 6–7 tobacco-related cancers [29]. *KRAS* G12C also serves as a marker of lung cancer sensitivity to immune therapy, because smoking-induced lung malignancies have a high tumor mutation burden (TMB) [30]. Non-G12C *KRAS* mutations are not necessarily associated with smoking history and cannot be targeted by currently approved drugs.

EGFR, *BRAF*, *MET*, *HER2*, *ALK*, *RET*, *ROS1*, and *NTRK1-3* targeted drugs render the best overall survival when applied in the first line [31]. Current laboratory facilities rarely provide comprehensive genetic profiling of lung carcinomas within an acceptable time frame, which is around two weeks or fewer. Consequently, many patients carrying druggable mutations start their therapy with non-specific interventions. Significant shortening of the turn-around time for the comprehensive analysis of actionable mutations is a primary need for lung cancer management [6].

Germline genetic testing is rarely applied to lung cancer patients, although the substantial incidence of these tumors in young non-smoking subjects remains an enigma. There are a few dozen subjects across the world, mainly of North American ancestry, who developed this disease due to inheritance of the *EGFR* T790M mutation [32]. In the practical sense, oncologists have to be aware of Li-Fraumeni syndrome, i.e.,

TP53 pathogenic variants, as a likely cause of young-onset lung cancer. Li-Fraumeni syndrome includes predisposition to early-onset breast, soft-tissue, brain, and hematological malignancies, so the combination of lung cancer with these tumors within a given patient or family calls for germline genetic testing. Of particular note, Li-Fraumeni related lung cancers almost always carry somatic *EGFR* mutations [33, 34].

Breast cancer

While molecular testing for lung cancer is focused mainly on the analysis of somatic mutations, germline DNA sequencing forms the backbone for genetic examination of breast cancer patients. Women with clinical features of hereditary cancer predisposition, e.g., younger than 50–55 years, or having family history of breast or ovarian cancer, or with triple-negative receptor phenotype, or with bilateral appearance of breast cancer disease, have to undergo testing for germline pathogenic variants in *BRCA1* and *BRCA2* genes. Historically, *BRCA1/2* analysis was viewed as a purely cancer predisposition test aimed to estimate the risk of the development of the second tumor as well as to reveal mutation carriers among family members. The discovery of the vulnerability of *BRCA1/2*-driven tumors to DNA double-strand break-inducing agents, such as platinum compounds and PARP inhibitors, shifted the focus towards predictive value of *BRCA1/2* testing [35–37].

Several issues regarding germline DNA analysis in breast cancer patients remain disputable. The increasing availability of NGS calls into question current attitudes towards the selection of breast cancer patients based on clinical criteria for hereditary syndromes. Some experts suggest universal *BRCA1/2* testing for all consecutive patients with breast cancer [38–40]. It is beyond doubt that this practice will eventually take place, although some essential nuances, e.g., clinical decisions with regard to chance finding of germline *BRCA1/2* pathogenic variants in family history-negative elderly women, have been neither discussed nor properly investigated.

The technical drawbacks of existing technologies for NGS analysis are rarely acknowledged in medical literature. For example, many NGS services cannot reliably detect so-called large gene rearrangements (LGRs), i.e., gross deletions or duplications involving one or several exons of *BRCA1/2* genes [41]. Importantly, patients with *BRCA1/2* LGRs are the best responders to PARP inhibitors, so this is a significant deficiency in that current DNA testing practices are likely to miss women with the most prolonged benefit from targeted therapy [42, 43].

The extension of NGS panels beyond *BRCA1/2* is a particularly difficult topic. *CHEK2* and *ATM* genes are the most common and established contributors to breast cancer predisposition after *BRCA1/2*; however, the clinical significance of the testing of these genes is unclear because they appear to be associated with only a two-fold increase of the risk of malignant disease [44–46]. Furthermore, *ATM*- and *CHEK2*-associated tumors apparently do not have a therapeutic window for PARP inhibitors [47, 48]. Similar concerns are applicable towards *BLM* and *NBS1* gene testing [49, 50].

A recent study provided novel insights into the testing of moderately penetrant breast cancer predisposing genes. Many genes associated with hereditary cancer syndromes render a relatively insignificant increase in cancer risk in heterozygous mutation carriers but are associated with fatal medical conditions in subjects with biallelic gene abnormalities. The inclusion of *BLM* in the breast cancer NGS diagnostic panel led to the identification of a woman with Bloom syndrome, who remained surprisingly healthy until she developed a malignancy. These chance findings may have profound impact both for the well-being of particular patients and for medical research. Indeed, compensatory mechanisms in subjects with otherwise fatal mutations deserve detailed investigations, as they may suggest novel treatments for life-threatening conditions [51].

The analysis of *TP53* germline mutations may reveal subjects with Li-Fraumeni syndrome, thus calling for genetic analysis of family members; it is particularly relevant to women with young-onset breast cancer [52]. Tumors arising in these patients have a high frequency of *HER2* gene amplification and overexpression [53]. Li-Fraumeni syndrome is a severe condition, so many carriers of the *TP53* mutation cannot transmit this defect to children due to limited life span or decreased chances to find a spouse. At the

same time, a significant portion of subjects with this disease have de novo alteration in the *TP53* gene, so they do not have a family history of cancer disease [54].

PTEN mutations are associated with Cowden syndrome [55]. It is of interest that clinical activity of the AKT inhibitor, capivasertib, has already been demonstrated for breast patients with somatic *PTEN* mutations, therefore, this drug deserves studies in subjects with hereditary *PTEN*-related tumors [56].

Several "*BRCA1/2*-like" genes, i.e., members of DNA repair by homologous recombination pathway, have been incorporated in breast cancer genetic testing relatively recently. Perhaps only *PALB2* may be regarded as a true equivalent of *BRCA* genes [40, 57]. Some *RAD* и *FANC* family members, e.g., *RAD51C* and *RAD51D*, demonstrated more or less consistent association with breast cancer, although neither the degree of the increase in cancer risk nor the somatic status of the remaining allele of the involved gene, and consequently, tumor sensitivity to PARP inhibitors, have been investigated in sufficient detail [58, 59]. Similar limitations apply to *BRIP1* and *BARD1* genes [40, 45, 59]. The impact of other genes from *RAD* и *FANC* families is even less studied. Overall, it is important to continue the analysis of rapidly growing data sets in order to clean diagnostic NGS gene lists from irrelevant genes while encouraging sequencing of novel breast cancer predisposing loci.

Somatic analysis of breast tumors is limited to the testing of only a few genes in metastatic hormone receptor-positive HER2-negative carcinomas. Some of these tumors are intrinsically resistant to endocrine therapy due to activation of PI3K/PTEN/AKT pathway. Patients with *PIK3CA* mutations may benefit from the addition of PI3K inhibitors to fulvestrant [60]. Capivasertib has somewhat larger indications, as it also demonstrated activity in women with somatic mutations affecting *PTEN* or *AKT* [61]. Importantly, a breast cancer study involving comprehensive genetic profiling, i.e., exhaustive analysis of all potential drug targets, demonstrated no benefit from extended testing for somatic mutations [62].

Ovarian cancer

Ovarian cancer, being a common disease, has an unusually high proportion of patients who developed this disease due to germline genetic defects. As many as 25–40% of women with high-grade serous carcinoma of the ovary carry pathogenic alleles in *BRCA1* or *BRCA2* genes [63, 64]. Unlike for breast cancer, the selection of patients based on clinical criteria, such as young onset or family history, is discouraged; hence, all consecutive patients with high-grade ovarian cancer disease need to be tested for germline *BRCA1/2* mutations [65]. The addition of new genes to this list is even more questionable than for breast cancer. Given that current NGS panels tend to pool together all cancer-predisposing genes in the same test, not a mere DNA analysis but the medical interpretation of the obtained data is likely to possess some problem in the future.

Approximately 30% of high-grade serous ovarian carcinomas do not have apparent alterations in *BRCA1/2* or similar genes but resemble *BRCA1/2*-driven tumors by genomic architecture as well as by sensitivity to PARP inhibitors and platinum compounds. This feature was historically defined as BRCAness, although in the current literature the term "homologous recombination deficiency" (HRD) is more common. HRD testing is utilized for the selection of patients for the treatment by PARP inhibitors. HRD analysis is a highly complex, expensive, and time-consuming test that involves NGS-based genome scanning followed by the bioinformatics analysis of the integrity of chromosomal arms [66].

Colorectal cancer

Molecular testing for colorectal cancer is no less complex than that for lung cancer, although it is often mistakenly perceived by oncologists as a relatively straightforward pipeline. The utmost challenge lies in the comprehensive detection of mutations in *KRAS* and *NRAS* genes. These substitutions occur at frequencies of approximately 50% and 5%, respectively, with surprisingly little impact of ethnic or geographic variations [67]. *RAS* testing guides the choice between anti-EGFR antibodies and bevacizumab when considering the addition to chemotherapy backbone. While patients with wild-type *KRAS* and *NRAS* derive significant benefit from cetuximab or panitumumab, erroneous administration of these drugs to

subjects with mutations, which were missed by laboratory tests, is associated with the risk of stimulation of tumor growth [68].

Initial clinical trials on anti-EGFR antibodies relied on PCR testing for a limited repertoire of *RAS* hotspot mutations [69]. Subsequent studies utilized Sanger sequencing, which may be prone to false-negative results when the proportion of the tumor cells in the sample is low [68]. This disadvantage can be overcome by NGS testing or by the use of relatively sophisticated laboratory procedures combining allele-specific PCR, high-resolution melting (HRM) analysis, pyrosequencing, etc. [67]. Importantly, unlike for lung cancer, molecular analysis of colorectal tumors is not a particularly time-sensitive procedure. Clinical studies demonstrate that the choice between anti-EGFR antibodies and bevacizumab can be safely postponed until the second cycle of chemotherapy; therefore, one month is an acceptable turn-around time for *RAS* testing [70].

The analysis of *BRAF* codon 600 mutations is not complicated. *BRAF* V600E substitutions occur in 4–8% of colorectal carcinomas and are associated with significantly worsened disease outcomes [67, 71]. BRAF inhibition alone or in combination with MEK downregulation is not effective due to collateral activation of EGFR-driven signaling cascade. The efficacy of combined administration of BRAF-targeted drugs and anti-EGFR therapeutic antibodies has been demonstrated in several clinical trials involving different agents; however, formal approval for colorectal cancer treatment has been granted only to encorafenib and cetuximab [72].

Microsatellite instability (MSI), i.e., accumulation of multiple alterations in tandem nucleotide repeats, reflects the deficient mismatch repair (dMMR). This mechanism of tumor development is relevant to approximately 5–10% of colorectal carcinomas. Two distinct routes underlie the emergence of MSI. Some colorectal cancer patients have developed their disease due to heterozygous germline defects in one of the dMMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM*). This condition is called Lynch syndrome (hereditary non-polyposis colorectal cancer); the majority of patients belonging to this category are aged below 50 years and/or have family history of colorectal or endometrial cancer. In addition to young-onset patients, MSI is characteristic of elderly subjects. Indeed, colorectal carcinomas arising in patients aged above 70–80 years frequently have somatic inactivation of the *MLH1* gene due to methylation of its promoter.

MSI tumors have an excessive number of mutations and, consequently, are highly immunogenic, have relatively low relapse rates after surgery, and can be efficiently managed by therapeutic inhibitors of immune checkpoints. The incidence of MSI demonstrates pronounced interstudy variations, depending on the proportion of localized and metastatic tumors, population-specific contribution of Lynch syndrome in cancer incidence, prevalence of elderly people among analyzed patients, and, possibly, technical nuances of MSI detection [73]. MSI is often combined with activating events in MAPK pathway genes, particularly *KRAS*, *NRAS*, and *BRAF* mutations. Up to a third of *KRAS/NRAS/BRAF* mutation-negative tumors carry druggable rearrangements in receptor tyrosine kinases [74].

POLE mutation testing has been introduced into clinical practice relatively recently. *POLE* encodes for DNA polymerase, so the tumors with altered *POLE* accumulate an excessive number of mutations. Clinical significance of *POLE* mutations is essentially similar to that for MSI-H, as they are associated with high tumor responsiveness to immune therapy and may indicate the presence of hereditary cancer syndrome [75].

HER2 amplification followed by gene overexpression is detected in approximately 1–2% of colorectal cancers. It is essential for treatment decisions to ensure that HER2 activation plays a driver but not a passenger role in a given tumor, i.e., at least to exclude the presence of *KRAS* mutations [25]. *HER2*-positive *KRAS*-negative tumors can be efficiently managed by a number of HER2-targeted drugs [76, 77].

Pancreatic cancer

KRAS mutations are detected in approximately 80–90% of pancreatic carcinomas [78]. The majority of them are currently not druggable, although *KRAS* G12C substitution, which is rare in pancreatic

malignancies, can be managed by sotarasib or adagrasib [79, 80]. *KRAS* mutation-negative tumors often carry genetic alterations associated with sensitivity to available drugs, particularly *BRAF* V600E substitutions and rearrangements involving receptor tyrosine kinases (*NTRK1-3*, *ALK*, etc.). Approximately 1–2% of pancreatic tumors are microsatellite-unstable [81].

The contribution of germline pathogenic variants is a controversial topic. It is beyond doubt that heterozygous inactivating variants in *BRCA2*, and possibly *PALB2* genes, are associated with the development of pancreatic tumors in some individuals, and these tumors arise via inactivation of the remaining allele of the involved gene, being, therefore, highly sensitive to platinum compounds and PARP inhibitors. The impact of *BRCA1*, which is similar to *BRCA2* with regard to the spectrum of associated cancer types and the biological role, is less proven, both for the increase of the disease risk and for the tumor sensitivity to DNA double-strand inducing agents [82, 83]. Other genetic causes of pancreatic cancer are exceptionally rare.

Biliary tract tumors

Activating mutations and rearrangements affecting the *FGFR2* gene are the most common events in this variety of tumors: they are detected in approximately 1 out of 5 biliary tract carcinomas. HER2 activation in this cancer type occurs via gene amplification and overexpression as well as via point mutations; overall, HER2 upregulation is observed in approximately 4% of biliary tumors. In addition, 2% of biliary carcinomas carry V600E substitution in the *BRAF* oncogene, and 2% of cases are microsatellite unstable. Intrahepatic cholangiocarcinomas, but not other tumors from this category, have frequent involvement of *FGFR2* receptor tyrosine kinase and *IDH1/2* genes. In total, more than a third of biliary malignancies are amenable to targeted therapy [84].

Melanoma

Approximately 60% of cutaneous melanomas carry *BRAF* V600E substitution. These tumors are often associated with excessive ultraviolet exposure and, therefore, a high mutation burden and increased antigenic load. The optimal sequence of BRAF inhibitors and immune oncology drugs depends on the particular clinical situation [85, 86]. The incidence of *NRAS* mutations in melanomas of the skin approaches 15% [87]. These alterations are not currently druggable; however, it is advisable to perform *NRAS* testing: the presence of *NRAS* mutations confirms *BRAF*-negative status of the tumor, while the absence of this event may call, at least in some circumstances, for the search of other alterations in MAPK signaling pathway [88]. Approximately 15% of mucous and acral melanomas carry activating events in KIT receptor tyrosine kinase; unfortunately, only a portion of *KIT* mutations are druggable by available KIT inhibitors [89].

Thyroid cancer

Medullary thyroid carcinomas are characterized by the high occurrence of activating mutations in *RET* receptor tyrosine kinase. Importantly, this histological diagnosis calls for germline *RET* testing irrespective of other clinical features, such as age or family history, as 10–25% of consecutive medullary thyroid carcinomas are hereditary in nature. In addition to germline mutations, a significant portion of these tumors arise due to somatic events affecting the same oncogene. Papillary thyroid cancers have a totally distinct spectrum of druggable genetic events. Approximately half of these carcinomas carry *BRAF* V600E substitutions. A significant portion of papillary tumors contain druggable rearrangements: *RET* fusions are relatively common, while *NTRK* and *ALK* translocations occur at moderate frequency [90, 91].

Perhaps the most striking molecular feature of thyroid cancer is a commonality of scenarios involving *RET* oncogene activation, especially given recent invention of RET inhibitors. While medullary cancers develop via mutation-driven activation of RET kinase, papillary tumors often arise due to the emergence of rearrangements affecting this oncogene [90, 91].

Endometrial carcinomas

Endometrial carcinomas have the highest rate of MSI among common tumor types: this event is detected in approximately 20% of malignancies belonging to this category. Although the majority of MSI-positive uterine cancers are sporadic, the possible presence of Lynch syndrome and, consequently, germline testing should be considered in patients with young-onset disease and/or a family history of endometrial or colorectal cancers. *POLE* mutations are also a mandatory component of DNA analysis in endometrial cancer patients. A subset of endometrial tumors carry amplified and overexpressed *HER2* oncogene and, therefore, are amenable to therapeutic HER2 inhibition [92, 93].

Prostate cancer

Similar to pancreatic cancer, the involvement of *BRCA2* germline mutations in the pathogenesis of prostate cancer is beyond any reasonable doubt, while the role of the *BRCA1* gene is far less clear [82, 94, 95]. Castrate-resistant prostate cancer patients are recommended to undergo testing for somatic mutations in homologous recombination repair (HRR) genes. The HRR test is sometimes confused with the HRD assay, given that both tests are intended to reveal tumors with sensitivity to PARP inhibitors and extend their spectrum beyond BRCA-driven carcinomas. As already explained above, the HRD test relies on the genomic scanning of the tumor karyotype and identifies instances of "BRCA-like" chromosomal instability. In contrast, the HRR assay is not capable of revealing the consequences of deficient homologous recombination, but instead simply supports the analysis for somatic mutations in genes presumably involved in this pathway.

The most well-known HRR panels contains up to 15 genes, including *BRCA1* and *BRCA2* [94, 96]. The reliance on HRR gene analysis has significant limitations. First of all, even the detection of a somatic mutation in a well-known gene, like *BRCA2*, does not guarantee the inactivation of the remaining gene allele, i.e., functional BRCA2 inactivation [43]. Furthermore, several genes, which have been included in HRR panels, do not play a role in rendering tumor responsiveness to DNA double-strand break inducing agents [97]. *ATM* and *CHEK2* are relevant examples in this respect, because they usually do not undergo somatic biallelic inactivation even in tumors with germline pathogenic variants. Furthermore, there are several lines of evidence suggesting that ATM and CHEK2 deficiency is not associated with tumor sensitivity to PARP inhibitors or platinum compounds [47, 48]. Molecular genetic testing for prostate cancer deserves to undergo substantial revision in the next few years.

Urothelial carcinomas

FGFR3 up-regulation is the most common druggable event in urothelial cancers. Somewhat surprisingly, activation of this receptor tyrosine kinase is observed in more than a quarter of localized cancers, while this estimate falls to about 15–20% in metastatic forms of this disease. The majority of alterations are point mutations affecting "hot codons". However, some carcinomas arise due to *FGFR3* gene rearrangements. Alterations in other genes belonging to the *FGFR* family are significantly less common. A significant portion of urothelial malignancies have evidence of activation of the *HER2* oncogene via point mutation or gene amplification followed by overexpression. MSI is detected in up to 1% of tumors of this type [98].

Agnostic administration of anticancer drugs

The term agnostic originates from the Greek word "gnosis" (knowledge). Agnostic choice of therapy means the reliance on molecular data irrespective of the knowledge of tumor histology. Although being intuitively attractive, this approach tends to ignore many important nuances, for example, the molecular context of druggable mutations. For instance, *BRAF* V600-mutated melanomas can be efficiently managed by BRAF inhibitors administered either alone or in combination with MEK antagonists. This treatment is ineffective in colorectal carcinomas because these malignancies express significant amounts of EGFR receptor and, therefore, are capable of escaping this treatment via the collateral pathway. However, appropriate modification of this therapy, i.e., the addition of anti-EGFR therapeutic antibodies to BRAF inhibitors, allows

to achieve tumor shrinkage. Despite all these limitations, the feasibility of the agnostic approach is beyond any reasonable doubt, and this attitude towards the use of cancer drugs will be increasingly utilized in the future [99].

Integrative genomic tests

The majority of molecular tests utilized in clinical oncology rely on the analysis of single activating or inactivating events on a gene-by-gene basis. There is a distinct category of assays which aim at the evaluation of integrative characteristics of the human genome. These assays are significantly more complex, deal with continuous variables, and use certain thresholds. Furthermore, they are more or less agnostic, i.e., relevant to a diverse spectrum of tumor types. Well-known examples of integrative tests include MSI, HRD, and TMB (Table 2). MSI and HRD have been described above in the sections devoted to colorectal and ovarian cancers, respectively.

Table 2. Integrative genomic tests in molecular oncology [30, 66, 73, 81, 82, 86, 92–94, 98]

Genomic feature	Definition	Methods	Tumor types	Drugs
MSI	Accumulation of mutations in microsatellite repeats as a result of mismatch repair deficiency	PCR (BAT25, BAT26, NR21, NR24, NR27) IHC for MMR proteins NGS (whole-genome, whole-exome, targeted panels)	Colorectal, endometrial, pancreatic, biliary, and urothelial cancers	Immune checkpoint inhibitors (pembrolizumab, nivolumab, etc.)
TMB	Total number of somatic mutations; TMB-high refers to more than 10 mutations per megabase	NGS (whole-genome, whole-exome, targeted panels)	Carcinogen-related cancers (smoking-induced lung cancer, ultraviolet-associated skin melanoma), *POLE*-, *POLD1*-, or *MUTYH*-associated cancers	Immune checkpoint inhibitors (pembrolizumab, nivolumab, etc.)
HRD	Failure to repair DNA double-strand breaks using homologous recombination	Analysis of germline and somatic mutations in HR genes Analysis of complex genomic rearrangements and genomic instability scores (HRD score, HRDetect, CHORD) Functional assays (RAD51 foci analysis)	High-grade serous ovarian carcinomas, triple-negative breast carcinomas, pancreatic cancer, prostate cancer	DNA-damaging cytotoxic drugs (platinum, mitomycin C, cyclophosphamide, doxorubicin, etc.), PARP inhibitors (olaparib, rucaparib, niraparib, talazoparib)

HRD: homologous recombination deficiency; IHC: immunohistochemistry; MSI: microsatellite instability; NGS: next-generation sequencing; PCR: polymerase chain reaction; TMB: tumor mutation burden

TMB reflects the total number of mutations in the human genome and correlates tightly with tumor antigenicity, and, consequently, with the efficacy of immune therapy. Initially, TMB was defined as a total number of coding events identified upon whole-exome sequencing. TMB is commonly estimated by multigene NGS assays consisting of several hundred genes. High TMB is characteristic of carcinogen-induced tumors, particularly lung cancer in smokers or melanomas caused by excessive ultraviolet irradiation. Some types of DNA repair deficiency, for example, dMMR, as well as mutations in DNA polymerase genes, e.g., *POLE*, cause an extraordinarily high increase of TMB [100, 101].

Complex genomic profiling

NGS technologies allow for characterization of individual tumor genome within several days. Many commercial services as well as in-house protocols aim at simultaneous analysis of all actionable genetic events in order to assist the choice of the best therapy and warn about hereditary cancer syndromes. The number of predictive genes does not exceed a few dozen, with only a few of them being sufficiently validated (*EGFR*, *HER2*, *BRAF*, *ALK*, *ROS1*, *RET*, *MET*, *NTRK1-3*, *FGFR1-4*, *IDH1/2*, *PIK3CA*, etc.). Similarly, only

a few dozen genes have been convincingly shown to be implicated in the pathogenesis of hereditary cancer syndromes. In addition to single-gene analysis, an "ideal" NGS-assay should consider integrative characteristics of the tumor genome, such as MSI, TMB, and HRD, with the latter test being particularly complicated.

Despite their obvious utility, current NGS diagnostic services have significant disadvantages. The vast majority of available NGS panels pool together truly actionable genes and genes with presumable significance [99]. Consequently, many reported genomic findings are not at all helpful but actually misleading. Technical drawbacks of currently utilized NGS procedures are essential: for example, many NGS tests are unable to reliably detect gene rearrangements or gross deletions and duplications.

Clinical feasibility of complex genomic profiling, i.e., the chances of finding an actionable event in a given tumor, is not always clearly understood by practicing oncologists or cancer patients. For example, NGS has limited added value in lung cancer patients with known driver mutations in MAPK pathway genes or with a history of heavy smoking. Prospective studies revealed low utility of complex genomic profiling in patients with breast cancer [62]. At the same time, complex genomic profiling seems highly relevant to rare tumors or cancers of unknown primary site [102]. Increasing availability of NGS will certainly facilitate the use of multigene testing, together with or instead of conventional single-target assays.

Liquid biopsy

All tumors carry a certain number of somatic mutations. As some tumor cells undergo decay, possibly due to apoptosis, they shed DNA into the bloodstream. Mutated DNA can be detected with the highest level of sensitivity and specificity: some available technologies, for example, specific modifications of droplet digital PCR or NGS, are capable of detecting a single mutated gene copy in the presence of a few thousand normal gene counterparts [3]. Historically, the analysis of circulating tumor DNA (ctDNA) proved to be useful as a replacement for tissue biopsy, for example, for the detection of treatment-induced *EGFR* T790M mutations [103]. The use of ctDNA for the analysis of primary chemonaive tumors looks less feasible, as all tumors undergo morphological investigation upon diagnosis and, therefore, are available for DNA and RNA testing.

The most impressive achievement of liquid biopsy is its ability to monitor the course of cancer disease. Several studies have confirmed that patients with residual ctDNA after surgery benefit from adjuvant therapy, while the clearance of ctDNA allows to omit the postsurgical use of anticancer drugs [104]. Liquid biopsy allows for almost immediate assessment of tumor sensitivity to a given therapy, thus providing an opportunity for timely modification of the treatment [105]. The most attractive feature of the analysis of tumor-derived DNA and proteins in human plasma is the promise for cancer screening: there are ongoing studies that appear to provide sound support to this concept [106].

Liquid biopsy has a significant disadvantage: it is currently performed at the limit of sensitivity and specificity of available technologies, so both false-negative and false-positive results are often observed for the time being. A recent animal study utilized interference with serum nucleases and liver-resident macrophages to minimize ctDNA decay and improve the performance of liquid biopsy. This approach resulted in a dramatic improvement in the sensitivity of ctDNA detection, and may be utilized in humans if proven to be safe [107].

Conclusions

Progress in understanding the mechanisms of cancer progression resulted in the dramatic breakthrough in the development of novel cancer treatments and the invention of a multitude of molecular diagnostic techniques. Cancer management has become a multidisciplinary field of medicine requiring constant interaction and mutual understanding between surgeons, medical oncologists, radiologists, morphologists, molecular geneticists, etc. Current professional requirements imply that practicing physicians should have significant fluency in molecular medicine, while laboratory diagnostic specialists need to be sufficiently familiar with the clinical value of molecular genetic tests. This combined expertise renders a great promise for improving treatment outcomes in cancer patients.

Abbreviations

ctDNA: circulating tumor DNA

dMMR: deficient mismatch repair

HRD: homologous recombination deficiency

HRM: high-resolution melting

HRR: homologous recombination repair

IHC: immunohistochemistry

LGRs: large gene rearrangements

MSI: microsatellite instability

NGS: next-generation sequencing

PCR: polymerase chain reaction

TKIs: tyrosine kinase inhibitors

TMB: tumor mutation burden

Declarations

Author contributions

EI: Conceptualization, Writing—original draft, Writing—review & editing. AS: Writing—review & editing. Both authors read and approved the submitted version.

Conflicts of interest

Evgeny Imyanitov who is the Guest Editor of *Exploration of Medicine* had no involvement in the decision-making or the review process of this manuscript. The other author declares that there are no conflicts of interest.

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References

1. Zhou Y, Tao L, Qiu J, Xu J, Yang X, Zhang Y, et al. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Signal Transduct Target Ther. 2024;9:132. [DOI] [PubMed] [PMC]
2. Sokolenko AP, Imyanitov EN. Molecular Diagnostics in Clinical Oncology. Front Mol Biosci. 2018;5: 76. [DOI] [PubMed] [PMC]
3. Ma L, Guo H, Zhao Y, Liu Z, Wang C, Bu J, et al. Liquid biopsy in cancer current: status, challenges and future prospects. Signal Transduct Target Ther. 2024;9:336. [DOI] [PubMed] [PMC]
4. Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. Clin Cancer Res. 2012;18:1167–76. [DOI] [PubMed] [PMC]
5. Imyanitov EN, Iyevleva AG, Levchenko EV. Molecular testing and targeted therapy for non-small cell lung cancer: Current status and perspectives. Crit Rev Oncol Hematol. 2021;157:103194. [DOI] [PubMed]
6. Imyanitov EN, Preobrazhenskaya EV, Orlov SV. Current status of molecular diagnostics for lung cancer. Explor Target Antitumor Ther. 2024;5:742–65. [DOI] [PubMed] [PMC]
7. Marin-Acevedo JA, Pellini B, Kimbrough EO, Hicks JK, Chiappori A. Treatment Strategies for Non-Small Cell Lung Cancer with Common *EGFR* Mutations: A Review of the History of EGFR TKIs Approval and Emerging Data. Cancers (Basel). 2023;15:629. [DOI] [PubMed] [PMC]
8. Imyanitov EN, Demidova IA, Gordiev MG, Filipenko ML, Kekeyeva TV, Moliaka YK, et al. Distribution of EGFR Mutations in 10,607 Russian Patients with Lung Cancer. Mol Diagn Ther. 2016;20:401–6. [DOI] [PubMed]
9. Robichaux JP, Le X, Vijayan RSK, Hicks JK, Heeke S, Elamin YY, et al. Structure-based classification predicts drug response in EGFR-mutant NSCLC. Nature. 2021;597:732–7. [DOI] [PubMed] [PMC]
10. Janning M, Süptitz J, Albers-Leischner C, Delpy P, Tufman A, Velthaus-Rusik JL, et al.; National Network Genomic Medicine Lung Cancer (nNGM). Treatment outcome of atypical EGFR mutations in the German National Network Genomic Medicine Lung Cancer (nNGM). Ann Oncol. 2022;33:602–15. [DOI] [PubMed]
11. Seo D, Lim JH. Targeted Therapies for EGFR Exon 20 Insertion Mutation in Non-Small-Cell Lung Cancer. Int J Mol Sci. 2024;25:5917. [DOI] [PubMed] [PMC]
12. Duruisseaux M, Besse B, Cadranel J, Pérol M, Mennecier B, Bigay-Game L, et al. Overall survival with crizotinib and next-generation ALK inhibitors in ALK-positive non-small-cell lung cancer (IFCT-1302 CLINALK): a French nationwide cohort retrospective study. Oncotarget. 2017;8:21903–17. [DOI] [PubMed] [PMC]
13. Solomon BJ, Liu G, Felip E, Mok TSK, Soo RA, Mazieres J, et al. Lorlatinib Versus Crizotinib in Patients With Advanced *ALK*-Positive Non-Small Cell Lung Cancer: 5-Year Outcomes From the Phase III CROWN Study. J Clin Oncol. 2024;42:3400–9. [DOI] [PubMed] [PMC]
14. Liu SV, Frohn C, Minasi L, Fernamberg K, Klink AJ, Gajra A, et al. Real-world outcomes associated with afatinib use in patients with solid tumors harboring NRG1 gene fusions. Lung Cancer. 2024;188: 107469. [DOI] [PubMed]
15. Schram AM, Goto K, Kim DW, Macarulla T, Hollebecque A, O'Reilly EM, et al.; eNRGy Investigators. Efficacy of Zenocutuzumab in *NRG1* Fusion-Positive Cancer. N Engl J Med. 2025;392:566–76. [DOI] [PubMed] [PMC]
16. Chagas GCL, Rangel AR, El Osta B. MET alterations in advanced non-small cell lung cancer. Curr Probl Cancer. 2024;49:101075. [DOI] [PubMed]
17. Sattler M, Salgia R. The expanding role of the receptor tyrosine kinase MET as a therapeutic target in non-small cell lung cancer. Cell Rep Med. 2025;6:101983. [DOI] [PubMed] [PMC]

18. Mitiushkina NV, Kholmatov MM, Tiurin VI, Romanko AA, Yatsuk OS, Sokolova TN, et al. Comparative analysis of expression of mutant and wild-type alleles is essential for reliable PCR-based detection of MET exon 14 skipping. Biochimie. 2019;165:267–74. [DOI] [PubMed]
19. Landi L, Chiari R, Tiseo M, D'Incà F, Dazzi C, Chella A, et al. Crizotinib in *MET*-Deregulated or *ROS1*-Rearranged Pretreated Non-Small Cell Lung Cancer (METROS): A Phase II, Prospective, Multicenter, Two-Arms Trial. Clin Cancer Res. 2019;25:7312–9. [DOI] [PubMed]
20. Wolf J, Hochmair M, Han JY, Reguart N, Souquet PJ, Smit EF, et al. Capmatinib in MET exon 14-mutated non-small-cell lung cancer: final results from the open-label, phase 2 GEOMETRY mono-1 trial. Lancet Oncol. 2024;25:1357–70. [DOI] [PubMed]
21. Dankner M, Maxwell J, Rose AAN. The evolving treatment landscape for BRAF-mutated non-small cell lung cancer. Transl Lung Cancer Res. 2024;13:930–5. [DOI] [PubMed] [PMC]
22. Dankner M, Wang Y, Fazelzad R, Johnson B, Nebhan CA, Dagogo-Jack I, et al. Clinical Activity of Mitogen-Activated Protein Kinase-Targeted Therapies in Patients With Non-V600 BRAF-Mutant Tumors. JCO Precis Oncol. 2022;6:e2200107. [DOI] [PubMed] [PMC]
23. Peters S, Stahel R, Bubendorf L, Bonomi P, Villegas A, Kowalski DM, et al. Trastuzumab Emtansine (T-DM1) in Patients with Previously Treated HER2-Overexpressing Metastatic Non-Small Cell Lung Cancer: Efficacy, Safety, and Biomarkers. Clin Cancer Res. 2019;25:64–72. [DOI] [PubMed]
24. Song Z, Lv D, Chen SQ, Huang J, Li Y, Ying S, et al. Pyrotinib in Patients with HER2-Amplified Advanced Non-Small Cell Lung Cancer: A Prospective, Multicenter, Single-Arm Trial. Clin Cancer Res. 2022;28:461–7. [DOI] [PubMed]
25. Sweeney CJ, Hainsworth JD, Bose R, Burris HA, Kurzrock R, Swanton C, et al. MyPathway Human Epidermal Growth Factor Receptor 2 Basket Study: Pertuzumab + Trastuzumab Treatment of a Tissue-Agnostic Cohort of Patients With Human Epidermal Growth Factor Receptor 2-Altered Advanced Solid Tumors. J Clin Oncol. 2024;42:258–65. [DOI] [PubMed] [PMC]
26. Liu SM, Tu HY, Wei XW, Yan HH, Dong XR, Cui JW, et al. First-line pyrotinib in advanced HER2-mutant non-small-cell lung cancer: a patient-centric phase 2 trial. Nat Med. 2023;29:2079–86. [DOI] [PubMed]
27. Goto K, Goto Y, Kubo T, Ninomiya K, Kim SW, Planchard D, et al. Trastuzumab Deruxtecan in Patients With *HER2*-Mutant Metastatic Non-Small-Cell Lung Cancer: Primary Results From the Randomized, Phase II DESTINY-Lung02 Trial. J Clin Oncol. 2023;41:4852–63. [DOI] [PubMed] [PMC]
28. Torres-Jiménez J, Espinar JB, de Cabo HB, Berjaga MZ, Esteban-Villarrubia J, Fraile JZ, et al. Targeting $KRAS^{G12C}$ in Non-Small-Cell Lung Cancer: Current Standards and Developments. Drugs. 2024;84:527–48. [DOI] [PubMed]
29. Mitiushkina NV, Kholmatov MM, Venina AR, Tiurin VI, Yanus GA, Sokolova TN, et al. PCR-based detection of EGFR, ALK, KRAS and BRAF mutations in Russian patients with lung adenocarcinoma: a single-center experience. Neoplasma. 2018;65:972–9. [DOI] [PubMed]
30. Ghazali N, Garassino MC, Leighl NB, Bestvina CM. Immunotherapy in advanced, *KRAS* G12C-mutant non-small-cell lung cancer: current strategies and future directions. Ther Adv Med Oncol. 2025;17:17588359251323985. [DOI] [PubMed] [PMC]
31. Scott JA, Lennerz J, Johnson ML, Gordan LN, Dumanois RH, Quagliata L, et al. Compromised Outcomes in Stage IV Non-Small-Cell Lung Cancer With Actionable Mutations Initially Treated Without Tyrosine Kinase Inhibitors: A Retrospective Analysis of Real-World Data. JCO Oncol Pract. 2024;20:145–53. [DOI] [PubMed] [PMC]
32. Oxnard GR, Chen R, Pharr JC, Koeller DR, Bertram AA, Dahlberg SE, et al. Germline *EGFR* Mutations and Familial Lung Cancer. J Clin Oncol. 2023;41:5274–84. [DOI] [PubMed] [PMC]
33. Mezquita L, Jové M, Nadal E, Kfoury M, Morán T, Ricordel C, et al. High Prevalence of Somatic Oncogenic Driver Alterations in Patients With NSCLC and Li-Fraumeni Syndrome. J Thorac Oncol. 2020;15:1232–9. [DOI] [PubMed]

34. Sokolova TN, Breder VV, Shumskaya IS, Suspitsin EN, Aleksakhina SN, Yanus GA, et al. Revisiting multiple erroneous genetic testing results and clinical misinterpretations in a patient with Li-Fraumeni syndrome: lessons for translational medicine. Hered Cancer Clin Pract. 2021;19:2. [DOI] [PubMed] [PMC]
35. Petrucelli N, Daly MB, Pal T. BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993–2025. [PubMed]
36. Patel MM, Adrada BE. Hereditary Breast Cancer: BRCA Mutations and Beyond. Radiol Clin North Am. 2024;62:627–42. [DOI] [PubMed]
37. Yadav S, Couch FJ, Domchek SM. Germline Genetic Testing for Hereditary Breast and Ovarian Cancer: Current Concepts in Risk Evaluation. Cold Spring Harb Perspect Med. 2024;14:a041318. [DOI] [PubMed] [PMC]
38. Whitworth PW, Beitsch PD, Patel R, Rosen B, Compagnoni G, Baron PL, et al. Clinical Utility of Universal Germline Genetic Testing for Patients With Breast Cancer. JAMA Netw Open. 2022;5:e2232787. [DOI] [PubMed] [PMC]
39. Culver JO, Freiberg Y, Ricker C, Comeaux JG, Chang EY, Banerjee V, et al. Integration of Universal Germline Genetic Testing for All New Breast Cancer Patients. Ann Surg Oncol. 2023;30:1017–25. [DOI] [PubMed] [PMC]
40. Rezoug Z, Totten SP, Szlachtycz D, Atayan A, Mohler K, Albert S, et al. Universal Genetic Testing for Newly Diagnosed Invasive Breast Cancer. JAMA Netw Open. 2024;7:e2431427. [DOI] [PubMed] [PMC]
41. Abel HJ, Duncavage EJ. Detection of structural DNA variation from next generation sequencing data: a review of informatic approaches. Cancer Genet. 2013;206:432–40. [DOI] [PubMed] [PMC]
42. Moiseyenko VM, Chubenko VA, Moiseyenko FV, Zagorskaya LA, Zaytseva YA, Gesha NE, et al. “Lazarus Response” to Olaparib in a Virtually Chemonaive Breast Cancer Patient Carrying Gross BRCA2 Gene Deletion. Cureus. 2018;10:e2150. [DOI] [PubMed] [PMC]
43. Mateo J, de Bono JS, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for the Treatment of Patients With Metastatic Castration-Resistant Prostate Cancer and Alterations in *BRCA1* and/or *BRCA2* in the PROfound Trial. J Clin Oncol. 2024;42:571–83. [DOI] [PubMed]
44. Mundt E, Mabey B, Rainville I, Ricker C, Singh N, Gardiner A, et al. Breast and colorectal cancer risks among over 6,000 CHEK2 pathogenic variant carriers: A comparison of missense versus truncating variants. Cancer Genet. 2023;278-279:84–90. [DOI] [PubMed]
45. Öfverholm A, Törngren T, Rosén A, Arver B, Einbeigi Z, Haraldsson K, et al. Extended genetic analysis and tumor characteristics in over 4600 women with suspected hereditary breast and ovarian cancer. BMC Cancer. 2023;23:738. [DOI] [PubMed] [PMC]
46. Yadav S, Boddicker NJ, Na J, Polley EC, Hu C, Hart SN, et al. Contralateral Breast Cancer Risk Among Carriers of Germline Pathogenic Variants in *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2*. J Clin Oncol. 2023;41:1703–13. [DOI] [PubMed] [PMC]
47. Póti Á, Gyergyák H, Németh E, Rusz O, Tóth S, Kovácsházi C, et al. Correlation of homologous recombination deficiency induced mutational signatures with sensitivity to PARP inhibitors and cytotoxic agents. Genome Biol. 2019;20:240. [DOI] [PubMed] [PMC]
48. Iyevleva AG, Aleksakhina SN, Sokolenko AP, Baskina SV, Venina AR, Anisimova EI, et al. Somatic loss of the remaining allele occurs approximately in half of CHEK2-driven breast cancers and is accompanied by a border-line increase of chromosomal instability. Breast Cancer Res Treat. 2022;192:283–91. [DOI] [PubMed]
49. Buslov KG, Iyevleva AG, Chekmariova EV, Suspitsin EN, Togo AV, Kuligina ESh, et al. NBS1 657del5 mutation may contribute only to a limited fraction of breast cancer cases in Russia. Int J Cancer. 2005;114:585–9. [DOI] [PubMed]

50. Sokolenko AP, Iyevleva AG, Preobrazhenskaya EV, Mitiushkina NV, Abysheva SN, Suspitsin EN, et al. High prevalence and breast cancer predisposing role of the BLM c.1642 C>T (Q548X) mutation in Russia. Int J Cancer. 2012;130:2867–73. [DOI] [PubMed]
51. Suspitsin E, Eliseyeva D, Chiryaeva O, Belogubova E, Aleksakhina S, Sokolenko A, et al. Asymptomatic Bloom syndrome diagnosed by chance in a patient with breast cancer. Fam Cancer. 2024;23:659–64. [DOI] [PubMed]
52. Rogoża-Janiszewska E, Malińska K, Górski B, Scott RJ, Cybulski C, Kluźniak W, et al. Prevalence of germline TP53 variants among early-onset breast cancer patients from Polish population. Breast Cancer. 2021;28:226–35. [DOI] [PubMed] [PMC]
53. Narasimhamurthy M, Le A, Boruah N, Moses R, Kelly G, Bleiweiss I, et al. Clinicopathologic Features of Breast Tumors in Germline TP53 Variant-Associated Li-Fraumeni Syndrome. Am J Surg Pathol. 2025;49:195–205. [DOI] [PubMed]
54. Schneider K, Zelley K, Nichols KE, Schwartz Levine A, Garber J. Li-Fraumeni Syndrome. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993–2025. [PubMed]
55. Magaña M, Landeta-Sa AP, López-Flores Y. Cowden Disease: A Review. Am J Dermatopathol. 2022; 44:705–17. [DOI] [PubMed]
56. Turner NC, Oliveira M, Howell SJ, Dalenc F, Cortes J, Gomez Moreno HL, et al.; CAPItello-291 Study Group. Capivasertib in Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2023;388: 2058–70. [DOI] [PubMed] [PMC]
57. Preobrazhenskaya EV, Shleykina AU, Gorustovich OA, Martianov AS, Bizin IV, Anisimova EI, et al. Frequency and molecular characteristics of PALB2-associated cancers in Russian patients. Int J Cancer. 2021;148:203–10. [DOI] [PubMed]
58. Torres-Esquius S, Llop-Guevara A, Gutiérrez-Enríquez S, Romey M, Teulé À, Llort G, et al. Prevalence of Homologous Recombination Deficiency Among Patients With Germline RAD51C/D Breast or Ovarian Cancer. JAMA Netw Open. 2024;7:e247811. [DOI] [PubMed] [PMC]
59. Potrony M, Morales-Romero B, Moreno L, Pastor B, Grau E, Badenas C, et al. Considerations for hereditary breast and ovarian cancer syndrome molecular diagnosis: experience from the clinical practice. Breast Cancer Res Treat. 2025;210:507–19. [DOI] [PubMed]
60. Rugo HS, Lerebours F, Ciruelos E, Drullinsky P, Ruiz-Borrego M, Neven P, et al. Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. Lancet Oncol. 2021;22:489–98. [DOI] [PubMed]
61. Howell SJ, Casbard A, Carucci M, Ingarfield K, Butler R, Morgan S, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive, HER2-negative breast cancer (FAKTION): overall survival, updated progression-free survival, and expanded biomarker analysis from a randomised, phase 2 trial. Lancet Oncol. 2022;23:851–64. [DOI] [PubMed] [PMC]
62. Andre F, Filleron T, Kamal M, Mosele F, Arnedos M, Dalenc F, et al. Genomics to select treatment for patients with metastatic breast cancer. Nature. 2022;610:343–8. [DOI] [PubMed]
63. Sokolenko AP, Gorodnova TV, Bizin IV, Kuligina ES, Kotiv KB, Romanko AA, et al. Molecular predictors of the outcome of paclitaxel plus carboplatin neoadjuvant therapy in high-grade serous ovarian cancer patients. Cancer Chemother Pharmacol. 2021;88:439–50. [DOI] [PubMed]
64. Marchetti C, Ataseven B, Perrone AM, Cassani C, Fruscio R, Sassu CM, et al. Clinical characteristics and survival outcome of early-stage, high-grade, serous tubo-ovarian carcinoma according to BRCA mutational status. Gynecol Oncol. 2024;187:170–7. [DOI] [PubMed]
65. Imyanitov EN, Kuligina ES, Sokolenko AP, Suspitsin EN, Yanus GA, Iyevleva AG, et al. Hereditary cancer syndromes. World J Clin Oncol. 2023;14:40–68. [DOI] [PubMed] [PMC]

66. Witz A, Dardare J, Betz M, Michel C, Husson M, Gilson P, et al. Homologous recombination deficiency (HRD) testing landscape: clinical applications and technical validation for routine diagnostics. Biomark Res. 2025;13:31. [DOI] [PubMed] [PMC]
67. Martianov AS, Mitiushkina NV, Ershova AN, Martynenko DE, Bubnov MG, Amankwah P, et al. *KRAS, NRAS, BRAF, HER2* and MSI Status in a Large Consecutive Series of Colorectal Carcinomas. Int J Mol Sci. 2023;24:4868. [DOI] [PubMed] [PMC]
68. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369:1023–34. [DOI] [PubMed]
69. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26:1626–34. [DOI] [PubMed]
70. Palmieri LJ, Mineur L, Tougeron D, Rousseau B, Granger V, Gornet JM, et al. Withholding the Introduction of Anti-Epidermal Growth Factor Receptor: Impact on Outcomes in RAS Wild-Type Metastatic Colorectal Tumors: A Multicenter AGEO Study (the WAIT or ACT Study). Oncologist. 2020;25:e266–75. [DOI] [PubMed] [PMC]
71. Malapelle U, Angerilli V, Intini R, Bergamo F, Cremolini C, Grillo F, et al. Detecting BRAF mutations in colorectal cancer in clinical practice: An Italian experts' position paper. Crit Rev Oncol Hematol. 2025;206:104574. [DOI] [PubMed]
72. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, et al. Encorafenib, Binimetinib, and Cetuximab in *BRAF* V600E-Mutated Colorectal Cancer. N Engl J Med. 2019;381:1632–43. [DOI] [PubMed]
73. Imyanitov E, Kuligina E. Molecular testing for colorectal cancer: Clinical applications. World J Gastrointest Oncol. 2021;13:1288–301. [DOI] [PubMed] [PMC]
74. Mulkidjan RS, Saitova ES, Preobrazhenskaya EV, Asadulaeva KA, Bubnov MG, Otradnova EA, et al. *ALK*, *ROS1*, *RET* and *NTRK1-3* Gene Fusions in Colorectal and Non-Colorectal Microsatellite-Unstable Cancers. Int J Mol Sci. 2023;24:13610. [DOI] [PubMed] [PMC]
75. Ambrosini M, Rousseau B, Manca P, Artz O, Marabelle A, André T, et al. Immune checkpoint inhibitors for POLE or POLD1 proofreading-deficient metastatic colorectal cancer. Ann Oncol. 2024;35:643–55. [DOI] [PubMed]
76. Meric-Bernstam F, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M, VanderWalde A, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol. 2019;20:518–30. [DOI] [PubMed] [PMC]
77. Raghav K, Siena S, Takashima A, Kato T, Van den Eynde M, Pietrantonio F, et al. Trastuzumab deruxtecan in patients with HER2-positive advanced colorectal cancer (DESTINY-CRC02): primary results from a multicentre, randomised, phase 2 trial. Lancet Oncol. 2024;25:1147–62. [DOI] [PubMed]
78. Norton C, Shaw MS, Rubnitz Z, Smith J, Soares HP, Nevala-Plagemann CD, et al. KRAS Mutation Status and Treatment Outcomes in Patients With Metastatic Pancreatic Adenocarcinoma. JAMA Netw Open. 2025;8:e2453588. [DOI] [PubMed] [PMC]
79. Bekaii-Saab TS, Yaeger R, Spira AI, Pelster MS, Sabari JK, Hafez N, et al. Adagrasib in Advanced Solid Tumors Harboring a *KRAS*^{G12C} Mutation. J Clin Oncol. 2023;41:4097–106. [DOI] [PubMed] [PMC]
80. Strickler JH, Satake H, George TJ, Yaeger R, Hollebecque A, Garrido-Laguna I, et al. Sotorasib in *KRAS* p.G12C-Mutated Advanced Pancreatic Cancer. N Engl J Med. 2023;388:33–43. [DOI] [PubMed] [PMC]
81. Doi T, Ishikawa T, Moriguchi M, Itoh Y. Current status of cancer genome medicine for pancreatic ductal adenocarcinoma. Jpn J Clin Oncol. 2025;55:443–52. [DOI] [PubMed]
82. Foulkes WD, Polak P. Probing the relevance of BRCA1 and BRCA2 germline pathogenic variants beyond breast and ovarian cancer. J Natl Cancer Inst. 2024;116:1871–4. [DOI] [PubMed]

83. Tischkowitz M, Balmaña J, Foulkes WD, James P, Ngeow J, Schmutzler R, et al.; ACMG Professional Practice and Guidelines Committee. Management of individuals with germline variants in PALB2: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23:1416–23. [DOI] [PubMed]
84. Mitiushkina NV, Tiurin VI, Anuskina AA, Bordovskaya NA, Shestakova AD, Martianov AS, et al. Molecular Analysis of Biliary Tract Cancers with the Custom 3' RACE-Based NGS Panel. Diagnostics (Basel). 2023;13:3168. [DOI] [PubMed] [PMC]
85. Ascierto PA, Casula M, Bulgarelli J, Pisano M, Piccinini C, Piccin L, et al. Sequential immunotherapy and targeted therapy for metastatic BRAF V600 mutated melanoma: 4-year survival and biomarkers evaluation from the phase II SECOMBIT trial. Nat Commun. 2024;15:146. [DOI] [PubMed] [PMC]
86. Atkins MB, Lee SJ, Chmielowski B, Tarhini AA, Cohen GI, Truong TG, et al. Combination Dabrafenib and Trametinib Versus Combination Nivolumab and Ipilimumab for Patients With Advanced *BRAF*-Mutant Melanoma: The DREAMseq Trial-ECOG-ACRIN EA6134. J Clin Oncol. 2023;41:186–97. [DOI] [PubMed] [PMC]
87. Frank GA, Aleksakhina SN, Zavalishina LE, Kekeyeva TV, Venina AR, Ivantsov AO, et al. BRAF and NRAS mutations in Russian melanoma patients: results of a nationwide study. Melanoma Res. 2016; 26:442–7. [DOI] [PubMed]
88. Dirven I, Calliauw E, Awada G, Vounckx M, Kessels JI, Neyns B. Successful treatment of *MAP2K1* mutant stage IV-M1d melanoma with trametinib plus low-dose dabrafenib: a case report. Front Med (Lausanne). 2024;11:1436774. [DOI] [PubMed] [PMC]
89. Hodi FS, Corless CL, Giobbie-Hurder A, Fletcher JA, Zhu M, Marino-Enriquez A, et al. Imatinib for melanomas harboring mutationally activated or amplified KIT arising on mucosal, acral, and chronically sun-damaged skin. J Clin Oncol. 2013;31:3182–90. [DOI] [PubMed] [PMC]
90. San Román Gil M, Pozas J, Molina-Cerrillo J, Gómez J, Pian H, Pozas M, et al. Current and Future Role of Tyrosine Kinases Inhibition in Thyroid Cancer: From Biology to Therapy. Int J Mol Sci. 2020;21: 4951. [DOI] [PubMed] [PMC]
91. Fallahi P, Ferrari SM, Galdiero MR, Varricchi G, Elia G, Ragusa F, et al. Molecular targets of tyrosine kinase inhibitors in thyroid cancer. Semin Cancer Biol. 2022;79:180–96. [DOI] [PubMed]
92. Kuhn E, Gambini D, Runza L, Ferrero S, Scarfone G, Bulfamante G, et al. Unsolved Issues in the Integrated Histo-Molecular Classification of Endometrial Carcinoma and Therapeutic Implications. Cancers (Basel). 2024;16:2458. [DOI] [PubMed] [PMC]
93. Ribeiro-Santos P, Martins Vieira C, Viana Veloso GG, Vieira Giannecchini G, Parenza Arenhardt M, Müller Gomes L, et al. Tailoring Endometrial Cancer Treatment Based on Molecular Pathology: Current Status and Possible Impacts on Systemic and Local Treatment. Int J Mol Sci. 2024;25:7742. [DOI] [PubMed] [PMC]
94. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2020;382:2091–102. [DOI] [PubMed]
95. Markowski MC, Antonarakis ES. *BRCA1* Versus *BRCA2* and PARP Inhibitor Sensitivity in Prostate Cancer: More Different Than Alike? J Clin Oncol. 2020;38:3735–9. [DOI] [PubMed] [PMC]
96. de Bono JS, Mehra N, Scagliotti GV, Castro E, Dorff T, Stirling A, et al. Talazoparib monotherapy in metastatic castration-resistant prostate cancer with DNA repair alterations (TALAPRO-1): an open-label, phase 2 trial. Lancet Oncol. 2021;22:1250–64. [DOI] [PubMed]
97. Stopsack KH. Efficacy of PARP Inhibition in Metastatic Castration-resistant Prostate Cancer is Very Different with Non-BRCA DNA Repair Alterations: Reconstructing Prespecified Endpoints for Cohort B from the Phase 3 PROfound Trial of Olaparib. Eur Urol. 2021;79:442–5. [DOI] [PubMed] [PMC]
98. Mitiushkina NV, Tiurin VI, Anuskina AA, Bordovskaya NA, Nalivalkina EA, Terina DM, et al. Use of 3' Rapid Amplification of cDNA Ends (3' RACE)-Based Targeted RNA Sequencing for Profiling of Druggable Genetic Alterations in Urothelial Carcinomas. Int J Mol Sci. 2024;25:12126. [DOI] [PubMed] [PMC]

99. Aleksakhina SN, Ivantsov AO, Imyanitov EN. Agnostic Administration of Targeted Anticancer Drugs: Looking for a Balance between Hype and Caution. Int J Mol Sci. 2024;25:4094. [DOI] [PubMed] [PMC]
100. Addeo A, Friedlaender A, Banna GL, Weiss GJ. TMB or not TMB as a biomarker: That is the question. Crit Rev Oncol Hematol. 2021;163:103374. [DOI] [PubMed]
101. Imyanitov E, Sokolenko A. Integrative Genomic Tests in Clinical Oncology. Int J Mol Sci. 2022;23: 13129. [DOI] [PubMed] [PMC]
102. Ross JS, Sokol ES, Moch H, Mileshkin L, Baciarello G, Losa F, et al. Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Origin: Retrospective Molecular Classification Considering the CUPISCO Study Design. Oncologist. 2021;26:e394–402. [DOI] [PubMed] [PMC]
103. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al.; AURA3 Investigators. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med. 2017;376: 629–40. [DOI] [PubMed] [PMC]
104. Kotani D, Oki E, Nakamura Y, Yukami H, Mishima S, Bando H, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat Med. 2023;29:127–34. [DOI] [PubMed] [PMC]
105. Moiseyenko FV, Kuligina ES, Zhabina AS, Belukhin SA, Laidus TA, Martianov AS, et al. Changes in the concentration of EGFR-mutated plasma DNA in the first hours of targeted therapy allow the prediction of tumor response in patients with EGFR-driven lung cancer. Int J Clin Oncol. 2022;27: 850–62. [DOI] [PubMed] [PMC]
106. Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, Cohain AT, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science. 2020;369: eabb9601. [DOI] [PubMed] [PMC]
107. Martin-Alonso C, Tabrizi S, Xiong K, Blewett T, Sridhar S, Crnjac A, et al. Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies. Science. 2024;383:eadf2341. [DOI] [PubMed] [PMC]