Checkpoint Inhibitors in Solid Tumors

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The emergence of cancer immunotherapy has already shown some remarkable results, having changed the treatment strategy in clinical practice for solid tumors. Despite these promising long-term responses, patients seem to lack the ability to respond to immune checkpoint inhibitors, thus demonstrating a primary resistance to immunotherapy. Moreover, a significant number of patients who initially respond to treatment eventually acquire resistance to immunotherapy. Both resistance mechanisms are a result of a complex interaction among different molecules, pathways, and cellular processes. Several resistance mechanisms, such as tumor microenvironment modification, autophagy, genetic and epigenetic alterations, tumor mutational burden, neo-antigens, and modulation of gut microbiota have already been identified, while more continue to be uncovered.

Keywords: solid tumors ; cancer ; immunotherapy ; resistance mechanism

1. Introduction

The concept of immune therapy to fight against cancer was first described in 1890 by W. Colley, who observed cancer remissions after inoculating sarcoma patients with erysipelas cultures [1]. Significant progress has been made since then and has provided an in-depth understanding of tumor biology and interactions within the immune system. Further knowledge of the relationship between the immune system and cancer can be derived under the scope of a process named immune-editing, which comprises three distinct phases: elimination, equilibrium, and escape [2]. An intact immune system is capable of recognizing cancer cell’s antigens as “non-self", and thus unleashing sufficient immune responses in order to induce elimination. Cancer cells surviving the elimination phase may rest in a dormant situation, unable to progress under the opposing forces of immune cells. In the escape phase, cancer cells are capable of altering their main characteristics with a loss of immunogenicity and/or antigenicity. Additionally, malignant cells can gain additional immunosuppressive properties, such as expression of programmed death-ligand 1 (PD-L1) or secretion of suppressive cytokines, which result in an additional reduction of their immunogenicity [3][4].

The principal mode of action of the immune system is the recognition by cells of the innate immune system, the release of various cytokines, and complement activation and concomitant phagocytosis. Tumor development is associated with cytotoxic innate and adaptive immune cells, but as the tumor evolves from neoplastic tissue to detectable tumors, tumor cells use different mechanisms in order to create an immunosuppressive recapitulate peripheral immune tolerance [5].

This immune activation is under strict control by inhibitory checkpoints that prevent overstimulation, resulting in autoimmunity and extensive damage to healthy cells [6]. Unfortunately, tumor cells develop resistance mechanisms in order to avoid the presence of an efficient immune system. Tumor cells escape from immune response through two main mechanisms: avoiding the immune-cancer cell recognition and triggering an immunosuppressive tumor microenvironment (TME). Firstly, cancer cells rapidly decrease the expression of tumor antigens on the cell surface, thus avoiding being recognized by cytotoxic T cells. Moreover, tumor cell-derived factors initiate an immune-tolerant TME through (i) secretion of inhibitory checkpoint molecules (ii) induction of the recruitment of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) and (iii) expression of small suppressive molecules [4][7]. In order to evade immune destruction, tumor cells express inhibitory checkpoints that induce immune suppression, a mechanism that has been thoroughly investigated, which leads to the development of several molecules able to restrain cancer-induced immuno-suppression (i.e., anti-CTLA4, anti-PD1, and anti-PDL1). These immune checkpoint inhibitors now form a new landscape in cancer therapeutics, having shown remarkable response and survival rates in a variety of tumors. However, many cancer patients do not derive benefit from immune checkpoint inhibitors, an observation that has led to the assumption that resistance to immunotherapy may be present [8][9].

Resistance to immunotherapy can be categorized as primary and acquired, depending on the presence or absence of initial response and subsequent relapse after the response that has been achieved. The mechanisms involved in the development of resistance include tumor-cell intrinsic and extrinsic factors that can either interfere with the antigen processing and presentation or enable tumors to recruit immune-suppressing cells that antagonize the activity of effectors cells [6]. With the advent of the immune checkpoint blockade, cancer therapeutics shifted from a tumor-cell focused approach to a broader concept of factors contributing to tumor formation. This has led to the recognition of the tumor microenvironment not only as a significant player supporting tumor growth and metastasis but also as a contributing one to the development of therapeutic resistance. In the context of immunotherapy, TME is regarded as a critical mediator of tumor-induced immuno-suppression through a variety of mechanisms, resulting in the down-regulation of both the effector T-cell activity and the recruitment of immunosuppressive cells [10].

2. Immunotherapy in Solid Tumors

The significant advance of the checkpoint inhibitors (anti-CTLA-4, anti-PD-1/PD-L1) has indicated as a hopeful therapeutic option in several solid tumors. CTLA-4 antibodies, like ipilimumab and tremelimumab, interrupt CTLA-4 from interacting with B7 and boost T-cell activation. The expression of CTLA-4 on tumors has been linked with poor survival in nasopharyngeal carcinoma [11] and increased survival in non-small cell lung cancer (NSCLC) [12]. The expression of PDL1 on tumor cells is high. On ligand binding by PD-L1, the PD-1 receptor inhibits the activation and proliferation of T-cells through a phosphatase, SHP-2, which de-phosphorylates the antigen receptor expressed by these cells. High PD-1/PD-L1 levels may associate with poor prognosis in some cancers (melanoma, esophageal, renal cell carcinoma, ovarian cancer) and with better prognosis in others (e.g., angiosarcoma and gastric cancer) [13]. PD-1 antibodies (such as nivolumab and pembrolizumab) and PD-L1 (such as atezolizumab) break off the interaction of PD-1 with PD-L1. In the last decade, numerous checkpoint inhibitors (e.g., ipilimumab, nivolumab, pembrolizumab, atezolizumab) got approval in various solid tumors such as melanoma, lung, renal, and bladder [14].

More specifically, in 2011, ipilimumab was the first approved checkpoint inhibitor, in patients with metastatic melanoma (shows better overall survival-OS). Pembrolizumab and nivolumab were also among the approved PD-1 inhibitors for advanced melanoma. Synergistic therapy with ipilimumab plus nivolumab led to better PFS compared to each drug being used separately (11.5 vs. 2.9 months only with ipilimumab and 6.9 months only with nivolumab) [15].

The first immunotherapy permitted (2015) was nivolumab for the treatment of patients with metastatic NSCLC. Between patients with advanced non-squamous NSCLC that had progressed during or after platinum-based chemotherapy, OS was better by 41% (nivolumab vs. docetaxel) [16]. Moreover, first-line treatment with nivolumab along with ipilimumab resulted in a better OS than only chemotherapy, in patients with NSCLC, independent of the PD-L1 expression [17].

Furthermore, the combination of nivolumab plus ipilimumab versus sunitinib shows significantly better OS rates among intermediate and poor-risk patients with previously untreated advanced renal-cell carcinoma [18].

Pembrolizumab was also used for second-line treatment of patients with NSCLC that express PD-L1. Additionally, it is authorized for the handling of patients with advanced NSCLC, high PDL1 expression, and metastatic melanoma patients and patients with recurrent/metastatic of the head and neck squamous cell carcinoma [14].

Atezolizumab (PD-L1 inhibitor), is already used for the treatment of patients with locally advanced or metastatic urothelial carcinoma due to its favorable safety profile compared to chemotherapy [19]. Moreover, the administration of atezolizumab versus bevacizumab plus chemotherapy significantly improved PFS and OS between patients with metastatic non-squamous NSCLC, independently of PD-L1 expression and EGFR/ALK genetic background [20].

3. Ways to Overcome the Resistance Mechanism Against Checkpoint Inhibitors

In recent years, the field of immune-oncology has established an increased understanding of molecular behavior of cancer, leading to the development of several therapeutics strategies, based on re-activation of immune system, against solid tumors. Despite the demonstrated successes of checkpoint inhibitors (ant-PD-1, anti-PD-L1, ant-CTLA4 etc.), most patients with solid tumors do not respond.

It is a common belief that PD-L1 expression in tumor cells immunohistochemistry (IHC) with the Tumor Proportional Score (TPS) is the only checkpoint inhibitor that is used as a predictive biomarker approved for NSCLC patients in first- and second-line treatment [21][22]. Unfortunately, checkpoint inhibitors against PD-1/PD-L1 have not been shown to play an essential role in predicting the immune response in other solid tumors or different settings. Moreover, the lack of PD-L1 expression in several cancers (as a biomarker), at a single time point may not fully represent the complexity of cancer cell communication network within TME [23][24].

The last years, research efforts revealed the complex and highly heterogeneous structure of TME. As it was mentioned before in the current review, TME is a main resistance mechanism against ICI. The following can be used to reduce the resistance of TME: (a) Upregulation of chemokines (CXCL) 9 and 10. Doxorubicin may induce the activity of CXCL10. The goal of a phase I/II study is to evaluate the effect of doxorubicin hydrochloride when given together with pembrolizumab in patients with sarcoma (NCT02888665); (b) activation of the endosomal toll-like receptors (TLRs) 3, 7, 8 and 9 [25]; (c) epigenetic silencing of Th1 cell-type chemokines; (d) blockade of the CXCL12/CXCR4 axis; (e) inhibition of MDSC using PI3K inhibitors;and (f) use of antiangiogenic drugs [26]. Several ongoing clinical trials try to investigate the role of antiangiogenic agents in order to enhance the effect of ICI. For example in a phase I/II study they combined lenvatinib (VEGFR inhibitor) with pembrolizumab in patients with advanced solid tumors (NCT02501096) (g) use of low molecular weight heparins (LMWHs) [27] (h) combined radiation therapy and PD-1/PD-L1 blockade, leading to an increased CD8+/Treg ratio and decreases immunosuppressive MDSCs. The investigators in a randomized Phase II clinical trial hypothesize that in a significant subset of patients with recurrent NSCLC immunotherapy (pembrolizumab) after stereotactic body radiation therapy (SBRT) (NCT02492568) will be superior to treatment with immunotherapy alone [28]. In a recent study, MHC I/II molecules appear to downregulated in resistance mutant Kras and p53-deficient lung cancer cells. However, local radiotherapy leads to increasing levels of IFN-β and MHC I molecules on the cell surface of resistant cells. Thus, it is proved that adjuvant radiotherapy may help to overcome anti-PD-1 resistance, and then enhances the efficacy of anti-PD-1 checkpoint inhibitors [29]

An increasing amount of research data supports the hypothesis that targeting the structure of blood vessels can reduce the function of suppressive cells and promote the anti-tumor activity of immune effector cells within TME [30]. Currently, a plethora of clinical studies are underway in order to identify the impact of simultaneous inhibition of angiogenesis and checkpoint inhibitors. Moreover, many research teams are focusing on reprogramming TME in order to become more immune-stimulatory through a therapeutic scheme that combines anti-angiogenic agents and immune checkpoint inhibitors such as pembrolizumab and nivolomumab. In this contest, this combinatorial scheme appears to inhibit the negative immune signals and augment the ratio of anti-/pro-tumor immune cells. Furthermore, immunotherapy appears to restore immune-supportive TME and promote tumor-vascular normalization. These facts lead to an increase in the infiltration and activation of lymphocyte within the tumor [30].

In addition, several studies highlight the fact that anti-PD-L1 based immunotherapy appears to be more efficient when combined with chemotherapeutic agents [31]. On the other hand, different cancer types, such as breast or colorectal cancer, and melanoma, are identified through the higher expression of PD-L1 in both cancer and infiltrating immune cells [32]. Further, ongoing clinical studies are trying to evaluate the combinatorial scheme of anti-PD-1/-L1, MoAbs with other therapeutic agents such as copanlisib (PI3Kinase inhibitor) in elapsed/refractory solid tumors with expansions in mismatch-repair proficient (MSS) colorectal cancer patients (NCT03711058). In addition, other clinical trials combine platinum-based agents such as carboplatin with nivolumab in order to evaluate the pathological complete response defined as the absence of residual tumor in lung and lymph nodes comparing patients treated with chemo-immunotherapy versus chemotherapy alone. FOLFOX scheme also has been combined with ICI in several clinical trials (NCT03202758, NCT02375672, and NCT02997228). In a randomized phase 2 study will evaluate 2 novel immunotherapy combinations in which pembrolizumab is integrated with ramucirumab and paclitaxel in patients with advanced gastric and GEJ adenocarcinoma (NCT04069273) The main goal of this study is to examined the re-activation of the immune response against several types of cancer with therapeutic benefits for patients.

In addition, pembrolizumab as monotherapy could better suit a patient with a low tumor burden and a better performance status, than the combination of pembrolizumab and chemotherapy that would be more beneficial for a cancer patient with a higher tumor burden and a more inferior performance status, for whom a rapid and more probable response to treatment is crucial [33]. Furthermore, this very same problem could also arise with another anti-PD-1 drug, atezolizumab, apart from the positive results coming from the IMpower150 clinical trial. Moreover, atezolizumab also showed interesting results in a recent study from the IMpower110. In this study, 555 high PD-L1 expressing (TC3/IC3) naïve nonsquamous or squamous advanced NSCLC-affected patients without positive genetic biomarkers were respectively randomized (1:1) to receive atezolizumab monotherapy vs. cis/carboplatin + pemetrexed or atezolizumab monotherapy vs. cis/carboplatin + gemcitabine and results decisively favored atezolizumab over SoC chemotherapy: mOS: 20.2 vs. 13.1 months (HR for death: 0.595) [33].

The crucial role of autophagy as a regulator mechanism for energy and metabolic balance in tumor cells is well described previously. The last decade research efforts have led to the development of agents that modulate autophagy. Chloroquine (CQ) and its derivative, hydroxychloroquine (HCQ), is one of the most studied inhibitors that target the fusion of the autophagosome with a lysosome [31]. Several studies indicate that autophagy inhibition in cancer cells may be a putative an approach to improve the effect of ICI. High-dose IL-2 (HDIL-2) alone has been found to be beneficial for immunotherapy in an advanced murine metastatic liver tumor model especially after co-treatment with CQ. It is known that IL-2 reduces tumorgenesis through initiation of immune cell proliferation and infiltration in the liver and spleen [34]. In another study in renal cell carcinoma, autophagy inhibition by CQ also increased the effect of HDIL-2 on stimulation of T-cells, NK cells and DCs [35]. In addition, combination of 3-MA and IL-24 induced apoptosis in oral squamous cell carcinomas (OSCC) [36]. Furthermore, as it was mentioned before in the current review, ICI such as nivolumab, pembrolizumab or ipilimumab can trigger the cytoprotective autophagy in CRC cell lines. The combination of Hydroxychloroquine or HCQ (autophagy inhibitor) and checkpoint inhibitors trigger apoptotic cell death in MSHI-H CRC cell lines [37]. The clinical response of CQ and it derivative HCQ appears to vary widely. Both of them are not specific inhibitors of autophagy and affect also other cellular functions [31]. Thus, a plethora other agents that modulate autophagy (inhibitors or promoters) have already been developed [31]. The impact of autophagy on tumorgenesis and its active participation in antigen presentation from MHC-I and/or MHC-II make autophagy an attractive target for solid tumor ICI-depended therapy.

As it was mentioned, mutation in different genes and the signaling pathways that control have been targeted from many research teams in order to overcome the resistance against ICI. The phase I/II trial studies (NCT04317105) tries to investigate the side effects and best dose of copanlisib (dual inhibitor of PI3K α and δ isoforms) when given together with nivolumab and ipilimumab in treating patients with solid cancers that have spread to other places in the body (advanced) with mutation in PIK3CA and PTEN genes. A novel small inhibitor CGX1321 (inhibitor of WNT pathways) has already entered in human clinical trials as an anti-cancer agent. Keynote 596 uses single agent dose expansion phase in gastrointestinal (GI) tumors and roll-over cohort of CGX1321 and pembrolizumab in subjects who have progressed on single agent CGX1321 and Phase 1b consisting of CGX1321 in combination with pembrolizumab in colorectal tumors. Both phases of this study try to evaluate the safety, pharmacokinetics, and clinical activity of this combinatorial treatment (NCT02675946)

In addition, several inhibitor of the histone lysine methyltransferase EZH2 such as CPI-1205 are already developed. In a Phase I/II clinical study (NCT03525795), combine CPI-1205 with ipilimumab in patients with histologically or cytologically confirmed advanced solid tumors. To avoid this resistance caused by gut microbiota, antibiotics, prebiotics (dietary or chemical entities), and synbiotics can be used.

Moreover, the food industry have been applied recently bacteriophages, to eliminate unfavorable bacteria [38]. Furthermore, gut microbiota have begun to attract many research team as putative target for solid tumors. In clinical trial with clinicaltrials.gov: NCT03829111 the investigators try to combine checkpoint inhibitors such as nivolumab and Ipilimumab with probiotics. This phase I trial study tries to investigate the effect of CBM588 probiotic in patients with kidney cancer (stage IV) that are treated with nivolumab and ipilimumab.

In Table 1 are presented several ongoing clinical trials that try to combined checkpoint inhibitors with other agents in order to overcome the resistance against ICI.

Table 1. Clinical studies with combination of immunotherapy with chemotherapy in solid tumors.

Number of Study.	Type of Cancer	Phase	Agent/Compound
NCT04069273	**Adenocarcinomas of the esophagogastric junction**	**II**	**Ramucirumab + pembrolizumab + paclitaxel**
NCT02501096	**Advanced solid tumors**	**I/II**	**Lenvatinib + pembrolizumab**
NCT02646748	**Advanced solid tumors**	**I**	**Pembrolizumab+INCB combinations**
NCT04317105	**Advanced malignant solid neoplasm**	**I/II**	**Copanlisib, ipilimumab, nivolumab**
NCT03525795	**Advanced solid tumors**	**I/II**	**CPI-1205, ipilimumab**
NCT02617589	**Brain cancer**	**III**	**Nivolumab, temozolomide**
NCT02684006	**Clear cell**	**III**	**Avelumab + axitinib vs. sunitinib**
NCT02853331	**Clear cell**	**III**	**Pembrolizumab + axitinib vs. sunitinib**
NCT01472081	**Clear cell/non-clear cell**	**I**	**Nivolumab + sunitib/pazopanib**
NCT02420821	**Clear cell, sarcomatoid**	**III**	**Atezolizumab+bevacizumab vs. sunitib**
NCT03202758	**Colorectal cancer**	**I/II**	**Durvalumab, tremelimumab and FOLFOX**

Number of Study.	Type of Cancer	Phase	Agent/Compound
NCT02981524	Colorectal cancer	II	Cyclophosphamide followed by Pembrolizumab
NCT03657641	Colorectal cancer	I/II	Pembrolizumab + vicriviroc
NCT02375672	Colorectal cancer	II	Pembrolizumab + FOLFOX
NCT03711058	Colorectal cancer	I/II	Nivolumab + copanlisi, nivolumab
NCT02327078	Colorectal cancer	VII	Nivolumab + epacadostat
NCT03832621	Colorectal cancer	II	Nivolumab, ipilimumab, temozolomide
NCT02675946	Gastrointestinal cancers	I	CGX1321+pembrolizumab
NCT02496208	Genitourinary tumors	I	Cabozantinib + nivolumab/ipilimumab
NCT02997228	mCRC	III	Atezolizumab + bevacizumab + mFOLFOX6
NCT01950390	Melanoma	II	Ipilimumab + bevacizumab
NCT02802098	Metastatic breast cancer	I	Durvalumab+ bevacizumab, taxane+ bevacizumab
NCT00790010	Metastatic melanoma	I	Ipilimumab, bevacizumab
NCT02959554	Metastatic renal cell carcinoma	II	Nivolumab after sunitinib/pazopanib
NCT03149822	Metastatic renal cell carcinoma	I/II	Pembrolizumab + cabozantinib
NCT02681549	mNSCLC	II	Pembrolizumab + bevacizumab
NCT03976375	mNSCLC	III	Pembrolizumab, lenvatinib, docetaxel
NCT03838159	NSCLC	II	Paclitaxel, carboplatin, nivolumab
NCT03425006	NSCLC	II	Itacitinib, Pembrolizumab
NCT02492568	NSCLC	II	SBRT, pembrolizumab
NCT02443324	NSCLC, Biliary tract carcinoma, Urothelial carcinoma	I	Ramucirumab + pembrolizumab
NCT03153410	Pancreatic cancer	I	Pembrolizumab, GVAX, cyclophosphamide, IMC-CS4
NCT02648282	Pancreatic cancer	II	Pembrolizumab, GVAX, cyclophosphamide
NCT03563248	Pancreatic cancer	II	Nivolumab, losartan, FOLFIRINOX
NCT03829111	Renal Cell carcinoma	I	Nivolumab, Ipilimumab, Clostridium butyricum CBM 588 probiotic strain
NCT02888665	Sarcoma	I/II	Doxorubicin+ pembrolizumab
NCT03898180	Urothelial carcinoma	III	Lenvatinib + pembrolizumab

NCT: national clinical trial; mCRC: metatstatic colorectal cancer; mNSCLC: metastatic nonsquamous non–small cell lung cancer; FOLFOX: folinic acid, fluorouracil, and oxaliplatin; mFOLFOX6: leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin; GVAX: cancer vaccine which includes granulocyte-macrophage colony-stimulating factor (GM-CSF); IMC-CS4: a monoclonal antibody targeted to the colony-stimulating factor receptor (CSF-1 receptor or CSF-1R); FOLFIRINOX: 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; SBRT: Stereotactic body radiation therapy.

References

1. Decker, W.K.; Da Silva, R.F.; Sanabria, M.H.; Angelo, L.S.; Guimarães, F.; Burt, B.M.; Kheradmand, F.; Paust, S. Cancer Immunotherapy: Historical Perspective of a Clinical Revolution and Emerging Preclinical Animal Models. Front. Immunol. 2017, 8, 829.
2. Beatty, G.L.; Gladney, W.L. Immune escape mechanisms as a guide for cancer immunotherapy. Clin. Cancer Res. 2014, 21, 687–692.
3. Oiseth, S.J.; Aziz, M.S. Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. J. Cancer Metastasis Treat. 2017, 3, 250.

4. Gonzalez, H.; Hagerling, C.; Werb, Z. Roles of the immune system in cancer: From tumor initiation to metastatic progre ssion. Genome Res. 2018, 32, 1267–1284.
5. Pio, R.; Ajona, D.; Ortiz-Espinosa, S.; Mantovani, A.; Lambris, J.D. Complementing the Cancer-Immunity Cycle. Front. I mmunol. 2019, 10, 774.
6. Seliger, B.; Hu-Lieskovan, S.; Wargo, J.A.; Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunothe rapy. Cell 2017, 168, 707–723.
7. Kitamura, T.; Qian, B.-Z.; Pollard, J.W. Immune cell promotion of metastasis. Nat. Rev. Immunol. 2015, 15, 73–86.
8. Young, K.; Hughes, D.J.; Cunningham, D.; Starling, N. Immunotherapy and pancreatic cancer: Unique challenges and potential opportunities. Ther. Adv. Med. Oncol. 2018, 10, 1758835918816281.
9. Fares, C.; Van Allen, E.M.; Drake, C.G.; Allison, J.P.; Hu-Lieskovan, S. Mechanisms of Resistance to Immune Checkpoi nt Blockade: Why Does Checkpoint Inhibitor Immunotherapy Not Work for All Patients? Am. Soc. Clin. Oncol. Educ. Bo ok 2019, 39, 147–164.
10. Whiteside, T.L. The tumor microenvironment and its role in promoting tumor growth. Oncogene 2008, 27, 5904–5912.
11. Huang, P.-Y.; Guo, S.-S.; Zhang, Y.; Lu, J.-B.; Chen, Q.-Y.; Tang, L.-Q.; Zhang, L.; Liu, L.-T.; Zhang, L.; Mai, H.-Q. Tumo r CTLA-4 overexpression predicts poor survival in patients with nasopharyngeal carcinoma. Oncotarget 2016, 7, 13060 –13068.
12. Salvi, S.; Fontana, V.; Boccardo, S.; Merlo, D.F.; Margallo, E.; Laurent, S.; Morabito, A.; Rijavec, E.; Bello, M.G.D.; Mor a, M.; et al. Evaluation of CTLA-4 expression and relevance as a novel prognostic factor in patients with non-small cell l ung cancer. Cancer Immunol. Immunother. 2012, 61, 1463–1472.
13. Seidel, J.; Otsuka, A.; Kabashima, K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. Front. Oncol. 2018, 8, 8.
14. Soliman, H. nab-Paclitaxel as a potential partner with checkpoint inhibitors in solid tumors. OncoTargets Ther. 2016, 10, 101–112.
15. Larkin, J.; Sileni, V.C.; Gonzalez, R.; Grob, J.-J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rut kowski, P.; et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N. Engl. J. Med. 2015, 373, 23–34.
16. Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D.R.; Steins, M.; Ready, N.E.; Chow, L.Q.; Vokes, E.; Felip, E.; Holgado, E.; et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N. Engl. J. Med. 2015, 373, 1627–1639.
17. Hellmann, M.D.; Paz-Ares, L.; Caro, R.B.; Zurawski, B.; Kim, S.-W.; Costa, E.C.; Park, K.; Alexandru, A.; Lupinacci, L.; Jimenez, E.D.L.M.; et al. Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer. N. Engl. J. Med. 2019, 381, 2020–2031.
18. Motzer, R.J.; Rini, B.I.; McDermott, D.F.; Frontera, O.A.; Hammers, H.J.; Carducci, M.A.; Salman, P.; Escudier, B.; Beus elinck, B.; Amin, A.; et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carci noma: Extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. Lancet Oncol. 201 9, 20, 1370–1385.
19. Powles, T.; Durán, I.; Van Der Heijden, M.S.; Loriot, Y.; Vogelzang, N.J.; De Giorgi, U.; Oudard, S.; Retz, M.M.; Castella no, D.; Bamias, A.; et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or meta static urothelial carcinoma (IMvigor211): A multicentre, open-label, phase 3 randomised controlled trial. Lancet 2018, 3 91, 748–757.
20. Socinski, M.A.; Jotte, R.M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodríguez-Abreu, D.; Moro-Sibilo t, D.; Thomas, C.A.; Barlesi, F.; et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N. En gl. J. Med. 2018, 378, 2288–2301.
21. Reck, M.; Rodríguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csőszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuff e, S.; et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N. Engl. J. Med. 2 016, 375, 1823–1833.
22. Herbst, R.S.; Baas, P.; Kim, D.W.; Felip, E.; Pérez-Gracia, J.L.; Han, J.Y.; Molina, J.; Kim, J.H.; Arvis, C.D.; Ahn, M.J.; e t al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced nonsmall-cell lung cancer (KEY NOTE-010): A randomised controlled trial. Lancet 2016, 387, 1540–1550.
23. Signorelli, D.; Giannatempo, P.; Grazia, G.; Aiello, M.M.; Bertolini, F.; Mirabile, A.; Buti, S.; Vasile, E.; Scotti, V.; Pisapia, P.; et al. Patients Selection for Immunotherapy in Solid Tumors: Overcome the Naïve Vision of a Single Biomarker. Bio Med Res. Int. 2019, 2019, 9056417.

24. Yu, H.; Boyle, T.A.; Zhou, C.; Rimm, D.L.; Hirsch, F.R. PD-L1 Expression in Lung Cancer. J. Thorac. Oncol. 2016, 11, 964–975.

25. Da Silva, C.; Camps, M.G.; Li, T.M.; Zerrillo, L.; Löwik, C.W.; Ossendorp, F.; Cruz, L.J. Effective chemoimmunotherapy by co-delivery of doxorubicin and immune adjuvants in biodegradable nanoparticles. Theranostics 2019, 9, 6485–6500.

26. Barrueto, L.; Caminero, F.; Cash, L.; Makris, C.; Lamichhane, P.; Deshmukh, R.R. Resistance to Checkpoint Inhibition in Cancer Immunotherapy. Transl. Oncol. 2020, 13, 100738.

27. Bokas, A.; Papakotoulas, P.; Sarantis, P.; Papadimitropoulou, A.; Papavassiliou, A.G.; Karamouzis, M.V. Mechanisms of the Antitumor Activity of Low Molecular Weight Heparins in Pancreatic Adenocarcinomas. Cancers 2020, 12, 432.

28. Gong, J.; Le, T.Q.; Massarelli, E.; Hendifar, A.E.; Tuli, R. Radiation therapy and PD-1/PD-L1 blockade: The clinical development of an evolving anticancer combination. J. Immunother. Cancer 2018, 6, 46.

29. Wang, X.; Schoenhals, J.E.; Li, A.; Valdecanas, D.R.; Ye, H.; Zang, F.; Tang, C.; Tang, M.; Liu, C.-G.; Liu, X.; et al. Suppression of Type I IFN Signaling in Tumors Mediates Resistance to Anti-PD-1 Treatment That Can Be Overcome by Radiotherapy. Cancer Res. 2016, 77, 839–850.

30. Ciciola, P.; Cascetta, P.; Bianco, C.; Formisano, L.; Bianco, R. Combining Immune Checkpoint Inhibitors with Anti-Angiogenic Agents. J. Clin. Med. 2020, 9, 675.

31. Koustas, E.; Sarantis, P.; Kyriakopoulou, G.; Papavassiliou, A.G.; Karamouzis, M.V. The Interplay of Autophagy and Tumor Microenvironment in Colorectal Cancer-Ways of Enhancing Immunotherapy Action. Cancers 2019, 11, 533.

32. Yaghoubi, N.; Soltani, A.; Ghazvini, K.; Hassanian, S.M.; Hashemy, S.I. PD-1/PD-L1 blockade as a novel treatment for colorectal cancer. Biomed. Pharmacother. 2019, 110, 312–318.

33. Della Gravara, L.; Battiloro, C.; Cantile, R.; Letizia, A.; Vitiello, F.; Montesarchio, V.; Rocco, D. Chemotherapy and/or immune checkpoint inhibitors in NSCLC first-line setting: What is the best approach? Lung Cancer Manag. 2020, 9, LMT22.

34. Liang, X.; De Vera, M.E.; Buchser, W.; Chavez, A.R.D.V.; Loughran, P.; Stolz, N.B.; Basse, P.; Wang, T.; Van Houten, B.; Zeh, H.J.; et al. Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. Cancer Res. 2012, 72, 2791–2801.

35. Lotze, M.T.; Buchser, W.; Liang, X. Blocking the interleukin 2 (IL2)-induced systemic autophagic syndrome promotes profound antitumor effects and limits toxicity. Autophagy 2012, 8, 1264–1266.

36. Li, J.; Yang, D.; Wang, W.; Piao, S.; Zhou, J.; Saiyin, W.; Zheng, C.; Sun, H.; Li, Y. Inhibition of autophagy by 3-MA enhances IL-24-induced apoptosis in human oral squamous cell carcinoma cells. J. Exp. Clin. Cancer Res. 2015, 34, 97.

37. Koustas, E.; Papavassiliou, A.G.; Karamouzis, M.V. The role of autophagy in the treatment of BRAF mutant colorectal carcinomas differs based on microsatellite instability status. PLoS ONE 2018, 13, e0207227.

38. Li, W.; Deng, Y.; Chu, Q.; Zhang, P. Gut microbiome and cancer immunotherapy. Cancer Lett. 2019, 447, 41–47.