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Roles of Tumor-Educated Platelets (TEPs) in the biology of Non-Small Cell Lung Cancer (NSCLC): A systematic review. "Re-discovering the neglected biosources of the liquid biopsy family"

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ABSTRACT

Due to their interactions with the neoplasm, platelets undergo various proteomic and transcriptomic modifications, resulting in the development of what is known as the "Tumor-Educated Platelets (TEPs) phenotype". Consequently, in addition to their suitability for Liquid Biopsy (LB) applications, they play a pivotal role in the malignancy by communicating with Circulating Tumor Cells (CTCs), Tumor Microenvironment (TME), and the tumor itself through multiple mechanisms and at multiple levels, ultimately promoting the metastasis of cancer. Therefore, this Systematic Review of MEDLINE and the Cochrane Library present in-depth insights into these phenomena, with the aim of enhancing the understanding of the complex interplay between TEPs and Non-Small Cell Lung Cancer (NSCLC). This endeavor serves to provide context and drive medical research efforts, which are increasingly focused on developing novel diagnostic and therapeutic technologies that leverage the specific binding of these platelets to the disease.

1. Introduction

In 2023, Lung Cancer (LC) remains the deadliest global cancer, ranking second in incidence [1]. The World Health Organization (WHO) classifies LC into two primary types: Small Cell Lung Cancer (SCLC), comprising 15 %, and Non-Small Cell Lung Cancer (NSCLC), accounting for 80–85 % [2]. Late-stage diagnoses contribute to bleak LC prognoses [3], emphasizing the potential of early detection to improve Overall Survival (OS) [4].

Since 1999 [5], tissue biopsy has been the gold standard for LC diagnosis. However, it shows some limitations [6]: the spatial and temporal intratumoral genetic heterogeneity may lead to incomplete genomic profiles when characterizing tumors through a single biopsy. Additionally, the analytical procedures may extend turnaround times and can pose challenges for frail patients, unable to repeat the exam to monitor disease progression. To address these issues, modern Liquid Biopsy (LB) techniques are supplanting or complementing traditional methods [7] by analyzing blood constituents, with the aim to minimize the need for invasive genotyping. However, due to the shortcomings of conventional circulating markers, such as the high incidence of false-positive or false-negative results [8,9], there is a strong interest in identifying novel and more reliable markers for earlier, accurate diagnosis and prognosis [10,11].

For these reasons, classical markers are gradually giving way to more specific tests involving cellular components and circulating molecules. This transition follows several years of exploring various sources [12]. The new diagnostic methods focus on elements like Circulating Tumor Cells (CTCs), circulating tumor DNA (ctDNA), circulating free DNA (cfDNA), circulating free RNA (cfRNA), microRNA (miRNA),

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List of abbreviations

Ab	Antibodies
ADP	Adenosine Diphosphate
CCL(n)	chemokine (C-C motif) Ligand(n)
CD(n)	Cluster of differentiation (n)
cfDNA(s)	circulating free DNA(s)
cfRNA(s)	circulating free RNA(s)
circRNA(s)	circular RNA(s)
CLEC-(n)	C-type Lectin domain family – (n)
CTC(s)	Circulating Tumor Cell(s)
ctDNA(s)	circulating tumor DNA(s)
ddPCR	droplet digital PCR
ECM	Extracellular Matrix
ELISA	Enzyme-linked Immunosorbent Assay
EML4-ALK	*Echinoderm Microtubule-associated protein-Like 4 – Anaplastic Lymphoma Kinase*
EMT	Epithelial-Mesenchymal Transition
ESMO	European Society for Medical Oncology
EVs	Extracellular Vesicles
G-CSF	Granulocyte Colony-Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GP(nx)	Glycoprotein(nx)
HC	Healthy Control
IASLC	International Association for the Study of Lung Cancer
IL-(nx)	Interleukin-(nx)
LB	Liquid Biopsy
LC(s)	Lung Cancer(s)
lncRNA(s)	long non-coding RNA(s)
LPA	Lysophosphatidic Acid
MET	Mesenchymal-Epithelial Transition
MHC-(n)	Major Histocompatibility Complex – (n)
miRNA(s)	micro-RNA(s)
MKs	Megakaryocytes
MMPs	Matrix Metalloproteinases
NCCN	National Comprehensive Cancer Network
NGS	Next Generation Sequencing
NHS	National Health Systems
NK	Natural Killer
NSCLC	Non-Small Cell Lung Cancer
PCR	Polymerase Chain Reaction
PDGF	Platelet-Derived Growth Factor
PD-L(n)	Programmed Death-Ligand(n)
PEVs	Platelet-derived EVs
PGE(n)	Prostaglandin E(n)
PMPs	Platelet-derived Microparticles
PRP	Platelet-Rich Plasma
RCT	Randomized Controlled Trial
RBP(s)	RNA-Binding Protein(s)
RNAseq	RNA sequencing
RT-PCR	Reverse Transcription – PCR
SC	Symptomatic Control
scRNAseq	single-cell RNA sequencing
SCLC	Small Cell Lung Cancer
snRNA(s)	small nuclear RNA(s)
TCIPA	Tumor Cell Induced Platelet Aggregation
TDEx	Tumor-Derived Exosomes
TEPs	Tumor-Educated Platelets
TGF-(x/xn)	Transforming Growth Factor – (x)
TME	Tumor Microenvironment
TMVs	Tumor Microvesicles
TNF-(x)	Tumor Necrosis Factor – (x)
TPO	Thrombopoietin
TSP-(n)	Thrombospondin-(n)
TXA(n)	Thromboxane A(n)
WHO	World Health Organization

Tumor-Educated Platelets (TEPs), and even Extracellular Vesicles (EVs) including exosomes, microvesicles, microparticles, and oncosomes [13, 14]. However, Sánchez-Herrero et al. [14] highlight that, although promising, ctDNAs, cfDNAs, and especially cfRNAs are susceptible to degradation within the bloodstream. Therefore, they suggest to analyze molecules enclosed within vesicles such as exosomes or platelets, where protection against detrimental agents is more substantial, ensuring greater integrity of the analytes under investigation. Finally, circular RNA (circRNA), small nuclear RNA (snRNA), and long non-coding RNA (lncRNA) are also being studied nowadays via LB [15–18]: indeed, these can be obtained both from solid biopsies and from platelets, which contain a rich array of coding and noncoding RNAs, as well as protein markers. Consequently, platelets are gaining increased attention as biosources, potentially serving as dynamic reservoirs of tumor-related biomolecules, on par with, if not superior to, exosomes.

In oncology, platelets' story dates back to the 19th century [19–21], linking thrombocytosis and spontaneous coagulation with neoplastic development. These early findings suggested a form of communication with the tumor. The 20th century witnessed advancements in the knowledge of platelet biogenesis by Megakaryocytes (MKs) located in the bone marrow, spleen, and (as discovered later, in 2017) in the lungs. During the 1990s exploration of platelet transcriptome is settled, shedding light on processes like inflammation, activation, and tumor neoangiogenesis. Finally, since 2009 [5], it has been established that platelets may be "educated" by tumors, making them pivotal contributors to both local and systemic tumorigenesis.

In the realm of TEPs, one of the earliest descriptions is often attributed to Calverley et al. [22]. They are integral to the Tumor Microenvironment (TME), influencing tumorigenesis, tumor progression, metastasis, and treatment response [23–25]. As will be seen, they emerge from the omics alterations induced by the tumor. TEPs are often characterized as the "new, promising players in LB", akin to "dynamic libraries" [8,19,20,26,27], primarily due to the distinctive modifications they inherit from the neoplasm and their relatively short lifespan, "ensuring information no older than 10 days". Besides these changes, TEPs are valued for their abundance, simple isolation through double-centrifugation, yielding Platelet-Rich Plasma (PRP), and marker stability [19,28]. Furthermore, Liu et al. [6] suggest that while early detection may offer valuable implications for TEPs in LB, it may not be sufficient to significantly reduce overall patient mortality, since certain cancers, such as NSCLC, often metastasize from the earliest stages, thus making complete eradication challenging for oncologists. Instead, pharmacogenetics stands out as a field that could benefit greatly from LB, as it inherently provides a more comprehensive representation of the patient's overall status and offers the advantage of easier, periodic monitoring of treatments.

Hence, this Systematic Review aim to explore three key questions regarding TEPs' biology: how does the educational process influence platelets? Following activation, what are the mechanisms through which TEPs interact with the neoplasm? Lastly, how these cells can be deployed in the battle against LCs? The Results section will delve into TEP biology to provide answers to these questions.

2. Research methods

2.1. Search strings

- ***MEDLINE (via PubMed) – Nov. 10, 2022 – 49 articles***

(("*Lung Neoplasms*"[Mesh] OR "*Carcinoma, Non-Small-Cell Lung*"[Mesh] OR "*NSCLC*"[tiab] OR "*Small Cell Lung Carcinoma*"[Mesh] OR "*SCLC*"[tiab]) AND ("*Tumor-Educated Platelet*" OR "*Tumor-Educated Platelets*" OR "*Tumor Educated Platelet*" OR "*Tumor Educated Platelets*" OR "*TEP*"[tiab] OR "*TEPs*"[tiab]))

- ***Cochrane Library – Nov. 14, 2022 – 8 articles***

#1) *lung neoplasm*; **#2**) *NSCLC*; **#3**) *Non-small-cell lung cancer*; **#4**) *MeSH descriptor: [Carcinoma, Non-Small-Cell Lung] explode all trees*; **#5**) *MeSH descriptor: [Lung Neoplasms] explode all trees*; **#6**) *#1 OR #2 OR #3 OR #4 OR #5*; **#7**) *tep*; **#8**) *tumor educated platelet*; **#9**) *tumor educated platelets*; **#10**) *tumor-educated-platelet*; **#11**) *tumor-educated-platelets*; **#12**) *tumor-educated platelet*; **#13**) *tumor-educated platelets*; **#14**) *teps*; **#15**) *#7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14*; **#16**) *#6 AND #15*; **#17**) *pet*; **#18**) *#16 NOT #17*.

2.2. Data extraction

The articles are summarized in their entirety, in order to not omit any relevant information and set up the work of later merging their contents, divided by topic (i.e., the topics depicted in the [*Graphical Abstract*]).

2.3. PRISMA 2020 flow diagram

In summary, the following records were added:

- 49 records from PubMed, 26 removed as not about TEPs, and 2 as available only in Chinese.
- 8 records from Cochrane Library, 5 removed as not about TEPs and 2 (manually) as duplicates.
- 19 records as "personal sources" (other articles already available to the authors).
- 22 records as "extra" (bibliographic references of interest, isolated from the above records during the later stages).

In total, 30 screened studies +22 "extra" + 11 personal sources are included, as shown in [Fig. 1].

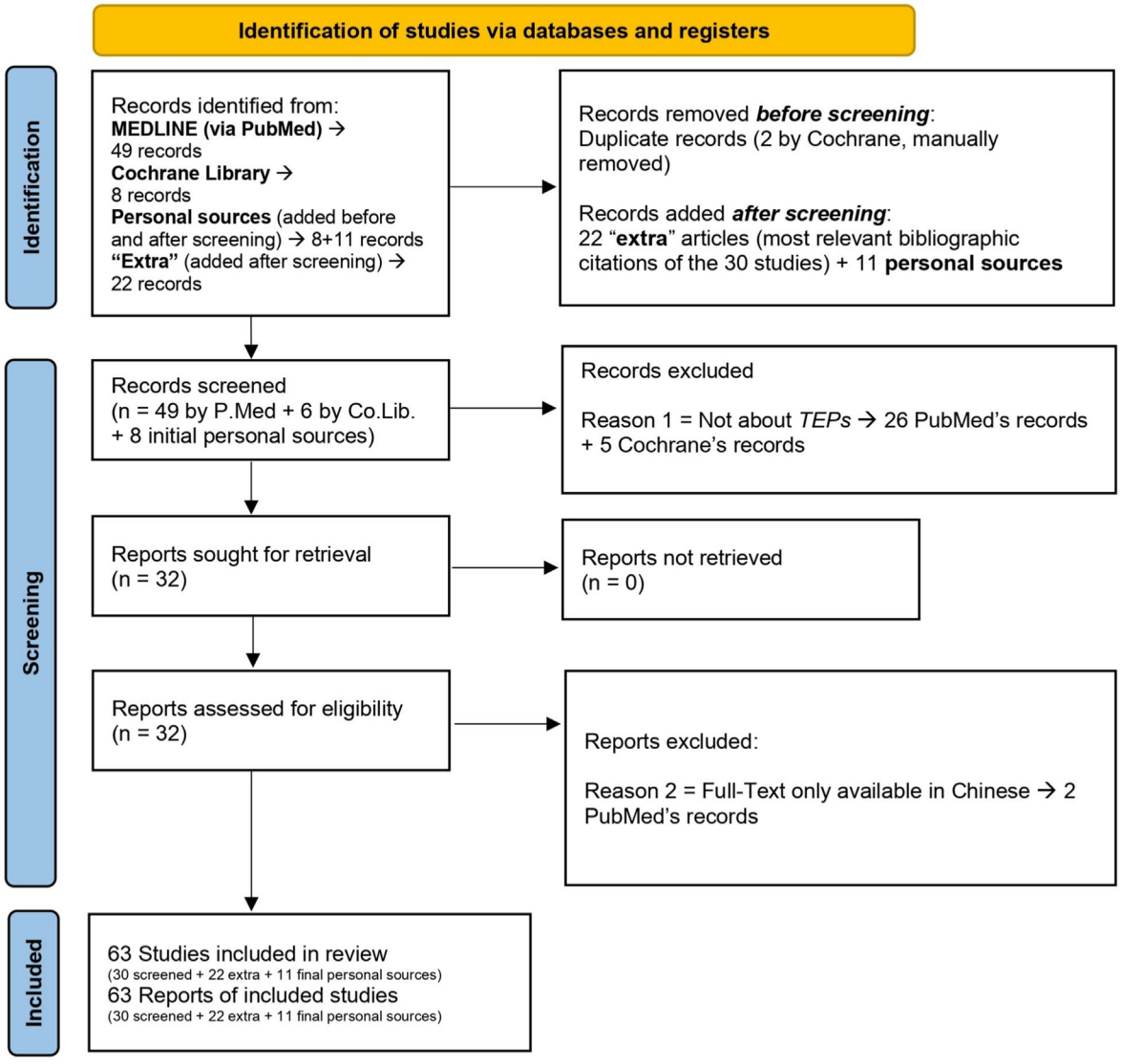

Fig. 1. Prisma 2020 flow diagram.

3. Results

3.1. Platelets' educational process

The educational process starts when platelets contact tumor cells or TME [5], resulting in various biochemical changes [29]: by quantitatively and qualitatively altering both the platelets' RNAs (messenger, mitochondrial, circulating, long noncoding, small nuclear/nucleolar, and miRNA [20]) and proteins (consisting of more than 5000 molecules [30] and including, on such alterations, especially adhesion molecules and the components of the spliceosome and translation machinery), this process will lead to the onset of the "TEPs phenotype". All these changes seem to occur through four main mechanisms:

- Platelet activation and aggregation

The first molecular insight into this interaction arises from Liu et al. [6], who assessed the role of the Cluster of Differentiation 97 (CD97) receptor, which is overexpressed in cancer cells and, when contacts platelets, causes their activation. Asghar et al. [31] conducted further investigations into this process, revealing a phenomenon known as Tumor Cell Induced Platelet Aggregation (TCIPA). TCIPA is triggered through interactions between platelets and the tumor or its derivatives, essentially through two primary pathways: the first one involves C-type Lectin domain family 2 (CLEC-2) and Glycoprotein VI (GPVI) receptors, resulting in complete platelet activation; the second one encompasses Thromboxane A2 (TXA2), thrombin, and Adenosine Diphosphate (ADP), which facilitate platelet activation. Additionally, integrins and platelet receptors, such as P-selectin, also contribute to TCIPA by participating in tumor-platelet interactions.

- ***Tumor-related factors uptake***

Since platelets can uptake circulating nucleic acids, proteins, and EVs, and since cancer cells can release tumor-related factors into circulation (either "circulating-free" or via EVs), thus, in a sense, platelets can be considered repositories for a significant portion of the so-called "tumor circulome" [32]. For many authors, this is the key mechanism of platelet education.

Roweth et al. [21] provided valuable insights on such phenomenon: they stated that the presence of mutant or tissue-specific RNA transcripts within platelets demonstrates their ability to efficiently store and transport ctRNAs, and one of the most notable examples specifically concerns *Echinoderm Microtubule-associated protein-Like 4 – Anaplastic Lymphoma Kinase* (*EML4-ALK*) rearrangements in NSCLC patients. However, it is believed that such RNAs primarily enter platelets through EVs, with direct uptake remaining a presumption based on comparisons with macrophages and other tumor-educable cells [33]. Additionally, they reported that platelets employ parts of the machinery required for receptor-mediated endocytosis and endosomal trafficking to internalize tumor-derived proteins.

Finally, several studies [21,34,35] described the uptake of circulating EVs, including both the generic Tumor Microvesicles (TMVs) or the more specific Tumor-Derived Exosomes (TDEx), by platelets: thrombocytes can assimilate and hereby protect these EVs from degradation; upon tumor-induced activation, the tumor-related factors contained within these vesicles give platelets a dynamic and specific omic profile, thus transforming them into TEPs. Nevertheless, the precise TEPs-TMVs interplay, as well as the fate of endocytosed transcripts, have yet to be determined.

- ***Splicing regulation***

Calverley et al. [22] were the first to observe differential splicing events of TEPs' RNA in metastatic LC, and similar findings were reported by Best et al. [36]. Therefore, it is hypothesized that the altered spliced RNA profile of TEPs results from tumor-specific signals, although the incorporation of exogenous RNA is also likely to contribute to the onset of such alterations. Unfortunately, these authors assert that the identity of such signals, their mechanisms, and how they diverge from physiological ones remain important unanswered questions. However, Best et al. [27] further added that quali-quantitative changes in RNA-Binding Proteins (RBPs) and/or in their binding sites, or interaction with miRNAs or circRNAs may be the cause of altered thrombocytes' splicing.

- ***Altered thrombopoiesis***

Different authors [21,23,27,37] show that, by releasing factors such as Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Granulocyte Colony-Stimulating Factor (G-CSF), Interleukin-1 (IL-1), and IL-6, the tumor stimulates the production of Thrombopoietin (TPO), which will result in thrombopoiesis and megakaryopoiesis; this will thus increase the number of activated and educable platelets, which significantly benefits tumor development. Moreover, Zaslavsky et al. [38] were the first to demonstrate that tumor affects platelet counts and content also through MKs alterations: in their study, bone marrow's MKs displayed elevated expression of Thrombospondin-1 (TSP-1, anti-angiogenic), which was passed to their platelet progeny. Indeed, although there is limited knowledge on this subject, it is known that MKs selectively package RNAs, proteins, and organelles into developing proplatelets, as well as that such phenomenon can be altered by various conditions, thus giving a distinct omic and functional profile to platelets formed in such settings. Therefore, the study of differential packaging of thrombocytes by MKs could provide insights into a novel mechanism of platelet education.

3.2. TEPs-tumor interactions

Once the TEP phenotype is established, these platelets will begin to interact in multiple ways and at multiple levels with the neoplasm, eventually influencing its growth, invasion, and dissemination. This process primarily involves two critical interactions: with CTCs and with the TME.

Asghar et al. [31] conducted a comprehensive analysis of the former interaction, starting with an examination of the role of TEPs in tumor intravasation: platelets actively contribute to tumor Epithelial-Mesenchymal Transition (EMT), Extracellular Matrix (ECM) remodeling, and metastasis – a sequential series of events encompassing the detachment of CTCs from the primary mass, vessel permeabilization, and tumor invasion. These processes occur through juxtacrine interactions involving selectins and glycoproteins, as well as via molecules released upon platelet activation, such as Transforming Growth Factor-β (TGF-β), Platelet-Derived Growth Factor (PDGF), and Prostaglandin E2 (PGE2). Notably, platelets located at the tumor's leading edge express Snail1, a well-known EMT-associated transcription factor. However, there is no direct evidence implicating platelets as the primary cause of cancer cell intravasation (i.e., specifically, entry into the circulation).

Regarding the role of TEPs in immunoevasion, Asghar's team underlines that CTCs have a very low lifespan, due to the immune system clearance and the mechanical damage caused by the shear stress of the bloodstream; therefore, for CTCs the interactions between their αvβ integrins with TEPs' GPIIb/IIIa and fibrin it's crucial, because, even through the TCIPA process, these give rise to a "hetero-aggregate" (indicated as "CTC-fibrin-TEPs complex") that traps those CTCs, thus facilitating metastasis through "passive" (steric hindrance in recognition by Natural Killer (NK) and T lymphocytes) and "active" (Major Histocompatibility Complex I (MHC-I) transfer to CTCs) immunogenicity, as well as providing mechanical protection from shear stress [6,23]. According to Asghar's studies, TEPs not only block NKs indirectly but also inhibit them directly, inducing downregulation of NK-activating receptors by their secreted factors; to confirm, he reports that thrombocytopenia increases the CTC-lysing ability of NK cells both *in-vivo* and

in-vitro. The cumulative effect of these tumor-platelet interactions initiates the coagulation cascade and promotes tumor dissemination.

Transitioning to the role of platelets in tumor extravasation, Asghar posits that this involves the interaction between adhesion receptors on endothelial cells and CTCs – an interaction that platelets are believed to regulate. The involvement of EMT and Mesenchymal-Epithelial Transition (MET) in cancer invasiveness and metastasis has also been a focal point in Asghar's investigations, considering that CTCs are often described as tumor cells undergoing EMT. However, the mechanisms sustaining the EMT state in CTCs for extended durations still require further elucidation, with the involvement of platelets being a plausible factor. According to Asghar's results, this process also involves leukocytes, which overexpress the chemokine C-C motif Ligand 5 (CCL5) and allow the recruitment of additional leukocytes to the site. To prove so, it's known that, in LCs, leukocytes can cause metastasis and increase tumor survival, but the inhibition of CCL5 caused the inhibition of metastasis. Furthermore, PGE2, Matrix Metalloproteinases (MMPs), Lysophosphatidic Acid (LPA), and other platelets and leukocyte factors (as TGF-β, PDGF, and ADP) aid in inducing EMT and intravasation as well as weakening the endothelium to allow extravasation. Finally, myeloid cells can also activate the endothelium, via Tumor Necrosis Factor-α (TNF-α), IL-1α, and IL-1β.

Turning the focus to the TEPs-TME interactions in NSCLC, it's convenient to start considering that, as known, there is a strong interplay between solid tumors and their microenvironment; different studies evaluated, indeed, the roles of the generical tumor circuloma [32] or the more concise EVs [39], but few have delved into the contribution of platelets in this context. Santarpia et al. [40] have shed light on this intriguing subject by elucidating the mechanisms underpinning platelet education: briefly, during their lifetime, platelets continuously exchange (primarily through EVs) nucleic acids and proteins with immune cells, endothelia, other platelets, and tumor cells; thus, the latter can "hack" thrombocytes themselves, leading to their education [5,35,41].

On the other hand, In 't Veld & Wurdinger [19] emphasize that platelets can respond to external stimuli by releasing Platelet-derived Microparticles (PMPs), a specific subset of Platelet-derived EVs (PEVs). PMPs account for a significant portion of circulating EVs (from 70 % to 90 %) and are generated following platelet activation. They play pivotal roles in processes such as angiogenesis, metastasis, and drug resistance. Antunes-Ferreira [20] adds that the designation and cargo of PEVs can vary based on their biogenesis and size. These vesicles are believed to carry cytokines, functional enzymes, coding and non-coding RNAs, and even platelet mitochondria. However, similar to other types of EVs, PEVs lack an official nomenclature, as the field is still evolving. Therefore, further research is essential to unravel their molecular intricacies and decipher their "communication code".

Finally, Kerr et al. [42] shown that, absorbing and transporting the tumor-related factors to different locations, TEPs can act as the "armed arm" of the neoplasm, stimulating the formation of new niches by promoting EMT, tumorigenesis, neoangiogenesis, and eventually metastasis [6,31].

3.3. TEPs' news: LB panels for clinical applications, next-generation techniques and anti-cancer therapies in development

Presently, international associations such as International Association for the Study of Lung Cancer (IASLC), European Society for Medical Oncology (ESMO), or National Comprehensive Cancer Network NCCN have included multigenic Next Generation Sequencing (NGS) panels in their guidelines [26], to guide targeted or personalized therapies; interestingly, these could use both solid and liquid biopsies, as the latest research seems to show [43,44]. Indeed, different authors stated that there are good prospects for using TEPs as biosources for LB, pending further data from studies on larger numbers of patients. In particular, as a result of all of the aforementioned alterations, TEPs may have a variety of clinical applications: for instance, Bracht et al. [34] demonstrate that transcriptomic changes in TEPs have enabled researchers to characterize ongoing neoplasia and monitor treatment progress through LB panels. The accuracy of these analyses is on par with, if not superior to, other blood biosources [6,21,34]. Liu et al. [6] further emphasizes the specificity of this analysis, being able not only to distinguish between cancer patients and Healthy Control (HC), but also between cancer patients and Symptomatic Controls (SC). Moreover, Sanchez-Herrero et al. [14] stated that, specifically for *EML4-ALK*+ NSCLC, TEPs' RNA outperforms cfRNA in detecting rearrangements. According to Liu [6], Roweth [21], and Sabrkhany [25], proteomic alterations might be an informative biosource for cancer diagnosis and monitoring, either alone or in combination with TEPs' transcriptome and with the "classical" markers, and both on late and early stages of tumor development [27]. However, it is stated that the pool of proteins in platelets may derive from MKs, systemic circulation, and thrombocytes themselves; therefore, is essential to determine which proteins are truly tumor-derived and which may fluctuate due to tumor-independent processes [19]. Finally, aside from inheriting proteins and transcripts from the tumor or the systemic circulation, the unique characteristic of platelets to harbor and splice ~5500 different RNAs provides an additional, valuable set of heterogeneous biomarkers, whose splicing levels can be analyzed by specific software to make oncological diagnoses [45].

However, beyond LB, some studies reported intriguing insights into innovative technologies developed for TEPs' investigations: e.g., Roweth et al. [21] state that one of the key points of the initial studies by Best et al. was to demonstrate the potential of machine learning in the medical sciences; from there on, in fact, more and more articles deal with new diagnostic software [46]. Moreover, although platelet levels of mutant RNA are often below the detection limit of conventional approaches (e.g., Reverse Transcription – Polymerase Chain Reaction (RT-PCR) and RNA sequencing (RNAseq)), new technologies such as droplet digital PCR (ddPCR, tested, in fact, by Xing et al. [47]) can analyze tumor RNA with greater precision, thus having the potential to further improve diagnosis via TEPs. Again, new single-cell RNAseq (scRNAseq) approaches are raising new questions about the homogeneity of such mutations within tumors and platelets [48]. Indeed, several studies seem to indicate that the number of young, reticulated platelets increases in cancer patients, resulting in an increased thrombotic potential [27]. Perhaps, these can uptake tumor-derived factors more efficiently or provide greater protection to CTCs. If the former hypothesis is confirmed, the molecular composition of platelets is expected to vary among different populations. Therefore, it would be of the utmost interest to test the dynamics of age-dependent platelets' education, especially through single-cell-RNAseq studies, to be able to assess the possible heterogeneity of such changes within the same or different populations. Additionally, identifying suitable membrane markers to enhance the isolation efficiency of specific platelet subtypes would be beneficial.

Finally, advancements in bioengineering [49,50] have enabled the *ex-vivo* modification of murine platelets for their application as antitumor agents: through the expression of anti-Programmed Death-Ligand 1 (PD-L1) Antibodies (Ab) and subsequent re-infusion into mice, researchers successfully diminished tumor recurrence following surgical resection. This approach yielded superior outcomes compared to infusing anti-PD-L1 Ab alone, primarily attributed to the reduced clearance of platelet-conjugated Ab and their accumulation at the neoplasm site. In addition, bioengineers [51] also tested some "decoy-platelets" that, by acting as "competitive inhibitors", prevented the binding of normal platelets on the neoplasm, ultimately leading to reduced metastasis. These investigations highlight the potential utility of artificially educated platelets as innovative therapeutic modalities. Nevertheless, the translation of these preclinical findings into clinical practice awaits further exploration.

4. Discussion

As evidenced, platelets play a pivotal role in cancer biology. Their

involvement spans across nearly every stage of cancer progression, from local expansion to the formation of CTC-fibrin-TEPs complexes used by metastatic cells for immune evasion, ultimately culminating in the preparation of metastatic niches. Remarkably, their absence or inhibition can significantly impede the development of metastases. This phenomenon arises from the tumor-induced alterations that educate platelets, enabling them to support the malignancy upon adopting the TEPs phenotype. However, the extensive omic changes orchestrated by the neoplasm have paved the way for the diagnostic and prognostic use of TEPs, since these may serve as biosources to detect concealed malignancies or the reactivation of dormant metastases. Consequently, characterizing TEPs holds the potential to forecast tumor progression, potentially leading to timely interventions that could save, or at the very least, extend countless lives. This is particularly pertinent for conditions like NSCLC, which are highly widespread and often late-onset. Furthermore, TEPs possess the distinct advantage of being easily characterizable through straightforward blood sampling, followed by a few isolation and extraction steps, culminating in PCR and/or Enzyme-Linked Immunosorbent Assay (ELISA). This streamlined process theoretically offers phenomenally rapid turnaround times and an excellent cost-benefit ratio, which means that all modern healthcare facilities may harness the potential of TEPs for patient monitoring and screening programs, providing an additional level of control and safety to such individuals without bankrupting National Health Systems (NHS) or imposing analysis timelines incompatible with the needs of cancer patients – indeed, solving the main needs and obtaining the main goal underlined in a lot of previous studies [6,7,27,47,52–55]. Additionally, TEPs are frequently found physically associated with CTCs, highlighting their significance in the metastatic process, and suggesting the possibility of using them to "target" neoplastic cells, for oncological therapies applications. Nevertheless, it's worth noting, as Antunes-Ferreira et al. [20] have emphasized, that the molecular interplay between these two cell types remains insufficiently characterized. Therefore, it is strongly recommended to conduct further studies exploring the various facets of their interaction, aiming for a comprehensive, multi-source, and more informative understanding of TEPs-CTCs interactions. This comprehensive approach may shed light on this phenomenon and lead to the discovery of new molecular markers with diagnostic or potentially therapeutic relevance.

Turning the discussion to the challenges associated with the analysis of TEPs, the articles reviewed in this study frequently point out the following issues:

- The analytical process for investigating TEPs' biomarkers is complex and not easily reproducible [56].
- As highlighted by Goswami et al. [53], omic analysis is not always available in routine clinical practice; consequently, protocols structured in this manner may not be readily accessible to all patients.
- Current studies often involve non-representative cohorts [57], both in terms of population diversity and variations in tumor stages and subtypes; moreover, these studies typically focus on a single biosource, although an increasing number of authors propose the evaluation of not only multi-marker panels (comprising RNA, protein, clinical parameters, etc.) but also multi-analyte panels (involving TEPs, CTCs, EVs, etc.) to aggregate the advantages and mitigate the limitations of individual biosources.
- It is imperative to investigate the effects of pre-analytical and confounding variables, potentially through dedicated studies addressing factors like SC, early malignancies, platelet activation, or concurrent therapies and diseases that may influence platelet function [58].

Furthermore, it's authors' opinion that another limitation hindering the clinical application of TEPs lies in the absence of internationally established, standardized laboratory procedures that would guarantee the reliability of the information obtained from these biological sources. To give an idea of the extent of this issue, among the studies cited in this Systematic Review, twenty-two of them analyzed the transcriptome of TEPs [2–5,7,9,15–18,22,23,35,36,41,45–47,53,56,57,59], while seven examined the proteome of TEPs [25,38,42,47,59–61]; notably, nearly all of these studies exhibit variations in the methods used for isolating TEPs, such as the number and parameters of blood centrifugations, storage durations and methods, as well as the techniques employed for analysis, including traditional methods, new-generation techniques, high-throughput analyses, or targeted approaches. These variations persist even among studies that investigate the same class of molecules.

It is worth noting that this issue closely resembles the challenges encountered in numerous studies involving the analysis and application of PRP in various contexts. PRP, as widely recognized, is increasingly employed in regenerative and aesthetic medical applications [62], either as a standalone treatment or as an adjunct to other medical devices and products, such as biomimetic scaffolds, bone paste, cellular components, and more [63], aimed at promoting the overall healing process. However, even within these diverse applications, there appears to be an excess of heterogeneity in the production methods for PRP, which often leads to conflicting and scarcely comparable results. In light of these challenges, it is hopeful that the international scientific community will address this issue by conducting a comprehensive analysis of the results reported in the literature or by initiating a Randomized Controlled Trial (RCT) specifically tailored to this concern. The ultimate objective would be to establish standardized procedures for the production of PRP that can be uniformly adopted by researchers and clinicians in their respective facilities. These standardized procedures, that might be unique to every field or tailored for the specific applications, based on the outcomes of the apposite analysis, could be used both for the analysis of PRP as a traditional biological sample for oncological diagnosis applications, or for its therapeutic use on patients under treatment.

In oncology, this will mean that, in few years, physicians may have a new, prognostic ally through which therapies might be timely, precisely, and cost-effectively followed, thus allowing providers to stay up to date with all sorts of evolution the patient's pathology may experience. Moreover, having grounded their roots in the clinical field, TEPs might start to be screened in the population at risk. Therefore, once settled in the oncological field through prognostic reasons, TEPs' usage might start to evolve them into proper diagnostic biosources, if the first results will follow the path started by preclinical studies – always, both alone and along with the other, more renowned analytes usually tested in the LB scenario.

5. Conclusion

As promising as the collected data may appear, presently it remains premature to designate TEPs as a "revolutionary tool" for the management of cancer patients. It is imperative to endorse further research endeavors, encompassing fundamental investigations (such as the exploration of TEPs' interactions with tumors), applied research (aimed at understanding the specific alterations characterizing various malignancies), and translational studies (e.g., focused on identifying the most effective methods for characterizing TEPs for clinical applications), with the aim to enhance the comprehension of such biosources. This process may be facilitated by integrating the knowledge derived from TEPs with data already obtained or attainable from other blood analytes: indeed, the integration of diverse data sources holds the potential to surmount any existing limitations, ultimately paving the way for the development and commercial release of suitable kits for the analysis of these circulating elements in cancer patients. For them, the availability of such diagnostic tools could represent a groundbreaking achievement, with the potential to significantly enhance their lives.

Authors' contributions

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All authors had full access to all study data and take responsibility for their integrity and for the accuracy of the data analysis. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio Russo reports a relationship with Bristol Myers Squibb Co that includes: consulting or advisory. Antonio Russo reports a relationship with Pfizer Inc that includes: consulting or advisory. Antonio Russo reports a relationship with Bayer Corporation that includes: consulting or advisory. Antonio Russo reports a relationship with Kyowa Kirin Inc that includes: consulting or advisory. Antonio Russo reports a relationship with Roche Diagnostics that includes: speaking and lecture fees. Antonio Galvano reports a relationship with Roche Diagnostics that includes: consulting or advisory. Antonio Galvano reports a relationship with Servier Italy SpA that includes: consulting or advisory. Valerio Gristina reports a relationship with AstraZeneca that includes: consulting or advisory. Valerio Gristina reports a relationship with Bristol Myers Squibb Co that includes: consulting or advisory. Valerio Gristina reports a relationship with IQVIA that includes: consulting or advisory. Valerio Gristina reports a relationship with MDS Medical Technologies SL that includes: consulting or advisory. Valerio Gristina reports a relationship with Novartis Pharmaceuticals Corporation that includes: consulting or advisory. Lorena Incorvaia reports a relationship with Ipsen that includes: speaking and lecture fees. Lorena Incorvaia reports a relationship with Bristol-Myers Squibb Co that includes: speaking and lecture fees. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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