*Review*

# Liquid Biopsy as a Tool for Differentiation of Leiomyomas and Sarcomas of Corpus Uteri

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**Abstract:** Utilization of liquid biopsy in the management of cancerous diseases is becoming more attractive. This method can overcome typical limitations of tissue biopsies, especially invasiveness, no repeatability, and the inability to monitor responses to medication during treatment as well as condition during follow-up. Liquid biopsy also provides greater possibility of early prediction of cancer presence. Corpus uteri mesenchymal tumors are comprised of benign variants, which are mostly leiomyomas, but also a heterogenous group of malignant sarcomas. Pre-surgical differentiation between these tumors is very difficult and the final description of tumor characteristics usually requires excision and histological examination. The leiomyomas and malignant leiomyosarcomas are especially difficult to distinguish and can, therefore, be easily misdiagnosed. Because of the very aggressive character of sarcomas, liquid biopsy based on early diagnosis and differentiation of these tumors would be extremely helpful. Moreover, after excision of the tumor, liquid biopsy can contribute to an increased knowledge of sarcoma behavior at the molecular level, especially on the formation of metastases which is still not well understood. In this review, we summarize the most important knowledge of mesenchymal uterine tumors, the possibilities and benefits of liquid biopsy utilization, the types of molecules and cells that can be analyzed with this approach, and the possibility of their isolation and capture. Finally, we review the typical abnormalities of leiomyomas and sarcomas that can be searched and analyzed in liquid biopsy samples with the final aim to pre-surgically differentiate between benign and malignant mesenchymal tumors.

**Keywords:** liquid biopsy; cell-free nucleic acids; circulating tumor cells; leiomyomas; sarcomas; leiomyosarcomas; exosomes

## 1. Introduction

Uterine mesenchymal tumors (UMT) are a very heterogeneous group comprised of benign variants, which are mostly leiomyomas (ULM) and also malignant sarcomas. ULM are very common, affecting over 50% of Caucasian women and up to 80% of African origin women and they are one of the main causes of hysterectomy [1,2]. The incidence may be even higher, particularly in developing countries, because ULM can be asymptomatic in more than 50% of women who are, therefore, unaware of their presence [1,2]. Although they do not primarily endanger patients´ lives, ULM can markedly decrease the quality of life if they become symptomatic [3]. In contrast, sarcomas are very rare, with an estimated incidence of three to seven per 100,000 women [4], comprising only up to 8% of all corpus uteri malignancies [5]. Sarcomas are also very aggressive. They spread rapidly and have a low five-year survival rate and high rates of recurrence with low therapeutic efficacy [5–7].

UMT heterogeneity causes complicated classification. The most common form of UMT is "conventional" or "typical" ULM. This covers 90–95% of all lesions and these are considered benign without signs of malignancy [1,3,8]. This group is also heterogeneous, so that "conventional" ULM can differ in the following characteristics: (1) in histological degenerative changes such as myxoid, hyaline, fatty, hemorrhagic, and cystic changes [8]; (2) their localization in the uterus [3]; and (3) the type of genetic driver alternations [9].

In addition to strictly benign ULM forms, the remaining 5–10% can mimic malignancy and, therefore, display some of typical malignant traits such as higher mitotic index, nuclear atypia or the presence of coagulant necrosis [8,10]. There are also smooth muscle proliferations with unusual growth patterns, including benign metastatic ULM or intravenous leiomyomatosis [11]. The most common variants of non-conventional ULM are atypical, bizarre-cellular and mitotically active [8,10]. The most unpredictable, but still considered non-malignant UMT, are the smooth muscle tumors of uncertain potential, abbreviated STUMP [12]. In addition, inflammatory myofibroblastic tumors are quite rare in the uterus, but can closely mimic both benign and malignant variants of UMT [13]. Debate also continues concerning whether uterine leiomyosarcomas (LMS) arise from malignant transformation of ULM [14–16], but, herein, we consider ULM and LMS two separate entities, as generally assumed [5].

The malignant sarcoma group is also very heterogeneous; comprising histologically different tumors of various origin. These are divided into nonepithelial and mixed epithelial-nonepithelial categories dependent on the type of cancerous cell and its presumed originating tissue [7]. However, a better explanation of the variability of these tumors is characterized by sarcoma division into the four previously traditional categories: (1) homologous sarcomas composed only of uterine tissue; (2) heterogeneous types with mixed uterine and non-uterine tissues, typically striated muscle, bone, and cartilage; (3) pure types which comprise only mesodermal structures; and (4) mixed types with mesodermal and non-mesodermal structures; mainly epidermal [17]. These categories could have been combined, for example, LMS were categorized as homologous pure tumors. Nonetheless, this traditional classification incorrectly included several mixed mesenchymal tumors such as carcinosarcomas which are currently listed as tumors of endometrial origin [18]. Moreover, the variability of sarcomas means that future reclassification cannot be excluded. According to present-day classification, the most common type of sarcomas are LMS, comprising up to 60%, followed by up to 20% endometrial stromal sarcomas (ESS), up to 10% adenosarcomas, and the remaining 10% comprises other types of sarcomas [19].

UMT heterogeneity makes it very difficult to pre-surgically distinguish particular types without histopathologic confirmation after excision; including diagnosis of mitotic index, coagulant necrosis, and cytological atypia [5,6]. It is still difficult to distinguish mesenchymal lesions, even with the help of standard imaging devices—variable types of ultrasonography, magnetic resonance or computed tomography (1) malignant sarcomas from benign ULM [1,3] and (2) one type of malignant tumor from other types [5], and even when helpful clinical indications, for example, usual sarcomas occurrence and ULM nonoccurrence after menopause are available [20]. Moreover, non-imaging clinical diagnostic approaches also cannot identify UMT type with certainty; these methods are more suited to identifying endometrial lesions [5,21].

Importantly, ULM are especially difficult to differentiate from the LMS [3,5], because imaging and collateral symptoms are very similar; notably those of abnormal uterine bleeding, abdominal and pelvic pain, dysmenorrhea, and urinary problems [1,7]. Furthermore, ULM and LMS have some common features connected with their formation, especially higher ER receptor expression and activity [22], higher incidence in African origin and obese women [2,7], and both tumor types are more frequent in women with diabetes mellitus [23]. The many similarities between ULM and LMS include also their same origin by transformation of the same cells [24]. Therefore, it would be very beneficial to utilize molecular approaches to distinguish between particular UMT types and this could overcome insufficiencies in pre-surgical clinical diagnosis, especially in ULM and LMS [25]. These factors have inspired the main focus of this article on the use of a noninvasive approach to

differentiate between these two tumor groups and we also present brief information on other sarcoma types. This mainly includes ESS, which can be differentiated from other UMT types, and, especially, LMS through molecular approaches [26].

## 2. Liquid Biopsy

The presence of tumorous cells released into the bloodstream was first described in the nineteenth century by Thomas Ashworth [27]. This can be considered the discovery of the phenomenon now known as liquid biopsy (LB). In general terms, LB is described as a noninvasive technique which enables analysis of molecular biomarkers sampled from body fluids. While this primarily concerns blood [28], it also includes cerebrospinal fluid, pleural effusion, broncho-alveolar lavage fluid, saliva, urine, and sputum [29]. The main concept of LB is to enable more simple, efficient, faster and less-expensive monitoring of disease status, response to treatment, metastasis progression, and early differentiation of particular tumor types. All this can be achieved by analyzing the body fluid targets which are described in greater detail in the following section. Finally, LB is a revolutionary technique that uses modern and precise medical principles, which can help clinicians in therapeutic decisions and make the diagnostic process less stressful for patients. LB has mainly arisen because of invasive method limitations which include the following: (1) it can have unexpected complications and cannot be performed when clinical conditions have worsened or when a tumor is inaccessible [30], (2) it is often unrepeatable and provides only information on the tumor at one point in time and precludes monitoring responses to medication during both treatment and follow up, (3) it cannot be used for screening or early prediction of cancerous disease, and (4) it is generally performed after incidental early-stage tumor identification or following clinical examination resulting from exacerbated patient symptomatology [28,31]. In addition, many patients fear invasive procedures, prefer noninvasive diagnosis, and medicated non-surgical treatment. Furthermore, over 50% women with UMT want to preserve their uterus and fertility [32].

In contrast, the test results from LB are more suitable for early diagnosis because these are usually available much earlier, they have potential to estimate the risk of metastasis progression and relapse more precisely, and they are usually less expensive [28,31]. Simply taking the blood or other body fluids and analyzing target molecules and cells is, therefore, beneficial in many ways, but despite the above mentioned benefits it is important to realize that LB is often used only for assessment of tumor characteristics, but when the LB diagnoses the tumor as malignant it still requires surgical excision [33,34]. In addition, the detection ability of LB still remains challenging and further studies are required to test its accuracy and ability to correctly identify various tumor types. It also remains uncertain if LB constantly provides a representative sampling of the whole tumor mass or only its parts. However, intratumoral and spatial heterogeneity can also often limit precise interpretation of the molecular profile in tissue biopsies [33]. Finally, there is, in some aspects a lack of established consensus to guide its utilization [33,34] and despite the LB approach being beneficial and becoming more and more popular, in some aspects, confirmation of its precise, advanced, and reliable use in clinical praxis is required.

## 3. Cells and Molecules That Can Be Analyzed from LB Samples

The following cells and molecules can be analyzed using the LB approach: circulating tumor DNA (ctDNA); circulating tumor cells (CTC); exosome vesicles; circulating RNAs, i.e., messenger RNA (mRNA); microRNA (miRNA); long non-coding RNA (lncRNA); and also proteins, peptides, and metabolites [29,31].

### *3.1. ctDNA*

The ctDNA is tumor derived and fragmented DNA which should preserve the abnormal molecular pattern present in the primary tumor [35–39]. Nonetheless, some studies also claim that ctDNA's pattern of abnormalities generally differs from those in primary tumors [40–42]. The ctDNA is primarily

released from tumors by apoptosis and necrosis (programmed and not programmed cell death), but also by tumor secretion [43]. Various factors affect the total composition of ctDNA circulating in the bloodstream; it is especially dependent on tumor status, burden, and histopathology [44].

### 3.2. CTC

CTC can be valuable disease indicators because they are exfoliated from both primary tumors and metastatic lesions. Their numbers and concentration can be used as a predictive marker, and as with exosomes, they contain variable nucleic acids, proteins, and metabolites which can be utilized in molecular analyses at the mutational, transcriptional, and epigenetical levels [45,46].

### 3.3. Exosomes

Exosomes are membrane-bound phospholipid vesicles, actively secreted by a variety of cells, including cancer cells, and they contain proteins as well as the following nucleic acids: miRNAs, lncRNAs, and mRNAs from tumors. Although the half-life of exosomes in bloodstream is still analyzed and debated, Boukoris et Mathivanan [47] considered exosomes highly stable in stored conditions, and therefore compared to freely circulating nucleic acids (CNA), those molecules encapsulated in exosomes should also be more stable in stored conditions. However, miRNAs, which can also be contained in exosomes, are generally considered very stable and their activity should remain the same, regardless if they are encapsulated in exosomes or incorporated in ribonuclear complexes [48].

### 3.4. mRNAs, miRNAs and lncRNAs

mRNAs, miRNAs and lncRNAs are present in the bloodstream through both active secretion in exosomes and apoptotic/necrotic cell lysis. However, apart from miRNAs, the half-life of other RNAs in the bloodstream is presumed to be very short and their utilization for LB, therefore, requires further analysis [49,50].

All the above elements, however, are very rare. For example, ctDNA comprises only 0.1% to 10% of all circulating free DNA (cfDNA) released into the bloodstream from all human cells. Especially leucocytes [51] and cfDNA concentration is itself quite low at 10 to 100 ng/mL [52]. The ctDNA concentration, therefore, ranges from 0.01 ng/mL to 10 ng/mL and while the half-life of unencapsulated particles ranges usually from 15 minutes to 2.5 h [31,53], ctDNA half-time is considered relatively long, i.e., at least two hours [51].

In addition, CTC have a frequency as low as 1 CTC per $10^6$–$10^7$ leucocytes which is usually less than 1 CTC per milliliter of blood [46]. Therefore, it is necessary to sample higher blood volumes, usually ranging from 5 to 9 mL to detect at least one CTC. Their occurrence also depends on the tumor stage, where lower numbers are more common in early-stage disease and this increases only slightly in patients with advanced metastases [54,55].

Rajagopal et al. [56] considered that increased exosome number is a potent biomarker for abnormal physiological states and that their total numbers increase in cancer patients as compared to healthy subjects. However, precise exosome quantification is difficult to determine because, in addition to total number, the variations in size, and protein and nucleic acid content, per vesicle can vary and "quantification" of these latter variations should be considered in further molecular analysis [57].

## 4. Capture, Enrichment, and Isolation of Freely Circulating Vesicles, Molecules and Cells

Traditional exosome capture and enrichment includes ultrafiltration and size exclusion chromatography, precipitation with polymers, and immunoaffinity purification by magnetic beads [58]. These methods can be utilized because exosomes have specific biological patterns, and their cellular origin provides typical surface markers. These include the CD9, CD63, and CD81 members of the tetraspanin family, heat-shock proteins such as HSP70, and the Rab protein family [59]. However, the exosome surface protein profile can vary with both the character and origin of the cell that

released them, and therefore establishing more specific membrane biomarkers and the genetic profile of exosomes would be beneficial for using exosomes as agents in novel targeted therapies [60].

Microfluid-based systems are now becoming more popular than traditional exosome capturing systems, because these can directly analyze blood samples and are, therefore, more practical for clinical praxis [45]. However, regardless of the capture technique used, it is very important to the utilization of exosomes in clinical diagnostics, and potentially also in tumor differentiation, that the nucleic acids and metabolites encapsulated in these vesicles can be analyzed following the electron microscope validation and isolation [60,61]. While most current analyzes have targeted the expression of RNA transcripts, which are abundantly present in these exosomes [61,62], there is scant knowledge of the genomic and epigenetic profile of DNAs encapsulated in these vesicles.

ctDNA isolation is relatively easier than capturing exosomes. The main ctDNA isolation limiting factor is its separation from other cfDNA molecules, however, both of the following targeted and untargeted approaches are now available for this separation [36].

(1) Targeted approaches are based on identifying the following mutations/abnormalities in the ctDNA elements: (a) previously accepted abnormal patterns in ctDNAs (e.g., in different cancer type or based on *in silico* prediction) and (b) the same pattern in the primary tumor and the released ctDNA. Here, the "standard" methods of molecular biology such as variable PCR modifications and pyrosequencing can be used [63–66], but precise and more sensitive methods including BEAMing, droplet digital PCR, and next generation sequencing (NGS) are more suitable because of the very low ctDNA levels as compared to cfDNAs [67–69].

(2) Nontargeted approaches require no prior knowledge of molecular alternatives and while these are generally based on NGS techniques, the digital karyotyping and PARE methods can also be utilized [36]. Whereas, both targeted and nontargeted approaches are used mainly for detecting point mutations, insertions and deletions, amplifications, translocations, and copy number alterations [36,67]; identifying abnormal epigenetic patterns and especially changes in promoter region methylation levels can be a suitable alternative to genomic alterations. It is supposed that ctDNA epigenetic patterns should also usually remain the same as in the tumors from which they were released and also be specific for both cancer type and progression [35,67,70–75].

The capture of CTC remains extremely challenging because of its rarity and short half-life. There are still affinity-based methods used which take advantage of antigens that are differentially expressed by CTC [46]. These include: (1) epCAM which is normally present on the surface of cells released from epithelial carcinomas [45,46]; (2) the MUC1 present in breast and ovary carcinomas [76,77]; and (3) the EphB4 which is typically over-expressed in advanced head and neck cancers [46]. However, this approach remains specific for limited cancer types because these markers are suitable for positive selection, and therefore knowledge of further CTC surface antigens typical for different cancer types is essential. A further limitation is imposed by epithelial cells which are undergoing epithelial-mesenchymal transition (EMT) and cannot be captured by positive selection [45,46]. Negative selection is used to avoid selection bias and is based on capturing and subsequent removal of non-CTC cells in the blood, most importantly, leucocytes. These are commonly depleted through immunomagnetic targeting and removal against CD45 and other leucocyte antigens [45,78]. To the best of our knowledge, no studies have focused on identifying CTC released from any type of uterine sarcoma or ULM. However, there are some options for the identification of CTC from different non-uterine sarcoma types, and this provides opportunities to determine CTC from uterine sarcomas. For example, Benini et al. [79] were able to identify CTC released from Ewing sarcomas by immunoseparation with CD99 antibody and magnetic microbeads. The origin of the cells was verified by the presence of fusions typical for this tumor. The expression of cell-surface vimentin provided an even better chance to identify CTC of various sarcomatous origins [80]. Most recently, Hayashi et al. [81] recorded CTC identification from various sarcoma types based on positive vimentin staining, negative CD45 staining, and nuclear morphology distinct from normal white blood cells.

The standard approaches for identifying CTC include using magnetic beads armed with antibodies for positive or negative separation and employing columns or cartridges or microchips coated with antibodies [82]. However, the CTC physical properties, such as electric charges, can also be used to enrich and isolate these cells with more practical microfluid cell sorters [83,84] or Microhall platforms [85,86]. Finally, microfiltration systems, such as CellSieve™, also provide great possibilities for the identification of variable CTC with only minimal cell processing [81]. Interestingly, CTC were previously thought to be released only from metastases, but it is now accepted that two CTC "types" exist. This is contrary to previous claims, because the first type acts as a metastatic factor and the other is only passively released into the bloodstream, and although it should not promote metastases formation, its role in tumor spread remains undetermined [87].

## 5. Known Abnormalities in UMT

LB methodology enables analysis of various molecular targets over longer periods and this makes it possible to monitor disease progression and response to treatment. Furthermore, it also provides the possibility of differentiating particular tumor types. This can be very beneficial for early diagnosis of cancer. For differentiation of UMT with the LB approach, there are two options: (1) assessment of the mutational/transcriptional/epigenetic pattern in solid tumors and subsequent identification of the very same abnormalities in LB samples and (2) determination of specific patterns of change in the candidate markers directly in the LB samples and their connection with particular cancer diseases.

Unfortunately, specific LB biomarkers with the potential to non-invasively distinguish particular UMT types have not been assessed. Further analysis is, therefore, required to find a successful marker, but this is quite promising because the presence of ctDNA released from ULM and malignant sarcomas has been noted in non-invasive prenatal testing (NIPT) [88–90]. Furthermore, Hemming et al. [91,92] described the presence of ctDNA from LMS in LB samples and these correlated with tumor progression. Moreover, many of the biomarkers of solid UMT described below have previously been reported in LB from patients with different cancer diseases and these provide the possibility of early diagnosis, prognosis, and therapeutic outcome. These include the identical microRNAs [93–97], methylation changes [95,98,99], mutational changes [100–102], and even similar chromosomal rearrangements [103–106]. We, therefore, suggest a way to identify LB biomarkers suitable for UMT differentiation by searching for abnormal patterns already established in solid UMT in LB samples. This possibility is very promising also because reports identify that solid tumor abnormalities should remain unchanged in CNA [35–39,67,70–75] and eventually also in vesicles and CTC. Therefore, all abnormal patterns listed below are noted in solid tumors and their summary can form the basis for designing LB-based analysis in patients with UMT.

## 6. miRNAs

The miRNAs are short non-coding RNAs which can bind to target mRNAs and induce gene silencing and repression of transcription [107]. The 2000 miRNA genes already identified in the human genome is expected to increase in number and their abnormal activity has been connected with the development of various cancerous diseases because they regulate the important cellular functions of development, differentiation, growth and metabolism [107]. Analysis of miRNA abnormal activity is currently attractive, however, in ULM and LMS tissues, it is still not sufficiently known. The most notable abnormally expressed microRNAs in ULM and LMS tissues are listed in Table 1. There are some interesting characteristics of miRNAs in these tumors including that treatment with 17β-oestradiol and medroxyprogesterone acetate should result in the regulation of variable miRNAs' activity, at least in the ULM and LMS cell lines [108]. Liu et al. [109] noted that various miRNAs are differentially active in African and Caucasian women. Wang et al. [110] considered that the abnormalities in microRNAs activity should be associated with tumor size as well as ethnicity. Moreover, ULM typically have an abnormal extracellular matrix, and miR-15b is more active in ULM tissue samples than in healthy myometrium (MM), and also more active in ULM cell lines than in MM cell lines [111]. This miRNA

is supposed to regulate RECK protein expression in normal physiological status to ensure negative regulation of matrix metalloproteinases [112,113]. In addition, the let-7 family miRNA family has the *HMGA2* gene as a target and *HMGA2* abnormal expression, especially from translocations, is considered a typical ULM mark [114].

The activity of most analyzed miRNAs has been assessed in ULM. MicroRNA activity is less understood in LMS and studies that could compare the activity of identical microRNAs in both tumor types are widely lacking. Exceptions, however, include studies by de Almeida et al. [115] and Schiavon et al. [116], which focused on assessing miRNA activity in both tumor types. In the former study, the authors identified 24 oncomirs with de-regulated expression profile in both ULM and LMS. The latter work recorded similar results in establishing widely different ULM and LMS expression patterns. Danielsson et al. [117] also suggested that different miRNA activity should contribute to separate ULM and LMS development, and they created a clustering system differentiating ULM and LMS based on miRNA activity. Unfortunately, however, they accomplished this only in cell lines. The most notably different ULM and LMS miRNAs recorded in these studies are listed in Table 1, under the "observed in LMS/ULM tissue" section, and these can be considered the best candidates for distinguishing between ULM and LMS on miRNA activity using LB. However, many of the analyzed ULM miRNAs were not analyzed in malignant LMS, even in the two separate studies. Therefore, the miRNAs summarized in Table 1 and reported as abnormal in ULM (listed under the "observed in ULM/MM tissues"), could prove beneficial if analyzed in LMS. There are also studies which assessed different miRNAs expression profiles in ESS. For example, Kowalewska et al. [118] reported four miRNAs which were differentially active in ESS and the control group, but it is quite interesting that they noted no significant differences in miRNA expression in LMS and control healthy tissues. The additional study by dos Anjos et al. [119] compared LMS, carcinosarcoma, and ESS miRNA profiles and identified both variable miRNA activity in these three tumor types and a variety of miRNAs connected with lower cancer-specific survival rates. This may, however, be due to tumor differences, whereas LMS are primarily smooth-muscle tumors. ESS, even of mesenchymal origin, are mixed tumors with a large proportion of endometrial elements and carcinosarcomas are classified as being of endometrial origin [18].

**Table 1.** Abnormally active microRNAs in ULM and LMS.

| ULM | | | LMS | | |
|---|---|---|---|---|---|
| **miRNA** | **Observed in** | **Expression in Tumorous Tissue** | **miRNA** | **Observed in** | **Expression in Tumorous Tissue** |
| Let-7 family * [110] | ULM/MM tissue | Up | miR-15a * [120] | Primary/metastatic LMS tissue | Up in metastases |
| miR-27a * [110] | ULM/MM tissue | Up | miR-92a * [120] | Primary/metastatic LMS tissue | Up in metastases |
| miR-21 * [110] | ULM/MM tissue | Up | miR-31 * [120] | Primary/metastatic LMS tissue | Up in primary |
| miR-23b * [110] | ULM/MM tissue | Up | miR-122-5p [116] | LMS/ULM tissue | Up |
| miR-200a * [121] | UtLM-hTert | Up | miR-206 * [116] | LMS/ULM tissue | Up |
| miR-542-3b [122] | ULM/MM tissue | Up | miR-373-3p * [116] | LMS/ULM tissue | Up |
| miR-377 [122] | ULM/MM tissue | Up | miR-144-3p [116] | LMS/ULM tissue | Up |
| miR-363 [123] | ULM/MM tissue | Up | miR-372-3p * [116] | LMS/ULM tissue | Up |
| miR-490 * [123] | ULM/MM tissue | Up | miR-34a-5p [116] | LMS/ULM tissue | Down |
| miR-137 [123] | ULM/MM tissue | Up | miR-27b-3p [116] | LMS/ULM tissue | Down |
| miR-15b * [111] | ULM/MM tissue; ULM/MM cell lines | Up | miR-135b-5p [116] | LMS/ULM tissue | Down |
| miR-30a [121] | ULM/MM tissue; UtlM-hTERT cell lines | Up | miR-9-5p [116] | LMS/ULM tissue | Down |
| miR-32 [110] | ULM/MM tissue | Down | miR-10b-5p * [115] | LMS/ULM tissue | Up |
| miR-29b * [110,124] | ULM/MM tissue | Down | miR-125b-1-3p [115] | LMS/ULM tissue | Up |
| miR-542-5p [122] | ULM/MM tissue | Down | miR-140-5p * [115] | LMS/ULM tissue | Up |

**Table 1.** *Cont.*

| ULM | | | LMS | | |
|---|---|---|---|---|---|
| miRNA | Observed in | Expression in Tumorous Tissue | miRNA | Observed in | Expression in Tumorous Tissue |
| miR-642 [122] | ULM/MM tissue | Down | miR-145-5p [115] | LMS/ULM tissue | Up |
| miR-93/106 [125] | ULM/MM tissue | Down | miR-130b-3p * [92] | LMS/ULM tissue | Down |
| miR-486-5p * [123] | ULM/MM tissue | Down | miR-148-3p [115] | LMS/ULM tissue | Down |
| miR-217 [123] | ULM/MM tissue | Down | miR-204-5p [92] | LMS/ULM tissue | Down |
| miR-4792 [123] | ULM/MM tissue | Down | miR-203a-3p [92] | LMS/ULM tissue | Down |
| miR-200a * [109] | ULM/MM tissue; UtlM-hTERT cell lines | Down | miR-152 * [126] | LMS/ULM tissue /SKLMS1cell lines | Down |
| mir-143 * [108] | ULM/MM tissue | Down | | | |
| miR-200c * [127] | ULM/MM tissue | Down | | | |
| miR-197 * [128] | ULM/MM tissue | Down | | | |
| miR-212 [121] | ULM/MM tissue; UtlM-hTERT cell lines | Down | | | |

* These were reported and analysed in LB samples in patients with different cancer diseases [93–97]; ULM—uterine leiomyomas, MM—healthy myometrium, LMS—uterine leiomyosarcomas.

## 7. DNA Mutations and Chromosomal Aberrations in ULM and Sarcomas

ULM often result from gene mutations, but this occurs only on a small scale. The most mutated is the *MED12* gene observed in almost 70% of ULM, and the second most frequent is *FH* gene mutations which comprise only approximately 1% [129,130]. In addition, non-sporadic germline mutations of this gene occurred more often in women with both ULM and LMS [131,132]. Interestingly, malignant uterine LMS also have *MED12* gene mutation in almost 70% of tumors [133], and this can be, therefore, considered a typical mark for these tumors. While this common mutation potentially distinguishes the two tumor groups from healthy tissue, unfortunately it cannot be used as a distinguishing factor of these tumors and is, therefore, unsuitable for LB diagnostics. In contrast to ULM, LMS have quite often mutated *TP53* gene [6,134]. Although mutations in this gene are typical for many cancerous diseases, LB determination of *TP53* mutation in women with previous image-diagnosed UMT should always provoke rapid tissue biopsy, subsequent histological examination, and close observation of the patient. Moreover, this gene should also be considered a potential candidate for gene therapy in women with LMS [135].

The *ATRX* gene is also relatively often mutated in LMS tissues [134], and also in STUMP [136]. Furthermore, the *RB1* gene is typically mutated in LMS [26] and in ULM with bizarre nuclei [137]. However, the percentage of mutations of both genes in LMS is less than 50% and the extent of *ATRX* mutation in leiomyomatous tissues remains undetermined. In addition, ULM with bizarre nuclei are quite rare and the *RB1* gene is often mutated in a wide range of cancers [138]. In summary, the mutation profiles of ULM and sarcomas are not well understood, and currently demonstrated abnormalities provide only limited possibilities for LB differentiation of these tumors. However, mutation changes in the *TP53*, *ATRX,* and *RB1* genes have been detected and analyzed in LB samples in patients with different cancer diseases [100–102]. In contrast to mutational changes, the ULM and LMS chromosomal aberrations are quite different and better understood, so current knowledge suggests that LB differentiation of these tumors based on chromosomal aberrations is more suitable than just relying on mutational changes. Moreover, this identification from LB samples is feasible, for example, with ultra-low passage whole genome sequencing [91,139,140]. The most important recognized chromosomal aberrations in ULM and LMS are listed in Table 2.

**Table 2.** Most typical chromosomal rearrangements in ULM, LMS, and ESS.

| Chromosomal Aberrations in ULM | | | Chromosomal Aberrations in LMS | | | Chromosomal Aberrations in ESS | | |
|---|---|---|---|---|---|---|---|---|
| Chromosome/Locus | Type | Affected Genes | Chromosome/Locus | Type | Affected Genes | Chromosome/Locus | Type | Affected Genes |
| 12q15 [141] | Translocation | *HMGA2* | 12q13-15 [142] | Amplification | *RB1* | (7;17)(p15;q21) [143] | Translocation | *JAZF1,SUZ12* |
| 14q24 [144] | Translocation | *RAD51B* | 10q21.3 * [145] | Loss | *PTEN* | (6;7)(p21;p15) [146] | Translocation | *JAZF1-PHF1* |
| 7(q22q32) [147] | Deletion | *CUX1* | 13q14.2-q14.3 * [145] | Loss | *LEU* | (6;10)(p21;p11) [146] | Translocation | *EPC1-PHF1* |
| 6p21 [148] | Translocation | *HMGA1* | 7q36.3 [142] | Gain | *PTPRN2* | (1;6)(p34;p21) [149] | Translocation | *MEAF6-PHF1* |
| 10q22 [150] | Translocation | *KAT6B* | 7q33-q35 [142] | Gain | *HAVCR1* | (X;17)(p11;q21) [151] | Translocation | *CXorf67-MBTD1* |
| 1(q31q43) [130] | Deletion | *FH* | 1p21.1 * [142] | Loss | *AMY2A* | (X;22)(p11;q13) [152] | Translocation | *ZC3H7B-BCOR* |
| Xq22 [153] | Deletion | *COL4A5/COl4A6* | 9p.21 | Gain | *CDKN2* | (10;17)(q22;p13) [154] | Translocation | *YWHAE-NUTM* |
| 1p36 [155] | Translocation/deletion | *AJAPI, NPHP* | 12q.15 [156] | Gain | *MDM2* | der(22)t(X;22)(p11;q13) [152] | Deletion | / |
| 8q12 [157] | Insertion/translocation | *PLAG1* | 1q21 * [158] | Amplification | *FLF, PRUNE* | del(16)(q22) [149] | Deletion | / |
| 19q * [159] | Deletion | / | 5p14-pter [158,160] | Amplification | / | | | |
| 12 [161] | Trisomy | / | 13q31 [158,160] | Amplification | / | | | |
| 10 [147] | Monosomy | / | 19p13 [158,160] | Amplification | / | | | |
| 22q * [159] | Deletion/monosomy | / | 20q13 [158,160] | Amplification | / | | | |

* Similar chromosomal rearrangements were reported in LB samples from patients with different cancer diseases [103–106]; ULM—uterine leiomyomas, LMS—uterine leiomyosarcomas, ESS—endometrial stromal sarcomas.

### 8. Methylation Changes in ULM and LMS

The methylation profiles of ULM and sarcoma promoter regions provide further possibilities for tumor differentiation based on LB, because changes in DNA methylation patterns can be identified in ctDNA and have been proposed as potential biomarkers for tumor staging, prognosis, and monitoring of the treatment response [31,98,162]. It has also been suggested that abnormal methylation patterns should remain identical in both the primary tumors and the ctDNAs released by the tumor [35,67,70–75]. Maekawa et al. [163] reported great variability in ULM methylation patterns. These authors reported 120 genes with different methylation pattern in ULM and MM, and 22 of these were estrogen receptor alpha (ERα) target genes, thus, indicating that their abnormal methylation could contribute to abnormal estrogen response. One of these genes is *DAPK1* which is often abnormally methylated in various cancer types [164] and importantly, it has also been reported abnormally methylated in the serum of women with ULM [165].

Following bisulfide-sequencing of the ERα receptor gene promoter region, Asada et al. [166] reported that seven CpGs in distal sites are often variably methylated in healthy and tumorous tissues. It is quite likely that methylation variations in distal ERα regions are dependent on menstrual cycle phases [167], and since this has already been assessed in solid tissues, it would be very interesting and beneficial to analyze the status in LB samples. ULM also have differentially methylated sex chromosomes [168] where the most notably hypomethylated was the *TSPYL2* gene. In addition, women of African origin have hypermethylated *DLEC1*, *KRT1,9* and *KLF11* genes in ULM as compared to MM [169].

Importantly, methylation markers have already been used by Sato et al. [170] to distinguish between ULM and LMS, especially by establishing a hierarchical clustering system able to discriminate between these tumors with 70% accuracy. The system is based on different methylation levels in ten genes. This study is also unique because it assesses the methylation status in both ULM and LMS tumor types. In contrast, the remaining methylation markers listed in Table 3 were assessed either for benign or malignant lesions, but not for both, and further analyses comparing the status of these genes in both tumor types would be beneficial to determine how well they coud distinguish between these tumor types. For LMS are typical aberrations altering the cell cycle [6], whereby promoter region hypermethylation is supposed to induces loss of function of the important cell cycle regulator *CDKN2* [171] and hypermethylation also most likely causes the *BRCA1* gene´s protein reduction in almost 30% of uterine LMS [172].

Malignant LMS have abnormal estrogen receptor activity and sensitivity, but these are lower than in benign ULM [22]. The LMS also have often hypomethylated *ESR1* target genes [26] and the polycomb group target genes [173]. Importantly, authors Miyata et al. [173] claimed that ULM and LMS can be distinguished by their global DNA methylation levels, and also presumed that the methylation pattern is different on a genome-wide level in ULM subtypes with different genetic driver aberration, i.e., *MED12* mutation, *HMGA2* activation, and *FH* mutation [9,174]. These results make tumor differentiation based on their ctDNA methylation levels very interesting, however, the bloodstream ctDNAs are very fragmented and poorly represented, and therefore ctDNA methylation analyses on either a genome-wide scale or a locus-specific level should preferably use very sensitive novel sequencing approaches [67,98]. The hyper- and hypomethylated genes in ULM and LMS tissues are listed in Table 3.

**Table 3.** Abnormally methylated genes in ULM and LMS.

| ULM | | LMS | |
|---|---|---|---|
| **Gene** | **Methylation Change** | **Gene** | **Methylation Change** |
| *IRS1* [163] | Hypermethylation | *MGMT* * [175] | Hypermethylation |
| *COL4A1* [163] | Hypermethylation | *BRCA1* * [172] | Hypermethylation |
| *GSTM5* [163] | Hypermethylation | *CDKN2* * [137] | Hypermethylation |
| *DAPK1* * [165] | Hypermethylation | *PTEN* [6] | Hypermethylation |
| *KLF11* [169] | Hypermethylation | *RASSF1A* *a [176] | Hypermethylation |
| *DLEC1* * [169] | Hypermethylation | *DAPK1* *a [177] | Hypermethylation |
| *KRT19* [169] | Hypermethylation | | |
| *ALX1* ‡ [170] | Hypermethylation | | |
| *CBLN1* ‡ [170] | Hypermethylation | | |
| *CORIN* ‡ [170] | Hypermethylation | | |
| *DUSP6* [170] | Hypermethylation | | |
| *FOXP1* ‡ [170] | Hypermethylation | | |
| *GATA2* ‡* [170] | Hypermethylation | | |
| *IGLON5* ‡ [170] | Hypermethylation | | |
| *NPTX2* ‡* [170] | Hypermethylation | | |
| *NTRK2* ‡ [170] | Hypermethylation | | |
| *STEAP4* ‡* [170] | Hypermethylation | | |
| *PRL* ‡ [170] | Hypomethylation | | |
| *PART1* [170] | Hypomethylation | | |
| *TSPYL2* [168] | Hypomethylation | | |
| *OCRL* [168] | Hypomethylation | | |

* These were reported and analyzed in LB samples in patients with different cancer diseases [95,98,99]; ‡ Methylation status of these genes was compared between ULM and LMS in a study by Sato et al. [170]; a Methylation changes were noted in variable types of leiomyosarcomas, including non-uterine. *DAPK1* gene was reported as hypermethylated in ULM and sarcomas as compared with healthy tissues, but it was not compared between ULM and LMS; ULM—uterine leiomyomas, LMS—uterine leiomyosarcomas.

## 9. lncRNAs and mRNAs

ULM expression profiles are relatively well assessed in studies analyzing gene activity on the mRNA levels in solid tumors [25,178–180]. The LMS mRNA expression profile is also relatively sufficiently known [6,25,181,182] and variably expressed genes are recognized also in primary and metastatic LMS [183] and in LMS as comparing to ESS [184]. Although mRNA molecules directly released into the bloodstream after apoptosis or necrosis can technically reflect intracellular processes in tumor cells and their levels can have some predictive value of tumor character, their very short half-life, instability, low abundance, and regular contamination with other intracellular mRNAs severely limits them as useful biomarkers [185]. In contrast, assessment of mRNA expression in CTC and exosomes appears more suitable because of the newly developed approaches [186–189].

Genome-wide analyses of ULM lncRNA expression have demonstrated that hundreds of these molecules are aberrantly active in ULM as compared to MM [190]. The number of abnormally active lncRNAs correlates with tumor size, and lncRNA H19 was considered as the most important abnormally active lncRNA in ULM [190]. lncRNA H19 contributes to *MED12* and *HMGA2* gene regulation. It is assumed that lncRNA H19 should also affects the activity of various extracellular matrix remodeling genes [191]. Therefore, further study of this lncRNA should shed more light on the regulation of important ULM marker expression and this lncRNA could potentially be used as

a reliable distinguishing marker of these tumors. Finally, further research of lncRNAs' abnormal activity in rare malignant sarcomas should also prove advantageous, but this is still currently lacking.

## 10. ULM and Sarcoma Metabolites

ULM are also the subject of metabolic studies because of their high occurrence, the search for efficacious medication, and their great current cost. The very interesting study performed by Heinonen et al. [192] assessed abnormal metabolomes and metabolic pathways in ULM, and they associated these with particular molecular subtypes, which harbor different driver genetic abnormalities. These were divided into the following four groups: those with the mutated *MED12* gene, those with *HMGA2* gene translocations, the group with *FH* gene biallelic inactivation, and *COL4A5-COL4A6* deletions [9]. In summary, Heinonen et al. [192] recorded 70 dysregulated metabolites in all ULM types, and the characteristic dysregulations in particular ULM subtypes included: (1) the FH subtype which has characteristic metabolic alterations in the tricarboxylic acid cycle and pentose phosphate pathways and increased levels of multiple lipids and amino acids and (2) the MED12 subtype which has markedly reduced levels of vitamin A and dysregulation of ascorbate metabolism. Interestingly, the retinoid acid receptor pathways have previously been observed to be very dysregulated in ULM, and this caused lower levels of biologically active retinoid acid and increased levels of its abnormal metabolites [9,193,194]. In addition, it has been suggested that premenopausal parous women with ULM share a wide range of metabolic syndrome features such as higher serum triglycerides levels (TG ≥ 150 mg/dL), low serum-high density lipoproteins (<50 mg/dL), and hyperglycemia (FPG ≥ 100 mg/dL) [195]. The obesity and high blood pressure observed in this syndrome have also been previously and repetitively associated with ULM occurrence [1–3]. However, while it is generally accepted [3] that vitamin D deficiency is strongly associated with ULM occurrence, its abnormal metabolites were not confirmed in these tumors [192].

The uterine sarcoma metabolite profile is poorly understood due to its rare occurrence. Paradoxically, some authors regard metabolomic markers more reliable than molecular markers in sarcoma diagnostics, especially in the high-grade type which are more difficult to distinguish histologically. Moreover, individual tumors often contain areas of different histologic grade, necrotic regions, and variable inflammatory cell infiltrate, and this heterogeneity hinders the search for molecular markers [196,197]. However, the preference for metabolomic markers over the molecular remains disputed and subjective.

Lastly, the same pattern of precursor ion differences has been found in LMS, myxofibrosarcomas, and undifferentiated pleiomorphic sarcomas. The two metabolite signals correlate with overall survival, *m/z* 180.9436 and *m/z* 241.0118; and one with metastasis-free survival, *m/z* 160.8417. In addition, FTICR-MSI identified *m/z* 241.0118 as inositol cyclic phosphate and *m/z* 160.8417 as carnitine [197].

## 11. Discussion and Conclusions

In this review, we based the differentiation of UMT on the LB approach because of the diverse action of non-coding RNAs, and mutational and methylation changes and differences in metabolic activity in ULM, other UMT, and MM. We also suggested searching for these previously known solid tumor abnormalities in LB samples. However, the differentiation of two or more tumor types by LB necessitates, in the final step, assessment of their altered patterns in the body fluid samples. For example, hypermethylation or mutational changes can be considered proof of ULM rather than LMS when a LB sample is abnormally hypermethylated or has particular mutations, and this abnormal pattern was previously observed with high predictive value in ULM but not in LMS. This explanation, however, is simplistic and follows only basic principles because the entire procedure for establishing a particular marker to its utilization in clinical testing is a long and multistep process. While it is debatable if there is sufficient knowledge of the differences in ULM, it is certainly lacking in sarcomas. Therefore, to enable LB to determine tumor character and to monitor the response to treatment and

tumor progression in malignant variants, it is essential to increase current knowledge of changes in ULM and also to perform these analyses in uterine sarcomas, especially LMS.

Moreover, we based our suggestions on the assumption that changes in CNA should remain the same as they are in the primary tumors which release them [35–39,67,70–75]. However, it should be mentioned, some studies suggest that the changes can vary [40–42]. In addition, it is necessary to realize that metastatic lesions can differ from primary tumors in their molecular patterns [183]. It would, therefore, be beneficial to perform more comparative analyses of primary and metastatic sarcomas to understand the changes in their progression. In addition, it would also be helpful to use more gene assays with high predictive value in LB screening, instead of sole ones. Finally, there is still no successful tool for differentiating UMT, so even achieving lower predictive value would be advantageous. An example here is that assays correctly assessing tumor type in eight out of 10 women would be better than none.

It is quite intriguing that there is no interest in developing techniques that could differentiate such frequent tumors as ULM and such aggressive tumors as sarcomas. The main reason appears to be the very low incidence of sarcomas and most UMT lesions are ULM. These latter are not life threatening and often asymptomatic. In addition, the statistical threat to life caused by uterine sarcomas is low, and almost negligible in comparison to the "deadliest" cancer types such as breast, lung and colon cancer. However, if sarcomatous lesions are primarily misdiagnosed as ULM, this can have fatal consequences, especially when women refuse surgical excision and prefer medicated treatment. Moreover, although uterine sarcomas are rare, these lesions still affect thousands of women globally and can be easily misdiagnosed. Therefore, early noninvasive diagnosis could provide certainty that lesions are really benign; and it could significantly prolong full-quality life even when the lesion is malign. Moreover, appropriate LB approaches can monitor both the metastatic progression of malignant lesions and response to treatment, and thus contribute to their better understanding.

Unfortunately, minimal analyses have focused directly on capturing and identifying circulating elements from UMT and most findings have been incidental and collateral. It is known that NIPT results can be affected by the presence of tumorous circulating elements [88]. The first study of these discrepancies we are aware of was conducted by Osoborne et al. [198]. Woman with metastatic neuroendocrine carcinoma gave birth to a healthy infant, and although both invasive testing during pregnancy and postnatal placental histology revealed no abnormalities, NIPT testing indicated trisomy in chromosomes 13 and 18. These trisomes, therefore, arose from release of tumor DNA into the bloodstream and not due to infant chromosomal aberrations. A summary of a three-year period of NIPT testing enabled identification of 55 samples with altered genomic profiles and thus not reportable NIPT results [89]. All these discrepancies are presumed to arise due to the presence of various neoplasm types, but importantly almost half of the identified discrepancies arose as a result of the presence of benign ULM and only one because of uterine sarcomas presence.

One study focused directly on identifying circulating ctDNAs from uterine LMS and confirmed ctDNA presence in five of 10 cases [91]. This number is considered quite low, but sarcomatous ctDNA was identified in five of six patients with progressive disease. Therefore, ctDNA identification correlated with tumor burden, and it was present in patients with both primary and metastatic lesions but not identified in disease-free or stable subjects. This was a pilot study, so repetition and finding new approaches and targets should increase the numbers of positively identified LMS ctDNAs.

In addition, Eastley et al. [199] confirmed the possibility of detecting and subsequently analyzing ctDNAs released from soft tissue tumors, including two leiomyosarcoma samples. The authors found *TP53* and *HRAS* mutations in both primary tumors and plasma samples. However, it is necessary to remember that the percentage of these mutations differs in tissue and plasma samples and the authors do not mention if these leiomyosarcomas arise from uterine tissues or not.

The understanding of CTC and exosome release from ULM and sarcomas is still poor, and to the best of our knowledge no miRNA profiles have been recovered from these tumors in LB samples despite their abundance in primary tumors. More studies should, therefore, focus on assessing non-coding

RNAs, especially miRNAs in LB samples and on the character of CTC and exosomes released from UMT. From a positive perspective, however, combined approaches have already been developed for the purposes of LB-based tumor diagnosis and prognosis, and these detect desired ctDNA changes in mesenchymal tumors, including uterine LMS [200].

In conclusion, there are no currently known LB biomarkers that can be used for early and noninvasive distinction between particular UMT types. However, (1) there are recorded nucleic acid molecules released from both benign and malignant variants into the bloodstream [89–92]; (2) many molecular biomarkers reported in solid UMT have been noted in LB samples in different cancer types and these can be used for early diagnosis, prognosis, and therapeutic outcomes [93–102]; and (3) it is presumed that abnormal patterns in solid tumors should remain the same in CNA [35–39,67,73–75]. On the basis of these facts, we can state that it is theoretically possible, and highly likely in practice, to distinguish clinically between UMT types using the LB approach when appropriate molecular markers are employed. Our review should, therefore, inspire further research and analysis of known abnormalities in solid tissues in LB samples. The implication of the markers in these processes could also help monitoring of both the progression of malignant variants and the response to treatment. It is, therefore, essential in the future to establish as many molecular markers as possible for each type of UMT and to evaluate them using LB approaches. This will be most beneficial when sufficiently large patient cohort screening can be performed with a variety of advanced molecular methods. Performing NGS-targeted approaches for identification of the markers highlighted in this review is suggested as a most suitable option. Moreover, genome-wide studies would also be welcome to identify useful markers, including epigenetic markers. Finally, any possible improvements in LB approaches for women with UMT will benefit both patients and clinicians, because clinicians would then be able to more precisely measure disease burden and better coordinate clinical treatment, providing greater certainty to affected women that their lesion remains benign.

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## Abbreviations

| | |
|---|---|
| cfDNA | circulating free DNA |
| CNA | freely circulating nucleic acids |
| CTC | circulating tumor cells |
| ctDNA | circulating tumor DNA |
| EMT | epithelial-mesenchymal transition |
| ERα | estrogen receptor alpha |
| ESS | endometrial stromal sarcomas |
| LB | liquid biopsy |
| LMS | uterine leiomyosarcomas |
| lncRNA | long non-coding RNA |
| mRNA | messenger RNA |
| miRNA | microRNA |
| MM | healthy myometrium |
| NGS | next generation sequencing |
| NIPT | non-invasive prenatal testing |
| STUMP | smooth muscle tumors of uncertain malignant potential |
| ULM | uterine leiomyomas |
| UMT | uterine mesenchymal tumors |

## References

1. Flake, G.P.; Andersen, J.; Dixon, D. Etiology and Pathogenesis of Uterine Leiomyomas: A Review. *Environ. Health Perspect.* **2003**, *111*, 1037–1054. [CrossRef] [PubMed]

2. Baird, D.D.; Dunson, D.B.; Hill, M.C.; Cousins, D.; Schectman, J.M. High Cumulative Incidence of Uterine Leiomyoma in Black and White Women: Ultrasound Evidence. *Am. J. Obstet. Gynecol.* **2003**, *188*, 100–107. [CrossRef] [PubMed]

3. Islam, M.S.; Protic, O.; Giannubilo, S.R.; Toti, P.; Tranquilli, A.L.; Petraglia, F.; Castellucci, M.; Ciarmela, P. Uterine Leiomyoma: Available Medical Treatments and New Possible Therapeutic Options. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 921–934. [CrossRef] [PubMed]

4. Brooks, S.E.; Zhan, M.; Cote, T.; Baquet, C.R. Surveillance, Epidemiology, and End Results Analysis of 2677 Cases of Uterine Sarcoma 1989–1999. *Gynecol. Oncol.* **2004**, *93*, 204–208. [CrossRef] [PubMed]

5. Santos, P.; Cunha, T.M. Uterine Sarcomas: Clinical Presentation and MRI Features. *Diagnostic Interv. Radiol.* **2015**, *21*, 4–9. [CrossRef] [PubMed]

6. Kobayashi, H.; Uekuri, C.; Akasaka, J.; Ito, F.; Shigemitsu, A.; Koike, N.; Shigetomi, H. The Biology of Uterine Sarcomas: A Review and Update. *Mol. Clin. Oncol.* **2013**, *1*, 599–609. [CrossRef] [PubMed]

7. Benson, C.; Miah, A.B. Uterine Sarcoma – Current Perspectives. *Int. J. Womens. Health* **2017**, *9*, 597–606. [CrossRef] [PubMed]

8. Arleo, E.K.; Schwartz, P.E.; Hui, P.; McCarthy, S. Review of Leiomyoma Variants. *Am. J. Roentgenol.* **2015**, *205*, 912–921. [CrossRef]

9. Mehine, M.; Kaasinen, E.; Heinonen, H.-R.; Mäkinen, N.; Kämpjärvi, K.; Sarvilinna, N.; Aavikko, M.; Vähärautio, A.; Pasanen, A.; Bützow, R.; et al. Integrated Data Analysis Reveals Uterine Leiomyoma Subtypes with Distinct Driver Pathways and Biomarkers. *Proc. Natl. Acad. Sci.* **2016**, *113*, 1315–1320. [CrossRef] [PubMed]

10. Zhang, Q.; Ubago, J.; Li, L.; Guo, H.; Liu, Y.; Qiang, W.; Kim, J.J.; Kong, B.; Wei, J.-J. Molecular Analyses of 6 Different Types of Uterine Smooth Muscle Tumors: Emphasis in Atypical Leiomyoma. *Cancer* **2014**, *120*, 3165–3177. [CrossRef]

11. Prat, J.; ʹNomonde, M. Uterine Sarcomas. *Int. J. Gynecol. Obstet.* **2015**, *131*, S105–S110. [CrossRef] [PubMed]

12. Kalogiannidis, I.; Stavrakis, T.; Dagklis, T.; Petousis, S.; Nikolaidou, C.; Venizelos, I.; Rousso, D. A Clinicopathological Study of Atypical Leiomyomas: Benign Variant Leiomyoma or Smooth-Muscle Tumor of Uncertain Malignant Potential. *Oncol. Lett.* **2016**, *11*, 1425. [CrossRef] [PubMed]

13. Pickett, J.L.; Chou, A.; Andrici, J.A.; Clarkson, A.; Sioson, L.; Sheen, A.; Reagh, J.; Najdawi, F.; Kim, Y.; Riley, D.; et al. Inflammatory Myofibroblastic Tumors of the Female Genital Tract Are Under-Recognized: A Low Threshold for ALK Immunohistochemistry Is Required. *Am. J. Surg. Pathol.* **2017**, *41*, 1433–1442. [CrossRef] [PubMed]

14. Indraccolo, U.; Luchetti, G.; Indraccolo, S.R. Malignant Transformation of Uterine Leiomyomata. *Eur. J. Gynaecol. Oncol.* **2008**, *29*, 543–544.

15. Patacchiola, F.; Palermo, P.; Di Luigi, G. Leiomyosarcoma: A Rare Malignant Transformation of a Uterine Leiomyoma. *Eur. J. Gynaecol. Oncol.* **2015**, *36*, 84–87.

16. Holzmann, C.; Saager, C.; Mechtersheimer, G.; Koczan, D.; Helmke, B.M.; Bullerdiek, J. Malignant Transformation of Uterine Leiomyoma to Myxoid Leiomyosarcoma after Morcellation Associated with ALK rearrangement and Loss of 14q. *Oncotarget* **2018**, *9*, 27595–27604. [CrossRef] [PubMed]

17. Blythe, J.G.; Bari, W.A. Uterine Sarcoma: Histology, Classification and Prognosis. In *Gynecology and Obstetrics*; Harper Row: Hagerstown, MD, USA, 2008; Volume 4, pp. 1–19. [CrossRef]

18. Artioli, G.; Wabersich, J.; Ludwig, K.; Gardiman, M.P.; Borgato, L.; Garbin, F. Rare Uterine Cancer: Carcinosarcomas. Review from Histology to Treatment. *Crit. Rev. Oncol. Hematol.* **2015**, *94*, 98–104. [CrossRef]

19. D'Angelo, E.; Prat, J. Uterine Sarcomas: A Review. *Gynecol. Oncol.* **2010**, *116*, 131–139. [CrossRef]

20. Chen, I.; Firth, B.; Hopkins, L.; Bougie, O.; Xie, R.-H.; Singh, S. Clinical Characteristics Differentiating Uterine Sarcoma and Fibroids. *JSLS J. Soc. Laparoendosc. Surg.* **2018**, *22*, e2017.00066. [CrossRef]

21. Falcone, T.; Parker, W.H. Surgical Management of Leiomyomas for Fertility or Uterine Preservation. *Obstet. Gynecol.* **2013**, *121*, 856–868. [CrossRef] [PubMed]

22. Bodner, K.; Bodner-Adler, B.; Kimberger, O.; Czerwenka, K.; Mayerhofer, K. Estrogen and Progesterone Receptor Expression in Patients with Uterine Smooth Muscle Tumors. *Fertil. Steril.* **2004**, *81*, 1062–1066. [CrossRef] [PubMed]

23. Felix, A.S.; Cook, L.S.; Gaudet, M.M.; Rohan, T.E.; Schouten, L.J.; Setiawan, V.W.; Wise, L.A.; Anderson, K.E.; Bernstein, L.; De Vivo, I.; et al. The Etiology of Uterine Sarcomas: A Pooled Analysis of the Epidemiology of Endometrial Cancer Consortium. *Br. J. Cancer* **2013**, *108*, 727–734. [CrossRef] [PubMed]

24. Singh, Z. Leiomyosarcoma: A Rare Soft Tissue Cancer Arising from Multiple Organs. *J. Cancer Res. Pract.* **2018**, *5*, 1–8. [CrossRef]

25. Mas, A.; Simón, C. Molecular Differential Diagnosis of Uterine Leiomyomas and Leiomyosarcomas. *Biol. Reprod.* **2018**, *0*, 1–9. [CrossRef] [PubMed]

26. Tsuyoshi, H.; Yoshida, Y. Molecular Biomarkers for Uterine Leiomyosarcoma and Endometrial Stromal Sarcoma. *Cancer Sci.* **2018**, *109*, 1743–1752. [CrossRef] [PubMed]

27. Ashworth, T. A Case of Cancer in Which Cells Similar to Those in the Tumours Were Seen in the Blood after Death. *Aust. Med. J.* **1869**, *14*, 146–149. [CrossRef]

28. Crowley, E.; Di Nicolantonio, F.; Loupakis, F.; Bardelli, A. Liquid Biopsy: Monitoring Cancer-Genetics in the Blood. *Nat. Rev. Clin. Oncol.* **2013**, *10*, 472–484. [CrossRef] [PubMed]

29. Lodewijk, I.; Dueñas, M.; Rubio, C.; Munera-Maravilla, E.; Segovia, C.; Bernardini, A.; Teijeira, A.; Paramio, J.M.; Suárez-Cabrera, C. Liquid Biopsy Biomarkers in Bladder Cancer: A Current Need for Patient Diagnosis and Monitoring. *Int. J. Mol. Sci.* **2018**, *19*, 2514. [CrossRef]

30. Perakis, S.; Speicher, M.R. Emerging Concepts in Liquid Biopsies. *BMC Med.* **2017**, *15*, 75. [CrossRef]

31. Palmirotta, R.; Lovero, D.; Cafforio, P.; Felici, C.; Mannavola, F.; Pellè, E.; Quaresmini, D.; Tucci, M.; Silvestris, F. Liquid Biopsy of Cancer: A Multimodal Diagnostic Tool in Clinical Oncology. *Ther. Adv. Med. Oncol.* **2018**, *10*, 175883591879463. [CrossRef]

32. Borah, B.J.; Nicholson, W.K.; Bradley, L.; Stewart, E.A. The Impact of Uterine Leiomyomas: A National Survey of Affected Women. *Am. J. Obstet. Gynecol.* **2013**, *209*, 319.e1–319.e20. [CrossRef]

33. Ilié, M.; Hofman, P. Pros: Can Tissue Biopsy Be Replaced by Liquid Biopsy? *Transl. lung cancer Res.* **2016**, *5*, 420–423. [CrossRef]

34. Arneth, B. Update on the Types and Usage of Liquid Biopsies in the Clinical Setting: A Systematic Review. *BMC Cancer* **2018**, *18*, 527. [CrossRef]

35. Warton, K.; Mahon, K.L.; Samimi, G. Methylated Circulating Tumor DNA in Blood: Power in Cancer Prognosis and Response. *Endocr. Relat. Cancer* **2016**, *23*, R157–R171. [CrossRef]

36. Gorgannezhad, L.; Umer, M.; Islam, M.N.; Nguyen, N.-T.; Shiddiky, M.J.A. Circulating Tumor DNA and Liquid Biopsy: Opportunities, Challenges, and Recent Advances in Detection Technologies. *Lab Chip* **2018**, *18*, 1174–1196. [CrossRef]

37. Wan, J.C.M.; Massie, C.; Garcia-Corbacho, J.; Mouliere, F.; Brenton, J.D.; Caldas, C.; Pacey, S.; Baird, R.; Rosenfeld, N. Liquid Biopsies Come of Age: Towards Implementation of Circulating Tumour DNA. *Nat. Rev. Cancer* **2017**, *17*, 223–238. [CrossRef]

38. Ng, S.B.; Chua, C.; Ng, M.; Gan, A.; Poon, P.S.; Teo, M.; Fu, C.; Leow, W.Q.; Lim, K.H.; Chung, A.; et al. Individualised Multiplexed Circulating Tumour DNA Assays for Monitoring of Tumour Presence in Patients after Colorectal Cancer Surgery. *Sci. Rep.* **2017**, *7*, 40737. [CrossRef]

39. Yang, N.; Li, Y.; Liu, Z.; Qin, H.; Du, D.; Cao, X.; Cao, X.; Li, J.; Li, D.; Jiang, B.; et al. The Characteristics of CtDNA Reveal the High Complexity in Matching the Corresponding Tumor Tissues. *BMC Cancer* **2018**, *18*, 319. [CrossRef]

40. Guo, Q.; Wang, J.; Xiao, J.; Wang, L.; Hu, X.; Yu, W.; Song, G.; Lou, J.; Chen, J. Heterogeneous Mutation Pattern in Tumor Tissue and Circulating Tumor DNA Warrants Parallel NGS Panel Testing. *Mol. Cancer* **2018**, *17*, 131. [CrossRef]

41. Kammesheidt, A.; Tonozzi, T.R.; Lim, S.W.; Braunstein, G.D. Mutation Detection Using Plasma Circulating Tumor DNA (CtDNA) in a Cohort of Asymptomatic Adults at Increased Risk for Cancer. *Int. J. Mol. Epidemiol. Genet.* **2018**, *9*, 1–12.

42. Toor, O.M.; Ahmed, Z.; Bahaj, W.; Boda, U.; Cummings, L.S.; McNally, M.E.; Kennedy, K.F.; Pluard, T.J.; Hussain, A.; Subramanian, J.; et al. Correlation of Somatic Genomic Alterations Between Tissue Genomics and CtDNA Employing Next-Generation Sequencing: Analysis of Lung and Gastrointestinal Cancers. *Mol. Cancer Ther.* **2018**, *17*, 1123–1132. [CrossRef]

43. Schwarzenbach, H.; Hoon, D.S.B.; Pantel, K. Cell-Free Nucleic Acids as Biomarkers in Cancer Patients. *Nat. Rev. Cancer* **2011**, *11*, 426–437. [CrossRef]

44. Leung, F.; Kulasingam, V.; Diamandis, E.P.; Hoon, D.S.B.; Kinzler, K.; Pantel, K.; Alix-Panabieres, C. Circulating Tumor DNA as a Cancer Biomarker: Fact or Fiction? *Clin. Chem.* **2016**, *62*, 1054–1060. [CrossRef]

45. Shao, H.; Chung, J.; Issadore, D. Diagnostic Technologies for Circulating Tumour Cells and Exosomes. *Biosci. Rep.* **2016**, *36*, e00292. [CrossRef]

46. Agarwal, A.; Balic, M.; El-Ashry, D.; Cote, R.J. Circulating Tumor Cells. *Cancer J.* **2018**, *24*, 70–77. [CrossRef]

47. Boukouris, S.; Mathivanan, S. Exosomes in Bodily Fluids Are a Highly Stable Resource of Disease Biomarkers. *Proteom.-Clin. Appl.* **2015**, *9*, 358–367. [CrossRef]

48. Tian, F.; Shen, Y.; Chen, Z.; Li, R.; Ge, Q. No Significant Difference between Plasma MiRNAs and Plasma-Derived Exosomal MiRNAs from Healthy People. *Biomed. Res. Int.* **2017**, *2017*, 1304816. [CrossRef]

49. Kawaguchi, T.; Komatsu, S.; Ichikawa, D.; Tsujiura, M.; Takeshita, H.; Hirajima, S.; Miyamae, M.; Okajima, W.; Ohashi, T.; Imamura, T.; et al. Circulating MicroRNAs: A Next-Generation Clinical Biomarker for Digestive System Cancers. *Int. J. Mol. Sci.* **2016**, *17*, 1459. [CrossRef]

50. De Souza, M.F.; Kuasne, H.; de Camargo Barros-Filho, M.; Cilião, H.L.; Marchi, F.A.; Fuganti, P.E.; Paschoal, A.R.; Rogatto, S.R.; de Syllos Cólus, I.M. Circulating MRNAs and MiRNAs as Candidate Markers for the Diagnosis and Prognosis of Prostate Cancer. *PLoS ONE* **2017**, *12*, e0184094. [CrossRef]

51. Diehl, F.; Schmidt, K.; Choti, M.A.; Romans, K.; Goodman, S.; Li, M.; Thornton, K.; Agrawal, N.; Sokoll, L.; Szabo, S.A.; et al. Circulating Mutant DNA to Assess Tumor Dynamics. *Nat. Med.* **2008**, *14*, 985–990. [CrossRef]

52. Fleischhacker, M.; Schmidt, B. Circulating Nucleic Acids (CNAs) and Cancer—A Survey. *Biochim. Biophys. Acta-Rev. Cancer* **2007**, *1775*, 181–232. [CrossRef]

53. Yao, W.; Mei, C.; Nan, X.; Hui, L. Evaluation and Comparison of in Vitro Degradation Kinetics of DNA in Serum, Urine and Saliva: A Qualitative Study. *Gene* **2016**, *590*, 142–148. [CrossRef]

54. Young, R.; Pailler, E.; Billiot, F.; Drusch, F.; Barthelemy, A.; Oulhen, M.; Besse, B.; Soria, J.-C.; Farace, F.; Vielh, P. Circulating Tumor Cells in Lung Cancer. *Acta Cytol.* **2012**, *56*, 655–660. [CrossRef]

55. Su, Z.; Zhao, J.; Ke, S.; Zhang, J.; Liu, X.; Wang, Y.; Sun, Q.; Pan, Q. Clinical Significance of Circulating Tumor Cells via Combined Whole Exome Sequencing in Early Stage Cancer Screening: A Case Report. *Exp. Ther. Med.* **2018**, *16*, 2527–2533. [CrossRef]

56. Rajagopal, C.; Harikumar, K.B. The Origin and Functions of Exosomes in Cancer. *Front. Oncol.* **2018**, *8*, 66. [CrossRef]

57. Koritzinsky, E.H.; Street, J.M.; Star, R.A.; Yuen, P.S.T. Quantification of Exosomes. *J. Cell. Physiol.* **2017**, *232*, 1587–1590. [CrossRef]

58. Li, P.; Kaslan, M.; Lee, S.H.; Yao, J.; Gao, Z. Progress in Exosome Isolation Techniques. *Theranostics* **2017**, *7*, 789–804. [CrossRef]

59. Taylor, D.D.; Gercel-Taylor, C. Exosomes/Microvesicles: Mediators of Cancer-Associated Immunosuppressive Microenvironments. *Semin. Immunopathol.* **2011**, *33*, 441–454. [CrossRef]

60. Yang, Y.; Hong, Y.; Cho, E.; Kim, G.B.; Kim, I.-S. Extracellular Vesicles as a Platform for Membrane-Associated Therapeutic Protein Delivery. *J. Extracell. Vesicles* **2018**, *7*, 1440131. [CrossRef]

61. Wu, Y.; Deng, W.; Klinke II, D.J. Exosomes: Improved Methods to Characterize Their Morphology, RNA Content, and Surface Protein Biomarkers. *Analyst* **2015**, *140*, 6631–6642. [CrossRef]

62. Sheridan, C. Exosome Cancer Diagnostic Reaches Market. *Nat. Biotechnol.* **2016**, *34*, 359–360. [CrossRef]

63. Lissa, D.; Robles, A.I. Methylation Analyses in Liquid Biopsy. *Transl. Lung Cancer Res.* **2016**, *5*, 492. [CrossRef]

64. Balgkouranidou, I.; Chimonidou, M.; Milaki, G.; Tsarouxa, E.G.; Kakolyris, S.; Welch, D.R.; Georgoulias, V.; Lianidou, E.S. Breast Cancer Metastasis Suppressor-1 Promoter Methylation in Cell-Free DNA Provides Prognostic Information in Non-Small Cell Lung Cancer. *Br. J. Cancer* **2014**, *110*, 2054–2062. [CrossRef]

65. Majchrzak-Celińska, A.; Paluszczak, J.; Kleszcz, R.; Magiera, M.; Barciszewska, A.-M.; Nowak, S.; Baer-Dubowska, W. Detection of MGMT, RASSF1A, P15INK4B, and P14ARF Promoter Methylation in Circulating Tumor-Derived DNA of Central Nervous System Cancer Patients. *J. Appl. Genet.* **2013**, *54*, 335–344. [CrossRef]

66. Kristensen, L.S.; Hansen, J.W.; Kristensen, S.S.; Tholstrup, D.; Harsløf, L.B.S.; Pedersen, O.B.; De Nully Brown, P.; Grønbæk, K. Aberrant Methylation of Cell-Free Circulating DNA in Plasma Predicts Poor Outcome in Diffuse Large B Cell Lymphoma. *Clin. Epigenetics* **2016**, *8*, 95. [CrossRef]

67. Elazezy, M.; Joosse, S.A. Techniques of Using Circulating Tumor DNA as a Liquid Biopsy Component in Cancer Management. *Comput. Struct. Biotechnol. J.* **2018**, *16*, 370–378. [CrossRef]

68. Zhang, R.; Chen, B.; Tong, X.; Wang, Y.; Wang, C.; Jin, J.; Tian, P.; Li, W. Diagnostic Accuracy of Droplet Digital PCR for Detection of EGFR T790M Mutation in Circulating Tumor DNA. *Cancer Manag. Res.* **2018**, *10*, 1209–1218. [CrossRef]

69. Calapre, L.; Warburton, L.; Millward, M.; Ziman, M.; Gray, E.S. Circulating Tumour DNA (CtDNA) as a Liquid Biopsy for Melanoma. *Cancer Lett.* **2017**, *404*, 62–69. [CrossRef]

70. Widschwendter, M.; Zikan, M.; Wahl, B.; Lempiäinen, H.; Paprotka, T.; Evans, I.; Jones, A.; Ghazali, S.; Reisel, D.; Eichner, J.; et al. The Potential of Circulating Tumor DNA Methylation Analysis for the Early Detection and Management of Ovarian Cancer. *Genome Med.* **2017**, *9*, 116. [CrossRef]

71. Le, A.; Szaumkessel, M.; Tan, T.; Thiery, J.-P.; Thompson, E.; Dobrovic, A. DNA Methylation Profiling of Breast Cancer Cell Lines along the Epithelial Mesenchymal Spectrum—Implications for the Choice of Circulating Tumour DNA Methylation Markers. *Int. J. Mol. Sci.* **2018**, *19*, 2553. [CrossRef]

72. Xu, R.; Wei, W.; Krawczyk, M.; Wang, W.; Luo, H.; Flagg, K.; Yi, S.; Shi, W.; Quan, Q.; Li, K.; et al. Circulating Tumour DNA Methylation Markers for Diagnosis and Prognosis of Hepatocellular Carcinoma. *Nat. Mater.* **2017**, *16*, 1155–1161. [CrossRef]

73. Sun, K.; Lun, F.F.M.; Jiang, P.; Sun, H. BSviewer: A Genotype-Preserving, Nucleotide-Level Visualizer for Bisulfite Sequencing Data. *Bioinformatics* **2017**, *33*, 3495–3496. [CrossRef]

74. Lun, F.M.F.; Chiu, R.W.K.; Sun, K.; Leung, T.Y.; Jiang, P.; Chan, K.C.A.; Sun, H.; Lo, Y.M.D. Noninvasive Prenatal Methylomic Analysis by Genomewide Bisulfite Sequencing of Maternal Plasma DNA. *Clin. Chem.* **2013**, *59*, 1583–1584. [CrossRef]

75. Wong, I.H.N.; Liew, C.-T.; Ng, M.H.L.; Lo, Y.M.D.; Zhang, J.; Hjelm, N.M.; Wong, N.; Johnson, P.J.; Lai, P.B.S.; Lau, W.Y. Detection of Aberrant P16 Methylation in the Plasma and Serum of Liver Cancer Patients. *Cancer Res.* **1999**, *59*, 71–73.

76. Cheng, J.-P.; Yan, Y.; Wang, X.-Y.; Lu, Y.-L.; Yuan, Y.-H.; Jia, J.; Ren, J. MUC1-Positive Circulating Tumor Cells and MUC1 Protein Predict Chemotherapeutic Efficacy in the Treatment of Metastatic Breast Cancer. *Chin. J. Cancer* **2011**, *30*, 54–61. [CrossRef]

77. Kuhlmann, J.D.; Wimberger, P.; Bankfalvi, A.; Keller, T.; Scholer, S.; Aktas, B.; Buderath, P.; Hauch, S.; Otterbach, F.; Kimmig, R.; et al. ERCC1-Positive Circulating Tumor Cells in the Blood of Ovarian Cancer Patients as a Predictive Biomarker for Platinum Resistance. *Clin. Chem.* **2014**, *60*, 1282–1289. [CrossRef]

78. Lin, H.-C.; Hsu, H.-C.; Hsieh, C.-H.; Wang, H.-M.; Huang, C.-Y.; Wu, M.-H.; Tseng, C.-P. A Negative Selection System PowerMag for Effective Leukocyte Depletion and Enhanced Detection of EpCAM Positive and Negative Circulating Tumor Cells. *Clin. Chim. Acta.* **2013**, *419*, 77–84. [CrossRef]

79. Benini, S.; Gamberi, G.; Cocchi, S.; Garbetta, J.; Alberti, L.; Righi, A.; Gambarotti, M.; Picci, P.; Ferrari, S. Detection of Circulating Tumor Cells in Liquid Biopsy from Ewing Sarcoma Patients. *Cancer Manag. Res.* **2018**, *10*, 49–60. [CrossRef]

80. Satelli, A.; Brownlee, Z.; Mitra, A.; Meng, Q.H.; Li, S. Circulating Tumor Cell Enumeration with a Combination of Epithelial Cell Adhesion Molecule-and Cell-Surface Vimentin-Based Methods for Monitoring Breast Cancer Therapeutic Response. *Clin. Chem.* **2015**, *61*, 259–266. [CrossRef]

81. Hayashi, M.; Zhu, P.; McCarty, G.; Meyer, C.F.; Pratilas, C.A.; Levin, A.; Morris, C.D.; Albert, C.M.; Jackson, K.W.; Tang, C.-M.; et al. Size-Based Detection of Sarcoma Circulating Tumor Cells and Cell Clusters. *Oncotarget* **2017**, *8*, 78965. [CrossRef]

82. Khetani, S.; Mohammadi, M.; Nezhad, A.S. Filter-Based Isolation, Enrichment, and Characterization of Circulating Tumor Cells. *Biotechnol. Bioeng.* **2018**, *115*, 2504–2529. [CrossRef]

83. Chung, J.; Shao, H.; Reiner, T.; Issadore, D.; Weissleder, R.; Lee, H. Microfluidic Cell Sorter ($\mu$ FCS) for On-Chip Capture and Analysis of Single Cells. *Adv. Healthc. Mater.* **2012**, *1*, 432–436. [CrossRef]

84. Sarioglu, A.F.; Aceto, N.; Kojic, N.; Donaldson, M.C.; Zeinali, M.; Hamza, B.; Engstrom, A.; Zhu, H.; Sundaresan, T.K.; Miyamoto, D.T.; et al. A Microfluidic Device for Label-Free, Physical Capture of Circulating Tumor Cell Clusters. *Nat. Methods* **2015**, *12*, 685–691. [CrossRef]

85. Issadore, D.; Chung, J.; Shao, H.; Liong, M.; Ghazani, A.A.; Castro, C.M.; Weissleder, R.; Lee, H. Ultrasensitive Clinical Enumeration of Rare Cells Ex Vivo Using a Micro-Hall Detector. *Sci. Transl. Med.* **2012**, *4*, 141ra92. [CrossRef]

86. Muluneh, M.; Issadore, D. A Multi-Scale PDMS Fabrication Strategy to Bridge the Size Mismatch between Integrated Circuits and Microfluidics. *Lab Chip* **2014**, *14*, 4552–4558. [CrossRef]

87. Yao, N.; Jan, Y.-J.; Cheng, S.; Chen, J.-F.; Chung, L.W.; Tseng, H.-R.; Posadas, E.M. Structure and Function Analysis in Circulating Tumor Cells: Using Nanotechnology to Study Nuclear Size in Prostate Cancer. *Am. J. Clin. Exp. Urol.* **2018**, *6*, 43–54.

88. Hui, L. Noninvasive Prenatal Testing for Aneuploidy Using Cell-Free DNA – New Implications for Maternal Health. *Obstet. Med.* **2016**, *9*, 148–152. [CrossRef]

89. Dharajiya, N.G.; Grosu, D.S.; Farkas, D.H.; McCullough, R.M.; Almasri, E.; Sun, Y.; Kim, S.K.; Jensen, T.J.; Saldivar, J.-S.; Topol, E.J.; et al. Incidental Detection of Maternal Neoplasia in Noninvasive Prenatal Testing. *Clin. Chem.* **2018**, *64*, 329–335. [CrossRef]

90. Giles, M.E.; Murphy, L.; Krstić, N.; Sullivan, C.; Hashmi, S.S.; Stevens, B. Prenatal CfDNA Screening Results Indicative of Maternal Neoplasm: Survey of Current Practice and Management Needs. *Prenat. Diagn.* **2017**, *37*, 126–132. [CrossRef]

91. Hemming, M.L.; Klega, K.S.; Acker, K.E.; Nag, A.; Thorner, A.; Nathenson, M.; Raut, C.P.; Crompton, B.D.; George, S. Identification of Leiomyosarcoma Circulating Tumor DNA through Ultra-Low Passage Whole Genome Sequencing and Correlation with Tumor Burden: A Pilot Experience. *J. Clin. Oncol.* **2018**, *36*, 11565. [CrossRef]

92. Hemming, M.L.; Klega, K.; Rhoades, J.; Ha, G.; Acker, K.E.; Andersen, J.L.; Thai, E.; Nag, A.; Thorner, A.R.; Raut, C.P.; et al. Detection of Circulating Tumor DNA in Patients With Leiomyosarcoma With Progressive Disease. *JCO Precis. Oncol.* **2019**, *3*, 1–11. [CrossRef]

93. Izzotti, A.; Carozzo, S.; Pulliero, A.; Zhabayeva, D.; Ravetti, J.L.; Bersimbaev, R. Extracellular MicroRNA in Liquid Biopsy: Applicability in Cancer Diagnosis and Prevention. *Am. J. Cancer Res.* **2016**, *6*, 1461–1493.

94. Giannopoulou, L.; Zavridou, M.; Kasimir-Bauer, S.; Lianidou, E.S. Liquid Biopsy in Ovarian Cancer: The Potential of Circulating MiRNAs and Exosomes. *Transl. Res.* **2019**, *205*, 77–91. [CrossRef]

95. De Groot, J.S.; Moelans, C.B.; Elias, S.G.; Fackler, M.J.; Van Domselaar, R.; Suijkerbuijk, K.P.M.; Witkamp, A.J.; Sukumar, S.; Van Diest, P.J.; Van Der Wall, E. DNA Promoter Hypermethylation in Nipple Fluid: A Potential Tool for Early Breast Cancer Detection. *Oncotarget* **2016**, *7*, 24778. [CrossRef]

96. Takashima, Y.; Kawaguchi, A.; Iwadate, Y.; Hondoh, H.; Fukai, J.; Kajiwara, K.; Hayano, A.; Yamanaka, R. MicroRNA Signature Constituted of MiR-30d, MiR-93, and MiR-181b Is a Promising Prognostic Marker in Primary Central Nervous System Lymphoma. *PLoS ONE* **2019**, *14*, e0210400. [CrossRef]

97. Komatsu, S.; Kiuchi, J.; Imamura, T.; Ichikawa, D.; Otsuji, E. Circulating MicroRNAs as a Liquid Biopsy: A next-Generation Clinical Biomarker for Diagnosis of Gastric Cancer. *J. Cancer Metastasis Treat.* **2018**, *4*, 36. [CrossRef]

98. Li, L.; Fu, K.; Zhou, W.; Snyder, M. Applying Circulating Tumor DNA Methylation in the Diagnosis of Lung Cancer. *Precis. Clin. Med.* **2019**, *2*, 45–56. [CrossRef]

99. Barault, L.; Amatu, A.; Bleeker, F.E.; Moutinho, C.; Falcomatà, C.; Fiano, V.; Cassingena, A.; Siravegna, G.; Milione, M.; Cassoni, P.; et al. Digital PCR Quantification of MGMT Methylation Refines Prediction of Clinical Benefit from Alkylating Agents in Glioblastoma and Metastatic Colorectal Cancer. *Ann. Oncol.* **2015**, *26*, 1994–1999. [CrossRef]

100. Martínez-Ricarte, F.; Mayor, R.; Martínez-Sáez, E.; Rubio-Pérez, C.; Pineda, E.; Cordero, E.; Cicuéndez, M.; Poca, M.A.; López-Bigas, N.; Ramon Y Cajal, S.; et al. Molecular Diagnosis of Diffuse Gliomas through Sequencing of Cell-Free Circulating Tumour DNA from Cerebrospinal Fluid. *Author Manuscr. Publ. OnlineFirst* **2018**, *24*, 2812–2819. [CrossRef]

101. Vandekerkhove, G.; Todenhöfer, T.; Annala, M.; Struss, W.J.; Wong, A.; Beja, K.; Ritch, E.; Brahmbhatt, S.; Volik, S.V.; Hennenlotter, J.; et al. Circulating Tumor DNA Reveals Clinically Actionable Somatic Genome of Metastatic Bladder Cancer. *Clin. Cancer Res.* **2017**, *23*, 6487–6497. [CrossRef]

102. Cimadamore, A.; Gasparrini, S.; Massari, F.; Santoni, M.; Cheng, L.; Lopez-Beltran, A.; Scarpelli, M.; Montironi, R. Emerging Molecular Technologies in Renal Cell Carcinoma: Liquid Biopsy. *Cancers* **2019**, *11*(2), 196. [CrossRef]

103. Kirkizlar, E.; Zimmermann, B.; Constantin, T.; Swenerton, R.; Hoang, B.; Wayham, N.; Babiarz, J.E.; Demko, Z.; Pelham, R.J.; Kareht, S.; et al. Detection of Clonal and Subclonal Copy-Number Variants in Cell-Free DNA from Patients with Breast Cancer Using a Massively Multiplexed PCR Methodology. *Transl. Oncol.* **2015**, *8*, 407–416. [CrossRef]

104. Best, M.G.; Sol, N.; Zijl, S.; Reijneveld, J.C.; Wesseling, P.; Wurdinger, T. Liquid Biopsies in Patients with Diffuse Glioma. *Acta Neuropathologica.* **2015**, *129*, 849–865. [CrossRef]

105. Miller, A.M.; Shah, R.H.; Pentsova, E.I.; Pourmaleki, M.; Briggs, S.; Distefano, N.; Zheng, Y.; Skakodub, A.; Mehta, S.A.; Campos, C.; et al. Tracking Tumour Evolution in Glioma through Liquid Biopsies of Cerebrospinal Fluid. *Nature* **2019**, 654–658. [CrossRef]

106. Yin, C.Q.; Yuan, C.H.; Qu, Z.; Guan, Q.; Chen, H.; Wang, F.B. Liquid Biopsy of Hepatocellular Carcinoma: Circulating Tumor-Derived Biomarkers. *Dis. Markers* **2016**. [CrossRef]

107. Hammond, S.M. An Overview of MicroRNAs. *Adv. Drug Deliv. Rev.* **2015**, *87*, 3–14. [CrossRef]

108. Pan, Q.; Luo, X.; Chegini, N. Differential Expression of MicroRNAs in Myometrium and Leiomyomas and Regulation by Ovarian Steroids. *J. Cell. Mol. Med.* **2008**, *12*, 227–240. [CrossRef]

109. Liu, Z.; Guo, H.; Wu, J.; Zavadil, J.; Ghanny, S.; Ghuo, S.; Wei, J. Differential Expression of MiRNAs in Uterine Leiomyoma and Adjacent Myometrium of Different Races. *Am J Clin Exp Obstet Gynecol.* **2015**, *2*, 45–256.

110. Wang, T.; Zhang, X.; Obijuru, L.; Laser, J.; Aris, V.; Lee, P.; Mittal, K.; Soteropoulos, P.; Wei, J.-J. A Micro-RNA Signature Associated with Race, Tumor Size, and Target Gene Activity in Human Uterine Leiomyomas. *Genes, Chromosom. Cancer* **2007**, *46*, 336–347. [CrossRef]

111. Guan, Y.; Guo, L.; Zukerberg, L.; Rueda, B.R.; Styer, A.K. MicroRNA-15b Regulates Reversion-Inducing Cysteine-Rich Protein with Kazal Motifs (RECK) Expression in Human Uterine Leiomyoma. *Reprod. Biol. Endocrinol.* **2016**, *14*, 45. [CrossRef]

112. Xin, C.; Buhe, B.; Hongting, L.; Chuanmin, Y.; Xiwei, H.; Hong, Z.; Lulu, H.; Qian, D.; Renjie, W. MicroRNA-15a Promotes Neuroblastoma Migration by Targeting Reversion-Inducing Cysteine-Rich Protein with Kazal Motifs (RECK) and Regulating Matrix Metalloproteinase-9 Expression. *FEBS J.* **2013**, *280*, 855–866. [CrossRef]

113. Takagi, S.; Simizu, S.; Osada, H. Reck Negatively Regulates Matrix Metalloproteinase-9 Transcription. *Cancer Res.* **2009**, *69*, 1502–1508. [CrossRef]

114. Kristjánsdóttir, K.; Fogarty, E.A.; Grimson, A. Systematic Analysis of the *Hmga2* 3′ UTR Identifies Many Independent Regulatory Sequences and a Novel Interaction between Distal Sites. *RNA* **2015**, *21*, 1346–1360. [CrossRef]

115. De Almeida, B.C.; Garcia, N.; Maffazioli, G.; Dos Anjos, L.G.; Baracat, E.C.; Carvalho, K.C. Oncomirs Expression Profiling in Uterine Leiomyosarcoma Cells. *Int. J. Mol. Sci.* **2018**, *19*, 52. [CrossRef]

116. Schiavon, B.N.; Carvalho, K.C.; Coutinho-Camillo, C.M.; Baiocchi, G.; Valieris, R.; Drummond, R.; da Silva, I.T.; De Brot, L.; Soares, F.A.; da Cunha, I.W. MiRNAs 144-3p, 34a-5p, and 206 Are a Useful Signature for Distinguishing Uterine Leiomyosarcoma from Other Smooth Muscle Tumors. *Surg. Exp. Pathol.* **2019**, *2*, 5. [CrossRef]

117. Danielson, L.S.; Menendez, S.; Attolini, C.S.-O.; Guijarro, M.V.; Bisogna, M.; Wei, J.; Socci, N.D.; Levine, D.A.; Michor, F.; Hernando, E. A Differentiation-Based MicroRNA Signature Identifies Leiomyosarcoma as a Mesenchymal Stem Cell-Related Malignancy. *Am. J. Pathol.* **2010**, *177*, 908–917. [CrossRef]

118. Kowalewska, M.; Bakula-Zalewska, E.; Chechlinska, M.; Goryca, K.; Nasierowska-Guttmejer, A.; Danska-Bidzinska, A.; Bidzinski, M. MicroRNAs in Uterine Sarcomas and Mixed Epithelial–Mesenchymal Uterine Tumors: A Preliminary Report. *Tumor Biol.* **2013**, *34*, 2153–2160. [CrossRef]

119. Gonzalez dos Anjos, L.; de Almeida, B.; Gomes de Almeida, T.; Mourão Lavorato Rocha, A.; De Nardo Maffazioli, G.; Soares, F.; Werneck da Cunha, I.; Chada Baracat, E.; Candido Carvalho, K.; Gonzalez dos Anjos, L.; et al. Could MiRNA Signatures Be Useful for Predicting Uterine Sarcoma and Carcinosarcoma Prognosis and Treatment? *Cancers (Basel)* **2018**, *10*, 315. [CrossRef]

120. Ravid, Y.; Formanski, M.; Smith, Y.; Reich, R.; Davidson, B. Uterine Leiomyosarcoma and Endometrial Stromal Sarcoma Have Unique MiRNA Signatures. *Gynecol. Oncol.* **2016**, *140*, 512–517. [CrossRef]

121. Zavadil, J.; Ye, H.; Liu, Z.; Wu, J.; Lee, P.; Hernando, E.; Soteropoulos, P.; Toruner, G.A.; Wei, J.-J. Profiling and Functional Analyses of MicroRNAs and Their Target Gene Products in Human Uterine Leiomyomas. *PLoS ONE* **2010**, *5*, e12362. [CrossRef]

122. Marsh, E.E.; Lin, Z.; Yin, P.; Milad, M.; Chakravarti, D.; Bulun, S.E. Differential Expression of MicroRNA Species in Human Uterine Leiomyoma versus Normal Myometrium. *Fertil. Steril.* **2008**, *89*, 1771–1776. [CrossRef]

123. Georgieva, B.; Milev, I.; Minkov, I.; Dimitrova, I.; Bradford, A.P.; Baev, V. Characterization of the Uterine Leiomyoma MicroRNAome by Deep Sequencing. *Genomics* **2012**, *99*, 275–281. [CrossRef]

124. Marsh, E.E.; Steinberg, M.L.; Parker, J.B.; Wu, J.; Chakravarti, D.; Bulun, S.E. Decreased Expression of MicroRNA-29 Family in Leiomyoma Contributes to Increased Major Fibrillar Collagen Production. *Fertil. Steril.* **2016**, *106*, 766–772. [CrossRef]

125. Chuang, T.-D.; Luo, X.; Panda, H.; Chegini, N. MiR-93/106b and Their Host Gene, MCM7, Are Differentially Expressed in Leiomyomas and Functionally Target F3 and IL-8. *Mol. Endocrinol.* **2012**, *26*, 1028–1042. [CrossRef]

126. Pazzaglia, L.; Novello, C.; Conti, A.; Pollino, S.; Picci, P.; Benassi, M.S. MiR-152 down-Regulation Is Associated with MET up-Regulation in Leiomyosarcoma and Undifferentiated Pleomorphic Sarcoma. *Cell. Oncol.* **2017**, *40*, 77–88. [CrossRef]

127. Chuang, T.-D.; Panda, H.; Luo, X.; Chegini, N. MiR-200c Is Aberrantly Expressed in Leiomyomas in an Ethnic-Dependent Manner and Targets ZEBs, VEGFA, TIMP2, and FBLN5. *Endocr. Relat. Cancer* **2012**, *19*, 541–556. [CrossRef]

128. Wu, X.; Ling, J.; Fu, Z.; Ji, C.; Wu, J.; Xu, Q. Effects of MiRNA-197 Overexpression on Proliferation, Apoptosis and Migration in Levonorgestrel Treated Uterine Leiomyoma Cells. *Biomed. Pharmacother.* **2015**, *71*, 1–6. [CrossRef]

129. Makinen, N.; Mehine, M.; Tolvanen, J.; Kaasinen, E.; Li, Y.; Lehtonen, H.J.; Gentile, M.; Yan, J.; Enge, M.; Taipale, M.; et al. MED12, the Mediator Complex Subunit 12 Gene, Is Mutated at High Frequency in Uterine Leiomyomas. *Science* **2011**, *334*, 252–255. [CrossRef]

130. Harrison, W.J.; Andrici, J.; Maclean, F.; Madadi-Ghahan, R.; Farzin, M.; Sioson, L.; Toon, C.W.; Clarkson, A.; Watson, N.; Pickett, J.; et al. Fumarate Hydratase–Deficient Uterine Leiomyomas Occur in Both the Syndromic and Sporadic Settings. *Am. J. Surg. Pathol.* **2016**, *40*, 599–607. [CrossRef]

131. Stewart, L.; Glenn, G.M.; Stratton, P.; Goldstein, A.M.; Merino, M.J.; Tucker, M.A.; Linehan, W.M.; Toro, J.R. Association of Germline Mutations in the Fumarate Hydratase Gene and Uterine Fibroids in Women with Hereditary Leiomyomatosis and Renal Cell Cancer. *Arch. Dermatol.* **2008**, *144*, 1584–1592. [CrossRef]

132. Ylisaukko-oja, S.K.; Kiuru, M.; Lehtonen, H.J.; Lehtonen, R.; Pukkala, E.; Arola, J.; Launonen, V.; Aaltonen, L.A. Analysis of Fumarate Hydratase Mutations in a Population-Based Series of Early Onset Uterine Leiomyosarcoma Patients. *Int. J. Cancer* **2006**, *119*, 283–287. [CrossRef]

133. Kämpjärvi, K.; Mäkinen, N.; Kilpivaara, O.; Arola, J.; Heinonen, H.-R.; Böhm, J.; Abdel-Wahab, O.; Lehtonen, H.J.; Pelttari, L.M.; Mehine, M.; et al. Somatic MED12 Mutations in Uterine Leiomyosarcoma and Colorectal Cancer. *Br. J. Cancer* **2012**, *107*, 1761–1765. [CrossRef]

134. Mäkinen, N.; Aavikko, M.; Heikkinen, T.; Taipale, M.; Taipale, J.; Koivisto-Korander, R.; Bützow, R.; Vahteristo, P. Exome Sequencing of Uterine Leiomyosarcomas Identifies Frequent Mutations in TP53, ATRX, and MED12. *PLOS Genet.* **2016**, *12*, e1005850. [CrossRef]

135. Zhang, W.-W.; Li, L.; Li, D.; Liu, J.; Li, X.; Li, W.; Xu, X.; Zhang, M.J.; Chandler, L.A.; Lin, H.; et al. The First Approved Gene Therapy Product for Cancer Ad-*P53* (Gendicine): 12 Years in the Clinic. *Hum. Gene Ther.* **2018**, *29*, 160–179. [CrossRef]

136. Slatter, T.L.; Hsia, H.; Samaranayaka, A.; Sykes, P.; Clow, W.B.; Devenish, C.J.; Sutton, T.; Royds, J.A.; PC, P.; Cheung, A.N.; et al. Loss of ATRX and DAXX Expression Identifies Poor Prognosis for Smooth Muscle Tumours of Uncertain Malignant Potential and Early Stage Uterine Leiomyosarcoma. *J. Pathol. Clin. Res.* **2015**, *1*, 95–105. [CrossRef]

137. Bennett, J.A.; Weigelt, B.; Chiang, S.; Selenica, P.; Chen, Y.-B.; Bialik, A.; Bi, R.; Schultheis, A.M.; Lim, R.S.; Ng, C.K.Y.; et al. Leiomyoma with Bizarre Nuclei: A Morphological, Immunohistochemical and Molecular Analysis of 31 Cases. *Mod. Pathol.* **2017**, *30*, 1476–1488. [CrossRef]

138. Dyson, N.J. *RB1*: A Prototype Tumor Suppressor and an Enigma. *Genes Dev.* **2016**, *30*, 1492–1502. [CrossRef]

139. Hovelson, D.H.; Liu, C.-J.; Wang, Y.; Kang, Q.; Henderson, J.; Gursky, A.; Brockman, S.; Ramnath, N.; Krauss, J.C.; Talpaz, M.; et al. Rapid, Ultra Low Coverage Copy Number Profiling of Cell-Free DNA as a Precision Oncology Screening Strategy. *Oncotarget* **2017**, *8*, 89848–89866. [CrossRef]

140. Klega, K.; Imamovic-Tuco, A.; Ha, G.; Clapp, A.N.; Meyer, S.; Ward, A.; Clinton, C.; Nag, A.; Van Allen, E.; Mullen, E.; et al. Detection of Somatic Structural Variants Enables Quantification and Characterization of Circulating Tumor DNA in Children With Solid Tumors. *JCO Precis. Oncol.* **2018**, *2018*, 1–13. [CrossRef]

141. Velagaleti, G.V.N.; Wang, X.; Erickson-Johnson, M.R.; Medeiros, F.; Oliveira, A.M.; Tonk, V.S.; Hakim, N.M.; Zhang, H. Fusion of HMGA2 to COG5 in Uterine Leiomyoma. *Cancer Genet. Cytogenet.* **2010**, *202*, 11–16. [CrossRef]

142. CHO, Y.; BAE, S.; KOO, M.; KIM, K.; CHUN, H.; KIM, C.; RO, D.; KIM, J.; LEE, C.; KIM, Y. Array Comparative Genomic Hybridization Analysis of Uterine Leiomyosarcoma. *Gynecol. Oncol.* **2005**, *99*, 545–551. [CrossRef]

143. Koontz, J.I.; Soreng, A.L.; Nucci, M.; Kuo, F.C.; Pauwels, P.; van den Berghe, H.; Cin, P.D.; Fletcher, J.A.; Sklar, J. Frequent Fusion of the JAZF1 and JJAZ1 Genes in Endometrial Stromal Tumors. *Proc. Natl. Acad. Sci.* **2001**, *98*, 6348–6353. [CrossRef]

144. Schoenmakers, E.F.P.M.; Huysmans, C.; Van De Ven, W.J.M. Allelic Knockout of Novel Splice Variants of Human Recombination Repair Gene RAD51B in t(12;14) Uterine Leiomyomas. *Cancer Res.* **1999**, *59*, 19–23.

145. Beck, A.H.; Lee, C.-H.; Witten, D.M.; Gleason, B.C.; Edris, B.; Espinosa, I.; Zhu, S.; Li, R.; Montgomery, K.D.; Marinelli, R.J.; et al. Discovery of Molecular Subtypes in Leiomyosarcoma through Integrative Molecular Profiling. *Oncogene* **2010**, *29*, 845–854. [CrossRef]

146. Micci, F.; Panagopoulos, I.; Bjerkehagen, B.; Heim, S. Consistent Rearrangement of Chromosomal Band 6p21 with Generation of Fusion Genes *JAZF1/PHF1* and *EPC1/PHF1* in Endometrial Stromal Sarcoma. *Cancer Res.* **2006**, *66*, 107–112. [CrossRef]

147. Sandberg, A.; Bridge, J. Updates on the Cytogenetics and Molecular Genetics of Bone and Soft Tissue Tumors: Alveolar Soft Part Sarcoma. *Cancer Genet. Cytogenet.* **2002**, *136*, 1–9. [CrossRef]

148. Sornberger, K.S.; Ligon, A.H.; Pedeutour, F.; Morton, C.C.; Weremowicz, S.; Williams, A.J.; Quade, B.J.; Vanni, R. Expression of HMGIY in Three Uterine Leiomyomata with Complex Rearrangements of Chromosome 6. *Cancer Genet. Cytogenet.* **1999**, *114*, 9–16. [CrossRef]

149. Micci, F.; Gorunova, L.; Gatius, S.; Matias-Guiu, X.; Davidson, B.; Heim, S.; Panagopoulos, I. MEAF6/PHF1 Is a Recurrent Gene Fusion in Endometrial Stromal Sarcoma. *Cancer Lett.* **2014**, *347*, 75–78. [CrossRef]

150. Moore, S.; Herrick, S.; Ince, T. Uterine Leiomyomata with t (10; 17) Disrupt the Histone Acetyltransferase MORF. *Cancer Res.* **2004**, *64*, 5570–5577. [CrossRef]

151. Dewaele, B.; Przybyl, J.; Quattrone, A.; Finalet Ferreiro, J.; Vanspauwen, V.; Geerdens, E.; Gianfelici, V.; Kalender, Z.; Wozniak, A.; Moerman, P.; et al. Identification of a Novel, Recurrent *MBTD1-CXorf67* Fusion in Low-Grade Endometrial Stromal Sarcoma. *Int. J. Cancer* **2014**, *134*, 1112–1122. [CrossRef]

152. Panagopoulos, I.; Thorsen, J.; Gorunova, L.; Haugom, L.; Bjerkehagen, B.; Davidson, B.; Heim, S.; Micci, F. Fusion of the *ZC3H7B* and *BCOR* Genes in Endometrial Stromal Sarcomas Carrying an X;22-Translocation. *Genes, Chromosom. Cancer* **2013**, *52*, 610–618. [CrossRef]

153. Garcia-Torres, R.; Cruz, D.; Orozco, L.; Heidet, L.; Gubler, M.C. Alport Syndrome and Diffuse Leiomyomatosis. Clinical Aspects, Pathology, Molecular Biology and Extracellular Matrix Studies. A Synthesis. *Nephrologie* **2000**, *21*, 9–12.

154. Regauer, S.; Emberger, W.; Reich, O.; Pfragner, R. Cytogenetic Analyses of Two New Cases of Endometrial Stromal Sarcoma—Non-Random Reciprocal Translocation t(10;17)(Q22;P13) Correlates with Fibrous ESS. *Histopathology* **2008**, *52*, 780–783. [CrossRef]

155. Van Rijk, A.; Sweers, M.; Huys, E.; Kersten, M.; Merkx, G.; van Kessel, A.G.; Debiec-Rychter, M.; Schoenmakers, E.F. Characterization of a Recurrent t (1; 2)(P36; P24) in Human Uterine Leiomyoma. *Cancer Genet. Cytogenet.* **2009**, *193*, 54–62. [CrossRef]

156. Cho, Y.L.; Koo, M.S.; Bae, S.; Lee, C.-H.; Kim, K.M.; Ro, D.Y.; Kim, J.H.; Ahn, W.S.; Chun, H.-J.; Kim, C.K.; et al. Array Comparative Genomic Hybridization Analysis of Uterine Leiomyosarcoma. *Gynecol. Oncol.* **2005**, *99*, 545–551. [CrossRef]

157. Panagopoulos, I.; Gorunova, L.; Brunetti, M.; Agostini, A.; Andersen, H.K.; Lobmaier, I.; Bjerkehagen, B.; Heim, S.; Panagopoulos, I.; Gorunova, L.; et al. Genetic Heterogeneity in Leiomyomas of Deep Soft Tissue. *Oncotarget* **2017**, *8*, 48769–48781. [CrossRef]

158. Yang, J.; Du, X.; Chen, K.; Ylipää, A.; Lazar, A.J.F.; Trent, J.; Lev, D.; Pollock, R.; Hao, X.; Hunt, K.; et al. Genetic Aberrations in Soft Tissue Leiomyosarcoma. *Cancer Lett.* **2009**, *275*, 1–8. [CrossRef]

159. Buza, N.; Carr, R.J.; Hui, P.; Xu, F.; Wu, W.; Li, P. Recurrent Chromosomal Aberrations in Intravenous Leiomyomatosis of the Uterus: High-Resolution Array Comparative Genomic Hybridization Study. *Hum. Pathol.* **2014**, *45*, 1885–1892. [CrossRef]

160. Hu, J.; Khanna, V.; Jones, M.; Surti, U. Genomic Alterations in Uterine Leiomyosarcomas: Potential Markers for Clinical Diagnosis and Prognosis. *Genes, Chromosom. Cancer* **2001**, *31*, 117–124. [CrossRef]

161. Vanni, R.; Van Roy, N.; Lecca, U.; Speleman, F. Interphase Cytogenetic Analysis of Karyotypically Normal Uterine Leiomyoma Excludes Undetected Trisomy 12. *Cancer Genet. Cytogenet.* **2003**, *63*, 131. [CrossRef]

162. Gai, W.; Sun, K. Epigenetic Biomarkers in Cell-Free DNA and Applications in Liquid Biopsy. *Genes.* **2019**, *10*. [CrossRef]

163. Maekawa, R.; Sato, S.; Yamagata, Y.; Asada, H.; Tamura, I.; Lee, L.; Okada, M.; Tamura, H.; Takaki, E.; Nakai, A.; et al. Genome-Wide DNA Methylation Analysis Reveals a Potential Mechanism for the Pathogenesis and Development of Uterine Leiomyomas. *PLoS ONE* **2013**, *8*, e66632. [CrossRef]

164. Eisenberg-Lerner, A.; Kimchi, A. DAPk Silencing by DNA Methylation Conveys Resistance to Anti EGFR Drugs in Lung Cancer Cells. *Cell Cycle* **2012**, *11*, 2051. [CrossRef]

165. Häfner, N.; Diebolder, H.; Jansen, L.; Hoppe, I.; Dürst, M.; Runnebaum, I.B. Hypermethylated DAPK in Serum DNA of Women with Uterine Leiomyoma Is a Biomarker Not Restricted to Cancer. *Gynecol. Oncol.* **2011**, *121*, 224–229. [CrossRef]

166. Asada, H.; Yamagata, Y.; Taketani, T.; Matsuoka, A.; Tamura, H.; Hattori, N.; Ohgane, J.; Hattori, N.; Shiota, K.; Sugino, N. Potential Link between Estrogen Receptor-$\alpha$ Gene Hypomethylation and Uterine Fibroid Formation. *Mol. Hum. Reprod.* **2008**, *14*, 539–545. [CrossRef]

167. Hori, M.; Iwasaki, M.; Shimazaki, J.; Inagawa, S.; Itabashi, M. Assessment of Hypermethylated DNA in Two Promoter Regions of the Estrogen Receptor $\alpha$ Gene in Human Endometrial Diseases. *Gynecol. Oncol.* **2000**, *76*, 89–96. [CrossRef]

168. Sato, S.; Maekawa, R.; Yamagata, Y.; Asada, H.; Tamura, I.; Lee, L.; Okada, M.; Tamura, H.; Sugino, N. Potential Mechanisms of Aberrant DNA Hypomethylation on the x Chromosome in Uterine Leiomyomas. *J. Reprod. Dev.* **2014**, *60*, 47–54. [CrossRef]

169. Navarro, A.; Yin, P.; Monsivais, D.; Lin, S.M.; Du, P.; Wei, J.-J.; Bulun, S.E. Genome-Wide DNA Methylation Indicates Silencing of Tumor Suppressor Genes in Uterine Leiomyoma. *PLoS ONE* **2012**, *7*, e33284. [CrossRef]

170. Sato, S.; Maekawa, R.; Yamagata, Y.; Tamura, I.; Lee, L.; Okada, M.; Jozaki, K.; Asada, H.; Tamura, H.; Sugino, N. Identification of Uterine Leiomyoma-Specific Marker Genes Based on DNA Methylation and Their Clinical Application. *Sci. Rep.* **2016**, *6*, 30652. [CrossRef]

171. Kawaguchi, K.; Oda, Y.; Saito, T.; Yamamoto, H.; Tamiya, S.; Takahira, T.; Miyajima, K.; Iwamoto, Y.; Tsuneyoshi, M. Mechanisms of Inactivation of Thep16INK4a Gene in Leiomyosarcoma of Soft Tissue: Decreased P16 Expression Correlates with Promoter Methylation and Poor Prognosis. *J. Pathol.* **2003**, *201*, 487–495. [CrossRef]

172. Xing, D.; Scangas, G.; Nitta, M.; He, L.; Xu, A.; Ioffe, Y.J.M.; Aspuria, P.J.; Hedvat, C.Y.; Anderson, M.L.; Oliva, E.; et al. A Role for BRCA1 in Uterine Leiomyosarcoma. *Cancer Res.* **2009**, *69*, 8231–8235. [CrossRef]

173. Miyata, T.; Sonoda, K.; Tomikawa, J.; Tayama, C.; Okamura, K.; Maehara, K.; Kobayashi, H.; Wake, N.; Kato, K.; Hata, K.; et al. Genomic, Epigenomic, and Transcriptomic Profiling towards Identifying Omics Features and Specific Biomarkers That Distinguish Uterine Leiomyosarcoma and Leiomyoma at Molecular Levels. *Sarcoma* **2015**, *2015*, 1–14. [CrossRef]

174. Kaasinen, E.; Alkodsi, A.; Mehine, M.; Heinonen, H.-R.; Mäkinen, N.; Aavikko, M.; Kampjärvi, K.; Taipale, M.; Vahteristo, P.; Lehtonen, R.; et al. Abstract 4435: Genome-Scale DNA Methylation Changes Delineate Uterine Leiomyoma Subgroups. In *Molecular and Cellular Biology, Genetics*; American Association for Cancer Research: Philadelphia, PA, USA, 2016; Volume 76, p. 4435. [CrossRef]

175. Bujko, M.; Kowalewska, M.; Danska-Bidzinska, A.; Bakula-Zalewska, E.; Siedechi, J.A.; BIDZINSKI, M. The Promoter Methylation and Expression of the O6-Methylguanine-DNA Methyltransferase Gene in Uterine Sarcoma and Carcinosarcoma. *Oncol. Lett.* **2012**, *4*, 551. [CrossRef]

176. Seidel, C.; Bartel, F.; Rastetter, M.; Bluemke, K.; Wurl, P.; Taubert, H.; Dammann, R. Alterations of Cancer-Related Genes in Soft Tissue Sarcomas: Hypermethylation OfRASSF1A Is Frequently Detected in Leiomyosarcoma and Associated with Poor Prognosis in Sarcoma. *Int. J. Cancer* **2005**, *114*, 442–447. [CrossRef]

177. Kawaguchi, K.; Oda, Y.; Saito, T.; Yamamoto, H.; Takahira, T.; Kobayashi, C.; Tamiya, S.; Tateishi, N.; Iwamoto, Y.; Tsuneyoshi, M. DNA Hypermethylation Status of Multiple Genes in Soft Tissue Sarcomas. *Mod. Pathol.* **2006**, *19*, 106–114. [CrossRef]

178. Skubitz, K.M.; Skubitz, A.P.N. Differential Gene Expression in Uterine Leiomyoma. *J. Lab. Clin. Med.* **2003**, *141*, 297–308. [CrossRef]

179. Arslan, A.A.; Gold, L.I.; Mittal, K.; Suen, T.-C.; Belitskaya-Levy, I.; Tang, M.-S.; Toniolo, P. Gene Expression Studies Provide Clues to the Pathogenesis of Uterine Leiomyoma: New Evidence and a Systematic Review. *Hum. Reprod.* **2005**, *20*, 852–863. [CrossRef]

180. Hoffman, P.J.; Milliken, D.B.; Gregg, L.C.; Davis, R.R.; Gregg, J.P. Molecular Characterization of Uterine Fibroids and Its Implication for Underlying Mechanisms of Pathogenesis. *Fertil. Steril.* **2004**, *82*, 639–649. [CrossRef]

181. Skubitz, K.M.; Skubitz, A.P.N. Differential Gene Expression in Leiomyosarcoma. *Cancer* **2003**, *98*, 1029–1038. [CrossRef]

182. Roberts, M.E.; Aynardi, J.T.; Chu, C.S. Uterine Leiomyosarcoma: A Review of the Literature and Update on Management Options. *Gynecol. Oncol.* **2018**, *151*, 562–572. [CrossRef]

183. Davidson, B.; Abeler, V.M.; Førsund, M.; Holth, A.; Yang, Y.; Kobayashi, Y.; Chen, L.; Kristensen, G.B.; Shih, I.-M.; Wang, T.-L. Gene Expression Signatures of Primary and Metastatic Uterine Leiomyosarcoma. *Hum. Pathol.* **2014**, *45*, 691–700. [CrossRef]

184. Davidson, B.; Abeler, V.M.; Hellesylt, E.; Holth, A.; Shih, I.M.; Skeie-Jensen, T.; Chen, L.; Yang, Y.; Wang, T.-L. Gene Expression Signatures Differentiate Uterine Endometrial Stromal Sarcoma from Leiomyosarcoma. *Gynecol. Oncol.* **2013**, *128*, 349–355. [CrossRef]

185. Rapisuwon, S.; Vietsch, E.E.; Wellstein, A. Circulating Biomarkers to Monitor Cancer Progression and Treatment. *Comput. Struct. Biotechnol. J.* **2016**, *14*, 211–222. [CrossRef]

186. Porras, T.B.; Kaur, P.; Ring, A.; Schechter, N.; Lang, J.E. Challenges in Using Liquid Biopsies for Gene Expression Profiling. *Oncotarget* **2018**, *9*, 7036–7053. [CrossRef]

187. Gorges, T.M.; Kuske, A.; Röck, K.; Mauermann, O.; Müller, V.; Peine, S.; Verpoort, K.; Novosadova, V.; Kubista, M.; Riethdorf, S.; et al. Accession of Tumor Heterogeneity by Multiplex Transcriptome Profiling of Single Circulating Tumor Cells. *Clin. Chem.* **2016**, *62*, 1504–1515. [CrossRef]

188. Ring, A.; Mineyev, N.; Zhu, W.; Park, E.; Lomas, C.; Punj, V.; Yu, M.; Barrak, D.; Forte, V.; Porras, T.; et al. EpCAM Based Capture Detects and Recovers Circulating Tumor Cells from All Subtypes of Breast Cancer except Claudin-Low. *Oncotarget* **2015**, *6*, 44623–44634. [CrossRef]

189. Prendergast, E.N.; De Souza Fonseca, M.A.; Dezem, F.S.; Lester, J.; Karlan, B.Y.; Noushmehr, H.; Lin, X.; Lawrenson, K. Optimizing Exosomal RNA Isolation for RNA-Seq Analyses of Archival Sera Specimens. *PLoS ONE* **2018**, *13*. [CrossRef]

190. Guo, H.; Zhang, X.; Dong, R.; Liu, X.; Li, Y.; Lu, S.; Xu, L.; Wang, Y.; Wang, X.; Hou, D.; et al. Integrated Analysis of Long Noncoding RNAs and MRNAs Reveals Their Potential Roles in the Pathogenesis of Uterine Leiomyomas. *Oncotarget* **2014**, *5*, 8625–8636. [CrossRef]

191. Cao, T.; Jiang, Y.; Wang, Z.; Zhang, N.; Al-Hendy, A.; Mamillapalli, R.; Kallen, A.N.; Kodaman, P.; Taylor, H.S.; Li, D.; et al. H19 LncRNA Identified as a Master Regulator of Genes That Drive Uterine Leiomyomas. *Oncogene* **2019**, *38*, 5356–5366. [CrossRef]

192. Heinonen, H.-R.; Mehine, M.; Mäkinen, N.; Pasanen, A.; Pitkänen, E.; Karhu, A.; Sarvilinna, N.S.; Sjöberg, J.; Heikinheimo, O.; Bützow, R.; et al. Global Metabolomic Profiling of Uterine Leiomyomas. *Br. J. Cancer* **2017**, *117*, 1855–1864. [CrossRef]

193. Zaitseva, M.; Vollenhoven, B.J.; Rogers, P.A.W. Retinoic Acid Pathway Genes Show Significantly Altered Expression in Uterine Fibroids When Compared with Normal Myometrium. *MHR Basic Sci. Reprod. Med.* **2007**, *13*, 577–585. [CrossRef]

194. Malik, M.; Webb, J.; Catherino, W.H. Retinoic Acid Treatment of Human Leiomyoma Cells Transformed the Cell Phenotype to One Strongly Resembling Myometrial Cells. *Clin. Endocrinol. (Oxf.)* **2008**, *69*, 462–470. [CrossRef]

195. Tak, Y.J.; Lee, S.Y.; Park, S.K.; Kim, Y.J.; Lee, J.G.; Jeong, D.W.; Kim, S.C.; Kim, I.J.; Yi, Y.H. Association between Uterine Leiomyoma and Metabolic Syndrome in Parous Premenopausal Women. *Medicine (Baltim.)* **2016**, *95*, e5325. [CrossRef]

196. Lou, S.; Balluff, B.; de Graaff, M.A.; Cleven, A.H.G.; Briaire-de Bruijn, I.; Bovée, J.V.M.G.; McDonnell, L.A. High-Grade Sarcoma Diagnosis and Prognosis: Biomarker Discovery by Mass Spectrometry Imaging. *Proteomics* **2016**, *16*, 1802–1813. [CrossRef]

197. Lou, S.; Balluff, B.; Cleven, A.H.G.; Bovée, J.V.M.G.; McDonnell, L.A. Prognostic Metabolite Biomarkers for Soft Tissue Sarcomas Discovered by Mass Spectrometry Imaging. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 376–383. [CrossRef]

198. Osborne, C.M.; Hardisty, E.; Devers, P.; Kaiser-Rogers, K.; Hayden, M.A.; Goodnight, W.; Vora, N.L. Discordant Noninvasive Prenatal Testing Results in a Patient Subsequently Diagnosed with Metastatic Disease. *Prenat. Diagn.* **2013**, *33*, 609–611. [CrossRef]

199. Eastley, N.C.; Ottolini, B.; Neumann, R.; Luo, J.-L.; Hastings, R.K.; Khan, I.; Moore, D.A.; Esler, C.P.; Shaw, J.A.; Royle, N.J.; et al. Circulating Tumour-Derived DNA in Metastatic Soft Tissue Sarcoma. *Oncotarget* **2018**, *9*, 10549–10560. [CrossRef]

200. Przybyl, J.; Chabon, J.J.; Spans, L.; Ganjoo, K.N.; Vennam, S.; Newman, A.M.; Forgó, E.; Varma, S.; Zhu, S.; Debiec-Rychter, M.; et al. Combination Approach for Detecting Different Types of Alterations in Circulating Tumor DNA in Leiomyosarcoma. *Clin. Cancer Res.* **2018**, *24*, 2688–2699. [CrossRef]