

## Review Article

# The Extraordinary Progress in Very Early Cancer Diagnosis and Personalized Therapy: The Role of Oncomarkers and Nanotechnology

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The impact of nanotechnology on oncology is revolutionizing cancer diagnosis and therapy and largely improving prognosis. This is mainly due to clinical translation of the most recent findings in cancer research, that is, the application of bio- and nanotechnologies. Cancer genomics and early diagnostics are increasingly playing a key role in developing more precise targeted therapies for most human tumors. In the last decade, accumulation of basic knowledge has resulted in a tremendous breakthrough in this field. Nanooncology, through the discovery of new genetic and epigenetic biomarkers, has facilitated the development of more sensitive biosensors for early cancer detection and cutting-edge multifunctionalized nanoparticles for tumor imaging and targeting. In the near future, nanooncology is expected to enable a very early tumor diagnosis, combined with personalized therapeutic approaches.

## 1. Introduction

Personalized oncology provides new hopes for reducing cancer mortality by selectively targeting anticancer drugs to malignant cells, at the level of the target-sites on the cell surface and within the tumor microenvironment [1, 2]. Nanooncology has succeeded in increasing specificity and effectiveness of cancer treatments, both facilitating drugs uptake and delivery and reducing systemic toxicity and adverse events.

Molecular diagnostics based on the development in sequencing and genotyping of tumor DNA plays a crucial role in personalized therapy [3]. Mutations are a prerequisite for tumor development, often found in cell signaling proteins as a result of deletions/point mutations in gene-coding regions or in promoter-enhancer sequences, DNA insertions, copy-number variations, and chromosomal translocations. However, not every mutation leads to cancer. Somatic mutations are typically present in the tyrosine kinase (TK) domains within DNA sequences encoding for growth factor

receptors involved in signaling pathways, that is, the EGFR, HER2/ERBB2, KRAS, KIT, FGFR, VEGF-R, ABL, PI3K, mTOR, PDGF-R, and HGFR/MET. The activating mutations of the EGFR in a subset of patients with advanced non-small-cell lung cancer (NSCLC) who respond to the EGFR tyrosine kinase inhibitor (TKI) gefitinib or erlotinib are paradigmatic [4].

Personalized oncology started with the HER2 antibody trastuzumab in breast cancer, followed by the BCR-ABL1 inhibitor imatinib in chronic myeloid leukemia (CML) and EGFR-TKIs and ALK-MET inhibitor crizotinib in NSCLC; the most recent success is the BRAF inhibitor vemurafenib in melanoma [1].

The dramatic breakthroughs in the clinical use of TKIs for cancer treatment depended on the predictive specific biomarkers. Metastatic colorectal cancers (CRC), expressing EGFR, allow for predictive response to targeted therapy with specific anti-EGFR monoclonal antibodies (mAbs) [5, 6]; however, CRC carrying KRAS mutations failed to respond.

In addition, current cancer immunotherapy is predominantly based on antitumor specific mAbs [5], after failure of interleukin-2 (IL-2), and the potentially life-threatening adverse events of adoptive cell-transfer of autologous tumor-infiltrating reactive lymphocytes; despite this, therapy achieved 40% tumor regression in advanced metastatic melanoma [7].

The checkpoint regulators CTLA-4 and PD-1 expressed in immune cells were successfully targeted for immunotherapies: in metastatic melanoma patients the anti-CTLA-4 ipilimumab significantly extended the median OS to 10.1 m, in phase III, compared to 6.4 m for the control group, enabling an unprecedented durable tumor regression of 46,6% at 1y. and 23,5% at 2y. [8]. Ipilimumab is becoming a first-line treatment, doubling survival at 1-2y. in the metastatic melanoma; its association with anti-PD-1 mAb nivolumab ameliorated the 2-year survival to 80%, in phase I [9].

Nanotechnology is revolutionizing cancer management, because it can partially change its diagnosis, clinical course, and prognosis. The cancer molecular profiling before treatment can be highly prognostic and predictive of therapeutic responses or recurrence, by allowing the most suitable treatment for each individual cancer to be administrated.

## 2. Molecular Diagnostics for Personalized Oncology

Mutations in TK genomic domains of a number of growth factor receptors were dubbed “driver” mutations, as they induce malignant transformation. Despite initial successes, targeted therapy may fail in a proportion of patients, especially in tumors with driver mutations. Genetic heterogeneity and insurgence of drug resistance due to secondary mutations, and tumor relapse, may occur, either early or late during tumor development, in a minority of tumor cell subclones, which engender insensitivity to the drug and tumor progression.

Examples are BRAF and MEK mutations in melanoma [10] or gatekeeper mutations of EGFR, such as pT790M mutation in NSCLC, that causes reduced drug binding and may coexist with EGFR pT790M and MET amplification, and the P13KCA mutation or overexpression of GAS6 ligand and its homologous receptor AXL [1]. The resistance to EGFR TK inhibitors in EGFR-mutated NSCLC patients not correlated to PT790M mutation is modulated by FAS and NF $\kappa$ B signaling [11].

The understanding of metabolic and signaling pathways implicated in most of the encountered resistance mechanisms is becoming clear, hence allowing development of new therapeutic approaches.

Advanced melanoma, which is particularly resistant to chemotherapy and rapidly metastasizes, is representative of a multidrug resistant (MDR) cancer type. BRAF-V600E mutations have been found in over 60% of cases of melanoma. Although initially responsive to specific inhibitors, resistance rapidly develops in the form of mutated gene amplification. BRAF is a component of MAPK pathway, which relays proliferative signals, triggered at cell membrane receptors, through the cytoplasm to the nucleus.

The mitogen-activated protein kinase MEK is also commonly overactivated in melanoma; thus, a combination of BRAF and MEK inhibitors was conceived as dual mechanisms to inhibit the MAPK pathway in BRAF-V600 mutation-bearing melanomas. Monotherapy with vemurafenib or dabrafenib achieved OR of 57% and 50% and progression-free survival (PFS) of 6.9 m and of 5.1 m, respectively, in phase III clinical trials. Dual-therapy consisting of dabrafenib + trametinib obtained a good OR of 75% and PFS of 9.4 m in phase II [12, 13]. Nevertheless, failure to respond and dual resistance to both drugs rapidly developed [14, 15].

Prognostic markers assays are relevant in molecular diagnostics for many types of leukemia and cancer (Table 1). Among these, there are the antioncogene TP53, the IGHV mutations, and the expression of CLLUI in chronic lymphocytic leukemia (CLL) and in breast cancer, to predict the recurrence risk of lymph node-negative tamoxifen-treated patients, or methods to quantify estrogen levels and expression of progesterone receptors, HER2 and Ki-67, which also have prognostic value [1]. Cell surface receptors (EGFR, HER2, and KIT) are useful targets for therapy, whereas the cytoplasmic BRAF results are less accessible.

Mutations of surface receptors are often present in NSCLC. EGFR mutations are in-frame with microdeletions at exon 19 [16] and rearrangement of the EML4 gene with parts of the ALK gene results in enhanced proliferation and transforming activity *in vitro* [17]. KRAS mutations are usually in codon 1 or 12, whereas the vast majority of BRAF mutations correspond to a hotspot transversion mutation T1799A at exon 15. All the above-mentioned mutations are key predictive biomarkers for developing effective strategies of targeted therapy.

The role of noncoding DNA in cancer has revealed novel types of promising biomarkers. Genetic and epigenetic biomarkers in cancer management are beginning to affect prognosis and outcome for most human tumors [18].

*2.1. Genetic Oncomarkers in Tumor and Metastasis Detected by Advanced Genomics.* Therapeutic targeting in solid tumors is primarily based on the identification of cancer-specific gene mutations [19]. Targeted delivery of drugs specifically directed to individual tumor cells resulted in successful treatment of NSCLC displaying EGFR mutations or carrying the EGFR-amplified gene. NSCLC patients treated with gefitinib showed a significant survival increase [20] whereas patients with small-cell lung carcinoma (SCLC) failed to respond. Integration of new proteomics assays for identifying biomarkers with statistical analysis for standardizing clinical data resulted in the implementation of personalized medicine, both providing a predictive response to treatments and indicating the likelihood of adverse events [21].

NSCLC frequently express genomic mutations in HER2 (28%), BRAF (2–5%), ALK (5%), and KRAS (30%) [22–25]. The response to specific TKIs varies according to tumor type and genetic makeup. Tyrosine kinases are undergoing evaluation as first-line treatment alone or combined with chemotherapy and radiotherapy. In NSCLC, FGFR1 amplifications, PK3CA and DDR2 mutations, and ROS1 and

TABLE 1: Genetic and epigenetic cancer biomarkers for targeting therapy.

Cancer	Clinical markers	Molecular biomarkers			Anticancer targeting	Biblio.
		Genetic	Epigenetic	Epigenetic		
Colorectal	CEA HER1/EGFR	KRAS, APC	<i>CpG, MLHI</i>	Cetuximab, panitumumab, FOLFFOX + bevacizumab	[1-3, 5, 6, 54-56, 66, 74, 78, 79, 88-90, 117, 144, 145]	
		WNT, Tp53 BRAF SMAD4	<i>TEPI2, SEPT9</i> > <i>miR-22, 155</i> > <i>miR-17-3p, 92</i>			
Gastric GIST	CEA, CA10-9 RCAS	KIT, HER2	> <i>miR-106a</i>	Imatinib, trastuzumab Sunitinib, ramucirumab Cetuximab, panitumumab FOLFIRI + bevacizumab	[1, 3, 55, 65, 144, 145]	
		MET, PDGF-R E-cadherin, VEGF mTOR, BAX, TFF1	< <i>miR-31</i> <i>MSI</i>			
Breast	CEA, Ki 67 CA-15,3, CA-27-29, estrogen and progest. recep.	HER2, HER3	<i>GSTPI</i>	Tamoxifen, docetaxel Paclitaxel, DXT Trastuzumab + T-DMI Pertuzumab, lapatinib Bevacizumab	[1-3, 55, 56, 59, 60, 70, 117, 122, 126, 144-146]	
		VEGF, EGFR NY-BR-1 ING-1, CLLU1 mTOR, IGHV	<i>CDK13</i> > <i>miR-21, 155</i> <i>RARβ2</i> <i>MIN17-1, 31</i>			
Non-small-cell lung	CEA, CA-19,9 CA-15,3	EGFR, HER2	<i>CDKN2A</i>	Gefitinib, imatinib Erlotinib, bevacizumab Crizotinib, afatinib Pembrolizumab	[1, 3, 4, 16, 23, 25, 26, 55, 56, 72, 117, 119, 143-145]	
		KRAS, Tp53 ALK-MET, BRAE, PK3CA RET, FGFR1, DDR2, ROS1	> <i>miR-21, 155</i>			
Prostate	PSA, PSMA, CEA, AFP androgen receptor	NKX3-1 RBI, BRCA2 ETS, Tp53 PIK3CA, SPOP TMPRSS2-ERG	<i>PTEN</i> > <i>miR-21</i> > <i>miR-141</i> <i>APC, MAPK</i> <i>ENDRB</i> <i>GSTPI</i>	Metformin Zoledronic acid Docetaxel	[1-3, 55, 56, 60, 64, 100, 101, 115, 117, 144-146, 148, 150]	
		Tp53, CLLU1, IGHV, ZAP-70	< <i>miR-15a, 16, 1</i>			
Chronic lymph. Leukemia	CD52, CAL-101	CLLU1, IGHV, ZAP-70		Fostamatinib, ibrutinib Alenutuzumab	[2, 3, 59, 60, 117]	
Chronic myeloid leukemia	BCR-ABL ATG48	KIT BCR-ABL1, PML/RARα PZFR/RARα, CBP/MOZ	> <i>miR-15, 16</i>	Imatinib, dasatinib Midostaurin, nilotinib	[1, 3, 27, 55, 59, 60, 117, 145]	
Acute lymph. Leukemia	CD20, Ig/T-cell receptor	BCR-ABL1 IKZF1	> <i>miR-155, 21</i> <i>PTK7</i> < <i>miR-92a</i>	Asparaginase, imatinib	[1, 3, 60, 117, 145]	
Acute myeloid leukemia	CD33	AML1/ETO PML/RARα	> <i>miR-191</i> > <i>miR-199a</i>	ATRA (all-trans-retinoic) Gemtuzumab	[1, 3, 60, 116, 117]	
B and T non-Hodgkin lymphoma	B2M, Myc	ALK, mTOR CARD11	> <i>miR-155</i> > <i>mir-210, 21</i>	DXT, CYCLO, vincristine Rituximab, bortezomib	[3, 5, 55, 60, 116, 144, 152]	
Hodgkin lymphoma	CD30, HRS cell	SNPs	> <i>miR-21, 155, 210</i>	Brentuximab Rituximab	[116, 117]	

TABLE I: Continued.

Cancer	Clinical markers	Molecular biomarkers			Anticancer targeting	Biblio.
		Genetic	Epigenetic	Anticancer targeting		
Melanoma	S100B, TA-90 Tyrosinase CTLA-4	VEGF, BRAF	MAPK	Sorafenib, dabrafenib	[1-3, 7-9, 12, 13, 61, 62, 75, 146]	
		KIT, MET-ALK PI3K/mTOR	MEK PTEN	Imatinib, vemurafenib Trametinib, ipilimumab Pembrolizumab		
Ovarian	CEA, CA-125	PTEN, BRCA1/2	GATA4	Metformin	[18, 55, 68, 117, 120, 144, 145]	
		TP53, BRAF	WIFI, NTRK1 >miR-20, 200, 205, let-7f	Abagovomab Cediranib + olaparib		
Cervical	CEA, CA-125	TP53, HER2, HPV	MAPK	Gardasil, bevacizumab	[55, 69, 144]	
Pancreatic	CEA, BIA CA-19-9	HER1	>miR-155, 21	Sunitinib, everolimus	[55, 126, 144, 145, 152]	
		mTOR	>miR-196a	Vismodegib		
Bladder	CEA, BTA NMP22	TSC1	HIC1, SLIT2	BCG, metformin	[2, 28, 71, 84, 85]	
		HSP90	RASAL, NDN	Everolimus		
HCC	AFP, AFU, GP73, SCCA	EZH2	MGMT>miR-122	Sorafenib, metformin	[54, 55, 67, 80, 82, 83, 104]	
		TP53, CDKN2A	CTNNB1	Ramucirumab		
Glioblastoma Glioma	GBM, EGFR CD133, Ki67	FGFR-TACC-3	MGMT	Temozolomide, gefitinib	[55, 56, 91- 93, 117]	
		EGFR-SEPI4	>miR-21	Erlotinib, bevacizumab		
Renal	CD10, CK7,20AO PI, CA9 ADFP, AQP1	VEGF, TP53, mTOR	VHL	Sunitinib, sorafenib	[105- 112, 114, 117, 145]	
		KIT, BRAF PDGF, PI3K	SETD2, BAPI >miR-7g, 21	Bevacizumab, axitinib Pazopanib, everolimus		

RET rearrangements [26] increase the number of potentially treatable patients.

Targeted therapy improved survival in interstitial stromal gastric cancer presenting KIT mutations and in HER2-over-expressing breast cancer [1, 2]. In myeloid leukemia, specific KIT-driven mutations in codon 822 are predictive of a positive response to imatinib, while other mutations require different treatments [27]. Everolimus therapy resulted in longer remission in the 5% of patients with advanced bladder cancer carrying a TSC1 gene mutation [28].

Molecular diagnostics now employs next-generation DNA sequencing [29], that is, oligonucleotide arrays, to reveal transcription profiling and large-scale single-nucleotide polymorphism assays to detect DNA deletions or amplifications [3]. The latest assays for sequencing the complete genome and nanopores [30] are specifically adapted to the clinical requirements, characteristic of a particular cancer. DNAs or RNAs, cDNAs, miRNAs, and small RNAs are specifically selected through novel techniques for genome resequencing and genetic variation. Sequencing of the complete sets of exons of the entire exome in a genome by target-capture hybridization identifies common and rare variants, along with infrequent mutations that are predisposing to cancer. Genome-wide-association studies (GWAS) have enabled investigation of sequence variations, which occur in chromosomal regions from different individuals, and the detection by chip-based methods of single-nucleotide polymorphisms in the human genome [2].

This has facilitated innovative approaches concerning the genesis of cancer and allowed the discovery of BRCA1-2 gene mutations, known to predispose to cancer [3]. Stromal cells are involved in the genesis and regulation of metastasis [31]. Translation of recent discoveries in metastatic cells and their interactions with the stromal cells is required to bypass the main obstacles in cancer therapy [32].

The microenvironment plays a role in renormalization of tumor cells, suggesting that the recovery of normal functions of stromal cells may be an advantageous strategy in cancer treatment [33]. Tumor suppression achieved by the microenvironment of embryonic stem cells has highlighted new potential therapeutic targets [34].

Expression profiling of genes involved in cell-cell and cell-matrix contacts has identified changes involved in metastasis. WNT, Notch, and BMP pathways regulate the dormancy of metastasis [35] and GATA3 inhibits metastasis via miRNA-29b expression [36]. In preclinical models, miRNA-126 promotes breast cancer metastasis affecting the tumor microenvironment [37]. Investigating the epithelial mesenchymal transition (EMT), and gene expression changes during transition [38], has enabled the understanding of the modulation of genes involved in ECM remodeling, cell adhesion, inflammation, oxidative stress, BMP, and TGF- $\beta$  and metastasis. This led to the exploration of other adverse events that facilitate malignancy, invasion, and migration, characteristic of tumors with cancer stem-cell- (CSC-) like phenotype, which promotes EMT [39].

EMT promotes resistance to chemo- and radiotherapy; hence, its elucidation has opened the way to therapeutic manipulation of the most aggressive cancers. A robust

EMT-signature was developed to predict resistance to EGFR and PI3K/AKT pathway inhibitors in NSCLC patients [40] in whom erlotinib-resistance was dependent on AXL-kinase activation [41]. AXL-inhibitor SGI-7079 reverses erlotinib-resistance.

**2.2. Targets in Cancer Stem Cells.** CSC are a stable cell population, whose origin is still a source of fierce debate, and their golden standards are self-renewal *in vitro* and *in vivo*, preserving a constant reservoir. CSC both interact with the tumor microenvironment and ensure to themselves changes that are the ideal niche for their preservation and continuity overtime. Although present in small number in the tumors, CSC show even within the same tumor a heterogeneity that depends on the stage, the degree, and the level of differentiation. CSC act as tumor initiating cells (TIC) and are involved in metastasis and in mediating tumor progression and relapse. A number of markers have been defined that enabled the isolation and characterization of CSC exhibiting different phenotypes in diverse types of tumors [42, 43] allowing targeting therapies [44]. CSC are highly carcinogenic and very resistant to conventional anticancer chemotherapy and radiotherapy [45]; hence, specific targets are mandatory for directing anticancer agents for effective therapies to successfully eradicate cancers. CSC are mainly involved in resistance: to DNA damage induced by high levels of DNA repair [46], to drug penetration, by enhanced expression of ATP-binding cassette drug transporters [47], and to apoptosis [48].

The location of CSC in “niches” of the microenvironment and hypoxia protects them from anticancer agents [49]. Various signaling pathways of normal stem cells are also required for the maintenance of CSC renewal in different human malignancies, such as Hyppo, Hedgehog, Notch, WNT, and PI3K/AKT [45]. Snail transcription factor and TGF- $\beta$ , involved in EMT, play a primary role in CSC activation.

Vitamin D3 and phytochemicals act on the CSC signaling pathway, such as green tea catechins and the salinomycin, which selectively kills breast CSC [50], and provide potentially effective compounds that reduce tumor growth [51]. Chemotherapy that preferentially kills most of the sensitive tumor cells selects the CSC-subset [52]. New agents specifically targeting for CSC are actively sought after [48], but not yet available.

**2.3. Searching for Epigenetic Cancer Biomarkers.** The long-term disruption of epigenetic control in cancer cells is due to reduced levels of DNA methylation [53], alteration in histones or their acetylation [18], and changes in lncRNAs and miRNAs [54, 55]. As DNA methylation is the initial control point of regulating gene transcription, as well as controlling anti-oncogene expression and transcription of silencing RNAs that predispose to cancer, epigenetics is principally implicated in the induction and maintenance of carcinogenesis [56]. The dependency on epigenetic alterations, such as changes in DNA methylation, for the survival of cancer cells, is well known and demonstrated *in vivo* in preclinical cancer models [18]. In the majority of cancers, loss of DNA methylation is



prevalent; conversely CpG islands of tumor suppressing genes (TSGs) are hypermethylated, leading to their silencing [57].

Silencing of regulatory genes has been suggested as alternative to mutations that impair genes structures or inactivate their functionality. Silencing of BRCA1-2 in ovarian and breast carcinomas, and of CDKN2A in squamous cell lung cancer, is typical and supports the “two-hit” hypothesis of Knudson that requires a mutation in both TSGs alleles for their silencing to produce a tumorigenic phenotype [58].

The first evidence for the involvement of miRNA in cancer was revealed by the discovery of frequent deletions of miRNA-15 and miRNA-16 in chronic lymphocytic leukemia (CLL) [59].

Epigenetic personalized medicine has advanced cancer therapy [60]. Epigenetic alterations are increasingly being discovered. Such alterations affect chromatin and gene expression, via DNA methylation, histone acetylation, and deacetylation (e.g., HDAC), respectively, silencing and reactivating gene expression. Indeed, HDAC and DNA methyltransferase (DNMT) inhibitors are successfully employed in MDS and various forms of leukemia [60]. Epigenetic biomarkers have greatly enhanced cancer detection and diagnosis (Table 1), such as for skin [61, 62], oral squamous cell carcinoma (OSCC) [63], prostate [64], gastric [65], colorectal [66], liver [67], ovarian [68], cervical [69], breast [70], bladder [71], respiratory tract [72], and lung [23] cancers.

In addition to TSGs, DNA methylation may also silence genes implicated in cell growth, regulation, and differentiation: hundreds of methylated genes have been reported in human cancers, associated with poor disease outcome and clinical response [73]. New methods for DNA methylation fingerprinting have enabled growth of epigenetic marks; nonetheless, very few methylated genes are utilized as cancer biomarkers in diagnostics [74]. Epigenetic modifications in BRAF and RAS genes are frequently observed in deregulation of the MAPK signaling pathway that drives melanoma and can therefore be utilized as genetic cancer biomarkers [75].

Abnormal DNA methylation, histone modifications, and impaired expression of induced miRNAs drive OSCC [76] presenting MLH1 mutations and hypermethylation and silencing of MGMT and RASSF genes. Hypermethylated HS3ST2, NPY, and HOXA9 genes allow early detection of OSCC, while epigenetic deregulation of NOTCH4 identifies disease recurrence [63].

Genetic and epigenetic changes that occur at different steps in normal cells [18] cooperatively determine the “hallmarks of cancer” leading to malignant transformation [77]. The extraordinary variability of prostate cancer observed by large-scale genomic studies is exemplary of molecular features required for stratifying patients into subgroups for predicting the outcome and selecting the most appropriate therapy. Gene expression profiles and related functional therapeutic information define the best therapy [64].

Prostate cancer frequently presents mutations, such as deletions in TSGs, NKX3-1, PTEN, BRCA2, and RB1, and structural genomic rearrangements which may lead to recurrent fusion of the androgen-responsive genes for TMPRSS2 and transcription factors (TFs). This tumor subtype is androgen-independent, displays a more aggressive

phenotype, and has an unfavorable prognosis [3]. Several point mutations have been identified in high-risk prostate cancer patients, providing information on the mechanisms that promote genetic instability and on the routes through which specific genes and associated pathways may function as potential targetable biomarkers for new therapies.

CRC is a paradigmatic example of tumor progression toward full malignancy, in which both genetic and epigenetic markers have been identified [78]. Comparative sequence analysis of adenomas, the most frequent precancerous lesion for adenocarcinomas, suggests that mutations arise primarily affecting genes and pathways that control cellular growth and differentiation and transform normal cells into carcinomas capable of metastasis [79]. The WNT pathway is involved in the adenoma-carcinoma-transition, and an inverse relationship has been observed between WNT and the APC gene, which is involved in early transformation to adenomas and is mutated in about 80% of CRC. APC mutations result in constitutive activation of WNT signaling. In CRC, other mutated oncogenes include KRAS and BRAF, which increase cell proliferation to the same extent as APC mutations, but mutations also occur in SMAD4 and TGFBR2 genes or as 18q locus deletions. Loss of TGF- $\beta$  signaling transforms the intermediate differentiated adenoma into a full adenoma and drives late events in tumor development, including mutations in TP53, present in 50% of CRC. Most CRC occur sporadically; however, they may also arise due to genetically transmitted mutations or following inflammatory bowel disease. Subtypes are defined according to molecular features based on the tumor epigenetic traits, namely, microsatellite instability (MSI), chromosomal instability (CIN), MLH1 promoter methylation, and CpG dinucleotide island methylator phenotype (CIMP) [18]. All three subtypes carry mutations in protein-coding genes in addition to impaired gene functions, and changes in miRNA expression affecting gene expression.

In HCC significant differential methylation has been revealed by genome-wide methylation profiling and pyrosequencing analysis [80]. Large-scale screening of differentially methylated regions in promoter CpG islands has shown that the changes in methylation particularly affect the signaling networks of BMP4, CDKN2A, GSTP1, and NFATC1, which are all involved in cell development, expression, and death.

A genetic basis for epigenetic disruption in cancer has been universally recognized. It is thus reasonable to assume that disruption of epigenetic control by mutations of key regulators of the extensive transcriptome amplifies the effect of a single genetic alteration. Large-scale sequencing of HCC has revealed mutations in epigenetic regulators, both associated and unassociated with HBV and HCV infections [81]. Mutated genes, most leading to loss of gene function, have been found in nearly all HCC cases [82]. Point mutations and microdeletions were infrequent in the control genes TP53, CDKN2A, and AXIN1, with the exception of CTNNB1, encoding  $\beta$ -catenin. Epigenetic alterations in TP53 and CTNNB1 genes offer new therapeutic targets and biomarkers; for example, for the deregulated expression of the regulatory gene EZH2 involved in epigenetic silencing of the WNT/ $\beta$ -catenin pathway and of TSG miRNA, the former is associated with progression and aggressive phenotype and the latter with

prometastatic evolution of HCC. Expression of specific miRNAs is also associated with HCV-related HCC-pathogenesis [83], the molecular network of which includes TSGs, namely, TP53, PTEN, and all-trans-RA, whose disruption is directly involved in tumor development.

Cervical carcinoma is another type of cancer associated with viral infection, in which recurrent somatic mutations of the MAPK1 gene are particularly relevant and may be indicative of ERBB2 activation suggesting that selected cervical carcinoma patients could therapeutically benefit from ERBB2 inhibition [69]. Gene expression levels in HPV integration sites are significantly higher in tumors with HPV integration.

Both hypermethylation and mutation of the TSG *neudin* (NDN) were detected in most primary urothelial carcinomas, accompanying loss of NDN expression [84]. A total of 50% of transitional bladder cancers show genetic aberration of chromatin-remodeling genes, among which *UTX* is preferentially altered in low-stages and low-grades cancers, providing implications for classification and diagnostics [85]. In lung adenocarcinomas, chromatin patterns change according to development and progression of the disease [86]; therefore, defining chromatin patterns according to the lung adenocarcinoma lifecycle would provide new reliable epigenetic biomarkers in diagnosis, prognosis of tumor progression, and therapy.

**2.4. Novel Emerging Cancer Biomarkers.** Protooncogenes play a very important role in growth and development of normal cells; they can, however, mutate and undergo abnormal activation, which progress towards carcinogenesis. This observation led to the discovery of driving mutations in a number of tumors. Among the mutations in several known oncogenes [87] and antioncogenes acting as TSGs, such as TP53 [88], those located in the PK domains of EGFR, HER2, KIT, FLT3, DNMT (3A and 1), and KRAS GTPase are particularly relevant [60].

In leukemia, chromosomal translocations leading to fusion genes with altered gene function are typical (Table 1). The fusion gene BCR/ABL is formed of the BCR region of chromosome 22 with *c-ABL* of chromosome 9; it occurs in nearly all CML patients and in 20% of patients with ALL and constitutively activates ABL kinase. Imatinib, which specifically blocks BCR-ABL enzyme inhibiting ALB kinase activation, is one of the greatest successes achieved in CML therapy. In Burkitt's lymphoma, specific translocation of the *c-Myc* gene (8:14) occurs, leading to increased expression of target genes and inhibition of NF $\kappa$ B pathway genes [3]; in another non-Hodgkin lymphoma, mutations affect NF $\kappa$ B via *CARD11* gene expression. Therefore, drugs specifically targeting NF $\kappa$ B have been developed, such as bortezomib (a proteasome inhibitor) considered the first effective treatment against relapsed and refractory lymphoma, along with chemotherapy.

Transforming genetic fusions have also been recently reported for several solid malignancies and colon adenocarcinomas [89, 90] representing promising targets for clinically untreatable cancers.

A subset of glioblastomas, containing the transforming fusion gene involving the TK domain of FGFR and the

transforming acid coiled-coil domain of TACC [91], can be treated with FGFR kinase inhibitors which corrects the aneuploidy; in mice harboring intracranial FGFR-TACC-3, oral administration of an FGFR TKI inhibits glioma development [92]. Translocations fusing the EGFR coding-region to several partners have been frequently found in glioblastoma as functional fusion gene [93]. EGFR-SEP14 fusions activate STAT3 signaling and confer sensitivity to EGFR inhibitors and independence to mitogens.

Genome-wide sequencing has identified four subgroups within medulloblastoma (the major cause of death from childhood cancer), characterized by unique targetable somatic copy-number aberrations (SCNAs) involving tandem duplication of the *SNCAIP* gene, recurrent translocations occurring through chromothripsis-like process and leading to PVT1-MYC and PVT1-NDRG1 fusions, and recurrent targeting of TGF- $\beta$  and NF $\kappa$ B signaling [94].

The prognostic role of the MYCN oncogene amplification in neuroblastoma cells has been evaluated in numerous clinical trials [95]. In about 20% of more than 1000 investigated neuroblastomas, MYCN has been found abnormally expressed. Since both the status and function of the MYCN gene and chromosomal aberrations have been reported to improve risk assessment of patients, it can help selecting the most appropriate therapy for each subgroup of patients. Using high density SNP analysis novel drivers of neuroblastoma development have been revealed, namely, the BARD1 gene variants, which are a high-risk indicator in carriers [96].

With the advent of GWAS and next-generation sequencing, allowing the in-depth study of the neuroblastoma genome, genes associated with tumor progression have been identified: ALK gene, candidate for therapy with small-molecule inhibitors; mTOR Aurora kinase and TRKB [95]. The LMO1 locus showed aberrant expression in more than 10% of the most aggressive cases of neuroblastoma, suggesting that this locus polymorphism is associated with susceptibility to tumor development and influences malignant progression [97]. Chromosomal deletions and sequence alterations of the chromatin-remodeling genes ARID1A-B were found in 10% of neuroblastomas, providing new biomarkers to identify rearranged DNA fragments in sera allowing for minimal residual disease detection and therapeutic follow-up [98]. In addition, methylation of PCDH8 and SFN can be used as outcome indicators in high-risk patients [99]. In 40% of gliomas, MGMT, involved in DNA repair, was hypermethylated in the promoter region [53] and associated with glioma sensitivity to radiotherapy and alkylating agents. MGMT protects cells against mutations by removing alkyl groups from guanine. These are imported with the alkylating agents nitrosamide 5-hydroxymethylcytosine (5HmC), catalyzed by three genes (TET1-TET3), with  $\alpha$ -ketoglutarate as cofactor, under the control of isocitrate dehydrogenases IDH1 and IDH2.

Silencing of TET1 by miRNA leads to a decrease in 5HmC, while IDH1 and IDH2 mutations have been found in glioma, myeloid leukemia, and melanoma and may therefore serve as diagnostic markers in a number of cancers [56, 60].

DNA methylation biomarkers are used for early cancer detection but can also predict and/or monitor therapeutic responses and disease recurrence. Examples include

the tissue-factor pathway inhibitor 2 (TEPI2), useful for screening CRC in stools and hypermethylation of septin 9 (SEPT9) in blood samples [79]. In prostate cancer tissue and urine, DNA methylation biomarkers such as APC or ENDRB, together with serum prostate specific antigen (PSA) and quantitative evaluation of glutathione-S-transferase p1 (GSTP1), have improved diagnosis [100, 101].

DNA methylation can be also used as a molecular prognostic biomarker in stage I NSCLC. Methylation of p16 CDKN2A and H-cadherin (CDK13), RASFA, and APC was shown to strongly correlate with early local and mediastinum lymph-nodal recurrences and short survival [72].

**2.5. Toward a Very Early Cancer Diagnosis.** Very early detection of cancer and timely monitoring of recurrence are of paramount importance in establishing prognosis and outcome of the disease and, in general, determine the cures that patients should undergo. Initially, noninvasive diagnostic tests are carried out, typically the fecal occult blood test (FOBT) for CRC [78], of blood in urine for bladder or renal cancer, or hemoptysis for lung cancer. However, all these symptoms lack specificity, as they are also present in other pathological conditions. Any positive result requires more invasive clinical investigations, undesirable for patients, often posing risks and requiring radiation exposure.

The detection of DNA methylation biomarkers based on nucleic acids analysis allows for early diagnosis, prognosis, and prediction of therapeutic response [56]. The discovery of marker genes of DNA methylation and the understanding of their role in cancer is leading to the development of new, more sensitive, and specific genomic-based assays. The identification of tumor specific DNA methylation at distal sites from the original neoplasia, such as blood and other bodily fluids, is particularly suitable for early diagnosis and clinical management of cancer patients, as it does not require invasive procedures for sample collection. Two CRC-specific methylation tests are now commercially available: the plasma methylated DNA for SEPT9 promoter (ColoVantage) and the fecal methylated DNA for the methylated vimentin gene (Colo Sure). DNA methylation markers offer a powerful diagnostic tool for screening individuals at risk of developing lung cancer [72].

Circulating DNA, either free or complexed to red blood cells, platelets, or nucleosomes, has been found in the blood during the course of various disease states [60] and can be a very early cancer biomarker, whereas its decrease and disappearance could be used to monitor the success of the therapy. One limit comes from that DNA mutations, epigenetic markers, and signaling pathways require sufficient material from the affected tissues.

Although promising as noninvasive cancer biomarkers, analyzing cell-free circulating nucleic acids has not reached clinical routine. A major hurdle remains which is the detection sensitivity, even though amplified genomic DNA and RNA from single cells can be used to characterize rare circulating cancer cells [102].

Circulating tumor cells (CTC) have been shown to be responsible for tumor metastasis and resistance to anticancer therapies. They are indeed highly pathognomonic of the

stage of tumor spread and indicative of metastasis in cancer patients. New clinical tests using minute quantities of CTC are ongoing, for early detection at the preneoplastic and/or premetastatic stage, as for prostate [64] and lung cancers [103].

Other molecules, such as lipids, carbohydrates, proteins, and smaller metabolite products related to cancer, can actually be oncomarkers with prognostic value of cancer progression, therapeutic response, and disease outcome [60]. As an example, comparing the emerging biomarkers from exhaled breath, known as volatile organic compounds (VOC), and those of exhaled breath condensate (containing small molecules and nucleic acids) is noninvasive test, allowing a highly specific diagnosis.

Serum miRNAs play a very important role in activation or suppression of target gene expression (Table 1). Its detection is minimally invasive; however, it may not be specific for the indicated cancer [54, 55].

AFP is among the known genetic biomarkers for HCC [104] (Table 1); in addition, other four potential protein biomarkers have been identified: ALNL, FLNB, C4A, and AFT. The first two characterize the HCC group before treatment better than AFP and provide available screening biomarkers in other cancers as well [67].

CA9 has been the first reliable diagnostic biomarker of clear cell renal cell carcinoma (ccRCC) [105]; now, for early detection of kidney cancer, the urine biomarkers aquaporin 1 (AQP1) and adipophilin (ADRP) [106] and increased plasma levels of LCPI, NNMT, and NM23A have been evaluated [107]. Kidney cancer biomarkers play an important role in determining diagnosis and prognosis and are predictive for treatment effectiveness and outcome [108], enabling discrimination between the ccRCC and papillary (pRCC) type 1 (with favorable outcome) and type 2 that rapidly metastasizes [109]. Mucin 1, E-cadherin, VEGF-R2 and VEGF-R3, and cytokeratins (CK) 20 and 7 are differentially expressed in type 1 and type 2 pRCCs, and alpha-methylacyl-CoA racemase mRNA is overexpressed in most pRCCs; all are useful diagnostic biomarkers. CD44, Ki67, CA9, and PCNA, which play a role in apoptosis, cell cycle, cell migration, and angiogenesis, offer prognostic value for ccRCCs. Most ccRCCs bear mutations in the VHL gene [110] that lead to its inactivation or silencing by methylation, causing accumulation of the transcription factor HIF (hypoxia inducible factor), hence upregulation of angiogenic and cell proliferation genes. Overexpressed proteins, such as PDGF and VEGF, are strong proangiogenic factors and are therapeutic targets that have led to the first reliable targeted treatments with bevacizumab and VEGF TKIs [111]. IMP-3 and VEGF-R2 correlate with poor prognosis in pRCC. IMP3, being expressed 10 times more in pRCC than in ccRCC, is particularly unfavorable and indicates a high risk of developing metastases [112].

Integrated data analysis of ccRCC evolution toward malignancy shows a shift in regulation of metabolic pathways controlled by genetic and epigenetic genes [113].

In RCCs, hypomethylation of DNA and miRNA-21 promoter, mutations in the chromosome 3p21 locus of epigenetic genes, chromatin remodeling, BAP1, and SETD2 all cause



inactivation of the TSG PBRM1 and predict an adverse outcome [114, 115].

In B-cell lymphoma, the serum levels of miRNA-155, miRNA-210, and miRNA-21 are significantly elevated [116]; miRNA-21 is also associated with prostate cancer [115] and represents a potential diagnostic biomarker in neoplasia [117]. In most cancers, alterations in chromosomal regions encoding for miRNAs which result in their altered expression have been reported, pointing to a causal role of miRNAs in cancer pathogenesis and metastasis [118]. To identify and validate circulating miRNAs for use as diagnostic and prognostic biomarkers, screening investigations were undertaken, revealing that high levels of miRNA-21 and miRNA-155 can be early indicators of lung cancer [119]. miRNA-205 and Lt-7f can be diagnostic-prognostic biomarkers of ovarian cancer [120].

Numerous genetic and epigenetic biomarkers that may accompany lung cancer development, such as miRNAs and autoantibodies against tumor-associated antigens (TAA), can be detected up to 5 years before NSCLC diagnosis [121]. Future studies should focus on the identification of miRNA signatures as biomarkers and on the development of targeted therapeutics based on miRNAs, as recently attempted for breast cancer [122]. Circulating regulatory noncoding RNAs, such as miRNAs, lncRNAs, and exosomes, are very promising [123].

Exosomes are nanodimensioned extracellular secreted vesicles, which are primarily involved in intracellular communications and transport of proteins, lipids, and other cell components, such as DNA and noncoding RNAs, functioning as signaling molecules, between cells. Free or exosome-encapsulated biomarkers are present in liquid biopsies and can be detected, even in traces, once concentrated and purified, or amplified from blood, urine, saliva, and other body fluids and exudates [124]. The latest generation of microarrays for gene expression profiling and miRNA transcriptomes has allowed a better assessment of changes in heterogeneity within and between tumors, for early prediction of therapeutic response, establishment of resistance, and metastatic evolution of the disease.

### 3. Nanotechnology in Cancer Diagnostics

Routine diagnostics lacks specificity and sensitivity. Engineering of nanoparticles (NPs) to specifically target cancer cells, combining diagnostic imaging with chemotherapeutic and/or physical anticancer agents, has facilitated the creation of multifunctional nanoplatforms for monitoring cancer therapy [125].

Novel nanodevices convert data from interactions between molecules and subcellular structures, based on mutations and alterations in pathways driving cells to malignant transformation, into transduced or amplified electromagnetic signals [126, 127]. At the same time, they can be combined with more effective therapeutic treatments due to their reduced toxicity, as they only target the transformed cells [128].

This has been achieved, in the latest biosensors and nanovehicles (Table 2) [129], utilizing new nanomaterials and

exploiting electric, magnetic, optical, acoustical, mechanical, chemical, and physical properties of unusual compounds in order to increase the sensitivity of diagnostic tools and the effectiveness of the therapeutic devices [130]. In addition, newly discovered genetic and epigenetic oncomarkers have been exploited [18] as molecular targets, either for early cancer detection [131] or for providing more effective and targeted therapies [132].

Modern nanooncology provides both more accurate tumor imaging and more efficient therapy by drug delivery or activation of other anticancer agents, selectively targeted to cancer cells on the basis of their genetic profile [126].

**3.1. Gold, Silica, and Magnetic Nanoparticles.** Gold nanoparticles (GNPs) are powerful imaging labels and contrast agents, due to their unusual optical properties, biocompatibility, absence of cytotoxicity, resistance to photobleaching, and absorbance at near-infrared light region (NIR) of surface-enhanced Raman scattering (SERS) [133] and have been successfully used in both cancer diagnosis and therapy [127]. The absorption and scattering properties displayed by GNPs, conjugated to a ScFv antibody, have been used for *in vivo* targeting of EGFR in NSCLC cells, enabling spectroscopic detection of microscopic tumors.

Recently, plasmon resonance for *in vivo* molecular imaging of carcinogenesis [134] has been experimentally used in breast cancer cells, allowing precancerous epithelium detection and selective photothermal therapy of OSCC cells by anti-EGFR antibody-conjugated NPs [135]. Gold nanoparticles combined with gold nanorods conjugated with specific antibody facilitated extremely sensitive immunoassay detection of PSA in prostate cancer cells by dynamic light scattering (DLS) [136, 137].

Metal, magnetic, and silica nanoparticles (SiNPs) present unique thermophysical properties, such as encapsulated phase change, which make them particularly suitable for cancer-specific marker diagnosis [129]. This type of NPs behaves as a highly sensitive multiplex in oncomarker detection. Phase-change materials (PCM) are able to absorb thermal energy without increasing temperature during melting. Heat diffusion from the environment to the core of PCM is time-consuming and increases the melting peak at constant temperature. SiNPs take advantage of the thermic and physical features of silica, during phase changes. This allows restriction of any changes in metal composition inside the silica-encapsulated NPs, thus providing thermal barcodes with melting peaks directly related to the concentration of the target molecules. SiNPs labeled with fluorescence resonance energy transfer (FRET) dyes have been used for simultaneous and multiplexed detection in order to monitor the presence of cancer cells. In preclinical models of colon carcinoma, the efficient accumulation, in target tissues, of silica-polyethylene glycol nanoparticles (SPNs) functionalized with two metastasis-specific peptides results in specific imaging of tumor foci, providing a powerful fluorescent platform for image-detection of submillimetric colorectal micrometastases [138]. Aptamer-conjugated silica NPs specifically target T-cell leukemia and B-cell lymphoma.

TABLE 2: Biosensors and biochips for early cancer detection.

Nanotechnology	Biomarkers	Cancer type	Biological sample	References
<i>Gold nanoparticles</i>	PSA, EGFR	Prostate, lung, breast epithelial carcinoma	Serum precancerous epithelium, cancer cells	[121, 127, 133–137, 143, 148]
(i) GNPs conjugated gold nanorods			Cell lysate	
<i>Silica and magnetic nanoparticles</i>	EGFR, Her-2/neu uPAR	Colorectal, prostate, breast, leukemia and lymphoma cells, pancreas	Micrometastases cancer cells and tissue	[126, 127, 129, 131, 135, 138, 139, 142, 143, 148]
(i) Aptamer-conjugated SiNPs				
(ii) Magnetic iron oxide (ION) NPs				
(iii) Superparamagnetic ION NPs (SPIONs)				
<i>Quantum dots</i>	PMSA, AFP, CEA, Her-2/neu CA-125, CA-19.9 EpCAM, DNA-methyl	Prostate, liver, pancreas, breast, lung	Serum, cancer cells, saliva, CTC	[121, 126, 127, 129, 140, 143, 148]
QD-functionalized magnetic nanoparticles				
<i>Carbon nanotubes</i>	PSA, AFP FR, Her-2/neu CA-125, CA-19.9	Prostate, liver, breast, lung, pancreas, melanoma	Serum, cancer cells CTC, VOC	[121, 127, 140, 143, 148, 151]
(i) Dendrimer-modified multiwalled CNT (MWNT)				
(ii) Single-walled (SWNT)				
<i>Nanowires (NW)</i>	PSA, IL-6 CEA, ER, VEGF	Prostate, breast epithelial cells, lung, cervical	Serum, cancer cells	[121, 127, 129, 142, 143, 148]
(i) Silicon SiNW	EpCAM, CK-7 IL-10, OPN CA-125 mRNA			
(ii) Gold AuNW				
<i>Cantilevers</i>	PSA, AFP, CEA, BRCAl DNA/RNA	Prostate; liver, breast, melanoma	Serum, cancer cells	[127, 129, 148, 151]
Microcantilevers				
<i>Nanopores</i>	PSA, hepsin, $\alpha$ -hemolysin DNA-methyl. -sequencing	Prostate	Serum, cancer cells	[30, 127, 129, 141]
Si3Na4 functionalized nanopores				
<i>Nanorods</i>	PSA IL-10, VEGF	Prostate, lung	Serum, cancer cells	[121, 127, 143]
(i) Gold nanorods				
(ii) Micropipettes				

Magnetic NPs are particularly recommended in cancer cell imaging to develop diagnostic chips and in therapy to specifically release drugs within selectively targeted malignant cells [139]. Magnetic NPs are able to aggregate in the presence of target molecules, facilitating cancer biomarker measurement. Following surgery, locally implanted magnetic NPs can be left *in loco*; hence, this innovative method for detecting *in vivo* cancer biomarkers, such as EGFR and HER2/neu, may be also suitable for continuous monitoring. The ability of magnetic particles to aggregate to target cells *via* affinity ligands has been exploited as a chip-based NMR system and, with miniaturization and multiplexing, for simultaneously identifying various biomarkers in small liquid samples.

Magnetic nanosensors are three orders of magnitude more sensitive than current clinical assays and recent microchips have been developed that can reveal up to 64 different proteins at the same time.

**3.2. Quantum Dots and Carbon Nanotubes.** Quantum dots (QD) are semiconductors composed of light-emitting nanocrystals displaying a wide-band adsorption with emission bands scattered from UV to NIR [140]. QD-conjugated multifunctional NPs have been successfully used for combining DOX (doxorubicin) with fluorescent PSMA imaging for targeting prostate cancer cells [127]. Ultrasensitive nanomicrochips, based on QD-labeled antibodies for other specific biomarkers, such as CEA, CA-125, and HER2/neu, have been recently developed, enabling oncomarker detection in serum and saliva. QD-functionalized magnetic NPs have also facilitated cancer targeting and imaging. In this way, it has been possible to identify and discard CTC from the blood by magnetic separation, using immobilized biotinylated EpCAM antibodies targeting malignant cells. In addition, the ultrasensitive MS-qFRET nanotechnological assay has been developed to detect and quantify DNA methylation in blood samples. As DNA methylation is directly involved in carcinogenesis through silencing of TSGs, this method can predict the effectiveness of a specific cancer treatment.

Carbon nanotubes (CNT) are helical nanotubes of graphitic carbon particularly recommended for label-free detection of several oncomarkers and, once conveniently modified, allow a series of extremely specific and sensitive diagnostic assays, for example, a multifunctional dendrimer-modified multiwalled CNT for targeting the folic acid (FA) receptor, which is overexpressed in cancer cells, a single-walled CNT coupled with electrochemiluminescent silica NPs for detecting PSA in the blood, and, finally, a multilayered enzyme-coated CNT for high sensitive chemiluminescent immunoassay of serum AFP. CNT, due to their intrinsic ability to penetrate the cell membrane in order to release drugs within targeted transformed cells and to convert optical energy into thermal energy, can be exposed to NIR in order to thermally destroy cancer cells. Conjugation of QD to CNT has enabled localizing cancer cells in the patients, by QD imaging, and subsequent cell destruction via drug release or thermal inactivation [140]. This approach can open up new horizons on multimodal nanoplatforms in oncology.

**3.3. Nanowires and Cantilevers.** In<sub>2</sub>O<sub>3</sub> nanowires (NW), silicon nanowires (SiNW), and gold-conducting polymer NW (AuNW) can be individually assembled or modified for detecting cancer biomarkers [127, 129], in particular, SiNWs for VEGF detection; peptide nucleic acid- (PNA-) modified SiNW for RNA cancer biomarkers; SiO<sub>2</sub>-NW IL-10 for alkaline phosphatase sandwich immune-assay of interleukin-10; and osteopontin (OPN) lung cancer biomarkers. AuNW polymers were used as templates for CK-7, epithelial cell marker, enzymatic immunoassay, and polypyrrole- (Ppy-) NW, which were integrated into the field effect transistor (FET) system, as a semiconducting channel for the cancer antigen 125 (CA 125) assay.

Cantilevers are biosensors for detecting cancer proteins and DNA/RNA biomarkers, based on nanometer-scale produced bends, according to changes in their environment or surface. A cantilever microassay for PSA detection consists of laser measurement of bend deflection caused by antibody-antigen specific binding. In this assay, anti-PSA antibody-conjugation to specific linkers at the microcantilever surface permits reduction of background signals [129] and allows measurement of very low antigen concentrations, monitoring the surface stress by the reflected laser beam. The latest microassays for sensing cancer biomarkers have been developed exploiting resonant microcantilevers, allowing measurements of shifts in frequency that originate from antibody-antigen interactions. As decrease in shift depends on molecular interactions and is proportional to the target concentration, microcantilevers coated with piezoelectric film have been developed, which allow exact determination of CEA concentrations. Another powerful immunological method using integrated rotating resonance microcantilevers for detecting AFP in undiluted serum at nanogram/mL level has allowed the investigation of liver cancer at a very early stage. Nanomechanical analysis for detecting cancer cells *in vivo* has been reported, which measures tumor cell-stiffness with respect to normal cells, by atomic force microscopy.

**3.4. Nanopores and Nanorods.** Nanopores have great potential for detecting biomolecules such as DNA, antigens, ions, and drugs [30]. Nanopores may consist of synthetic or artificial materials or may be protein-based. They are based on the principle that ionic conductance changes when micropores submitted to an electric field are passed through by small molecules; thus, the electrochemical impedance, which varies according to the concentration and the nature of the molecules, can be measured, allowing for the stochastic sensing and biophysical studies of individual molecules. Nanopore membrane functionalized with antibodies enables PSA and hepsin detection while the  $\alpha$ -hemolysin protein nanopore enables detecting immobilized DNA. Another possible application of nanopores is the detection of aberrant DNA methylation by epigenetic analysis [141]. Protein or synthetic nanopores in Si<sub>3</sub>N<sub>4</sub> membranes have been used for methylated DNA detection and for ultrarapid DNA sequencing.

Finally, other ultrasensitive sensors for cancer biomarkers are the gold nanorods and micropipettes. Gold nanorods can be used as molecular imaging agent and, in conjugation

with anti-PSA antibodies, can facilitate DLS analysis for the PSA immunoassay. Micropipettes, due to the capability of capturing antibodies on their surface, can be exploited for detecting IL-10 and VEGF [127].

### 3.5. Multifunctional Nanoplatfoms for Cancer Theranostics.

Over the past twenty years, a prodigious effort has been made in designing and developing nanometer-sized targeted probes for cancer diagnosis and therapy, which combine several features and diverse functions: stability in the circulation, accumulation to specific districts, responsiveness to local stimuli, and effective intracellular drug delivery and multimodality of action [127, 130]. Magnetic iron oxide (ION) nanoparticles used as an MRI contrast agent in cancer imaging provide morphological details of the tumor and monitor in real-time the therapeutic response. Highly lymphotropic superparamagnetic iron oxide nanoparticles (SPIONs), which at once combine high-resolution MRI with optical imaging, have been successfully used for micrometastasis screening in patients with prostate cancer that have undergone surgical lymph node resection or biopsy [135].

Recently, multifunctional ultrasmall SPIONs (USPIONs) based on appropriate and biodegradable coating of N-phosphonomethyl iminodiacetic acid (PMIDA) and using functional hydrophilic, biocompatible, and spacer molecules, such as rhodamine B isothiocyanate, have been designed to combine cancer-targeted MRI/optical imaging and release of pH-sensitive drugs [142]. By combining the amine-derivatized with USPION-PMIDA support, very effective multimodal and theranostics nanoagents have been produced, which, when modified with both folic acid and methotrexate (MTX), simultaneously provide tumor imaging and intracellular drug targeting and delivery in tumors overexpressing folic acid receptors (FR) [128].

## 4. Conclusion

Diagnosis of cancer is often achieved at a late stage when the adverse effects and drawbacks limit conventional cancer therapy [126]. Novel molecular biomarkers offer new hope [143]; for example, miRNAs allow very early, precise, and innovative cancer diagnosis and therapy and improve prognosis [144, 145]. Customized anticancer treatments started with specific antitumor mAbs and TKIs [5, 6]; however, mAbs are frequently ineffective as second-line therapy [146], while TKIs easily engender resistance. To be effective, personalized oncology requires an anticancer approach at molecular level specifically directed to a single cancer cell. Hence, comprehensive diagnostics of the molecular signature of the tumor to be treated is essential. Orthotopic tumor models of patient-derived xenograft (PDX) in immune-compromised mice allow predicting drug responsiveness and personalization of the anticancer treatment [147].

Current nanooncology is focused on satisfying the above-mentioned diagnostic and therapeutic requirements. It is based on functionalized nanosensors and biochips for early and ultrasensitive detection of novel cancer biomarkers [127, 148] and/or on the latest NPs directed to specific cancer

molecular targets, multifunctionalized for diagnostics imaging and targeting therapy [125, 128, 132].

More complex systems have been developed to enhance drug delivery to key molecular structures in cancer cells and/or neighboring tissues but also are able to operate as direct agents themselves, such as for thermal inactivation [149].

Second-generation polymeric nanoparticles have been approved or are undergoing evaluation. Modification of NPs facilitates the binding to a large number of cell ligands and optimizes physicochemical characteristics for drug targeting, release, and clearance [150]. The field of cancer theranostics is now dawning. Recently developed nanoparticles, functionalized to provide multifunctional nanoplatfoms in melanoma [151], are potentially utilizable for many other cancer types [152, 153], including cancers that present a stem-cell phenotype [154]. Theranostics, being molecularly directed, can enable tumor detection at the level of a single cell(s) and can be simultaneously combined with tumor diagnostic imaging for monitoring very effective targeted therapy [155]. Dual diagnostic and therapeutic approach joined to specific targeting permits a real-time detection of nanovectors localized at the target-sites and direct visual appreciation of therapeutic effect on tumor cells, providing a feedback to diagnosis. To date, it was carried out only in animal models and cells, displaying a significant reduction and also the complete disappearance of the tumor mass [153]. Undoubtedly in the near future theranostics could provide the powerful tool for eradicating cancer and metastasis of any type, at every stage, offering the necessary and alternative follow-up supporting liquid biopsy, which represents a great future challenge for early tumor diagnostic.

Liquid biopsies have many clinical applications: the detection of circulating cell-free DNA (cfDNA) [124], CTC [64, 103], miRNAs, and exosomes [123]. Although with different modality and variable benefits it permits a very early and predictive cancer detection, in a noninvasive manner, through the characterization of the genetic profile of tumors that, especially under the selective pressure of treatment, often change their mutation pattern or acquire new mutations.

Very early stage cancer detection helps to reduce mortality, but this is conceivable only through widespread screening, since most of cancers are at the beginning asymptomatic.

Personalized oncology meets some of major expectations in diagnostics and therapy. However, genetic heterogeneity, inside and between tumors and metastasis, together with the instauration of resistance to antitumor agents represents the major challenge in targeting therapy of cancer and will require additional efforts.

At present, there is a lack of standard biomarkers for cancer diagnosis. Identification biomarkers at a very early stage of tumorigenesis could be further improved and other biomarkers specific for particular cancer type require confirmation. The development of biosensors for detecting serum biomarkers has provided high sensitive and specific noninvasive tool, making them extremely promising for use in early cancer diagnostics. Introduction of nanomaterials will adjust sensitivity of biosensors in microfluids rendering them more appropriate for high-throughput assay. In the



near future, last generation biosensors will help to decrease mortality rate.

## Abbreviations

ABL:	Abelson murine leukemic viral oncogene homolog	HPV:	Human papilloma virus
ADRP:	Adipophilin	KIT:	v-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
AFP:	Alpha-fetoprotein	KRAS:	Kirsten rat sarcoma viral oncogene homolog
ALK:	Anaplastic lymphoma receptor tyrosine kinase	LMO1:	LIM domain only 1
ALL:	Acute lymphatic leukemia	IL-2:	Interleukin-2
AML:	Acute myeloid leukemia	LCPI:	L-plastin, lymphocyte cytosolic protein 1
AMACR:	Alpha-methylacyl-CoA racemase	lncRNAs:	Long noncoding RNAs
APC:	Adenomatous polyposis coli	mAbs:	Monoclonal antibodies
AQP1:	Aquaporin 1	MAPK:	Mitogen-activated protein kinase
BCR:	Breakpoint cluster	MDR:	Multidrug resistance
BMP:	Bone morphogenetic protein	MDS:	Myelodysplastic syndromes
BRAF:	v-Raf murine sarcoma viral oncogene homolog B1	5mC:	5-Methylcytosine
BAP1:	BRCA1 associated protein	MGMT:	O6-Methylguanine-methyltransferase
CA-125:	Cancer antigen 125	miRNAs:	MicroRNAs
CDKN2A:	Cyclin-dependent kinase	MLH1:	mutl homolog 1
CEA:	Carcinoembryonic antigen	MSC:	Mesenchymal stem cells
cfDNA:	Circulating cell-free DNA	MSI:	Microsatellite instability
CIN:	Chromosomal instability	mTOR:	Mammalian target of rapamycin
CK:	Cytokeratin	MTX:	Methotrexate
CLL:	Chronic lymphocytic leukemia	NDN:	Necdin gene
CML:	Chronic myeloid leukemia	NF $\kappa$ B:	Nuclear factor kappa B
CIMP:	CpG dinucleotide island methylator phenotype	NIR:	Near-infrared light region
CRC:	Colorectal cancer	NM23A:	Nonmetastatic cells 1 protein
CSCC:	Cervical squamous cell carcinoma	NMR:	Nuclear magnetic resonance
CSC:	Cancer stem cell	NNMT:	Nicotinamide, N-methyltransferase
CTC:	Circulating tumor cells	NMR:	Nuclear magnetic resonance
CTLA-4:	Cytotoxic T-lymphocyte antigen-4	NPs:	Nanoparticles
DLS:	Dynamic light scattering	NSCLC:	Non-small-cell lung cancer
DNMT:	DNA methyltransferase	OR:	Overall response
ECM:	Extracellular matrix	OSCC:	Oral squamous cell carcinoma
EGFR:	Epidermal growth factor receptor	PCM:	Phase-change materials
EML4:	Echinoderm microtubule-associated protein-like 4	PCNA:	Proliferating cell nuclear antigen
EMT:	Epithelial mesenchymal transition	PD-1:	Programmed death-1
FA:	Folic acid	PDGF-R:	Platelet derived growth factor receptor
FR:	Folic acid receptors	PFS:	Progression-free survival
FET:	Field effect transistor	PI3K:	Phosphoinositide-3-kinase
FGFR:	Fibroblast growth factor receptor	PMSA:	Prostate membrane specific antigen
FISH:	Fluorescent <i>in situ</i> hybridization	PSA:	Prostate specific antigen
FOBT:	Fecal occult blood test	PTEN:	Phosphatase and tensin homolog
FRET:	Fluorescence resonance energy transfer	RA:	Retinoid acid
GNPs:	Gold nanoparticles	RASSF:	RAS-associated domain family
GSTP1:	Glutathione-S-transferase p1	SCC-Ag:	Squamous cell carcinoma antigen
HCC:	Human hepatocellular carcinoma	SCLC:	Small-cell lung cancer
HDAC:	Histone deacetylase	SCNAs:	Somatic copy-number alterations
HER2/ERBB2:	Human epidermal growth factor receptor ERBB2	SEPT9:	Septin 9
HGFR/MET:	Hepatocyte growth factor receptor c-MET	SERS:	Surface-enhanced Raman scattering
HIF:	Hypoxia inducible factor	SNP:	Single-nucleotide polymorphism
5HmC:	5-Hydroxymethylcytosine	SPIONs:	Superparamagnetic iron oxide nanoparticles
		SiPNs:	Silica nanoparticles
		SPOP:	Speckle-type POZ protein
		TEPI2:	Tissue-factor pathway inhibitor 2
		TGF- $\beta$ :	Tumor growth factor beta
		TK:	Tyrosine kinase
		TKIs:	Tyrosine kinase inhibitors
		TFs:	Transcription factors
		TSC1:	Tuberous sclerosis complex 1 gene

TSG: Tumor suppressor gene  
 USPIONS: Ultrasmall SPIONS  
 VEGF-R: Vascular growth factor receptor  
 VHL: Von Hippel-Lindau  
 VOC: Volatile organic compounds  
 WNT: Wingless-related type.

## Competing Interests

The authors declare that they have no competing interests.

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