Extracellular Vesicles Mediate Drug Resistance in Melanoma

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Melanoma represents only 1% of human skin cancers, but in several cases can lead to the death of the patient. Nowadays, there are different systemic therapies used for the treatment of human melanoma. Although these substantially improve patients' lifespan, they are still associated with resistance. Extracellular vesicles (EVs), tiny vesicles released by tumor cells involved in intercellular communication, play an important role in melanoma pathogenesis and progression. They are crucially involved in several mechanisms of cancer drug resistance in several types of cancer, and there is a strong indication that EVs released by melanoma cells might play a role in the development of resistance, modulating the response towards anti-cancer drugs.

cancer melanoma extracellular vesicles

1. Systemic Therapies for Melanoma

According to the American Cancer Society, melanoma represents only 1% of skin cancers in humans, but in several cases it can lead to the death of the patients. The first-line defense for the treatment of this neoplasia is surgical resection. It has been demonstrated that resection of melanoma is associated with a decreased number of circulating extracellular vesicles (EVs) in humans [1][2].

Nowadays there are different systemic therapies used for the treatment of human melanoma, such as neoadjuvant/adjuvant therapy or primary treatment:

- Chemotherapy: cisplatin, temozolomide, vincristine and vinblastine are some of the chemotherapeutic drugs most frequently used to treat advanced-stage melanoma [3]. This happened in the past when this was the only systemic therapy used to treat stage IV melanoma patients. Nowadays, even if a reduction in tumor size occurs with the administration of these drugs, there is no evidence of survival advantages [4]. Currently, chemotherapy is almost no longer administrated in most melanoma patients and represents only a last-line treatment in those cases in which a resistance towards immune check-point inhibitors (ICI) and target therapy occurs [5]. However, chemotherapy can still be used when it represents the only alternative available and for those melanoma patients without BRAF mutation who develop a toxicity reaction after the administration of ICI [4];

- Small Molecule Targeted Therapy: such as BRAF-inhibitors (i.e., vemurafenib, dabrafenib), MEK-inhibitors (i.e., trametinib) and the combination of BRAF and MEK-inhibitors [3]. About half of cutaneous melanoma patients carry BRAF V600 mutation. An increase in the overall survival and progression-free survival occurs after the

administration of vemurafenib and dabrafenib in patients with BRAFV600E mutation [6][7]. Additionally, an increase in the response rate, in the overall survival and in the progression free survival, occurs after the administration of BRAF and MEK-inhibitors in combination [4].

- Immune Checkpoint Inhibitors (ICI): such as CTLA-4 inhibitors (i.e., ipilimumab) and anti-PD1 therapy (i.e., nivolumab, pembrolizumab). The combination of nivolumab and ipilimumab leads to the best outcome, and the Food and Drug Administration (FDA) approved this cocktail as the best treatment for both advanced BRAF-negative melanoma patients [3][8][9] and for melanoma patients with BRAF mutation [4]. Up to now, since PD-1 antibodies have been approved, ipilimumab is used in combination with the antibodies or by itself as a second treatment option [4].

The scientific community is trying to both improve the effectiveness of these melanoma therapies by limiting their side effects and identifying the best candidate patients for each different type of therapy [4]. Considering the key role of EVs in melanoma pathogenesis and progression, and in light of their crucial involvement in several mechanisms of cancer drug resistance, there is a strong indication that EVs released by melanoma cells might play a role in the development of resistance, modulating the response towards anti-cancer drugs.

The results of the most relevant published works on this topic, divided into different types of melanoma treatments, will be listed and discussed in the following paragraphs. However, to better understand the role of EVs in melanoma drug resistance, further studies are needed.

2. Mechanisms of Extracellular Vesicle-Mediated Resistance towards Chemotherapeutic Drugs

As mentioned above, chemotherapy currently represent only a last-line treatment, although it may rarely still be administrated [5]. Therefore, it is relevant to mention the published works investigating the EV-mediated mechanisms of resistance to these systemic drugs, which may be worthy of further investigation.

It is well known that chemotherapy drugs are associated with resistance [10][11][12], and an increased number of EVs released by melanoma cells has been reported as a consequence of chemotherapy [13].

Cisplatin is an alkylating agent able to interfere with the DNA replication once inside tumor cells. The uptake and the efflux of cisplatin is regulated by several mechanisms and one of them is the pH variation. An acidic environment leads to the selection of cells with elevated levels of proton-pump activity, actively eliminating the drug and becoming resistant cells. The acidic environment itself contributes by lowering the extracellular pH, decreasing the pH inside EVs [14][15] and increasing the secretion of EVs from human melanoma cells. On the contrary, the administration of proton pump inhibitors (PPI) (anti-acidic drugs used for the inhibition of the H^+K^+ATPases) could reduce the release of EVs from tumor cells [16]. Hence, the hypothesis that acidic vesicles can play a fundamental role in the resistance towards cytotoxic drugs through both the sequestration and neutralization of alkaline drugs (i.e., cisplatin), as well as through the elimination of these molecules towards a vesicles-mediated mechanism [17][18][19].

Federici et al., in 2014, tried to better understand the process that leads human melanoma cells to the development of resistance towards cisplatin through exposing human melanoma cell lines to the drug. Results confirmed not only the important role played by acidification in cisplatin uptake by melanoma cells, but also highlighted the key role of EVs in the development of resistance. By the analysis of the content of EVs collected from the conditioned culture medium, cisplatin was found at differing levels depending on the pH of the medium. Higher levels of cisplatin were found in association with lower pH condition. Thus, their results strongly indicated the involvement of EVs in the secretory pathway leading to the elimination of the cisplatin and contributing to the development of drug resistance [20].

Drug sequestration and efflux are not the only methods used by EVs to decrease the response of melanoma cells to chemotherapeutic agents. It has been observed that in both human melanoma cell lines treated with temozolomide (TMZ) and in a murine melanoma cell line treated with cisplatin, alkylating drugs can induce the increase in EVs' shedding and uptake by melanoma cell lines in a dose-dependent manner. In this case, however, the authors found that the EV-mediated resistance was not "direct", through a melanoma cell-to-cell communication via EVs. In fact, no alteration in the sensitivity to TMZ or cisplatin was observed after the treatment of naïve melanoma cell lines with EVs isolated from the same cell line pre-treated with the drugs [13]. However, when incubating macrophages obtained from bone marrow cells with EVs isolated from melanoma cell lines pre-treated with TMZ, they noticed a change in the polarization toward a M2 phenotype, with an upregulation of M2-marker genes. Since the M2 phenotype is commonly associated with the development and progression of the tumor, this suggests that EVs can contribute to melanoma progression and to the development of resistance towards these drugs also through a different, "indirect" mechanism of action [13][21] by the modulation of the tumor associated immune response.

3. Extracellular Vesicles Cargo in the Regulation of Target-Therapies

Around 50% of the patients with melanoma have a mutation in the Ser/Thr-Kinase BRAF (most of the time V600E). Therefore, the introduction of specific BRAF-inhibitors had represented a crucial point in the treatment of this neoplasia. However, the development of resistance with the reactivation of the MAPK pathway occurs quickly, highlighting the importance of focusing research on the mechanisms underlying this resistance [22].

Three studies that have specifically investigated EV-mediated drug resistance in melanoma towards BRAF-inhibitor therapy are discussed below.

Two of them demonstrated that, after treatment with two commonly used BRAF inhibitors (vemurafenib and dabrafenib), the EVs released by resistant melanoma cells contained factors linked to melanoma resistance and BRAF inhibitors, such as miR211-5p and PDGFR.

In the first of these two studies, Lunavat et al. (2017) observed that treatment with BRAF-inhibitors was able to change the miRNA cargo of EVs. They treated two melanoma cell lines with V^{600E} mutation (MML-1 and A375) and

a primary melanoma cell line obtained from MML-1 cells transplanted mice, inducing an increased levels of miR-211-5p in both cells and small EVs isolated from their conditioned medium [23]. It is demonstrated that miR211-5p is involved in the resistance to BRAF-inhibitors, since RNA-sequence data have shown higher levels of this miRNA in resistant cells compared to sensitive cells [24][25]. Moreover, Lunavat et al. observed that melanoma cells transfected with mRNA-211-5p showed a reduced sensitivity to vemurafenib treatment and that the inhibition of mRNA-211-5p in the resistant cell line affected proliferation negatively. However, it needs to be further investigated whether EVs are indeed capable of transferring this active miRNA to sensitive cells by performing more mechanistic studies.

Vella et. al. (2017) investigated whether EVs released by melanoma cells resistant to BRAF-inhibitors can modify the response of sensitive recipient cells after their exposure to these drugs. They used two melanoma cell lines: sensitive (LM-MEL-64) and resistant (LM-MEL-64R3). In the latter, resistance was due to the reactivation of the PI3K/AKT/AKT pathway, specifically linked to the higher phosphorylation of two RTKs (i.e., Receptors Tyrosine-Kinase), EGFR and PDGFRβ. In melanoma, after the exposure to BRAF-inhibitors, two different pathways can be reactivated, resulting in the development of resistance: the MAPK and the PI3K/AKT/AKT pathways [26]. With these experiments, the authors found that small EVs released from resistant melanoma cells had more PDGFRβ expression compared to sensitive cells and proved that EV-derived PDGFRβ can also be transferred from resistant to sensitive cell lines. They showed that when LM-MEL-64 exposed to LM-MEL-64R3 derived EVs were treated with PLX4720, the treatment did not reduce their growth compared to the ones treated with EVs from the sensitive cell line. Moreover, they demonstrated the reactivation, in the sensitive recipient cells, of the PI3K/AKT pathway in a dose-dependent manner; the more EVs the cells received, the higher the increase in pAKT detected. Authors also found that the development of resistance obtained through EVs carrying PDGFRβ was not just a prerogative of the cell line LM-MEL-64, but also of another melanoma cell line (M229AR) after the exposure to BRAF-inhibitors and linked to resistance [27]. All together, these results show that the role of PDGFRβ-carrying EVs is essential to preserving the functionality of one of the main mechanisms related to resistance after administration of MAPK-pathway inhibitors, the reactivation of the PI3K/AKT pathway.

In the last paper, Cesi et al. (2018) focused their attention on a truncated form of the receptor ALK (anaplastic lymphoma kinase), which they named ALK^{RES}, upregulated in several neoplasia, including melanoma. Eleven % of melanoma tissues present a truncated ALK transcript resulting in a smaller protein, which was shown to be oncogenic [28]. ALK^{RES} is involved in the development of BRAF-inhibitor resistance through the reactivation of MAPK pathways, while in the absence of the mutation, resistant cells respond to both BRAF and MEK inhibitors. Using an in vivo assay in A375-X1-resistant melanoma cell-transplanted mice, they first proved that treatment with combined BRAF and ALK inhibitors can stop tumor growth. Subsequently, they demonstrated that this resistance can be transmitted through EVs. EVs can transfer ALK^{RES}, and by proteomic analysis of the EV cargo they also demonstrated that ALK^{RES} remain functional after being transferred in recipient cells. Indeed, by the determination of the dose response to the BRAF-inhibitor PLX4032 following EVs' uptake by IC_{50} calculation, they found no significant differences after incubation with sensitive-cells-derived EVs, while significantly higher IC_{50} was measured after incubation with resistance-cells-derived EVs, demonstrating that the drug resistant phenotype can be mediated by EVs [22].

These three studies represent a fundamental starting point to further understand the role of EVs in target-therapy resistance mechanisms in melanoma and they prove the fundamental role of EVs in the development of melanoma-resistance regarding the use of BRAF-inhibitors that are worth further investigations.

4. The Role of Extracellular Vesicles in Immune Check-Point Inhibitors

The immunogenicity of a tumor is its ability to activate an adaptive immune response able to prevent its growth. However, the fundamental prerogative is that cells must express an adequate amount of antigen capable of raising immune activation instead of immune tolerance [29]. Melanoma is one of the most immunogenic tumors, and there are frequently many immune cells reflecting a reaction of the host towards the neoplasm. However, tumor progression can also occur in the presence of an antitumor response implemented by the immune system; melanoma is able to progress in the presence of an abundant lymphocytic infiltrate, suggesting that the immune response can fail in effectively controlling tumor growth. Human melanoma often develops in an environment rich in immune cells, especially lymphocytes, which release their cytokines, contributing to an anti-tumor response [30]. Melanoma cells have a unique immunogenic profile and provide a model for investigating the molecular interaction of neoplastic cells with those of the immune system. Several studies in this field contributed to the discovery of new target molecules on immune cells for the development of effective therapeutic strategies [29]. Several studies have been performed discovering new biomarkers (in liquid biopsies) able to predict response to treatment to ICI and a number of molecules (protein and RNA) have been identified [31][32].

The role of EVs in immune response has been extensively studied, including their role in melanoma development and progression [33][34]. By analysis of the literature, several studies can be found demonstrating the utility of EVs as predictive factors in melanoma: PD-1$^+$ [31][32], PD-L1$^+$ [32], CD8$^+$ [31] and uPAR$^+$ [35] EVs represent biomarkers able to predict the response to ICI. However, only a few studies tried to deeply investigate the mechanism through which EVs can contribute to the development of resistance towards ICI. Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells whose presence is associated with cancers. They can inhibit the activity of antitumor T cells [36] and are able to activate pathways associated with cell resistance [37][38]. Huber et al. showed by in vitro studies that melanoma EVs can mediate the transition of monocytes to MDSCs [39]. Incubating CD14$^+$ monocytes for 24 h with EVs isolated from melanoma cell cultures, authors observed the differentiation of the cells towards a new phenotype that they called EV-MDSCs. These cells were able to downregulate the expression of HLA-DRA (Major Histocompatibility Complex, Class II, DR Alpha), enhance the transcription of IL-6 and CCL2 and inhibit the activity of T cells. Similar results were obtained incubating CD14$^+$ monocytes for 24 h with EVs isolated from the plasma of patients with advanced melanoma [39]. Further investigating the underlying molecular mechanisms by genome-wide transcriptional analyses, the authors showed that under the phenotypic change of CD14+ cells, the transfer of miRNAs occurred. Since the lack of local T cells' immune reactivity is one of the causes of resistance towards ICI, they demonstrated that EVs can contribute to the development of resistance, also mediating this phenotypic change of CD14+ cells. Furthermore, Xiao et al. showed that EVs released from melanoma cells bring two different types of miRNAs (miR-191 and Let-7a) that can modulate the process, leading to: (a) melanoma

phenotyping switching (a process similar to the epithelial-to-mesenchymal-transition, EMT), (b) loss of adhesion factors and (c) regression toward a mesenchymal phenotype [40].

Melanoma-derived EVs express large amounts of PD-L1 on their surface [41] and these EVs are able to reach the lymph nodes inactivating the T cell response [42]. Serratí et al., besides investigating the role of PD-L1$^+$ and PD1$^+$ melanoma EVs, also tried to understand their involvement in the development of resistance toward ICI [32]. They isolated EVs from the blood of responders and non-responder melanoma patients, stained them and then incubated with PBMCs previously isolated from the blood of healthy donors, responders and non-responders. Using LND1 cells (a BRAF wt melanoma cell line) they created spheroids, adding PBMCs onto them to study the trafficking towards the tumor of these cells. They observed a reduction in the trafficking to the tumors for the PBMCs derived from melanoma patients compared with those obtained from healthy donors. Moreover, they showed a decrease in trafficking for the PBMCs that had been previously incubated with EVs from responders and, above all, for those cells incubated with EVs from non-responders. Interestingly, after the addition of nivolumab on spheroids, cell death was reduced for those cells previously incubated with EVs from responders and, once again, the phenomenon was even more evident for those incubated with Evs from non-responders [32].

Taken together, these results represent the proof that EVs are directly involved in the development of resistance in ICI-therapy.

References

1. Bollard, S.M.; Casalou, C.; Goh, C.Y.; Tobin, D.J.; Kelly, P.; McCann, A.; Potter, S.M. Circulating Melanoma-Derived Extracellular Vesicles: Impact on Melanoma Diagnosis, Progression Monitoring, and Treatment Response. Pharmaceuticals 2020, 13, 475.
2. DePeralta, D.; Michaud, W.; Hammond, M.; Boland, G. 44.03 Circulating Microvesicles, Exosomes, Are Enriched in Melanoma and Correlate with Tumor Burden. In Proceedings of the Academic Surgical Congress, Jacksonville, FL, USA, 2–4 February 2016.
3. Bomar, L.; Senithilnathan, A.; Ahn, C. Systemic Therapies for Advanced Melanoma. Dermatol. Clin. 2019, 37, 409–423.
4. Garbe, C.; Amaral, T.; Peris, K.; Hauschild, A.; Arenberger, P.; Basset-Seguin, N.; Bastholt, L.; Bataille, V.; del Marmol, V.; Dréno, B.; et al. European Consensus-Based Interdisciplinary Guideline for Melanoma. Part 2: Treatment—Update 2022. Eur. J. Cancer 2022, 170, 256–284.
5. Goldinger, S.M.; Buder-Bakhaya, K.; Lo, S.N.; Forschner, A.; McKean, M.; Zimmer, L.; Khoo, C.; Dummer, R.; Eroglu, Z.; Buchbinder, E.I.; et al. Chemotherapy after Immune Checkpoint Inhibitor Failure in Metastatic Melanoma: A Retrospective Multicentre Analysis. Eur. J. Cancer 2022, 162, 22–33.

6. Chapman, P.B.; Hauschild, A.; Robert, C.; Haanen, J.B.; Ascierto, P.; Larkin, J.; Dummer, R.; Garbe, C.; Testori, A.; Maio, M.; et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. N. Engl. J. Med. 2011, 364, 2507–2516.
7. Sosman, J.A.; Kim, K.B.; Schuchter, L.; Gonzalez, R.; Pavlick, A.C.; Weber, J.S.; McArthur, G.A.; Hutson, T.E.; Moschos, S.J.; Flaherty, K.T.; et al. Survival in BRAF V600–Mutant Advanced Melanoma Treated with Vemurafenib. N. Engl. J. Med. 2012, 366, 707–714.
8. Hodi, F.S.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Rutkowski, P.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Wagstaff, J.; Dummer, R.; et al. Nivolumab plus Ipilimumab or Nivolumab Alone versus Ipilimumab Alone in Advanced Melanoma (CheckMate 067): 4-Year Outcomes of a Multicentre, Randomised, Phase 3 Trial. Lancet. Oncol. 2018, 19, 1480–1492.
9. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.; et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N. Engl. J. Med. 2015, 373, 23–34.
10. Wagstaff, W.; Mwamba, R.N.; Grullon, K.; Armstrong, M.; Zhao, P.; Hendren-Santiago, B.; Qin, K.H.; Li, A.J.; Hu, D.A.; Youssef, A.; et al. Melanoma: Molecular Genetics, Metastasis, Targeted Therapies, Immunotherapies, and Therapeutic Resistance. Genes Dis. 2022, 9, 1608–1623.
11. Zhong, J.; Yan, W.; Wang, C.; Liu, W.; Lin, X.; Zou, Z.; Sun, W.; Chen, Y. BRAF Inhibitor Resistance in Melanoma: Mechanisms and Alternative Therapeutic Strategies. Curr. Treat. Options Oncol. 2022, 23, 1503–1521.
12. Shui, I.M.; Scherrer, E.; Frederickson, A.; Li, J.W.; Mynzhassarova, A.; Druyts, E.; Tawbi, H. Resistance to Anti-PD1 Therapies in Patients with Advanced Melanoma: Systematic Literature Review and Application of the Society for Immunotherapy of Cancer Immunotherapy Resistance Taskforce Anti-PD1 Resistance Definitions. Melanoma Res. 2022, 32, 393–404.
13. Andrade, L.N.d.S.; Otake, A.H.; Cardim, S.G.B.; da Silva, F.I.; Ikoma Sakamoto, M.M.; Furuya, T.K.; Uno, M.; Pasini, F.S.; Chammas, R. Extracellular Vesicles Shedding Promotes Melanoma Growth in Response to Chemotherapy. Sci. Rep. 2019, 9, 14482.
14. Simon, S.; Roy, D.; Schindler, M. Intracellular PH and the Control of Multidrug Resistance. Proc. Natl. Acad. Sci. USA 1994, 91, 1128–1132.
15. Mahoney, B.P.; Raghunand, N.; Baggett, B.; Gillies, R.J. Tumor Acidity, Ion Trapping and Chemotherapeutics. I. Acid PH Affects the Distribution of Chemotherapeutic Agents in Vitro. Biochem. Pharmacol. 2003, 66, 1207–1218.
16. Parolini, I.; Federici, C.; Raggi, C.; Lugini, L.; Palleschi, S.; De Milito, A.; Coscia, C.; Iessi, E.; Logozzi, M.; Molinari, A.; et al. Microenvironmental PH Is a Key Factor for Exosome Traffic in Tumor Cells. J. Biol. Chem. 2009, 284, 34211–34222.

17. Ouar, Z.; Lacave, R.; Bens, M.; Vandewalle, A. Mechanisms of Altered Sequestration and Efflux of Chemotherapeutic Drugs by Multidrug-Resistant Cells. Cell Biol. Toxicol. 1999, 15, 91–100.
18. Raghunand, N.; He, X.; Van Sluis, R.; Mahoney, B.; Baggett, B.; Taylor, C.W.; Paine-Murrieta, G.; Roe, D.; Bhujwalla, Z.M.; Gillies, R.J. Enhancement of Chemotherapy by Manipulation of Tumour PH. Br. J. Cancer 1999, 80, 1005–1011.
19. Raghunand, N.; Martínez-Zaguilán, R.; Wright, S.H.; Gillies, R.J. PH and Drug Resistance. II. Turnover of Acidic Vesicles and Resistance to Weakly Basic Chemotherapeutic Drugs. Biochem. Pharmacol. 1999, 57, 1047–1058.
20. Federici, C.; Petrucci, F.; Caimi, S.; Cesolini, A.; Logozzi, M.; Borghi, M.; D'Ilio, S.; Lugini, L.; Violante, N.; Azzarito, T.; et al. Exosome Release and Low PH Belong to a Framework of Resistance of Human Melanoma Cells to Cisplatin. PLoS ONE 2014, 9, e88193.
21. Bardi, G.T.; Smith, M.U.; Hood, J.L. Melanoma exosomes promote mixed M1 and M2 macrophage polarization. Cytokine 2018, 105, 63–72.
22. Cesi, G.; Philippidou, D.; Kozar, I.; Kim, Y.J.; Bernardin, F.; Van Niel, G.; Wienecke-Baldacchino, A.; Felten, P.; Letellier, E.; Dengler, S.; et al. A New ALK Isoform Transported by Extracellular Vesicles Confers Drug Resistance to Melanoma Cells. Mol. Cancer 2018, 17, 145.
23. Lunavat, T.R.; Cheng, L.; Einarsdottir, B.O.; Bagge, R.O.; Muralidharan, S.V.; Sharples, R.A.; Lässer, C.; Gho, Y.S.; Hill, A.F.; Nilsson, J.A.; et al. BRAFV600 Inhibition Alters the MicroRNA Cargo in the Vesicular Secretome of Malignant Melanoma Cells. Proc. Natl. Acad. Sci. USA 2017, 114, E5930–E5939.
24. Díaz-Martínez, M.; Benito-Jardón, L.; Alonso, L.; Koetz-Ploch, L.; Hernando, E.; Teixidó, J. MiR-204-5p and MiR-211-5p Contribute to BRAF Inhibitor Resistance in Melanoma. Physiol. Behav. 2017, 176, 139–148.
25. Michniewicz, A.G.; Czyz, M. Role of Mirnas in Melanoma Metastasis. Cancers 2019, 11, 326.
26. Welsh, S.J.; Rizos, H.; Scolyer, R.A.; Long, G.V. Resistance to Combination BRAF and MEK Inhibition in Metastatic Melanoma: Where to Next? Eur. J. Cancer 2016, 62, 76–85.
27. Vella, L.J.; Behren, A.; Coleman, B.; Greening, D.W.; Hill, A.F.; Cebon, J. Intercellular Resistance to BRAF Inhibition Can Be Mediated by Extracellular Vesicle–Associated PDGFRβ. Neoplasia 2017, 19, 932–940.
28. Wiesner, T.; Lee, W.; Obenauf, A.C.; Ran, L.; Murali, R.; Zhang, Q.F.; Wong, E.W.P.; Hu, W.; Scott, S.N.; Shah, R.H.; et al. Alternative Transcription Initiation Leads to Expression of a Novel ALK Isoform in Cancer. Nature 2015, 526, 453–457.
29. Passarelli, A.; Mannavola, F.; Stucci, L.S.; Tucci, M.; Silvestris, F. Immune System and Melanoma Biology: A Balance between Immunosurveillance and Immune Escape. Oncotarget 2017, 8,

106132–106142.

30. Porcellato, I.; Silvestri, S.; Menchetti, L.; Recupero, F.; Mechelli, L.; Sforna, M.; Iussich, S.; Bongiovanni, L.; Lepri, E.; Brachelente, C. Tumour-Infiltrating Lymphocytes in Canine Melanocytic Tumours: An Investigation on the Prognostic Role of CD3+ and CD20+ Lymphocytic Populations. Vet. Comp. Oncol. 2020, 18, 370–380.
31. Tucci, M.; Passarelli, A.; Mannavola, F.; Stucci, L.S.; Ascierto, P.A.; Capone, M.; Madonna, G.; Lopalco, P.; Silvestris, F. Serum Exosomes as Predictors of Clinical Response to Ipilimumab in Metastatic Melanoma. Oncoimmunology 2018, 7, e1387706.
32. Serratì, S.; Guida, M.; Di Fonte, R.; De Summa, S.; Strippoli, S.; Iacobazzi, R.M.; Quarta, A.; De Risi, I.; Guida, G.; Paradiso, A.; et al. Circulating Extracellular Vesicles Expressing PD1 and PD-L1 Predict Response and Mediate Resistance to Checkpoint Inhibitors Immunotherapy in Metastatic Melanoma. Mol. Cancer 2022, 21, 20.
33. Zhou, Q.; Yan, Y.; Li, Y.; Fu, H.; Lu, D.; Li, Z.; Wang, Y.; Wang, J.; Zhu, H.; Ren, J.; et al. Tumor-Derived Extracellular Vesicles in Melanoma Immune Response and Immunotherapy. Biomed. Pharmacother. 2022, 156, 113790.
34. Dieterich, L.C. Mechanisms of Extracellular Vesicle-Mediated Immune Evasion in Melanoma. Front. Immunol. 2022, 13, 4905.
35. Porcelli, L.; Guida, M.; De Summa, S.; Di Fonte, R.; De Risi, I.; Garofoli, M.; Caputo, M.; Negri, A.; Strippoli, S.; Serratì, S.; et al. UPAR+ Extracellular Vesicles: A Robust Biomarker of Resistance to Checkpoint Inhibitor Immunotherapy in Metastatic Melanoma Patients. J. Immunother. Cancer 2021, 9, e002372.
36. Gabrilovich, D.I. Myeloid-Derived Suppressor Cells. Cancer Immunol. Res. 2017, 5, 3–8.
37. Umansky, V.; Blattner, C.; Gebhardt, C.; Utikal, J. The Role of Myeloid-Derived Suppressor Cells (MDSC) in Cancer Progression. Vaccines 2016, 4, 36.
38. Bronte, V.; Brandau, S.; Chen, S.H.; Colombo, M.P.; Frey, A.B.; Greten, T.F.; Mandruzzato, S.; Murray, P.J.; Ochoa, A.; Ostrand-Rosenberg, S.; et al. Recommendations for Myeloid-Derived Suppressor Cell Nomenclature and Characterization Standards. Nat. Commun. 2016, 7, 12150.
39. Huber, V.; Vallacchi, V.; Fleming, V.; Hu, X.; Cova, A.; Dugo, M.; Shahaj, E.; Sulsenti, R.; Vergani, E.; Filipazzi, P.; et al. Tumor-Derived MicroRNAs Induce Myeloid Suppressor Cells and Predict Immunotherapy Resistance in Melanoma. J. Clin. Investig. 2018, 128, 5517–5530.
40. Xiao, D.; Barry, S.; Kmetz, D.; Egger, M.; Pan, J.; Rai, S.N.; Qu, J.; McMasters, K.M.; Hao, H. Melanoma Cell-Derived Exosomes Promote Epithelial-Mesenchymal Transition in Primary Melanocytes through Paracrine/Autocrine Signaling in the Tumor Microenvironment. Cancer Lett. 2016, 376, 318–327.

41. Van Niel, G.; D’Angelo, G.; Raposo, G. Shedding Light on the Cell Biology of Extracellular Vesicles. Nat. Rev. Mol. Cell Biol. 2018, 19, 213–228.
42. Poggio, M.; Hu, T.; Pai, C.C.; Chu, B.; Belair, C.D.; Chang, A.; Montabana, E.; Lang, U.E.; Fu, Q.; Fong, L.; et al. Suppression of Exosomal PD-L1 Induces Systemic Anti-Tumor Immunity and Memory. Cell 2019, 177, 414–427.e13.