Drug Discovery Today • Volume xxx, Number xx • xxxx 2023 REVIEWS

ELSEVIER

Platelets and their derived extracellular vesicles: The new generation of markers in non-small cell lung cancer management

Roberta Tasso [1,2], Silvia Marconi [3,*], Giovanni Rossi [4], Carlo Genova [5,6,\dag], Simona Coco [3,\dag]

[1] Dipartimento di Medicina Sperimentale (DIMES); Università degli Studi di Genova, Genova, Italy
[2] UO Oncologia Cellulare, IRCCS Ospedale Policlinico San Martino, Genova, Italy
[3] Lung Cancer Unit, IRCCS Ospedale
Policlinico San Martino, Genova, Italy
[4] UOC Oncologia Medica 2, IRCCS Ospedale Policlinico San Martino, 16132 Genova, Italy
[5] UOC Clinica di Oncologia Medica, IRCCS Ospedale Policlinico San Martino, Genova, Italy
[6] Dipartimento di Medicina Interna e Specialità Mediche (DiMI); Università degli Studi di Genova, Genova, Italy

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death. Circulating elements have gained significant interest in the diagnosis and prognosis of NSCLC patients. Among these, platelets (PLTs) and their derived extracellular vesicles (P-EVs) are emerging eligible biosources as both number and genetic material transfers (RNA, proteins, and lipids). PLTs are mainly produced by the shedding of megakaryocytes and together with P-EVs, participate in a variety of pathological processes including thrombosis, tumor progression, and metastasis. Here, we performed an extensive literature review focusing on PLTs and P-EVs as potential diagnostic, prognostic, and predictive markers for NSCLC patient management.

Keywords: non-small cell lung cancer; platelets; extracellular vesicles; tumor-educated platelets; prognosis; predictive marker

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death worldwide, although patient outcomes are improving due to targeted therapies and more recently immunotherapy.[1,2] Tissue biopsy is critical for diagnosis and subsequent treatment; however, in some cases, tumor tissue is inadequate for molecular analyses. Liquid biopsy from peripheral blood is an alternative source for detecting cancer biomarkers in the absence of adequate tumor tissue.[3] To date, numerous blood derivatives have been identified, and some of them, such as circulating tumor DNA (ctDNA), are already used in clinical practice, while others are currently under investigation and include circulating tumor cells (CTCs), extracellular vesicles (EVs), and more recently, tumor-educated platelets (TEPs).[3–5] Platelets (PLTs) are anucleate fragment cells derived from megakaryocytes and play a central role in hemostasis and thrombosis, as well as tumor growth. PLTs can also indirectly participate in cancer development by releasing EVs, which can in turn modulate recipient cells through the transfer of genetic material. Due to their easy and quick purification (i.e., differential centrifugation), together with their high concentration in the bloodstream, PLTs represent the next generation of blood-derived markers.

Here, we performed an extensive literature review focusing on PLTs and their derived EVs as diagnostic, prognostic, and predictive markers based on their number and features, as well as their

* *Corresponding author. Marconi, S. (silvia.marconi@hsanmartino.it)*
\dag These authors equally share the last co-authorship.

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

correlations with lung cancer progression and response to treatments.

Characteristics and roles of PLTs

PLT origin

The existence of PLTs in blood and their role in hemostasis and blood coagulation have been known for more than a century.[6] PLTs are disc-shaped anucleate fragments produced in the bone marrow and lung by their megakaryocyte precursors. PLTs generally have a half-life of approximately 5–7 days, after which they are degraded in the spleen.[7] Although the number of PLTs varies across individuals, the normal count is between 150,000 and 400,000/μl of blood and they are essential for maintaining the hemostasis and integrity of blood vessels.[8]

The role of PLTs in tumor progression

In addition to their crucial role in blood clotting, accumulating evidence demonstrates that PLTs are involved in diverse pathophysiological processes such as inflammation and many stages of carcinogenesis.[9] Trousseau was one of the first scientists to define the relationship between PLTs and cancer, in particular in the context of thrombosis, in the 19th century.[10] Indeed, thrombosis is a well-recognized complication in patients with cancer, especially NSCLC.[11] In addition, a growing number of studies have also shown that PLTs can participate in all stages of oncogenesis, acting both as 'defenders' protecting the tumor cells and as active 'players' promoting cancer progression (Figure 1). Indeed, when the CTCs leave the primary tumor site to penetrate blood vessels, they must resist the forces of blood flow and attack by immune cells. At this stage, CTCs directly interact with PLTs by means of different receptors (mainly the PLT activation receptor P-selectin, cluster of differentiation 62P [CD62P]), creating clusters that improve their adhesion to blood vessel walls and survival.[12] The ability of CTCs to form these clusters with PLTs, named tumor cell-induced PLT aggregation (TCIPA), is a mechanism triggered by tumor markers (e.g., tissue factor) that activate the coagulation cascade, generating thrombin that in turn activates PLTs.[12,13] Indeed, these fibrin–PLT aggregates around tumor cells confer many advantages to CTCs, preserving their cellular integrity through physical protection and creating a defensive shield that allows them to make themselves 'invisible' to the immune system.[9,14] In particular, PLTs can impair the antitumor activity of natural killer (NK) cells by transferring major histocompatibility complex class I to CTCs[15] and releasing factors such as transforming growth factor beta (TGF-β). Indeed, PLT-derived TGF-β, secreted during TCIPA formation, downregulates the release of immunoreceptor natural killer group 2, member D and interferon gamma, impairing NK lytic activity.[15] Besides their key role in CTC defense, TCIPA represents one of the early stages of metastatic spread and is involved in various

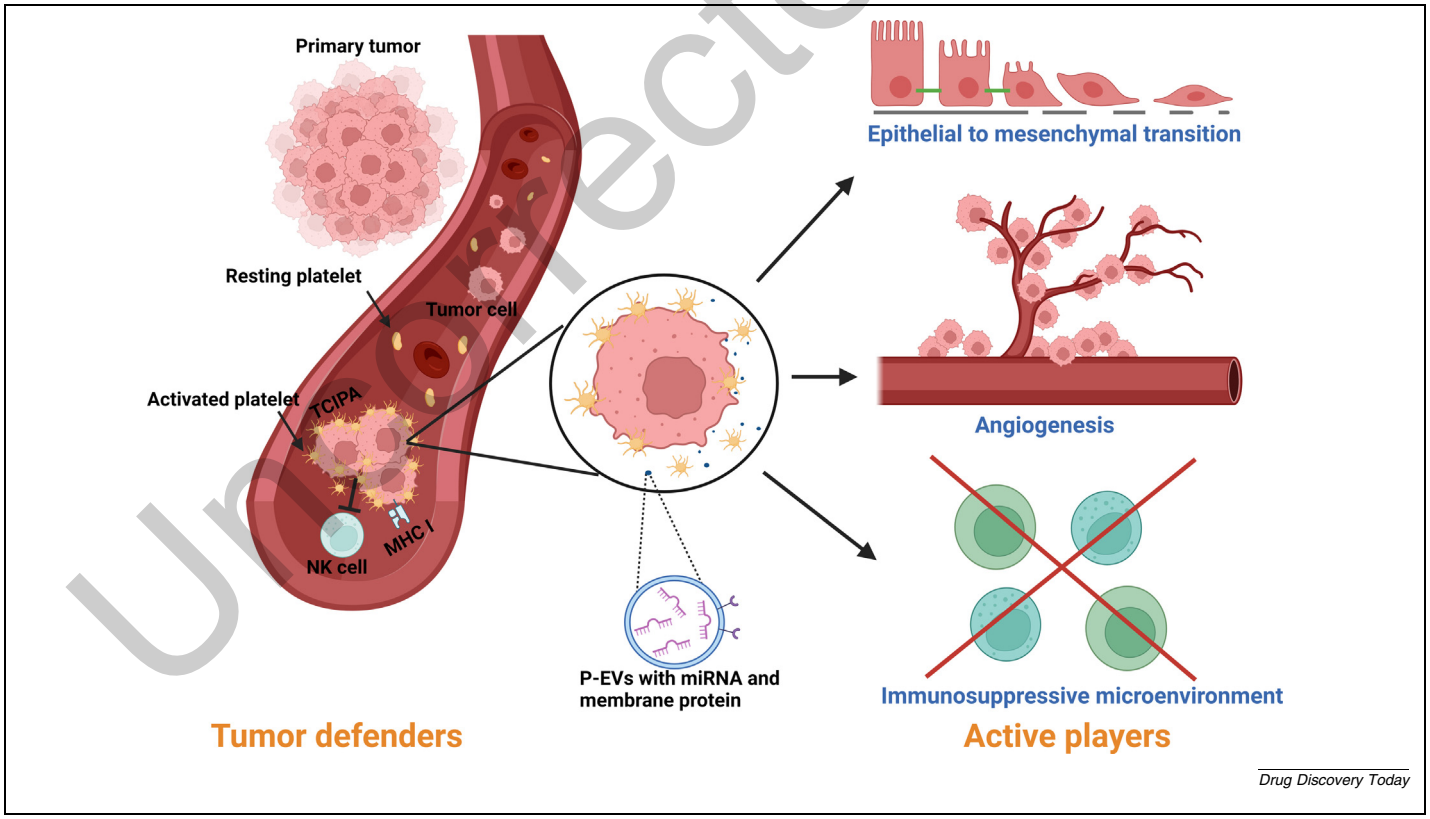

FIGURE 1

Schematic representation of the role of platelets (PLTs) and their derived extracellular vesicles (P-EVs) in cancer progression. In the bloodstream, circulating tumor cells (CTCs) can interact with PLTs producing tumor cell-induced platelet aggregation (TCIPA). These clusters protect tumor cells from the deleterious effects of shear forces and suppress natural killer (NK) cell immunological attacks through the transfer of major histocompatibility complex class I to the surface of CTCs. PLTs are also involved in three different steps of tumor progression: (i) epithelial-mesenchymal transition; (ii) blood vessel formation; (iii) immunosuppression of the tumor microenvironment.

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

tumor types including lung malignancies.[16] In this regard, bidirectional interactions between PLTs and cancer cells, involving the transfer of lipids, proteins, and RNA, have been widely described.[17] Indeed, tumor cells can educate PLTs by generating TEPs as result of the complex network between tumor cells and PLTs themselves, leading to the transformation of naive PLTs into protumorigenic-activated forms. In particular, the same cancer cells can give rise to TEPs both by direct contact[18] or by indirect mechanisms involving the release of EVs and signaling molecules. Indeed, PLTs are able to take up membrane vesicles containing tumor-associated biomolecules, mainly RNAs[19] and proteins,[20] from tumor cells and transfer them to other cell types,[21] promoting tumor growth and metastatic spread.[22] Furthermore, in response to external signals (e.g., activation of surface PLT receptors and lipopolysaccharide-mediated PLT activation) released by tumor cells, TEPs can also modulate the splicing of oncogenic pre-mRNAs.[23,24] In the same way, PLTs can in turn educate tumor cells, giving rise to PLT-educated tumors (PETs), resulting in promotion of a metastatic phenotype leading to the epithelial-mesenchymal transition by upregulating vimentin, SNAIL1, and SNAIL2.[17] In addition, TGF-β secreted by PLTs activate the TGF-β/Smad signaling pathway in cancer cells, resulting in transition to a more invasive phenotype.[25] Some evidence also supports the role of PLTs in the context of cancer neovascularization, carrying pro-angiogenic factors (e.g., vascular-endothelial growth factor, VEFG; PLT-derived growth factor, PDGF). In this regard, a recent study demonstrated, both *in vitro* and *in vivo*, that NSCLC lines interacting with PLTs can promote angiogenesis through the vascular endothelial growth factor (VEGF)/VEGF receptor 2 signaling pathway.[26] Finally, PLTs, along with their direct effects on tumor cells, can also modulate immune system components, contributing to the generation of an immunosuppressive microenvironment.[27] For instance, PLTs themselves can express programmed cell death ligand 1 (PD-L1), one of the major negative regulators of the adaptive immune antitumor response, and stimulate its expression (both mRNA and membrane protein) on tumor cells, promoting immunoevasion.[28] Likewise, tumor cells can transfer PD-L1 to the PLT surface by fibronectin, α5β1, and glycoprotein Ib alpha (GPIbα), and the resulting PD-L1 + TEPs reportedly inhibit CD4 + and CD8 + T cells.[18].

PLT enumeration and clinical application in NSCLC management

The assessment of blood components and their relative frequencies have widely been evaluated in multiple studies designed to identify prognostic and predictive factors in NSCLC, with particular regard to immune checkpoint inhibitors (ICIs) (Table 1).

PLT-to-lymphocyte ratio and efficacy of ICIs

The first published paper on this topic evaluated the correlation between the PLT-to-lymphocyte ratio (PLR) and the objective response (OR) in a population of NSCLC patients receiving nivolumab or docetaxel in second or further lines. Notably, high PLR was associated with fewer responses in both nivolumab- and docetaxel-treated patients. However, due to the small number of patients (nivolumab: $N = 28$; docetaxel: $N = 34$) evaluated in this retrospective study, it was not possible to demonstrate a statistically significant correlation with progression-free survival (PFS) and overall survival (OS).[29] The same authors subsequently enlarged the cohort including 187 patients, who received nivolumab in second-line settings. In this study, baseline PLR < 200 was significantly associated with a higher OR rate (ORR; 40.1% vs. 24.1%) and disease control rate (DCR), as well as longer PFS (7.0 vs. 4.0 months) and OS (15.0 vs. 11.0 months) compared to baseline PLR ≥ 200.[30] In another publication, Pavan and colleagues consistently reported decreased PFS (2.9 vs. 7.3 months) and OS (14.7 vs. 36.4 months) among NSCLC patients with high PLR receiving ICIs, compared to patients with low PLR.[31] In this context, two large meta-analyses, comprising 21 studies each and involving more than 4000 advanced patients, investigated the correlations between the efficacy of ICIs and PLR, confirming the detrimental effect of high PLR among ICI-treated patients.[32,33]

The PLR in NSCLC patients treated with immunotherapy is also correlated with the occurrence of immune-related adverse events (irAEs). In particular, in the aforementioned study by Pavan *et al.*, a low PLR value was also associated with an increase in irAEs with an OR of 2.8.[31] Furthermore, the PLR was also confirmed to be an independent predictor of the onset of irAEs in the multivariate analysis (OR = 2.3).

PLT enumeration and prognosis in early-stage NSCLC

Although the study of PLTs was successful in conjunction with the ICIs, other studies have evaluated the usefulness of PLTs in completely different settings for NSCLC, such as early-stage NSCLC. A retrospective study evaluated the neutrophil-to-lymphocyte ratio (NLR) and PLR in 1,637 patients with completely resected NSCLC. The median PLR observed at each time point (preoperative and postoperative days 1, 2, and 3) was used to divide patients with high and low inflammatory status. Notably, the preoperative PLR was significantly lower in stage I NSCLC compared to stage II–III disease. In multivariate analysis, an increased preoperative PLR was associated with a higher risk of both recurrence (hazard ratio, HR = 1.22) and death (HR = 1.33).[34] Similar results were also reported by Sulibhavi *et al.*, who observed that, in a population of 103 patients completely resected for stage I NSCLC, preoperative increased PLT count (above the median) was correlated with up to a 7.5-fold higher risk of recurrence.[35] Łochowski *et al.* observed, in a population of 532 patients who were radically treated with surgery for stage IA–IIIA NSCLC, that high PLR (>144) was an independent negative prognostic factor for survival at 2 years.[36].

Molecular biomarkers from PLTs

PLT-derived RNA signatures in lung cancer

Although PLTs lack a nucleus, they contain a rich repertoire of megakaryocyte-derived pre-mRNA transcripts that, upon stimulation, can be spliced into mature mRNA and translated into proteins.[37] Through large-scale sequencing studies, various types of PLT-derived RNAs have been characterized, including both coding and noncoding RNA (ncRNA) along with functional spliceosomes to transform pre-mRNAs.[37] To date, a number of studies have shown that the PLT RNA-based signature can mirror the tumor biology, and their expression may correlate with tumor

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

TABLE 1

List of the most relevant studies showing the platelet-to-lymphocyte ratio (PLT) and PLT number role in early-stage and advanced patients treated with immune checkpoint inhibitors (ICIs).

Refs	*N* pts	Type	Cut-off	Setting	Treatment	PFS	ORR	OS	Conclusions
Russo, 2018[29]	62	RS	PLR ≥ 160	Advanced NSCLC (2nd line)	*Nivolumab; docetaxel	2.0 vs 5.0 ms HR = 1.47 *P* = 0.017 *6.0 vs. 6.0 ms *P* = 0.437	4% vs 14% *P* = 0.04 *8% vs 47% *P* < 0.0004	4.0 vs. 12.0 ms HR = 1.67 P = 0.003 *6.0 vs. 10.0 ms *P* = 0.756	Poor outcome (i.e., in refractory patients) with high PLR in both groups
Russo, 2020[30]	187	RS	PLR ≥ 200	Stage IIIB-IV NSCLC (2nd line)	Nivolumab	7.0 vs. 4.0 ms HR = 0.67 P = 0.027	40% vs 24% *P* = 0.04	15.0 vs. 11.0 ms HR = 0.66 *P* = 0.05	Better outcome with high pretreatment PLR
Pavan, 2019[31]	184	RS	PLR ≥ 180	Advanced NSCLC (1st, 2nd line)	Nivolumab; pembrolizumab; atezolizumab	2.9 vs 7.3 ms HR = 1.71 *P* = 0.005	23% vs 28% *P* = 0.350	14.7 vs. 36.4 ms HR = 2.24 *P* < 0.001	Worse outcomes with high PLR
Zhou, 2022[32]	2312	MA (21[a])	Varies across studies	Advanced lung cancer	Any type of ICI	HR = 1.66 *P* < 0.01	OR = 0.61 *P* = 0.29	HR = 2.24 *P* = 0.01	Worse outcomes with high PLR in NSCLC PLR cut-off = 200 as reasonable
Zhang, 2020[33]	1845	MA (21[a])	PLR ≥ 169	Advanced NSCLC	Nivolumab; pembrolizumab; atezolizumab	HR = 1.57 *P* < 0.001	–	HR = 1.93 *P* < 0.001	Worse outcomes with high PLR No correlation between posttreatment PLR and OS
Lee, 2016[34]	1637	RS	PLR ≥ 180	Resected stage I/II/III NSCLC	Surgery	HR = 1.22 (RFF) *P* = 0.019	–	HR = 1.33 *P* = 0.004	Decreased RFS and OS With high preoperative PLR
Sulibhavi, 2020[35]	103	RS	PLT ≥ 253 × 10^3	Resected stage I NSCLC	Surgery	5-year RFF 72.0% vs. 91.8% *P* = 0.02	–	–	7.5-fold higher risk of recurrence with high preoperative PLTs
Łochowski, 2021[36]	532	RS	PLR ≥ 144.02	Resected stage IA–IIIA NSCLC	Surgery	–	–	HR = 1.00 (2-year OS) *P* = 0.001	Decreased OS at 2 years with high preoperative PLR (multivariate analysis)

Abbreviations: HR, hazard ratio; ICI, immune checkpoint inhibitor; ms, months; ORR, objective response rate; MA, meta-analysis: ([a]number of studies); NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; PLR, platelet-to-lymphocyte ratio; pts, patients; RFF, recurrence-free survival; RS, retrospective study.

growth and progression.[23,38] Also in the setting of lung malignancies, several PLT gene signatures have been identified as biomarkers for diagnosis and prognosis, suggesting that these circulating elements could be used as a source of eligible biomaterial for screening programs[38–42] (Table 2). In this field, Best and colleagues devised a diagnostic classification algorithm using the particle-swarm optimization, a computational method inspired by a swarm of birds. Using this approach, the authors tested over 750 individuals divided into 402 NSCLC patients and 377 non-oncological controls and based on differently spliced PLT-derived RNAs, obtained an accuracy of more than 80% for the detection of cancer patients.[43] In another study, the same authors identified TEP mRNA-based profiles able to predict epidermal growth factor receptor (*EGFR*), *KRAS*-, and *MET*-positive NSCLC with 87%, 90%, and 91% accuracy, respectively.[38].

PLTs have also been described as a source of tumor-derived mutant RNAs, particularly for anaplastic lymphoma kinase (*ALK*) rearrangements.[44] In a cohort of 26 patients treated with crizotinib, the presence of *ALK*-positive PLTs predicted a longer duration in treatment (7.2 vs. 1.5 months), as well as a higher response (70.6% vs. 11.1%) and DCR (88.2% vs. 44.4%) compared to *ALK*-negative PLTs.[45] By contrast, very few data have been reported on mutant PLT-DNA with *EGFR* alterations, detecting only the *EGFRvIII* isoform from RNA-derived TEPs.[46].

Besides TEP-gene signatures, several ncRNA species including long ncRNAs, microRNAs (miRNAs), and small nuclear RNAs as alternative biomaterial sources for biomarkers of NSCLC diagnosis, have been described.[46–48] On this subject, Dong *et al.*, showed that the small nucleolar RNA, C/D box 55 (SNORD55) and the spliceosome proteins U1, U2, and U5 play significant roles in the early diagnosis of NSCLC.[41,48] The authors established that both markers were significantly lower in TEPs from NSCLC, especially in early-stage patients compared with healthy controls achieving excellent accuracies [area under the curve (AUC) = 0.85 for SNORD55 and AUC = 0.70 for U1/U2/U5]. Notably,

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

TABLE 2

List of the most relevant RNA-based signatures as disease biomarkers and predictor of response to the therapy in NSCLC.

Refs	TEP-molecules	*N* pts/healthy controls (HCs)	Techniques	Conclusions
Best, 2015[38]	mRNA	228 cancers (60 NSCLC)/55 HCs	RNA-seq	TEP-mRNA profile discriminates:NSCLC vs. HCs (0.96 Acc for NSCLC)primary vs. metastasis (0.84 Acc for NSCLC)in case of metastases identifies the origin organ (0.64 Acc for NSCLC)
Xue, 2018[39]	mRNA	159 NSCLC/104 HCs	[a]RNA-seq datasets	20 TEP-mRNAs discriminate NSCLC vs. HCs
Sheng, 2018[40]	mRNA	402 NSCLC/231 HCs	[a]RNA-seq dataset	48 TEP-genes discriminate NSCLC vs. HCs (0.89 Acc; 0.92 Sen; 0.83 Spe)
Dong, 2020[41]	snRNA	382 lung cancers/361 HCs	qPCR	TEP-U1/U2/U5 discriminate:NSCLC vs. HCs (0.84 AUC; 0.86 Sen; 0.70 Spe)early-stage vs. HCs (0.83 AUC; 0.94 Sen; 0.61 Spe)early- vs. advanced-stage (0.70 AUC; 0.60 Sen, 0.74 Spe)
Goswami, 2020[42]	mRNA	10 NSCLC/7 HCs	[a]RNA-seq, microarray (N = 434, 57) datasets, qPCR	11 TEP-genes discriminate NSCLC vs. HCs (0.97 AUC) Artificial augmentation of gene expression data improves predictive model performance (0.99 AUC)
Best, 2017[43]	mRNA	402 NSCLC; 377 no-cancer	[a]RNA-seq (N = 115) dataset, RNA-seq	PSO-enhanced algorithm discriminates early- (0.89 AUC) and late-stage (0.94 AUC) vs HCs and pts with different non-cancerous inflammatory conditions
Nilsson, 2015[44]	mRNA	77 NSCLC	qPCR	TEP-mRNA showed a higher Acc (0.86 Acc; 0.65 Sen; 1 Spe) than plasma RNA (0.66 Acc; 0.21 Sen; 1 Spe) in detecting EML4-ALK rearrangements
Park, 2019[45]	mRNA	61 NSCLC	qPCR	TEP-RNA showed the highest Acc (0.80) than plasma RNA (0.79) and tumor tissue (0.75) in detecting *EML4-ALK* rearrangements
Luo, 2018[46]	mRNA, lncRNA	101 NSCLC/60 HCs	Microarray (N = 347) datasets, qPCR	'TEP-MAGI2-AS3 + TEP-ZFAS1' discriminates NSCLC vs HC (0.91 AUC in ADC; 0.92 AUC in SCC) better than plasma-free RNA (0.89 AUC in ADC; 0.90 AUC in SCC) *EGFR* mutations are undetected in TEP-DNA, whereas *EGFRvIII* is detected in TEP-RNA
Li, 2021[47]	lncRNA	329 NSCLC/300 HCs	Microarray, qPCR	'TEP-linc-GTF2H2-1 + TEP-RP3-466P17.2 + TPE-lnc-ST8SIA4-12' discriminate: NSCLC vs. HCs (0.92 AUC; 0.83 Sen; 0.87 Spe) ;early-stage vs. HCs (0.89 AUC; 0.94 Sen; 0.70 Spe) 'TEP-linc-GTF2H2-1 + CEA + Cyfra21-1 + NSE' discriminate early- vs. advanced-stage (0.90 AUC; 0.77 Sen; 0.85 Spe)
Dong, 2021[48]	snRNA	290 NSCLC/189 HCs	[a]RNA-seq (N = 2106) dataset, qPCR	TEP-SNORD55 discriminates:NSCLC vs. HCs (0.80 AUC; 0.79 Sen; 0.68 Spe)Early-stage vs. HCs (0.78 AUC; 0.91 Sen; 0.50 Spe) 'TEP-SNORD55 + CEA' improve the early diagnosis (0.83 AUC; 0.66 Sen; 0.90 Spe)
D'Ambrosi, 2021[49]	circRNA	29 NSCLC/30 HCs	RNA-seq, qPCR	TEP-circNRIP1 downregulated in NSCLC vs. HC
Liang, 2015[50]	miRNA	20 NSCLC/20 HCs	qPCR	TEP-miR-223 upregulated in NSCLC vs. HCs

Abbreviations: Acc, accuracy; AUC, area under the curve; CEA, carcinoembryonic antigen; Cyfra21-1, fragment of cytokeratin 19; circRNA, circulating RNA; HCs, healthy controls; miRNA, microRNA; mRNA, messenger RNA; NSCLC, non-small cell lung cancer; NSE, neuron-specific enolase; PSO, particle-swarm optimization; pts, patients; qPCR, quantitative polymerase chain reaction; RNA-seq, RNA sequencing; Sen, sensitivity; snRNA, Small nuclear RNA; Spe, specificity; TEP, tumor-educated platelet.

[a] Genomic data downloaded from public repository.

the combination of TEP U1/U2/U5 and the tumor marker carcinoembryonic antigen enhanced the diagnostic efficiency of tumor progression (AUC = 0.81).[41] Similarly, D'Ambrosi and colleagues showed that circular RNA nuclear receptor-interacting protein 1 was significantly downregulated in NSCLC PLTs compared with cancer-free controls.[49] In another study, the miR-223 in PLTs and their P-EVs were significantly overexpressed in lung cancer patients compared with controls. Moreover, miR-223 secreted by PLTs through EVs is able to promote tumor invasion.[50].

PLT–protein and lipid signature

As aforementioned, PLTs exhibit the ability to translate mature spliced RNA into proteins. However, Londin and colleagues in 2014, through PLT transcriptome and proteome profiling, showed a very low correlation between RNA and the corresponding proteins (Spearman correlation ~ 0.3),[51] leading to the hypothesis that not all PLT proteins are translated by *in situ* mRNAs. Exploiting *in vitro* co-culture experiments, it has been recently reported that PLTs can ingest, and subsequently express on their surface tumor-derived proteins such as PD-L1.[18] In this regard, several studies have shown that proteins and lipids

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

TABLE 3

List of the most relevant studies on the role of PLT-protein/lipid signatures as disease biomarkers in NSCLC.

Refs	*N* pts/healthy controls (HCs)	Techniques	Conclusions
Ercan, 2021[52]	41 cancers (19 lung cancers)/41 HC	2D-DIGE; MS; WB; ELISA	PLT endoplasmic reticulum proteins (P4HB; CALR; HSPA5) and F13A1 increase in lung cancer vs. HCs
Fu, 2015[53]	65 NSCLC/50 HC	ELISA	PLT-VEGF and serum TGF-β increase in NSCLC (at baseline) vs. HC PLT-VEGF and serum TGF-β after chemotherapy: decrease in PR and CR pts increase in SD and PD pts
De Castro, 2009[54]	50 advanced NSCLC/50 HC	ELISA; GC–MS; SDS-PAGE; WB	PLT-β-TG increases in NSCLC vs. HC Changes in the composition of fatty acid in PLTs (decrease of arachidonic; increase of palmitic acid) in NSCLC vs HCs
De Castro, 2009[55]	50 advanced NSCLC; 30 BILD/50 HC	ELISA; GC–MS	PLT-linoleic acid decreases in NSCLC vs. BILD

Abbreviations: BILD, benign inflammatory lung diseases; CALR, calreticulin; circNRIP1, circular RNA nuclear receptor-interacting protein 1; CR, complete response; 2D-DIGE, two-dimensional gele electrophoresis; ELISA, enzyme-linked immunosorbent assay; F13A1, coagulation factor XIII fragment; GC–MS, gas chromatography–mass spectrometry; HSPA5, endoplasmic reticulum chaperone BiP; MS, mass Spectrometry; P4HB, protein disulfide-isomerase; PD, progressive disease; PR, partial response; pts, patients; SD, stable disease; VEGF, vascular endothelial growth factor; WB, western blot; β-TG, β-thromboglobulin.

derived from TEPs are promising prognostic and predictive biomarkers in NSCLC, as well as screening markers (Table 3). Among the most noteworthy studies, Hinterleitner and colleagues evaluated the ability of PD-L1-expressing PLTs (PD-L1-PLTs) to predict PFS in one cohort of patients treated with immunotherapy and another cohort treated with chemotherapy. In chemotherapy-treated patients, low PD-L1-PLT level predicted long PFS, while in anti-PD-1-treated patients, low PD-L1-PLT expression was associated with worse PFS with up to fourfold higher risk of progression. Moreover, when compared with tissue PD-L1 expression, PD-L1-PLT was better in predicting response.[18] Alteration of the PLT proteome has been linked to cancer and response to therapy. In this area, Ercan and colleagues profiled the PLT proteome of two cancer types with high risk of thrombosis (i.e., brain and lung cancers) compared to healthy controls. Notably, multiple endoplasmic reticulum proteins were significantly elevated in lung cancer, but not in brain cancer, compared to healthy individuals.[52] In another study, Fu *et al.* analyzed the changes of PLT-VEGF together with the serum TGF-β1 levels in a cohort of 65 NSCLC patients before and after chemotherapy. Interestingly, the authors demonstrated that both markers were significantly higher in patients at baseline compared to healthy controls, and their concentrations were markedly increased after chemotherapy in the stable/progressive disease group.[53].

Emerging evidence also suggests that advanced NSCLC is associated with modifications in phospholipid fatty acids from red blood cells and PLTs.[54] In this regard, De Castro *et al.* investigated the diagnostic role of fatty acid from erythrocytes and PLTs in 50 advanced NSCLC patients, 15 patients with chronic obstructive pulmonary disease (COPD), and 50 healthy individuals. Interestingly, this study revealed that PLT linoleic acid had higher diagnostic value in distinguishing cancer patients versus benign inflammatory diseases/healthy controls.[55].

P-EVs

P-EVs comprise a heterogeneous population of small and medium-sized membrane-enclosed vesicles, namely exosomes and microvesicles, containing abundant molecular cargo.[56] The first evidence of their existence dates back to 1967,[57] when Peter Wolf described the nature and significance of PLT products in human plasma, described as PLT dust, a subcellular material of thrombocytic origin circulating in the plasma and serum of healthy subjects. Notably, P-EVs are involved in intercellular communication and signaling and are considered valuable biomarkers of disease.[58] In particular, P-EVs share some functional features with their PLT parents and are crucial in coagulation and clot formation,[59,60] albeit with a significantly higher clotting capacity than PLTs themselves.[61] Moreover, anomalies in P-EV concentration and function have been described among patients with bleeding disorders.[62] Most P-EVs are released by resting/activated PLTs or megakaryocytes, and the precise phenotyping to distinguish P-EVs from megakaryocyte-EVs (M−EVs) involves the use of cell-surface antigens. CD41/CD61 are considered constitutive markers for both EV types, whereas CD62P and CD107a are more specifically expressed by activated PLTs. Moreover, P-EVs express GPIIb/IIIa, GPIb, GPIIa, and lysosome-associated glycoprotein-1[61] (Figure 2).

P-EVs as biomarkers in NSCLC

The exponential growth in the field of EV research during the last 10 years has led to reconsideration of the impact of P-EVs on various pathological conditions, including NSCLC (Table 4). In particular, while PLTs cannot overcome tissue barriers, P-EVs can cross them, extending their capabilities beyond the blood and connecting with cells of the tumor microenvironment (TME). Notably, P-EVs interact with the vascular network associated with the tumor, playing an important role in inducing changes in local endothelial cells.[63] In 2005, Janowska-Wieczorek and colleagues demonstrated that P-EVs induced the mRNA expression of angiogenic factors such as matrix metalloproteinase 9, VEGF, interleukin 8, and hepatocyte growth factor, as well as adhesion to fibrinogen and human umbilical vein endothelial cells. The authors also observed that P-EVs were able to transfer integrin CD41 to different types of lung cancer cell lines, stimulating their proliferation and increasing their inva-

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

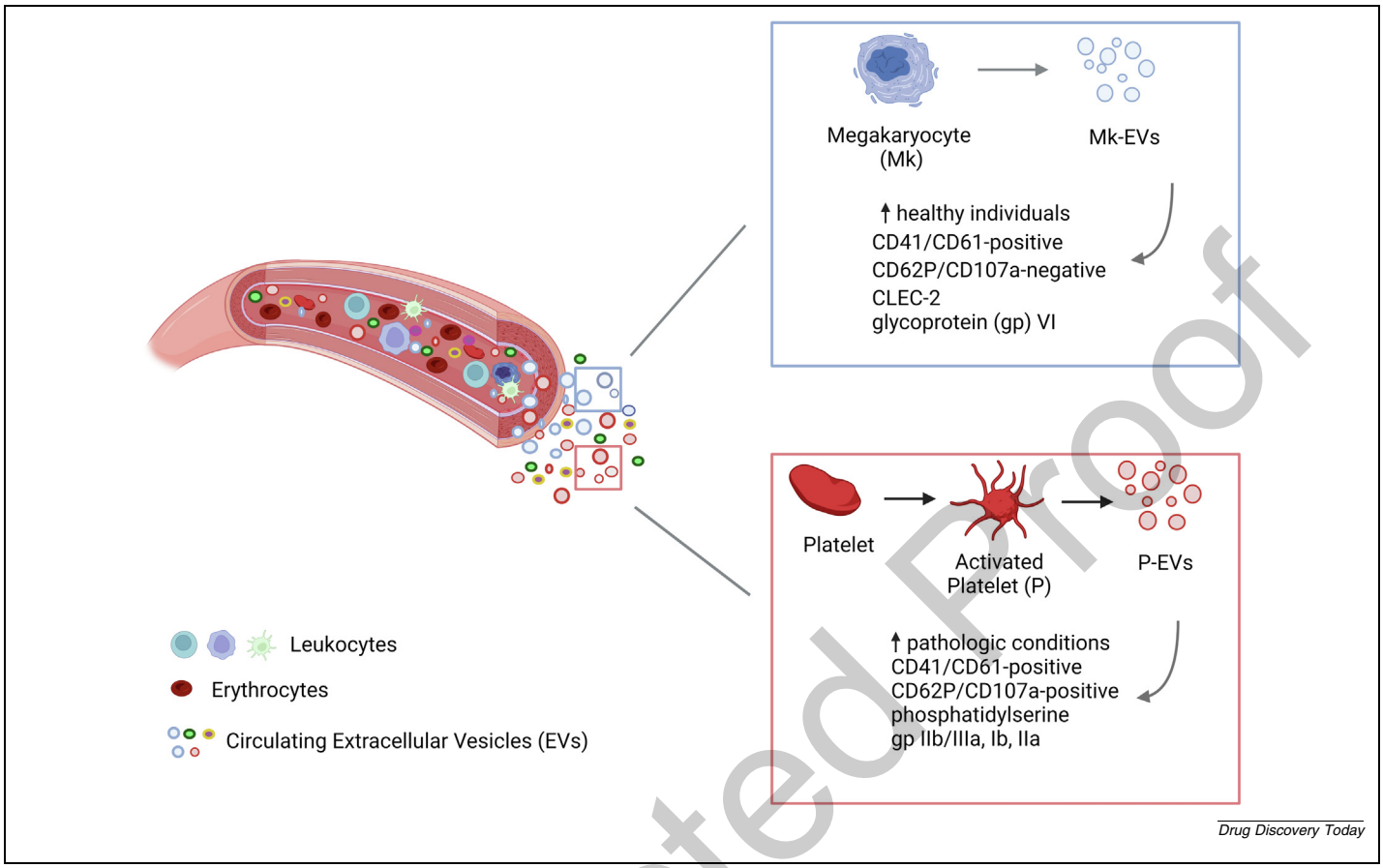

FIGURE 2

Schematic depicting the differences in terms of protein expression among platelet- and megakaryocyte-extracellular vesicles (P-EVs and Mk-EVs, respectively).

sion capacity.[64] In addition, a number of preclinical models of lung and colon carcinomas have also been used to investigate the horizontal transfer of RNAs and miRNAs, supporting their involvement in enhancing vascular permeability and cell invasion.[65].

Similar to PLT-derived scores, P-EV concentration has also been assessed in the blood of cancer patients, showing a significant increase compared to healthy controls.[66] In a selected cohort of 136 NSCLC patients, P-EV number after 3 months of treatment (either chemotherapy or targeted therapy) was significantly increased among patients with disease progression (progressive disease [PD]) as the best response compared to patients with controlled disease, confirming its negative involvement. Furthermore, high circulating P-EVs predicted poor 1-year survival ($p < 0.05$). By contrast, baseline P-EVs were not predictive of clinical outcomes.[67] In a similar study, 86 patients with advanced NSCLC were divided into OR ($N = 60$) and PD ($N = 26$) groups, and their circulating P-EV levels were evaluated before and after treatment with chemotherapy and/or ICI. The authors reported no differences in terms of P-EV number at baseline, whereas P-EV concentration was significantly higher in the PD group after treatment. These data, combined with the evaluation of NLR, were used to build a predictive model for the progression of advanced NSCLC.[68]

Similar results were found in another prospective study that analyzed circulating P-EVs from 50 NSCLC patients treated with ICIs. After treatment, the number of P-EVs in the PD group ($N = 18$) was significantly higher compared with the OR group ($n = 32$).[69] More recently, Genova and colleagues showed that PD-L1 + EVs from metastatic NSLCL patients treated with anti-PD1 in the first-line setting, expressed resting (CD41b, CD42a) or activated (CD62P) PLT markers.[70] Notably, CD62P was generally enriched in the EVs of nonresponding patients, leading to the speculation that activated PTLs may be involved in an anti-PD-1 resistance mechanism via the release of PD-L1-expressing EVs.

Discussion

Multianalyte liquid biopsy analysis is a promising approach for the future clinical practice of NSCLC patients. Currently, different soluble components in the peripheral blood such as CTCs, ctDNA, and tumor-derived EVs have been widely investigated.[71] More recently, PLTs and their P-EVs have emerged as important sources of potential cancer biomarkers, including several types of RNAs, lipids, and proteins.[72] However, while the analysis of ctDNA has already entered the clinical practice of lung neoplasms to evaluate genetic variations that can reflect the mutational landscape of the corresponding tumor tissue,[71] the

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

TABLE 4

List of the most relevant studies showing the role of P-EVs role as disease biomarkers in NSCLC.

Refs	N pts/ healthy controls (HC)	Setting	Treatment	Outcomes	Conclusions
Wang, 2017[67]	136 NSCLC/ 25 HC	All stages	Surgery; adjunctive or palliative chemotherapy; radiotherapy and/or target therapy	The levels of P-EVs predicted one-year prognostic outcomes	P-EVs are valuable prognostic biomarkers in advanced NSCLC
Liu, 2021[68]	86 NSCLC	Advanced	Chemotherapy; immunotherapy; target therapy	P-EVs number and NLR are independently associated with PD of advanced NSCLC	P-EVs are associated with progression of advanced NSCLC
Liu, 2021[69]	50 NSCLC	Advanced	Pembrolizumab or nivolumab + chemotherapy	P-EVs (80 events/mL) after ICI are associated with PD and independently predict the therapeutic effect of ICI	P-EVs after ICI independently predicted the therapeutic effects of ICIs, making it possible to monitor the therapeutic effect in real time and rapidly adjust treatment regimens
Genova, 2023[70]	182 NSCLC	Advanced	Pembrolizumab or nivolumab	PD-L1 + EV expresses markers of PLT activation (CD62P) and the latter is increased in PD pts	Activated PLTs may be involved in the anti-PD-1 resistance via the release of PD-L1-expressing EVs

Abbreviations: CD62P, cluster of differentiation 62P; ICIs, immune checkpoint inhibitors; IL-6, interleukin 6; NSCLC, non-small cell lung cancer; NLR, neutrophil/lymphocyte ratio; PD, progressive disease; PD-1, programmed cell death protein 1; P-EV, platelet-derived extracellular vesicles; PD-L1, programmed death-ligand 1; PLTs, platelets; pts, patients; TNF-α, tumor necrosis factor α.

application of the other circulating elements is still far from clinical use. This is mostly due to the lack of standardization of the preanalytical processes necessary to compare the results obtained in different studies. This is particularly true for the EVs, whose separation from the other components present in plasma/serum has not yet been definitively standardized among different laboratories. Moreover, their isolation requires specific and costly instrumentations and personnel training.[73]

In this scenario, PLTs could be an interesting alternative source of tumor biomarkers to be transferred into clinical practice, as they are abundant and their isolation is easier and faster than other circulating elements,[74] making them one of the most cost-effective tests in the liquid biopsy landscape. However, although the PLT isolation protocol is based on two-step centrifugations, the time and duration of these can often vary between studies, and the differences in these steps can affect the results.[75] Another complication relevant to the results of downstream omics analysis (PLT-RNA/protein/lipid signatures) could be blood cell contamination.[76] In addition, during handling, PLT activation can be a critical issue as it induces the release of P-EVs and pro-coagulation factors.[72] In this regard, Best and colleagues published in 2017 a standardized protocol for blood processing and PLT isolation that maintains high-quality RNA for omics studies.[43].

Nevertheless, in the last few years, the role of PLTs in NSCLC management is acquiring increasing relevance, both as PLT enumeration and ratios with other blood elements, and with regard to their role as molecular carriers. The former represents an easily evaluable biomarker, as it requires a simple blood count to be assessed and is consistently associated with poor outcomes in NSCLC patients across multiple settings and treatments. Despite the easy accessibility, PLT enumeration still has some limits in terms of predictivity. First, since several studies involve both early-stage and advanced NSCLC, the role of PLT enumeration and PLR appears to be mainly prognostic, rather than predictive of benefit of a specific therapeutic approach, such as ICIs. Hence, to clarify whether PLR has a prognostic or a predictive role in NSCLC, further *ad hoc* studies are required. Additionally, a solid biological explanation of the relationship between PLT enumeration and PLT-based ratio and prognosis in NSCLC needs to be elaborated upon in large multi-institutional studies.

Regarding the role of PLTs as carriers of tumor-derived molecules (e.g., mRNAs, ncRNAs, lipids, and proteins), although their analysis has provided relevant information on the tumor status, their analysis is more challenging. First, as with pre-analytical processing, downstream analyses have not yet been fully standardized and often lack a reliable normalization system among the studies. Nonetheless, several RNA-, protein-, and lipid-based signatures have been described that can distinguish healthy controls from cancer patients with excellent accuracies, although their performances, in terms of sensitivity and specificity, are not always entirely satisfactory. Consequently, before being translated into the clinic, these should be further investigated in prospective and independent cohorts of patients also including benign diseases (e.g., COPD) as well as high-risk individuals (e.g., heavy smokers). In addition, PLTs do not appear to be an adequate source of tumor-derived DNA, although current data are too sparse to draw concrete conclusions. By contrast, several studies have shown that RNA characterization of PLTs has greater sensitivity and specificity in detecting *ALK* rearrangements than circulating free RNA and tumor tissue-derived RNA, representing a valuable source for the noninvasive detection of gene rearrangements. Finally, due to the continuous development of new targeted therapies and ICI alone or in combination, the molecular characterization of PLTs in terms of gene and protein signatures might provide useful information in pre-

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

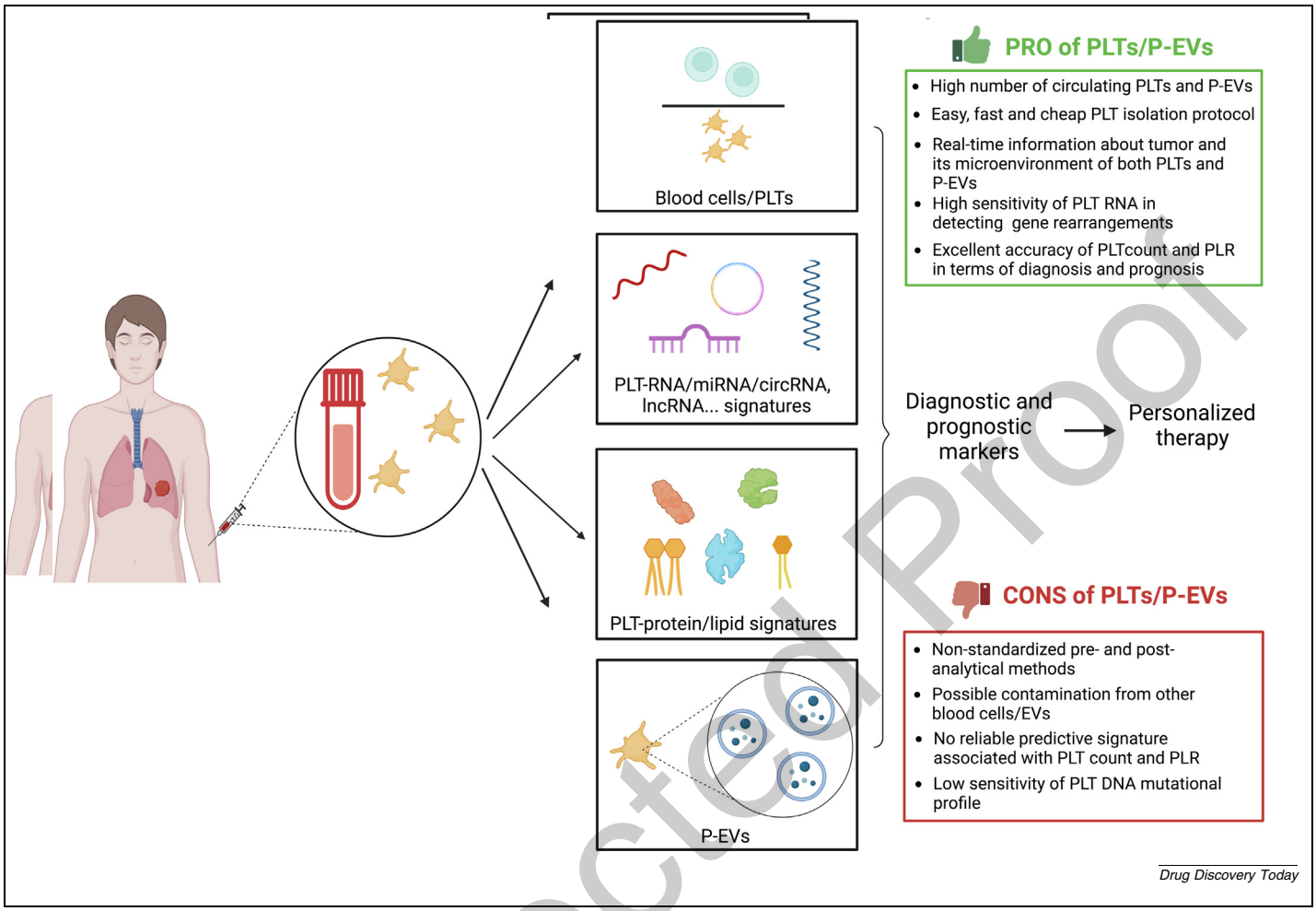

FIGURE 3

Schematic representation of platelets (PLTs) and their derived extracellular vesicles (P-EVs) as next-generation biomarkers in the management of non-small cell lung cancer (NSCLC) patients, both in terms of enumeration and molecular characterization, including the most relevant pros and cons compared to other liquid biopsy-derived elements already known in the context of NSCLC.

dicting response to specific antineoplastic agents and thus represents an extremely promising field of research.

P-EVs, similar to their PLT parents, might represent a future source of cancer-related biomarkers, due to their physiopathological roles and their ability to interact with the TME. In particular, unlike PLTs which fail to cross the blood vessel or can be trapped in thrombi, P-EVs can directly interact with different cell types within the TME, exerting their downstream functions. Consequently, extensive *in vitro* and *in vivo* investigations could provide insights into the oncogenic functions of PLT as well as alternative diagnostic and prognostic markers. However, their study is still in its infancy and many efforts should be made to clarify their precise role in the context of tumor progression and invasion. In addition, regarding other EV types, it remains difficult to make an exhaustive comparison of the results obtained by different research groups due to the lack of standardized methods to isolate and characterize P-EVs.

Concluding remarks

PLTs and their derived P-EVs represent a new generation of biomarkers for NSCLC, both in terms of enumeration and characterization, and the results achieved to date strongly encourage further development and proper standardization (Figure 3). Therefore, the scientific community should make an effort to standardize these methods to effectively use these alternative blood elements as liquid biopsy in the near future.

Declarations of interest

C.G. declares honoraria from Amgen, AstraZeneca, Bristol-Myers-Squibb, Eli-Lilly, Merck-Sharp-Dohme, Novartis, Roche, Sanofi, Takeda, and Thermo Fisher Scientific; and research grants from Bristol-Myers-Squibb and the Italian Ministry of Health. G.R. declares honoraria from AstraZeneca, Bristol-Myers Squibb, Roche, MSD, and Janssen. The other authors have no conflicts of interest to declare.

Data availability

No data was used for the research described in the article.

Acknowledgments

We thank Dr. Angela Alama for her invaluable support. We would like to thank the Italian Ministry of Health (Ricerca Cor-

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

rente 2022, RRC 2022; 5 × 1000 funds: 2018-2019; 2022) and Compagnia di San Paolo (2017-0529) for financially supporting our current research focused on the identification of prognostic and predictive markers by liquid biopsy.

References

1. Cella E et al. Immunotherapy-chemotherapy combinations for non-small cell lung cancer: current trends and future perspectives. *Expert Opin Biol Ther*. 2022;22:1259–1273. https://doi.org/10.1080/14712598.2022.2116273.
2. Rebuzzi SE et al. Novel emerging molecular targets in non-small cell lung cancer. *Int J Mol Sci*. 2021;22:2625. https://doi.org/10.3390/ijms22052625.
3. Rijavec E, Coco S, Genova C, Rossi G, Longo L, Grossi F. Liquid biopsy in non-small cell lung cancer: highlights and challenges. *Cancers (Basel)*. 2019;12:17. https://doi.org/10.3390/cancers12010017.
4. Bożyk A, Nicoś M. The overview of perspectives of clinical application of liquid biopsy in non-small-cell lung cancer. *Life (Basel)*. 2022;12:1640. https://doi.org/10.3390/life12101640.
5. Vanni I, Alama A, Grossi F, Dal Bello MG, Coco S. Exosomes: a new horizon in lung cancer. *Drug Discov Today*. 2017;22:927–936. https://doi.org/10.1016/j.drudis.2017.03.004.
6. Osler W. An account of certain organisms occurring in the liquor sanguinis. *Proc R Soc Lond*. 1874;22:391–398. https://doi.org/10.1098/rspl.1873.0074.
7. Holinstat M. Normal platelet function. *Cancer Metastasis Rev*. 2017;36:195–198. https://doi.org/10.1007/s10555-017-9677-x.
8. Daly ME. Determinants of platelet count in humans. *Haematologica*. 2011;96:10–13. https://doi.org/10.3324/haematol.2010.035287.
9. Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. *J Hematol Oncol*. 2018;11:125. https://doi.org/10.1186/s13045-018-0669-2.
10. Metharom P, Falasca M, Berndt MC. The history of Armand Trousseau and cancer-associated thrombosis. *Cancers (Basel)*. 2019;11:158. https://doi.org/10.3390/cancers11020158.
11. Tagalakis V, Levi D, Agulnik JS, Cohen V, Kasymjanova G, Small D. High risk of deep vein thrombosis in patients with non-small cell lung cancer: a cohort study of 493 patients. *J Thorac Oncol*. 2007;2:729–734. https://doi.org/10.1097/JTO.0b013e31811ea275.
12. Labelle M, Hynes RO. The initial hours of metastasis: the importance of cooperative host-tumor cell interactions during hematogenous dissemination. *Cancer Discov*. 2012;2:1091–1099. https://doi.org/10.1158/2159-8290.CD-12-0329.
13. Strasenburg W et al. Tumor cell-induced platelet aggregation as an emerging therapeutic target for cancer therapy. *Front Oncol*. 2022;12 https://www.frontiersin.org/articles/10.3389/fonc.2022.909767 909767.
14. Nieswandt B, Hafner M, Echtenacher B, Männel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res*. 1999;59:1295–1300.
15. Placke T et al. Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells. *Cancer Res*. 2012;72:440–448. https://doi.org/10.1158/0008-5472.CAN-11-1872.
16. Heinmöller E et al. Studies on tumor-cell-induced platelet aggregation in human lung cancer cell lines. *J Cancer Res Clin Oncol*. 1996;122:735–744. https://doi.org/10.1007/BF01209121.
17. Rodriguez-Martinez A et al. Exchange of cellular components between platelets and tumor cells: impact on tumor cells behavior. *Theranostics*. 2022;12:2150–2161. https://doi.org/10.7150/thno.64252.
18. Hinterleitner C et al. Platelet PD-L1 reflects collective intratumoral PD-L1 expression and predicts immunotherapy response in non-small cell lung cancer. *Nat Commun*. 2021;12:7005. https://doi.org/10.1038/s41467-021-27303-7.
19. Nilsson RJA et al. Blood platelets contain tumor-derived RNA biomarkers. *Blood*. 2011;118:3680–3683. https://doi.org/10.1182/blood-2011-03-344408.
20. Klement GL et al. Platelets actively sequester angiogenesis regulators. *Blood*. 2009;113:2835–2842. https://doi.org/10.1182/blood-2008-06-159541.
21. Dovizio M, Bruno A, Contursi A, Grande R, Patrignani P. Platelets and extracellular vesicles in cancer: diagnostic and therapeutic implications. *Cancer Metastasis Rev*. 2018;37:455–467. https://doi.org/10.1007/s10555-018-9730-4.
22. Haemmerle M, Stone RL, Menter DG, Afshar-Kharghan V, Sood AK. The platelet lifeline to cancer: challenges and opportunities. *Cancer Cell*. 2018;33:965–983. https://doi.org/10.1016/j.ccell.2018.03.002.
23. Calverley DC et al. Significant downregulation of platelet gene expression in metastatic lung cancer. *Clin Transl Sci*. 2010;3:227–232. https://doi.org/10.1111/j.1752-8062.2010.00226.x.
24. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1β-rich microparticles. *J Immunol*. 2011;186:5489–5496. https://doi.org/10.4049/jimmunol.1001623.
25. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell*. 2011;20:576–590. https://doi.org/10.1016/j.ccr.2011.09.009.
26. Li B et al. The crosstalk between lung cancer cells and platelets promotes tumor angiogenesis in vivo and in vitro. *J Cancer Res Clin Oncol*. 2022. https://doi.org/10.1007/s00432-022-04259-9.
27. Gkolfinopoulos S, Jones RL, Constantinidou A. The emerging role of platelets in the formation of the micrometastatic niche: current evidence and future perspectives. *Front Oncol*. 2020;10:374. https://doi.org/10.3389/fonc.2020.00374.
28. Asgari A et al. Platelets stimulate programmed death-ligand 1 expression by cancer cells: Inhibition by anti-platelet drugs. *J Thromb Haemost*. 2021;19:2862–2872. https://doi.org/10.1111/jth.15478.
29. Russo A et al. Baseline neutrophilia, derived neutrophil-to-lymphocyte ratio (dNLR), platelet-to-lymphocyte ratio (PLR), and outcome in non small cell lung cancer (NSCLC) treated with nivolumab or docetaxel. *J Cell Physiol*. 2018;233:6337–6343. https://doi.org/10.1002/jcp.26609.
30. Russo A et al. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and outcomes with nivolumab in pretreated non-small cell lung cancer (NSCLC): a large retrospective multicenter study. *Adv Ther*. 2020;37:1145–1155. https://doi.org/10.1007/s12325-020-01229-w.
31. Pavan A et al. Peripheral blood markers identify risk of immune-related toxicity in advanced non-small cell lung cancer treated with immune-checkpoint inhibitors. *Oncologist*. 2019;24:1128–1136. https://doi.org/10.1634/theoncologist.2018-0563.
32. Zhou K et al. Prognostic role of the platelet to lymphocyte ratio (PLR) in the clinical outcomes of patients with advanced lung cancer receiving immunotherapy: a systematic review and meta-analysis. *Front Oncol*. 2022;12. https://doi.org/10.3389/fonc.2022.962173 962173.
33. Zhang N, Jiang J, Tang S, Sun G. Predictive value of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in non-small cell lung cancer patients treated with immune checkpoint inhibitors: a meta-analysis. *Int Immunopharmacol*. 2020;85. https://doi.org/10.1016/j.intimp.2020.106677 106677.
34. Lee BM et al. Platelet-to-lymphocyte ratio and use of NSAIDs during the perioperative period as prognostic indicators in patients with NSCLC undergoing surgery. *Cancer Control*. 2016;23:284–294. https://doi.org/10.1177/107327481602300312.
35. Sulibhavi A et al. Peripheral blood lymphocytes and platelets are prognostic in surgical pT1 non-small cell lung cancer. *Ann Thorac Surg*. 2020;109:337–342. https://doi.org/10.1016/j.athoracsur.2019.09.006.
36. Łochowski M et al. The prognostic significance of preoperative platelet-to-lymphocyte and neutrophil-to-lymphocyte ratios in patients operated for non-small cell lung cancer. *Cancer Manag Res*. 2021;13:7795–7802. https://doi.org/10.2147/CMAR.S317705.
37. Nassa G et al. Splicing of platelet resident pre-mRNAs upon activation by physiological stimuli results in functionally relevant proteome modifications. *Sci Rep*. 2018;8:498. https://doi.org/10.1038/s41598-017-18985-5.
38. Best MG et al. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell*. 2015;28:666–676. https://doi.org/10.1016/j.ccell.2015.09.018.
39. Xue L, Xie L, Song X, Song X. Identification of potential tumor-educated platelets RNA biomarkers in non-small-cell lung cancer by integrated bioinformatical analysis. *J Clin Lab Anal*. 2018;32:e22450.
40. Sheng M, Dong Z, Xie Y. Identification of tumor-educated platelet biomarkers of non-small-cell lung cancer. *Onco Targets Ther*. 2018;11:8143–8151. https://doi.org/10.2147/OTT.S177384.
41. Dong X et al. Small nuclear RNAs (U1, U2, U5) in tumor-educated platelets are downregulated and act as promising biomarkers in lung cancer. *Front Oncol*. 2020;10:1627. https://doi.org/10.3389/fonc.2020.01627.
42. Goswami C et al. Correction to: molecular signature comprising 11 platelet-genes enables accurate blood-based diagnosis of NSCLC. *BMC Genomics*. 2020;21:877. https://doi.org/10.1186/s12864-020-07230-5.

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616

43. Best MG et al. Swarm intelligence-enhanced detection of non-small-cell lung cancer using tumor-educated platelets. *Cancer Cell*. 2017;32:238–252.e9. https://doi.org/10.1016/j.ccell.2017.07.004.
44. Nilsson RJA et al. Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer. *Oncotarget*. 2015;7:1066–1075.
45. Park CK et al. Feasibility of liquid biopsy using plasma and platelets for detection of anaplastic lymphoma kinase rearrangements in non-small cell lung cancer. *J Cancer Res Clin Oncol*. 2019;145:2071–2082. https://doi.org/10.1007/s00432-019-02944-w.
46. Luo CL et al. LncRNAs and EGFRvIII sequestered in TEPs enable blood-based NSCLC diagnosis. *Cancer Manag Res*. 2018;10:1449–1459. https://doi.org/10.2147/CMAR.S164227.
47. Li X et al. TEP linc-GTF2H2-1, RP3-466P17.2, and lnc-ST8SIA4-12 as novel biomarkers for lung cancer diagnosis and progression prediction. *J Cancer Res Clin Oncol*. 2021;147:1609–1622. https://doi.org/10.1007/s00432-020-03502-5.
48. Dong X et al. Tumor-educated platelet SNORD55 as a potential biomarker for the early diagnosis of non-small cell lung cancer. *Thorac Cancer*. 2021;12:659–666. https://doi.org/10.1111/1759-7714.13823.
49. D'Ambrosi S et al. The analysis of platelet-derived circRNA repertoire as potential diagnostic biomarker for non-small cell lung cancer. *Cancers (Basel)*. 2021;13:4644. https://doi.org/10.3390/cancers13184644.
50. Liang H et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. *Mol Cancer*. 2015;14:58. https://doi.org/10.1186/s12943-015-0327-z.
51. Londin ER et al. The human platelet: strong transcriptome correlations among individuals associate weakly with the platelet proteome. *Biol Direct*. 2014;9:3. https://doi.org/10.1186/1745-6150-9-3.
52. Ercan H et al. Alterations of the platelet proteome in lung cancer: accelerated F13A1 and ER processing as new actors in hypercoagulability. *Cancers (Basel)*. 2021;13:2260. https://doi.org/10.3390/cancers13092260.
53. Fu BH, Fu ZZ, Meng W, Gu T, Sun XD, Zhang Z. Platelet VEGF and serum TGF-β1 levels predict chemotherapy response in non-small cell lung cancer patients. *Tumor Biol*. 2015;36:6477–6483. https://doi.org/10.1007/s13277-015-3338-x.
54. de Castro J, Hernández-Hernández A, Rodríguez MC, Llanillo M, Sánchez-Yagüe J. Comparison of changes in erythrocyte and platelet fatty acid composition and protein oxidation in advanced non-small cell lung cancer. *Cancer Invest*. 2006;24:339–345. https://doi.org/10.1080/07357900600705250.
55. de Castro J, Rodríguez MC, Martínez-Zorzano VS, Llanillo M, Sánchez-Yagüe J. Platelet linoleic acid is a potential biomarker of advanced non-small cell lung cancer. *Exp Mol Pathol*. 2009;87:226–233. https://doi.org/10.1016/j.yexmp.2009.08.002.
56. Tao SC, Guo SC, Zhang CQ. Platelet-derived extracellular vesicles: an emerging therapeutic approach. *Int J Biol Sci*. 2017;13:828–834. https://doi.org/10.7150/ijbs.19776.
57. Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol*. 1967;13:269–288. https://doi.org/10.1111/j.1365-2141.1967.tb08741.x.
58. Taus F, Meneguzzi A, Castelli M, Minuz P. Platelet-derived extracellular vesicles as target of antiplatelet agents. What is the evidence? *Front Pharmacol*. 2019;10:1256.. https://doi.org/10.3389/fphar.2019.01256.
59. Melki I, Tessandier N, Zufferey A, Boilard E. Platelet microvesicles in health and disease. *Platelets*. 2017;28:214–221. https://doi.org/10.1080/09537104.2016.1265924.
60. van der Pol E, Harrison P. From platelet dust to gold dust: physiological importance and detection of platelet microvesicles. *Platelets*. 2017;28:211–213. https://doi.org/10.1080/09537104.2017.1282781.
61. Sinauridze EI et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thromb Haemost*. 2007;97:425–434.
62. Castaman G, Yu-Feng L, Battistin E, Rodeghiero F. Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *Br J Haematol*. 1997;96:458–463. https://doi.org/10.1046/j.1365-2141.1997.d01-2072.x.
63. Happonen KE et al. The Gas6-Axl protein interaction mediates endothelial uptake of platelet microparticles. *J Biol Chem*. 2016;291:10586–10601. https://doi.org/10.1074/jbc.M115.699058.
64. Janowska-Wieczorek A et al. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int J Cancer*. 2005;113:752–760. https://doi.org/10.1002/ijc.20657.
65. Michael JV et al. Platelet microparticles infiltrating solid tumors transfer miRNAs that suppress tumor growth. *Blood*. 2017;130:567–580. https://doi.org/10.1182/blood-2016-11-751099.
66. Ren JG et al. Elevated level of circulating platelet-derived microparticles in oral cancer. *J Dent Res*. 2016;95:87–93. https://doi.org/10.1177/0022034515592593.
67. Wang CC et al. Circulating microparticles are prognostic biomarkers in advanced non-small cell lung cancer patients. *Oncotarget*. 2017;8:75952–75967. https://doi.org/10.18632/oncotarget.18372.
68. Liu T et al. Predicting disease progression in advanced non-small cell lung cancer with circulating neutrophil-derived and platelet-derived microparticles. *BMC Cancer*. 2021;21:939. https://doi.org/10.1186/s12885-021-08628-4.
69. Liu T et al. Prediction of the therapeutic effects of pembrolizumab and nivolumab in advanced non-small cell lung cancer by platelet-derived microparticles in circulating blood 1533033821997817. *Technol Cancer Res Treat*. 2021;20. https://doi.org/10.1177/1533033821997817.
70. Genova C et al. Prognostic role of soluble and extracellular vesicle-associated PD-L1, B7–H3 and B7–H4 in non-small cell lung cancer patients treated with immune checkpoint inhibitors. *Cells*. 2023;12:832. https://doi.org/10.3390/cells12060832.
71. Keup C, Kimmig R, Kasimir-Bauer S. Combinatorial power of cfDNA, CTCs and EVs in oncology. *Diagnostics (Basel)*. 2022;12:870. https://doi.org/10.3390/diagnostics12040870.
72. Antunes-Ferreira M, Koppers-Lalic D, Würdinger T. Circulating platelets as liquid biopsy sources for cancer detection. *Mol Oncol*. 2021;15:1727–1743. https://doi.org/10.1002/1878-0261.12859.
73. Irmer B, Chandrabalan S, Maas L, Bleckmann A, Menck K. Extracellular vesicles in liquid biopsies as biomarkers for solid tumors. *Cancers (Basel)*. 2023;15:1307. https://doi.org/10.3390/cancers15041307.
74. Best MG, Wesseling P, Wurdinger T. Tumor-educated platelets as a noninvasive biomarker source for cancer detection and progression monitoring. *Cancer Res*. 2018;78:3407–3412. https://doi.org/10.1158/0008-5472.CAN-18-0887.
75. Siewiera K, Labieniec-Watala M, Wolska N, Kassassir H, Watala C. Sample preparation as a critical aspect of blood platelet mitochondrial respiration measurements-the impact of platelet activation on mitochondrial respiration. *Int J Mol Sci*. 2021;22:9332. https://doi.org/10.3390/ijms22179332.
76. Rikkert LG, van der Pol E, van Leeuwen TG, Nieuwland R, Coumans FAW. Centrifugation affects the purity of liquid biopsy-based tumor biomarkers. *Cytometry A*. 2018;93:1207–1212. https://doi.org/10.1002/cyto.a.23641.

Please cite this article in press as: Tasso R. et al., Drug Discovery Today (2023), https://doi.org/10.1016/j.drudis.2023.103616