International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 4, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

The Use of Combination Therapy to Overcome Diverse Challenges in Immune Checkpoint Inhibitors Treatment

Mohammed Maeed Alqhtani[1], Mohammed Saad Abdullah Alqahtani[2], Ahmed Abdullah Alshahrani[3], Yasser Saad Shalan[4], Ali Hamad M Almegames[5], Naif Saad M. Alkaraan[6], Abdulaziz Ali Muidh Al Karan[7], Abdullah Bunaydir Eid Alharbi[8], Mohmad Saad M Al Karani[9], Habiba Saleh G Alenzi[10], Ziyad Ali Almalki[11], Mohammed Abdullah Alshahrani[12], Khalid Abdulrahman A Alfantoukh[13], Khalid Ibrahim Khawaji[14], Hosam M. Alqahtani[15], Abdullah Mohammed Shalaan[16]

[1]Pharmacist Armed Forces Hospitals, Southern Region, Email: Mohammed.21211@Hotmail.Com
[2]Pharmacist Armed Forces Hospitals, Southern Region, Email: al_hatla1994@hotmail.com
[3]Pharmacist Armed Forces Hospitals, Southern Region, Email: Ahmad.A.alamoudi1@gmail.com
[4]Pharmacist Armed Forces Hospitals, Southern Region, Email: shlanyasr@gmail.com
[5]Nursing Technician Ministry of Health Riyadh Al-Muhammadiyah Health Center 1, Email: Ali2634@hotmail.com
[6]Medical laboratories Ministry of Health, Email: naif-0123@hotmail.com
[7]Pharamcy Ministry of Health - Khamis Mushayt General Hospital, Email: Abdulaziz.alkaraan@gmail.com
[8]Ministry of Health Specialist-Radiological Technology, Email: abbnalharbi@moh.gov.sa
[9]Pharmacist Ministry of Health, Email: Malkaran@moh.gov.sa
[10]Ministry of Health General-General Practice, Email: Habibasa@moh.gov.sa
[11]Family medicine Riyadh third health cluster Oiyaynah phc, Email: zaalmalki@gmail.com
[12]Orthopedic surgery specialist Armed Forces Hospital in the Southern area, Email: alshahrani9533@gmail.com
[13]Public Health Health Administration and Community Health, Email: Alfantoukh.02013@gmail.com
[14]Diriyah Hospital Laboratory Specialist, Email: Kkhauuaji@moh.gov.sa
[15]General Directorate Of Medical Service, Ministry Of Interior, Riyadh, Kingdom Of Saudi Arabia, Email: husam.qahtani@moimsd.gov.sa
[16]Dariyah General Hospital Radiological Technology, Email: ashalaan@moh.gov.Sa

Received: 14.08.2024 Revised: 19.09.2024 Accepted: 09.10.2024

ABSTRACT
The potential of combination strategies with ICIs is to deliver positive outcomes for solid tumors. Clinical trial data indicate that combination approaches improve response rates, duration of response, and overall survival. Despite being in the field of immune therapy for several years, ICIs can trigger mechanisms underlying resistance and diverse immune-related adverse events. This emphasizes the need for using combination therapy with cutaneous agents. A number of clinical trials have shown promising activity and tolerability for different combination strategies that include ICIs. The studies also confirm the concept that synergy exists between the actions of different treatments acting on different pathways, and this is reflected through a different spectrum of toxicities. Preclinical studies provided insights into potential rational strategies of combination therapy with ICIs. Main areas of research that have brought new insights in combined strategies included insights into the synergy of the type I interferon pathway and PD-1 inhibitors, mechanisms of resistance to ICIs, anti-tumor activities of myeloid-derived suppressor cells, and insights into how transferred immune cells work with checkpoint inhibitors. New insights into natural killer cell biology, as well as the discovery of NK cell checkpoints, expanded the portfolio of combination treatment areas. Advances in technology enabled in-depth state-of-the-art characterization and functional studies of the immune system at the single-cell level. A new era of combination therapy that is under development in several different cancers, other than hematologic malignancies, refers to the use of multiple drugs operating in the immune and non-immune pathways with anticancer agents.

Keywords: biology, multiple, suppressor cells, immune

1. Introduction to Immune Checkpoint Inhibitors (ICIs)

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of many solid and hematologic malignancies. The key mechanism of ICIs is to reactivate the immune response to native tumors and other viral-

associated malignancies by blocking immune checkpoint molecules, which function as 'brakes on the immune system[1]. This leads to potent antitumor immunity by amplifying T-cell effector function, increasing tumor-specific T-cell activity, and augmenting the ability of the immune system to recognize and destroy tumor cells.[2] Tumor cells are killed by ICIs through an active immunologic mechanism and can leave a 'memory' in the immune system to prevent tumor recurrence or progression.[3] ICIs are divided into several classes, including monoclonal antibodies that block the programmed death-1 (PD-1) receptor, its ligand PD-L1, or the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and bind to the immune checkpoint receptor.[4]

The development of ICIs has achieved significant enhancements in the results of cancer patients. ICIs have changed the clinical landscape of cancer therapy and are now considered the standard therapeutic option for multiple tumors.[5] The repertoire of immune-related adverse events (irAEs) for ICIs is wide and diverse. These agents are used in a variety of malignancies, including melanoma, non-small cell lung cancer, renal cell cancer, urothelial cancer, Hodgkin's lymphoma, Merkel cell cancer, head and neck squamous cell cancer, and cervical carcinoma.[6]

There are some potential immune responses to the effects exerted by ICIs on immune checkpoint protein delivery on the cell surface. Immune checkpoint proteins that usually reside inside effector cells, which act to block immune cells and tumors, can be detected in these vesicles and have good predictive power for the response in patients. In addition, the immune responses are focused primarily on cell types, especially subpopulations of lymphocytes and, to a lower degree, NK cells.[7]

1.1. Mechanisms of Action and Clinical Applications

Apart from the above concept, another breakthrough in the development of cancer treatment that has gained attention is immune checkpoint inhibitors (ICIs) 3. ICIs are one of the six major types of targeted drugs used for cancer treatment that modulate the action of the immune system.[8]Unlike other targeted therapies such as receptor tyrosine kinases, which are present in both healthy and cancer cells, immune checkpoint inhibitors reactivate the immune system, allowing the body to recognize and destroy cancer cells.[8]

Mechanistically, ICIs inhibit immune checkpoint proteins, which are inhibitory signals that dampen the immune response and limit T-cell signaling, thereby enhancing T-cell activation and proliferation. ICIs consist of several classes such as programmed cell death 1 (PD-1) inhibitors, programmed cell death 1 ligand (PD-L1) inhibitors, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors. [9, 10]

The efficacy of ICIs has been demonstrated on many cancers, including a clinical trial comparing the efficacy of the anti-CTLA-4 inhibitor and the gp-100 vaccine in patients with melanoma. [11] The study results showed that the inhibitor could reduce the risk of death by 32.5% and increase overall survival (OS) by as long as 10 months. Another study proved that the use of a PD-1 inhibitor also showed OS advantages in non-squamous non-small-cell lung cancer progression patients in the phase 3 trial. [12]

Two patients were illustrated regarding a combination of nivolumab and ipilimumab for advanced epithelioid mesothelioma. The two patients showed different responses when receiving the combination therapy. The first patient had long-term progression-free survival (PFS) of up to 27 months before the disease progressed, while the second patient had a complete response. In the second patient, one year after treatment, scans of the thorax and total body showed partial response, and mediastinal and hilar lymph nodes were significantly reduced following one year of treatment. The last scan showed stable disease (SD) after 20 months in the mediastinal, papillary, and hilar locoregional lymph nodes.[12]

Current studies are being conducted on Immune Checkpoint Inhibitors (ICIs), with a focus on extending their application in different situations such as in the early stages of the disease, as maintenance therapy, after allogeneic transplantation, and in combination with radiotherapy and chemotherapy. These studies also aim to address the different mechanisms of resistance encountered with ICIs. [13]

2. Challenges in Immune Checkpoint Inhibitors Therapy

Immune checkpoint inhibitors (ICIs) are monoclonal antibodies specifically targeting immune checkpoints such as programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).[14]ICIs have brought a revolution in the field of oncology and are being used for the treatment of a wide array of cancers. However, ICIs are associated with several challenges owing to their mechanism of action and anti-tumor immune response.[15]

First, resistance to ICIs can be one of the major challenges which can occur at two stages: primary resistance occurs when patients do not respond to ICI treatment from the beginning, and acquired resistance is the phenomenon where initially responsive tumors develop as non-responsive over time. [16]

Second, immune-related adverse events (irAEs) are a set of toxicities associated with the PD-1/PD-L1 and CTLA-4 inhibitors, primarily pertaining to autoimmune pathologies. **[17]** These irAEs can independently predict immune response, leading to better survival of cancer patients. Diversity in irAEs also makes them one of the important challenges in ICI therapy, thereby altering the course of management for patients and increasing treatment costs. [18, 19]

Third, the success of ICIs is seen primarily with tumors having a higher mutational burden compared to the remaining tumors. [20] In recent times, several major ICI clinical trials have showcased dismal results due to their low mutational burden. [21] Other reasons working against the ICIs are the presence of multiple immunosuppressive mechanisms within the tumor microenvironment.[22]

PD-1 can also be located on tumor-infiltrating lymphocytes (TILs), which can lead to impaired TIL function, hence reducing the efficacy of ICI treatment.[23]Despite the challenges, ICIs have revolutionized cancer care and are approved for the treatment of numerous cancers. [15, 24]Owing to their enhanced application, the limitations will accompany the ICIs' upfront therapy even in the coming years. [25]

Hence, to relieve the difficulties, there is a necessity to develop novel approaches to improve treatment outcomes and management of these toxicities.[26]Overcoming the barriers will primarily include the development of personalized strategies acting via a different set of mechanisms. Hence, in the coming sections, we focus on combinations overcoming the limitations associated and also the strategies implemented in current clinical settings. [27]

2.1. Primary and Acquired Resistance

Over the past few years, immune checkpoint inhibitors have revolutionized the management of cancer, resulting in the long-term control of various advanced malignancies.[28] These inhibitors have made a profound impact on survival; however, a substantial proportion of initially responsive tumors do not show the same long-term benefit. [29]One effective approach to dealing with poor outcomes involves the use of combination therapies [30]. There are two general forms of therapeutic resistance: primary and acquired, which can often present together.[31] Primary resistance is defined as a situation in which some patients do not show any clinical benefit from therapy from the start of treatment, and these patients have not yet responded to treatment. [31]Unfortunately, when resistance does not exist in tumor cells, the therapy acts as a remedy for those patients. [32]

Acquired resistance signifies patients who have initially responded to treatment but subsequently are no longer responsive.[33, 34] The phenomenon of both primary and acquired resistance progressing is typically due to multiple genetic alterations. Acquired resistance is known to have multiple occurrences that might lead to additional mutations as a result of selection pressure during treatment. [35]The various resistance mechanisms in tumor cells frequently lead to the same results, which is preventing T cells from recognizing, infiltrating, and ultimately killing tumor cells. Nonetheless, the immune landscape of the tumor microenvironment has a decisive influence on these mechanisms. [36]

2.2. Immune-Related Adverse Events (irAEs)

The immune system disregards self-immunity, comprised of inclusive and restrictive safeguards, and comprises many checks and balances to avoid these two opposed cases of violence to self. [37]

Immune checkpoint inhibitors are just silencers of those restrictive checkpoints. Correlating this back to clinical life, ICIs have shown dramatic benefits or seemingly miraculous results for patients with previously refractory cancers. **[38]** However, there are adverse events, and sometimes, as healthcare providers, we need to be alert for them or handle them. Sometimes, it's all about learning how best to take care of this technology and still move forward.[39]

Immune-Related Adverse Events (irAEs)

The most affected system in anti-PD-1 and anti-CTLA-4 ICI therapy is the skin, in terms of signs seen through phyto-evaluation as a spectrum of inflammatory reactions. Among them, dermatitis and rash are seen in more than 20%. [40] IAEs are primarily universal across the checkpoint inhibitors of all agents and modulate predominantly the immune system in many organs overall, which would be termed immune-related adverse effects rather than immune-related adverse events. [41] Overall, what can be negatively managed by corticosteroids whenever it's a grade more than 2, except for some reassessed management.[42]This is where many new recommendations are being given to consider antiviral prophylaxis when corticosteroids have been started in the first place, along with tapering the corticosteroids.[43]

So the whole strategy has changed with the initial bout of technologies, even from the management of these side effects. The issue regarding pneumonitis may present with different clinical presentations, which might be mistaken for infective or neoplastic conditions. [44] The time at which pneumonitis occurs is generally variable, extending up to two years or more. [45] If it happens to be the last agent, then further treatment would be difficult. It is generally considered not to restart therapy when treatment for pneumonitis is needed. Immune-related adverse events do impact the quality of life of patients, and ongoing research aims to understand their impact once we have left the treatment.[45]

Due to IAE, the patient does not appear to derive benefit from the anti-tumor response of the therapy. Therefore, understanding, monitoring, and prompt management are occasionally needed. Guidelines provide these recommendations for the treatment of IAE. [46, 47]

3. Rationale for Combination Therapy in Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) are remarkable not only for their efficacy but also for the durability of responses in various cancers. However, the benefits of ICI monotherapy are achieved in a minority of patients with advanced malignancies, highlighting the need to expand potential benefits beyond the classical patient population for ICIs. [48]ICIs also display very small effects when used in the adjuvant setting, or even agonistic effects that require mitigation by the addition of an anti-PD-(L)1 agent. [44]This aligns with the fact that mounting evidence suggests challenges related to ICI therapy. Evidence of tumor heterogeneity supports the use of combination therapy, as no one approach will ever be enough to address the diverse challenges that arise.[44, 48]The appeal of combination therapy is that it amplifies the attractive qualities of ICIs while simultaneously addressing the associated resistance and minimizing off-target effects through potential synergistic strategies. [49, 50]

The therapeutic rationale for the combinations of ICIs with chemotherapy, targeted therapy, or other immune therapies can be based on cardinal or complementary mechanisms.[49, 50]In principle, the cardinal or concerted mechanisms are represented by the combination of ICIs with different actions, achieving additive or concurrent and non-redundant enhancement of the immune response.[49] Combination therapies have the potential to exploit different mechanisms of action and should be better able to reduce or overcome existing mechanisms of resistance to monotherapies. [45]

These combinations can exploit different vulnerabilities of cancer cells and thus improve the response rate. However, the mechanism of action of a combination partner is potentially influenced by the mechanism of action of a checkpoint inhibitor. Thus, a mechanistic understanding is necessary to comprehend why combination therapies work.[45, 49]

3.1. Enhancing Efficacy and Overcoming Resistance

In multiple strategies, combination therapies improve the efficacy of immune checkpoint inhibitors. The primary objective of these combinations is to activate and expand a tumor-specific T cell population that is significantly robust. [51] Combining additional receptor-targeted agents with immune checkpoint inhibitors to treat these patients leads to high objective responses. [45]

Multiple synergistic immunologic and pleiotropic combinations can provide potential solutions to the resistance of anti-PD-1 and allow for synergistic prime shocks that escalate other immune checkpoints for immune checkpoint inhibitors.[44]Strategies of combining checkpoint-conquering with either concurrent or sequential collections also aim to increase therapeutic efficacy. [49]Thus, the gain of maximum impact on many independent intracellular signaling means that parallel inhibition of multiple signaling pathways in cells is likely to be responsible for the shared molecular achievements of parallel combination strategies. [49, 51]Either the intrinsic preexisting resistance of primary resistance mechanisms or the participating intracellular signaling can offset resistance to prevent acquired resistance by targeting signal strengthening and the main complementary pathways. [49, 50] Given the relevant roles that these pathways and their players play in many varieties of immune and resistance evasion, combinations discuss examples with promising clinical findings.

For each of these combinations, identified biomarkers of response or resistance mechanisms to qualify patients for these combinations after further validation are also included. These scans will track reports over time as new clinical periods appear and the field approaches more precision medicine via combinations. [44, 49, 50]

4. Types of Combination Therapies

Combination therapies have been developed based on the synergy between immune checkpoint inhibitors, and they can be categorized into the following types.

(1) Immunotherapy combined with a targeted therapy. They may be a rational and effective combination because of their complementary mechanisms of action: the targeted therapy reduces tumor volume and relieves immunosuppression of the microenvironment. [52]

(2) Immunotherapy combined with another immunotherapeutic agent. These inhibitors can target other immune checkpoints or act as adjuvants, and the combination may have an augmented antitumor effect and a longer response. [53]

(3) Immunotherapy combined with traditional therapy. Coupling immunotherapy with conventional therapies such as chemotherapy or radiotherapy can significantly improve the patient's immune response by altering properties of the tumor that may be more susceptible to inhibitors. [54]

(4) Immunotherapy combined with an innovative therapeutic strategy. Genetic modifications, bispecific T cell engagers, and drugs targeting immune cells also hold great promise for combination therapy. Currently, evidence accumulates for these various combinations. Given that the mechanism of action differs, combating resistance-related mechanisms would be highly appealing and may constitute the near future of therapeutic choices. [55, 56]

Currently, inhibitors combined with targeted therapy can be further divided into combinations of inhibitors with tyrosine kinase inhibitors or other matched gene therapy to target patients whose tumor harbors deterministic gene alterations. [57]Inhibitors can also be combined with other targeted agents, but these combinations are beyond the scope of this discussion. [58]In addition, different inhibitors targeting various immune checkpoints are different from other self-owned molecules produced by the drug, which makes treatment between various inhibitors not drug-attenuable. [59]

When combining inhibitors with another immunotherapy similar to inhibitors, however, the safety profiles and overlaps of side effects related to immune response should be given high consideration, and guidance suggests measures to be included for investigations on standards for treatments that include combinations of immunotherapy and anti-PD-1/PD-L1 antibodies only. [60]

With increasing study of the dosage pair and sequence in various combination strategies, the efficacy and safety of inhibitors used in combination have no advantage. Because individual therapy with a drug agent is arranged by a medical team based on patient condition and tumor characteristics, extensive and flexible combination strategies have been developed.[61] Combination therapy for inhibitors is gradually becoming complicated, but such versatility is beneficial because it matches the variable molecular characteristics of tumors and patient tumors and can be used for individualized management. [62]

4.1. Immunotherapy and Targeted Therapy Combinations

ICI is a superior selection for cancer because of low immune-related adverse effects and durable clinical effects. However, due to the low sensitivity of tumor cells to ICIs, the positive objective response rate (ORR) to ICIs alone is less than 30%, which cannot meet the global demand.[15]Therefore, improving the sensitivity of tumor cells to ICIs and enhancing the efficacy of ICIs have become the principles of cancer treatment, and combination therapy of ICI and diverse methods is developed based on these mechanisms. [63] Combination therapy of ICI and chemotherapy is based on the idea that chemotherapy destroys intratumoral immune-tolerant cells, increases the expression of HLA molecules, and gene transduction of esophageal cancer, breast cancer cells, and rectal cancer. [64, 65]

Through NGS, it is found that their mutation or signal changes are significantly related to their ORR or patient survival or can be used as a marker for disease prediction of ICI. This provides a basis for combining ICI with SERMIs, PARP inhibitors, and mTOR inhibitors that produce dsDNA breaks, which increase the release of cGAS, stimulator of interferon genes (STING) pathway ligands, and type I interferon (IFN), causing innate immune imbalances. [66, 67]

The combination therapy embraces BRAF/MEK inhibitors and ICIs, PI3K pathway inhibitors, and ICIs. For example, BRAF-mutant/EGFR-mutant non-small cell lung cancer (NSCLC) cells can activate the MAPK pathway and cause immunosuppression. [68, 69] The combination of BRAF inhibitors and MEK inhibitors can inhibit the phosphorylation of MEK and ERK in BRAF-mutant NSCLC, thereby downgrading immunosuppression and increasing tumor infiltration of killer T cells, and the use of ICIs produces better results. [70] PD-1 inhibition has been approved in combination with BRAF and MEK inhibitors for the first-line treatment of patients with advanced BRAF-mutant malignant melanoma due to the synergistic improvement of progression-free survival (PFS) and ORR. [71] Targeted drugs aim at cooperating with ICIs and are directed against patients' molecular targets. Because the level of innate immune ligands in the TME is related to the sensitivity of tumor cells to ICIs, and the expression of dsDNA breaks and ssDNA breaks is the leading factor in the presence of innate immune ligands, the combination of targeted drugs and ICI can make more tumor cells release innate immune ligands to promote ICI sensitivity. [72, 73]

Diverse combinations have different levels of approval, and because immune-related adverse events may be amplified by this combination, it is necessary to be careful when using it, and it does not have the possibility for patients to receive high-dose treatment. [18, 74] The ongoing clinical trials are in the data collection or follow-up stage. The association of diverse gene selection or gene expression value with ICI sensitivity is the index. The patients have no difference in other factors, and the selected affected gene selection patients obtain specific inhibitors for high expressive screening or mutated genes to test the effectiveness of specific inhibitors on ICI sensitivity. [75]

5. Clinical Evidence Supporting Combination Therapy

The combination of immune checkpoint inhibitors and other treatment modalities has been a hotspot of research. Currently, combination strategies using anticancer drugs such as molecular targeted therapy, traditional chemotherapy, and radiation have been investigated in phase II, III, or IV clinical trials. 56Combination with PD-1/PD-L1 and CTLA-4 inhibitors has also been tested in clinical trials. Some of these trials have been successful, and subjects receiving combination therapy have shown better outcomes.[76] A clinical study found that a combination regimen including the PD-1 inhibitor with apatinib was successful, while an estimated 9- and 18-month overall survival was recorded at 86% and 53%, respectively. [77]

Notably, treatment with the combination of sintilimab plus apatinib displayed promising efficacy in the treatment of advanced HCC, with manageable toxicity. Furthermore, considerable evidence from ongoing clinical studies and trials remains a focus. [78, 79]

Phase II trials are ongoing to evaluate the efficacy, safety, and pharmacokinetics of cemiplimab plus an anti-CEA/CD3 half-life extended bispecific antibody in patients with treatment-naïve or previously exposed non-small cell lung cancer. [80]Clinical trials are instrumental in providing evidence regarding the therapeutic benefits of combination therapy.[81] Traditional clinical studies for immune checkpoint inhibitors are typical randomized controlled trials, commonly employing a phase I, II, and III trial study design that involves an initial dose-finding study, followed by a larger two-randomization phase II trial and a larger phase III trial with a more prominent sample size. [82, 83]

Objectives of these trials are aligned with the determination of response rates, progression-free survival, and overall survival, and incorporate endpoints such as duration of response, disease control rate, landmark progression-free survival, and overall survival.[84]Participants in these trials are always confined to a specific population, including subjects with different levels of PD-L1 expression and treatment line, different genders, and ages. [85]The vast majority of subjects included in the trials have tumors with squamous and non-squamous histology, including those planned to undergo surgery or radiotherapy. [86]

Several combination strategies have demonstrated reasonable therapeutic benefits with immune checkpoint inhibitors in many cancers, increasing the overall survival and progression-free survival in cancer patients. Interestingly, these combination strategies showed efficacy in melanoma, non-small cell lung cancer, and metastatic colorectal cancer tumors. Combination therapy can overcome the limitations previously reported with monotherapy. [87, 88]

5.1. Key Clinical Trials and Outcomes

Substantial clinical trial data have shown that combination therapies involving immune checkpoint inhibitors can result in impressive efficacy and improved patient outcomes across various solid tumor histologies. [55] The combination therapies listed that have shown clinical benefit in terms of overall survival were often approved under conditional grounds and are prescribed upon clinical or shared decision-making discussions. The remainder are investigational only and may be available through an ethically approved clinical trial. [89]

Pembrolizumab and pembrolizumab plus chemotherapy are recommended first-line treatments for patients with metastatic non-small cell lung cancer in the first-line setting without targetable oncogenic driver mutations. [90]

The combination treatments have consistently improved overall survival and are not restricted by programmed death ligand 1 expression status. Frontline nivolumab was not superior to chemotherapy in patients with non-small cell lung cancer, however. Ongoing and future trials in the combinations of anti-PD-(L)1 plus chemotherapy or targeted therapies are also being conducted. [91, 53]

Conclusion and Appraisal of Clinical Trials

Clinical trial data show increased overall response rates, progression-free survival, and overall survival in immune checkpoint inhibitor combination therapeutic strategies. To date, pivotal studies have been conducted in combinations of anti-PD-(L)1 ingenol-3, anti-PD-(L)1 MEK, anti-PD-(L)1 chemotherapy, and anti-PD-(L)1 anti-CTLA-4 across a number of histologies.[55, 92] The combination of ipilimumab and nivolumab has been shown to improve survival in metastatic melanoma, squamous cell lung cancer, and renal cell carcinoma when addressing patient stratifications.[93]

There was no significant survival improvement in classic Hodgkin lymphoma identified yet, although overall response rates were doubled. Moreover, the roles and outcomes of anti-PD-(L)1 based combination therapy are currently being evaluated in patients with high/intermediate risk non-muscle invasive bladder cancer. [94]

Ongoing clinical research in this area aims to assess overall response rates and overall survival of two ongoing pivotal phase 3 trials in non-small cell lung cancer and anti-PD-(L)1 plus VEGF in ovarian cancer.[95]Registration trials in other solid tumor histologies were less mature and thus alone as of recently. There are several potential limitations, which should be acknowledged. In particular, the concept of anti-PD-(L)1 plus anti-CTLA-4 as a backbone was only studied in a single phase 3 trial and the scope of the concept is not fully understood. Anti-PD-(L)1 plus anti-CTLA-4 carries a higher rate of adverse events compared to anti-PD-(L)1 plus chemotherapy.**[96]**Patient characteristics are important; for instance, patients in one trial were overall younger than patients in the first-line non-small cell lung cancer study. Additionally, anti-CTLA-4 is known to be associated with a short exposure time due to high rates of adverse events. [97]

For glioblastoma, a similar pivotal trial was presented with a reporting median overall survival of 20.9 months for the combination therapy group compared to 18.4 months among those subjects in the maintenance temozolomide alone group. [98]

Post-hoc analysis suggested even better value when compared to subjects with methylated methylguanine-DNA-methyltransferase promoter status, where the median overall survival was 34.7 months compared to 21.2 months. Although interesting, the trial was relatively small and the anti-PD-L1 component may have provided

significant clinical activity, as had been shown in some of the checkpoint inhibitor monotherapy trials in this area.[99]

These findings are likely to contribute to a change in clinical practice with earlier treatment and with non-brain metastasis. The research in clinical trials evaluating anti-PD-(L)1 with or without potential immune-based agents will continue to evolve in an effort to better optimize patient outcomes.

6. Biomarkers and Patient Selection for Combination Therapy

The identification of predictive biomarkers can be crucial for patient selection for combination therapy, but not all identified biomarkers are applicable to this aspect. Once a combination strategy including the ICIs is determined, potential biomarkers are needed to determine who is most likely to benefit from the strategy. [100]

Depending on whether the identified biomarkers are expressed in tumors or the patient's immune system, they can be classified into the following three types: (1) genomic markers, (2) proteomic markers, such as immune cell evaluation, and (3) immune-based markers, such as TMB, PD-L1 expression, and microsatellite instability. In general, TMB and PD-L1 status can both be assessed in a biopsy sample, indicating that they are both expressed in tumors and immune systems. Several of these markers are known to be associated with an increase in immune activity or editing of the TME, reflecting the immune system's response [101, 102]

Correspondingly, markers of immune systems, such as immune cell composition, immune signature, and tumor immunogenicity, have been suggested as prognostic factors for the efficacy of ICIs, although they are not yet considered in clinical practice.[103]Regardless of the type of marker, they all aim to predict the future effect of treatment, and the ultimate goal is to identify who will benefit from a combination to maximize the benefit.[104]

Furthermore, as the TME is increasingly recognized as a potential determinant of therapeutic efficacy, the biological status of the tumor can, in principle, lead to patient stratification, and it is always promising to use these characteristics as biomarkers in combination [105]. As we delve further into the research, new markers with greater potential may become apparent. Identifying and utilizing them more appropriately is expected to increase precision in future clinical trial designs.

6.1. Predictive Biomarkers

Predictive biomarkers are of paramount importance for decision-making in immune checkpoint inhibitor (ICI) combinations. **[103]**They indicate an enhanced probability for a subject to respond to an ICI, and thus they are employed to stratify potential extraordinary responders who are most likely to benefit from ICIs against the candidates who may not secure much interest from ICI combinations. **[106]** This heralds the necessity for assessing more than one prior predictive marker, as a single marker may be restrictive in evaluating any treatment strategy. The several combinations of the available predictive biomarkers may guide the path experts need to follow to cure any disease. [107]

A predictive biomarker should be assessed before the therapy; quantifying it during the course of therapy might provide insight on early prediction of effectiveness and tailoring the combined regimen. Viable predictive biomarkers for ICI combinations to date fall into five categories. Predictive biomarkers' use in combination therapy predicts patient outcomes and is helpful in stratifying them into different classes. [108]

In order to use these biomarkers, a strategy is required, as two different biomarkers may predict the same outcome differently. [109] This approach may therefore enable scientists to release the promising aspects of these markers to provide maximum effects when used optimally; further, it necessitates standardizing their assessment and thorough validation by prospectively conducting phase III clinical trials in various patient populations representing utmost known diversity in ethnicity, geographical distribution, immunogenicity, and biological events of multiple races. [110]Also, a panel of newly identified T cell activation biomarkers may serve in delineating near-successful patients who might not benefit as much, as observed in the later stages of clinical trials with ipilimumab as a single agent. [111]

7. Future Directions and Emerging Strategies

While diversity of strategies is an asset, some specific plans worth considering are: eradicating cells with compromised immunogenic phenotypes and bolstering the immunogenic conversion of non-responding low-TMB lesions; targeting alternate cell-stroma interactions by combining checkpoint inhibitors with immune reprogramming agents; using oncolytic virus or MDP targeting; improving CAR and TCR T therapy delivery and duration by ICI strategies, and evidence merging other mechanisms in special cases of recurrence. [112, 113]

A major frontier of combination therapy is the incorporation of novel agents and therapeutics, only a few of which were mentioned by this review's final publication date. These include advances in some of the targets reviewed above, updates on ICI delivery and TMB-centered trials. In order to discover the most effective tumor-specific strategies, advancements in the understanding of tumor immune and oncogenesis require multidisciplinary and multi-stakeholder research collaboration. [114, 115]

With the advent of combination therapy, emphasis in the patient selection process is likely to evolve from simply selecting patients likely to respond to including patient selection for the most effective intervention. The

bridge from bench to bedside is the promise of this era – translational medicine – where the most relevant outcomes of these exponentially increasing target discovery projects can help reinvigorate the promising application of options in a wealth of new combinatorial studies. Evolution of regulatory attitudes toward ICI combinations may be needed to meet the clinical and scientific communities at the crossroads envisioned in this review. [91, 116]

Nonetheless, combination trials of the future should incorporate input from both relevant experts in the foreseeable future and consideration of endpoints, methods, and expectations for evidence of experts' preference models. One may move toward answering questions relating to the outcomes the patients themselves value, therefore providing a much broader view of the increasingly diverse range of players in cancer psychology. [117, 118]

7.1. Novel Combinations and Technologies

Combinations of ICIs with other therapeutic options have become a major strategy to boost the activity of this immunotherapeutic class of drugs and to offer effective treatment options to patients who do not respond to ICI monotherapy. [55, 87]Even though some ICI combinations do demonstrate some synergy, either by complementing the respective shortcomings of each compound or by simply boosting antitumoral immune responses in various ways, there is an urgent need to develop new, innovative compounds that can synergize to improve ICI activity even further. [55, 119]

One approach to achieve this is the adaptation of already well-established as well as novel technologies to narrow different resistance mechanisms and circumvent various issues of tumor immune evasion.[34, 120]Research has also been turned towards the development of novel therapeutic options, besides the already well-established chemotherapies and targeted agents that are currently used in a clinical setting to modulate immune checkpoints. [83]Even though immune-relevant targets are most frequently being exploited, other novel molecules involved in tumor immune evasion were found to be of interest as well.[121]

For instance, a variety of new suppression pathways beyond the PD-1/PD-L1 axis were described and subsequently linked with poor clinical outcomes in various malignancies.[122] Similar to this, the importance of tumor-infiltrating lymphocytes (TIL)-derived potential targets was increasingly acknowledged in recent years, including the identification of novel receptors for T-cell engagement that are able to bypass primary TCR activation.[123]The clinical potential of T-cell-engaging bispecific antibodies of this kind in the frame of ICIs has been shown for the anti-PD-1 in modern clinical trials. It functions by recommending the local release of innate-like immune cell-related cytokines, hence shifting the immune microenvironment towards T-cell activation and engagement. [124]

A similar approach is based on the concept of personalized therapeutic vaccines which are able to produce neoantigen-specific T-cell responses with already ongoing clinical trials evaluating their putative capacity in ICI combinations. [125] To maximize the clinical impact in terms of patient care, it is imperative to adapt the current and future landscape of ICIs and combinations to the specific alterations of the different types of cancer as well as their microenvironment. By implementing NGS and AI as tools for combination design, ICIs and novel combinations will not only broaden but also unify the treatment spectrum for cancer patients. [126]

8. Conclusion and future prospective

Combination therapy that uses various radiosensitizers, immune agents, or immunomodulators concurrently has demonstrated favorable efficacy in preclinical and clinical trials. This approach is being employed to tackle the disruption of the anti-tumor immune response, shortage of effective antigens, and heterogeneity of tumor cells. Moreover, combination therapy aims to improve the immune response-induced cytokine storm, overcome drug resistance, and increase the prevention of relapses. Hence, rationally designed combination strategies hold compelling potential for efficient clinical management of tumors. Indeed, combination approaches could overcome the adverse effect of monotherapy as well as broadening its application, leading to an enhanced cytotoxic T cell response against various cancers. With the attractive benefits of both molecularly precise agents and ICIs, it could be applicable in dealing with heterogenic tumor cells, cancer stem cells, desmoplastic stromal cells, and inflammatory cells within the TME. The effectiveness of combination therapy, including radiosensitizing and adherent-induced therapies, is currently supported by ongoing clinical studies.

During the era of precision medicine, the choice of monotherapy or, especially, combination therapy regimens should engage an interdisciplinary team, including oncologists, pathologists, clinical pharmacists, radiotherapists, and, importantly, patients themselves. Historically, such interdisciplinary teams have been increasingly organized in cancer hospitals. Emerging evidence and technologies may also offer an exciting new dimension for interpreting the effects of combination therapy in future clinical applications. If combination therapy is demonstrated to have therapeutic benefits to cancer patients, it could have general medical, public health, and economic impacts.

REFERENCES

1. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. Journal of hematology & oncology. 2021 Dec;14:1-29.
2. Kumar S, Singh SK, Rana B, Rana A. Tumor-infiltrating CD8+ T cell antitumor efficacy and exhaustion: molecular insights. Drug discovery today. 2021 Apr 1;26(4):951-67.
3. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annual Review of Pathology: Mechanisms of Disease. 2021 Jan 24;16(1):223-49.
4. Wojtukiewicz MZ, Rek MM, Karpowicz K, Górska M, Polityńska B, Wojtukiewicz AM, Moniuszko M, Radziwon P, Tucker SC, Honn KV. Inhibitors of immune checkpoints—PD-1, PD-L1, CTLA-4—new opportunities for cancer patients and a new challenge for internists and general practitioners. Cancer and Metastasis Reviews. 2021 Sep;40:949-82.
5. Pasello G, Pavan A, Attili I, Bortolami A, Bonanno L, Menis J, Conte P, Guarneri V. Real world data in the era of Immune Checkpoint Inhibitors (ICIs): Increasing evidence and future applications in lung cancer. Cancer treatment reviews. 2020 Jul 1;87:102031.
6. Esfahani K, Elkrief A, Calabrese C, Lapointe R, Hudson M, Routy B, Miller Jr WH, Calabrese L. Moving towards personalized treatments of immune-related adverse events. Nature reviews Clinical oncology. 2020 Aug;17(8):504-15.
7. de Miguel-Perez D, Russo A, Arrieta O, Ak M, Barron F, Gunasekaran M, Mamindla P, Lara-Mejia L, Peterson CB, Er ME, Peddagangireddy V. Extracellular vesicle PD-L1 dynamics predict durable response to immune-checkpoint inhibitors and survival in patients with non-small cell lung cancer. Journal of Experimental & Clinical Cancer Research. 2022 Jun 2;41(1):186.
8. Shiravand Y, Khodadadi F, Kashani SM, Hosseini-Fard SR, Hosseini S, Sadeghirad H, Ladwa R, O'Byrne K, Kulasinghe A. Immune checkpoint inhibitors in cancer therapy. Current Oncology. 2022 Apr 24;29(5):3044-60.
9. Singh V, Khurana A, Allawadhi P, Banothu AK, Bharani KK, Weiskirchen R. Emerging role of PD-1/PD-L1 inhibitors in chronic liver diseases. Frontiers in Pharmacology. 2021 Dec 23;12:790963.
10. Van Coillie S, Wiernicki B, Xu J. Molecular and cellular functions of CTLA-4. Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy. 2020:7-32.
11. Rossi E, Schinzari G, Maiorano BA, Indellicati G, Di Stefani A, Pagliara MM, Fragomeni SM, De Luca EV, Sammarco MG, Garganese G, Galli J. Efficacy of immune checkpoint inhibitors in different types of melanoma. Human vaccines & immunotherapeutics. 2021 Jan 2;17(1):4-13.
12. Pérol M, Felip E, Dafni U, Polito L, Pal N, Tsourti Z, Ton TG, Merritt D, Morris S, Stahel R, Peters S. Effectiveness of PD-(L) 1 inhibitors alone or in combination with platinum-doublet chemotherapy in first-line (1L) non-squamous non-small-cell lung cancer (Nsq-NSCLC) with PD-L1-high expression using real-world data. Annals of Oncology. 2022 May 1;33(5):511-21.
13. Kooshkaki O, Derakhshani A, Hosseinkhani N, Torabi M, Safaei S, Brunetti O, Racanelli V, Silvestris N, Baradaran B. Combination of ipilimumab and nivolumab in cancers: from clinical practice to ongoing clinical trials. International journal of molecular sciences. 2020 Jun 22;21(12):4427.
14. Obeagu EI, Obeagu GU. The Impact of Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) Genetic Variations on HIV Susceptibility and Progression. Elite Journal of Immunology. 2024;2(4):18-35.
15. Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan W, Shalaby MN, Thangavelu L, Kamrava S, Shomali N, Sohrabi AD. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. Cell communication and signaling. 2022 Apr 7;20(1):44.
16. Schoenfeld AJ, Hellmann MD. Acquired resistance to immune checkpoint inhibitors. Cancer cell. 2020 Apr 13;37(4):443-55.
17. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, Lambotte O, Mariette X, Prat A, Suárez-Almazor ME. Immune-related adverse events of checkpoint inhibitors. Nature reviews Disease primers. 2020 May 7;6(1):38.
18. Birnboim-Perach R, Benhar I. Using Combination therapy to overcome diverse challenges of Immune Checkpoint Inhibitors treatment. International Journal of Biological Sciences. 2024;20(10):3911.
19. Gougis P, Jochum F, Abbar B, Dumas E, Bihan K, Lebrun-Vignes B, Moslehi J, Spano JP, Laas E, Hotton J, Reyal F. Clinical spectrum and evolution of immune-checkpoint inhibitors toxicities over a decade—a worldwide perspective. EClinicalMedicine. 2024 Apr 1;70.
20. Sha D, Jin Z, Budczies J, Kluck K, Stenzinger A, Sinicrope FA. Tumor mutational burden as a predictive biomarker in solid tumors. Cancer discovery. 2020 Dec 1;10(12):1808-25.
21. Iyer KA, Ivanov J, Tenchov R, Ralhan K, Rodriguez Y, Sasso JM, Scott S, Zhou QA. Emerging Targets and Therapeutics in Immuno-Oncology: Insights from Landscape Analysis. Journal of Medicinal Chemistry. 2024 May 24.

22. Toor SM, Nair VS, Decock J, Elkord E. Immune checkpoints in the tumor microenvironment. InSeminars in cancer biology 2020 Oct 1 (Vol. 65, pp. 1-12). Academic Press.
23. Majidpoor J, Mortezaee K. The efficacy of PD-1/PD-L1 blockade in cold cancers and future perspectives. Clinical Immunology. 2021 May 1;226:108707.
24. Peterson C, Denlinger N, Yang Y. Recent advances and challenges in cancer immunotherapy. Cancers. 2022 Aug 17;14(16):3972.
25. Amhis N, Carignan J, Tai LH. Transforming pancreaticobiliary cancer treatment: Exploring the frontiers of adoptive cell therapy and cancer vaccines. Molecular Therapy Oncology. 2024 Sep 19;32(3).
26. Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, Kitui SK, Manyazewal T. New approaches and procedures for cancer treatment: Current perspectives. SAGE open medicine. 2021 Aug;9:20503121211034366.
27. Dey A, Mitra A, Pathak S, Prasad S, Zhang AS, Zhang H, Sun XF, Banerjee A. Recent advancements, limitations, and future perspectives of the use of personalized medicine in treatment of colon cancer. Technology in Cancer Research & Treatment. 2023 May;22:15330338231178403.
28. Carlino MS, Larkin J, Long GV. Immune checkpoint inhibitors in melanoma. The Lancet. 2021 Sep 11;398(10304):1002-14.
29. Johnson DB, Nebhan CA, Moslehi JJ, Balko JM. Immune-checkpoint inhibitors: long-term implications of toxicity. Nature Reviews Clinical Oncology. 2022 Apr;19(4):254-67.
30. Adam G, Rampášek L, Safikhani Z, Smirnov P, Haibe-Kains B, Goldenberg A. Machine learning approaches to drug response prediction: challenges and recent progress. NPJ precision oncology. 2020 Jun 15;4(1):19.
31. Marine JC, Dawson SJ, Dawson MA. Non-genetic mechanisms of therapeutic resistance in cancer. Nature Reviews Cancer. 2020 Dec;20(12):743-56.
32. Zhou HM, Zhang JG, Zhang X, Li Q. Targeting cancer stem cells for reversing therapy resistance: mechanism, signaling, and prospective agents. Signal transduction and targeted therapy. 2021 Feb 15;6(1):62.
33. Haas L, Elewaut A, Gerard CL, Umkehrer C, Leiendecker L, Pedersen M, Krecioch I, Hoffmann D, Novatchkova M, Kuttke M, Neumann T. Acquired resistance to anti-MAPK targeted therapy confers an immune-evasive tumor microenvironment and cross-resistance to immunotherapy in melanoma. Nature cancer. 2021 Jul;2(7):693-708.
34. Kim TK, Vandsemb EN, Herbst RS, Chen L. Adaptive immune resistance at the tumour site: mechanisms and therapeutic opportunities. Nature Reviews Drug Discovery. 2022 Jul;21(7):529-40.
35. Crucitta S, Cucchiara F, Mathijssen R, Mateo J, Jager A, Joosse A, Passaro A, Attili I, Petrini I, van Schaik R, Danesi R. Treatment-driven tumour heterogeneity and drug resistance: Lessons from solid tumours. Cancer treatment reviews. 2022 Mar 1;104:102340.
36. Pérez-Romero K, Rodríguez RM, Amedei A, Barceló-Coblijn G, Lopez DH. Immune landscape in tumor microenvironment: Implications for biomarker development and immunotherapy. International journal of molecular sciences. 2020 Aug 1;21(15):5521.
37. de Barra C. *Defining the Impact of Obesity and Obesity-Targeted Therapies on Natural Killer Cell Metabolism and Function* (Doctoral dissertation, National University of Ireland Maynooth).
38. Cai Z, Zhan P, Song Y, Liu H, Lv T. Safety and efficacy of retreatment with immune checkpoint inhibitors in non-small cell lung cancer: a systematic review and meta-analysis. Translational Lung Cancer Research. 2022 Aug;11(8):1555.
39. Choi J, Lee SY. Clinical characteristics and treatment of immune-related adverse events of immune checkpoint inhibitors. Immune network. 2020 Feb;20(1).
40. Nakamura Y, Namikawa K, Yoshikawa S, Kiniwa Y, Maekawa T, Yamasaki O, Isei T, Matsushita S, Nomura M, Nakai Y, Fukushima S. Anti-PD-1 antibody monotherapy versus anti-PD-1 plus anti-CTLA-4 combination therapy as first-line immunotherapy in unresectable or metastatic mucosal melanoma: a retrospective, multicenter study of 329 Japanese cases (JMAC study). ESMO open. 2021 Dec 1;6(6):100325.
41. Guidon AC, Burton LB, Chwalisz BK, Hillis J, Schaller TH, Amato AA, Warner AB, Brastianos PK, Cho TA, Clardy SL, Cohen JV. Consensus disease definitions for neurologic immune-related adverse events of immune checkpoint inhibitors. Journal for immunotherapy of cancer. 2021;9(7).
42. Olsen TA, Zhuang TZ, Caulfield S, Martini DJ, Brown JT, Carthon BC, Kucuk O, Harris W, Bilen MA, Nazha B. Advances in knowledge and management of immune-related adverse events in cancer immunotherapy. Frontiers in Endocrinology. 2022 Mar 22;13:779915.
43. Akhavanrezayat A, Uludag Kirimli G, Matsumiya W, Khojasteh H, Or C, Karaca I, Pham B, Ongpalakorn P, Lajevardi S, Lam B, Hwang JJ. The role of corticosteroids and immunomodulatory therapy in the management of infectious uveitis. Ocular Immunology and Inflammation. 2024 Feb 10:1-2.

44. Serce Unat D, Ulusan Bagci O, Unat OS, Kose S, Caner A. The Spectrum of infections in patients with lung cancer. Cancer Investigation. 2023 Jan 2;41(1):25-42.
45. Cherian SV, Patel D, Machnicki S, Naidich D, Stover D, Travis WD, Brown KK, Naidich JJ, Mahajan A, Esposito M, Mina B. Algorithmic approach to the diagnosis of organizing pneumonia: a correlation of clinical, radiologic, and pathologic features. Chest. 2022 Jul 1;162(1):156-78.
46. Okiyama N, Tanaka R. Immune-related adverse events in various organs caused by immune checkpoint inhibitors. Allergology International. 2022 Apr 1;71(2):169-78.
47. Yamamoto T. Skin manifestation induced by immune checkpoint inhibitors. Clinical, Cosmetic and Investigational Dermatology. 2022 May 10:829-41.
48. Karpathiou G, Péoc'h M, Sundaralingam A, Rahman N, Froudarakis ME. Inflammation of the pleural cavity: a review on pathogenesis, diagnosis and implications in tumor pathophysiology. Cancers. 2022 Mar 10;14(6):1415.
49. Lletí MS, Navalón MD, Pitarch MT, García-Bustos V. Etiology, diagnosis, and management of pneumonia in immunosuppressed patients. Revista Española de Quimioterapia. 2022;35(Suppl 1):89.
50. McKelvy BJ, Araujo-Filho JA, Godoy MC, de Groot PM, Truong MT, Moran CA. Infectious Diseases That May Mimic Lung Cancer. InThe Thorax: Medical, Radiological, and Pathological Assessment 2023 Jun 15 (pp. 827-851). Cham: Springer International Publishing.
51. Neacşu F, Vârban AŞ, Simion G, Șurghie R, Pătrașcu OM, Sajin M, Dumitru M, Vrînceanu D. Lung cancer mimickers–a case series of seven patients and review of the literature. Romanian Journal of Morphology and Embryology. 2022 Feb 18;62(3):697.
52. Xu Y, Xiong J, Sun X, Gao H. Targeted nanomedicines remodeling immunosuppressive tumor microenvironment for enhanced cancer immunotherapy. Acta Pharmaceutica Sinica B. 2022 Dec 1;12(12):4327-47.
53. Zhu S, Zhang T, Zheng L, Liu H, Song W, Liu D, Li Z, Pan CX. Combination strategies to maximize the benefits of cancer immunotherapy. Journal of hematology & oncology. 2021 Sep 27;14(1):156.
54. Liang JL, Luo GF, Chen WH, Zhang XZ. Recent advances in engineered materials for immunotherapy-involved combination cancer therapy. Advanced Materials. 2021 Aug;33(31):2007630.
55. Vafaei S, Zekiy AO, Khanamir RA, Zaman BA, Ghayourvahdat A, Azimizonuzi H, Zamani M. Combination therapy with immune checkpoint inhibitors (ICIs); a new frontier. Cancer Cell International. 2022 Dec;22:1-27.
56. Varayathu H, Sarathy V, Thomas BE, Mufti SS, Naik R. Combination strategies to augment immune check point inhibitors efficacy-implications for translational research. Frontiers in oncology. 2021 May 28;11:559161.
57. Testa U, Pelosi E, Castelli G. Genetic alterations in renal cancers: Identification of the mechanisms underlying cancer initiation and progression and of therapeutic targets. Medicines. 2020 Jul 29;7(8):44.
58. Morel D, Jeffery D, Aspeslagh S, Almouzni G, Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours—past lessons and future promise. Nature Reviews Clinical Oncology. 2020 Feb;17(2):91-107.
59. Kraehenbuehl L, Weng CH, Eghbali S, Wolchok JD, Merghoub T. Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. Nature reviews Clinical oncology. 2022 Jan;19(1):37-50.
60. Tarantino P, Ricciuti B, Pradhan SM, Tolaney SM. Optimizing the safety of antibody–drug conjugates for patients with solid tumours. Nature reviews Clinical oncology. 2023 Aug;20(8):558-76.
61. Sun L, Zhang L, Yu J, Zhang Y, Pang X, Ma C, Shen M, Ruan S, Wasan HS, Qiu S. Clinical efficacy and safety of anti-PD-1/PD-L1 inhibitors for the treatment of advanced or metastatic cancer: a systematic review and meta-analysis. Scientific reports. 2020 Feb 7;10(1):2083.
62. Malone ER, Oliva M, Sabatini PJ, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. Genome medicine. 2020 Dec;12:1-9.
63. Marei HE, Hasan A, Pozzoli G, Cenciarelli C. Cancer immunotherapy with immune checkpoint inhibitors (ICIs): potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. Cancer cell international. 2023 Apr 10;23(1):64.
64. Liu W, Zhang L, Xiu Z, Guo J, Wang L, Zhou Y, Jiao Y, Sun M, Cai J. Combination of immune checkpoint inhibitors with chemotherapy in lung cancer. OncoTargets and therapy. 2020 Jul 27:7229-41.
65. Sadagopan A, Michelakos T, Boyiadzis G, Ferrone C, Ferrone S. Human leukocyte antigen class I antigen-processing machinery upregulation by anticancer therapies in the era of checkpoint inhibitors: a review. JAMA oncology. 2022 Mar 1;8(3):462-73.
66. Shi C, Qin K, Lin A, Jiang A, Cheng Q, Liu Z, Zhang J, Luo P. The role of DNA damage repair (DDR) system in response to immune checkpoint inhibitor (ICI) therapy. Journal of Experimental & Clinical Cancer Research. 2022 Sep 7;41(1):268.

67. Jiang T, Wang P, Zhang J, Zhao Y, Zhou J, Fan Y, Shu Y, Liu X, Zhang H, He J, Gao G. Toripalimab plus chemotherapy as second-line treatment in previously EGFR-TKI treated patients with EGFR-mutant-advanced NSCLC: a multicenter phase-II trial. Signal Transduction and Targeted Therapy. 2021 Oct 15;6(1):355.
68. Salva de Torres C, Baraibar I, Saoudi González N, Ros J, Salva F, Rodríguez-Castells M, Alcaraz A, García A, Tabernero J, Élez E. Current and Emerging Treatment Paradigms in Colorectal Cancer: Integrating Hallmarks of Cancer. International Journal of Molecular Sciences. 2024 Jun 26;25(13):6967.
69. Adams R, Coumbe JE, Coumbe BG, Thomas J, Willsmore Z, Dimitrievska M, Yasuzawa-Parker M, Hoyle M, Ingar S, Geh JL, MacKenzie Ross AD. BRAF inhibitors and their immunological effects in malignant melanoma. Expert Review of Clinical Immunology. 2022 Apr 3;18(4):347-62.
70. Peiffer L, Farahpour F, Sriram A, Spassova I, Hoffmann D, Kubat L, Stoitzner P, Gambichler T, Sucker A, Ugurel S, Schadendorf D. BRAF and MEK inhibition in melanoma patients enables reprogramming of tumor infiltrating lymphocytes. Cancer Immunology, Immunotherapy. 2021 Jun;70:1635-47.
71. Liu Y, Zhang X, Wang G, Cui X. Triple combination therapy with PD-1/PD-L1, BRAF, and MEK inhibitor for stage III–IV melanoma: a systematic review and meta-analysis. Frontiers in Oncology. 2021 Jun 14;11:693655.
72. Lao Y, Shen D, Zhang W, He R, Jiang M. Immune checkpoint inhibitors in cancer therapy—how to overcome drug resistance?. Cancers. 2022 Jul 22;14(15):3575.
73. Dong Y, Wong JS, Sugimura R, Lam KO, Li B, Kwok GG, Leung R, Chiu JW, Cheung TT, Yau T. Recent advances and future prospects in immune checkpoint (ICI)-based combination therapy for advanced HCC. Cancers. 2021 Apr 18;13(8):1949.
74. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. Cancers. 2020 Mar 20;12(3):738.
75. Sathian B, Asim M, Banerjee I, Pizarro AB, Roy B, Van Teijlingen ER, do Nascimento IJ, Alhamad HK. Impact of COVID-19 on clinical trials and clinical research: a systematic review. Nepal journal of epidemiology. 2020 Sep;10(3):878.
76. Hayashi H, Nakagawa K. Combination therapy with PD-1 or PD-L1 inhibitors for cancer. International journal of clinical oncology. 2020 May;25(5):818-30.
77. Xia WL, Zhao XH, Guo Y, Cao GS, Wu G, Fan WJ, Yao QJ, Xu SJ, Guo CY, Hu HT, Li HL. Transarterial chemoembolization combined with apatinib with or without PD-1 inhibitors in BCLC stage C hepatocellular carcinoma: A multicenter retrospective study. Frontiers in Oncology. 2022 Sep 30;12:961394.
78. Dong H, Zhang Z, Ni M, Xu X, Luo Y, Wang Y, Zhang H, Chen J. The trend of the treatment of advanced hepatocellular carcinoma: combination of immunotherapy and targeted therapy. Current Treatment Options in Oncology. 2024 Sep 11:1-8.
79. Zhang L, Wang W, Ge S, Li H, Bai M, Duan J, Yang Y, Ning T, Liu R, Wang X, Ji Z. Sintilimab Plus Apatinib and Chemotherapy as Second-/Third-Line treatment for Advanced Gastric or Gastroesophageal Junction Adenocarcinoma: a prospective, Single-Arm, phase II trial. BMC cancer. 2023 Mar 5;23(1):211.
80. Gu Y, Zhao Q. Clinical Progresses and Challenges of Bispecific Antibodies for the Treatment of Solid Tumors. Molecular Diagnosis & Therapy. 2024 Nov;28(6):669-702.
81. Ackley SF, Zimmerman SC, Brenowitz WD, Tchetgen EJ, Gold AL, Manly JJ, Mayeda ER, Filshtein TJ, Power MC, Elahi FM, Brickman AM. Effect of reductions in amyloid levels on cognitive change in randomized trials: instrumental variable meta-analysis. Bmj. 2021 Feb 25;372.
82. de Miguel M, Calvo E. Clinical challenges of immune checkpoint inhibitors. Cancer cell. 2020 Sep 14;38(3):326-33..
83. Braun DA, Bakouny Z, Hirsch L, Flippot R, Van Allen EM, Wu CJ, Choueiri TK. Beyond conventional immune-checkpoint inhibition—novel immunotherapies for renal cell carcinoma. Nature reviews Clinical oncology. 2021 Apr;18(4):199-214.
84. Walia A, Tuia J, Prasad V. Progression-free survival, disease-free survival and other composite end points in oncology: improved reporting is needed. Nature Reviews Clinical Oncology. 2023 Dec;20(12):885-95.
85. Scott SC, Shao XM, Niknafs N, Balan A, Pereira G, Marrone KA, Lam VK, Murray JC, Feliciano JL, Levy BP, Ettinger DS. Sex-specific differences in immunogenomic features of response to immune checkpoint blockade. Frontiers in Oncology. 2022 Aug 3;12:945798.
86. Lee NY, Ferris RL, Psyrri A, Haddad RI, Tahara M, Bourhis J, Harrington K, Chang PM, Lin JC, Razaq MA, Teixeira MM. Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. The Lancet Oncology. 2021 Apr 1;22(4):450-62.

87. Chyuan IT, Chu CL, Hsu PN. Targeting the tumor microenvironment for improving therapeutic effectiveness in cancer immunotherapy: focusing on immune checkpoint inhibitors and combination therapies. Cancers. 2021 Mar 10;13(6):1188.
88. He M, Yang T, Wang Y, Wang M, Chen X, Ding D, Zheng Y, Chen H. Immune checkpoint inhibitor-based strategies for synergistic cancer therapy. Advanced Healthcare Materials. 2021 May;10(9):2002104.
89. El-Kadiry AE, Rafei M, Shammaa R. Cell therapy: types, regulation, and clinical benefits. Frontiers in Medicine. 2021 Nov 22;8:756029.
90. Yang Z, Chen Y, Wang Y, Hu M, Qian F, Zhang Y, Zhang B, Zhang W, Han B. Pembrolizumab plus chemotherapy versus chemotherapy monotherapy as a first-line treatment in elderly patients (≥ 75 years old) with non-small-cell lung cancer. Frontiers in Immunology. 2022 Feb 14;13:807575.
91. Plana D, Palmer AC, Sorger PK. Independent drug action in combination therapy: implications for precision oncology. Cancer discovery. 2022 Mar 1;12(3):606-24.
92. Palmer AC, Izar B, Hwangbo H, Sorger PK. Predictable clinical benefits without evidence of synergy in trials of combination therapies with immune-checkpoint inhibitors. Clinical Cancer Research. 2022 Jan 15;28(2):368-77.
93. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, Ferrucci PF. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. Journal of Clinical Oncology. 2022 Jan 10;40(2):127-37.
94. Zhou X, Yao Z, Bai H, Duan J, Wang Z, Wang X, Zhang X, Xu J, Fei K, Zhang Z, Tan F. Treatment-related adverse events of PD-1 and PD-L1 inhibitor-based combination therapies in clinical trials: a systematic review and meta-analysis. The Lancet Oncology. 2021 Sep 1;22(9):1265-74.
95. Passarelli A, Aieta M, Sgambato A, Gridelli C. Targeting immunometabolism mediated by CD73 pathway in EGFR-mutated non-small cell lung cancer: a new hope for overcoming immune resistance. Frontiers in immunology. 2020 Jul 14;11:1479.
96. Zhang L, Sun L, Zhou Y, Yu J, Lin Y, Wasan HS, Shen M, Ruan S. Association of survival and immune-related adverse events with anti-PD-1/PD-L1 and anti-CTLA-4 inhibitors, alone or their combination for the treatment of cancer: a systematic review and meta-analysis of 13 clinical trials. Frontiers in Oncology. 2021 Feb 25;11:575457.
97. Paller AS, Simpson EL, Siegfried EC, Cork MJ, Wollenberg A, Arkwright PD, Soong W, Gonzalez ME, Schneider LC, Sidbury R, Lockshin B. Dupilumab in children aged 6 months to younger than 6 years with uncontrolled atopic dermatitis: a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet. 2022 Sep 17;400(10356):908-19.
98. Feng W, Que S, Wang H, Dai E, Zhai S, Mo Q, Li Y, Wang J, Liang X. EP. 13B. 15 Radiotherapy Combined with Immunotherapy in the Treatment of Brain Metastases from Small Cell Lung Cancer. Journal of Thoracic Oncology. 2024 Oct 1;19(10):S670.
99. Habiburrahman M, Masrour N, Patel N, Piskorz A, Brown R, Brenton J, McNeish IA, Flanagan JM. Clinical validation of a DNA methylation biomarker to predict overall survival of relapsed ovarian cancer patients. medRxiv. 2024:2024-09.
100. Wolf DM, Yau C, Wulfkuhle J, Brown-Swigart L, Gallagher RI, Lee PR, Zhu Z, Magbanua MJ, Sayaman R, O'Grady N, Basu A. Redefining breast cancer subtypes to guide treatment prioritization and maximize response: Predictive biomarkers across 10 cancer therapies. Cancer Cell. 2022 Jun 13;40(6):609-23.
101. Ramalhete LM, Araújo R, Ferreira A, Calado CR. Proteomics for biomarker discovery for diagnosis and prognosis of kidney transplantation rejection. Proteomes. 2022 Jul 2;10(3):24.
102. Lin K, Huang J, Luo H, Luo C, Zhu X, Bu F, Xiao H, Xiao L, Zhu Z. Development of a prognostic index and screening of potential biomarkers based on immunogenomic landscape analysis of colorectal cancer. Aging (Albany NY). 2020 Apr 4;12(7):5832.
103. Brueckl WM, Ficker JH, Zeitler G. Clinically relevant prognostic and predictive markers for immune-checkpoint-inhibitor (ICI) therapy in non-small cell lung cancer (NSCLC). BMC cancer. 2020 Dec;20:1-6.
104. Müller L, Stoehr F, Mähringer-Kunz A, Hahn F, Weinmann A, Kloeckner R. Current strategies to identify patients that will benefit from TACE treatment and future directions a practical step-by-step guide. Journal of Hepatocellular Carcinoma. 2021 May 13:403-19.
105. Bejarano L, Jordão MJ, Joyce JA. Therapeutic targeting of the tumor microenvironment. Cancer discovery. 2021 Apr 1;11(4):933-59.
106. Zhang QU, Luo J, Wu S, Si H, Gao C, Xu W, Abdullah SE, Higgs BW, Dennis PA, van der Heijden MS, Segal NH. Prognostic and predictive impact of circulating tumor DNA in patients with advanced cancers treated with immune checkpoint blockade. Cancer discovery. 2020 Dec 1;10(12):1842-53.

107. Shum B, Larkin J, Turajlic S. Predictive biomarkers for response to immune checkpoint inhibition. InSeminars in cancer biology 2022 Feb 1 (Vol. 79, pp. 4-17). Academic Press.
108. Wang Y, Tong Z, Zhang W, Zhang W, Buzdin A, Mu X, Yan Q, Zhao X, Chang HH, Duhon M, Zhou X. FDA-approved and emerging next generation predictive biomarkers for immune checkpoint inhibitors in cancer patients. Frontiers in oncology. 2021 Jun 7;11:683419.
109. Rosellini M, Marchetti A, Mollica V, Rizzo A, Santoni M, Massari F. Prognostic and predictive biomarkers for immunotherapy in advanced renal cell carcinoma. Nature Reviews Urology. 2023 Mar;20(3):133-57.
110. Turner BE, Steinberg JR, Weeks BT, Rodriguez F, Cullen MR. Race/ethnicity reporting and representation in US clinical trials: a cohort study. The Lancet Regional Health–Americas. 2022 Jul 1;11.
111. Yamauchi T, Hoki T, Oba T, Jain V, Chen H, Attwood K, Battaglia S, George S, Chatta G, Puzanov I, Morrison C. T-cell CX3CR1 expression as a dynamic blood-based biomarker of response to immune checkpoint inhibitors. Nature communications. 2021 Mar 3;12(1):1402.
112. Gokemeijer J, Balasubramanian N, Ogasawara K, Grudzinska-Goebel J, Upreti VV, Mody H, Kasar S, Vepachedu VR, Xu W, Gupta S, Tarcsa E. An IQ consortium perspective on best practices for bioanalytical and immunogenicity assessment aspects of CAR-T and TCR-T cellular therapies development. Clinical Pharmacology & Therapeutics. 2024 Feb;115(2):188-200.
113. Błach J, Wojas-Krawczyk K, Nicoś M, Krawczyk P. Failure of immunotherapy—the molecular and immunological origin of immunotherapy resistance in lung cancer. International Journal of Molecular Sciences. 2021 Aug 21;22(16):9030.
114. Hontecillas-Prieto L, Flores-Campos R, Silver A, De Álava E, Hajji N, García-Domínguez DJ. Synergistic enhancement of cancer therapy using HDAC inhibitors: opportunity for clinical trials. Frontiers in genetics. 2020 Sep 11;11:578011.
115. Shi T, Song X, Wang Y, Liu F, Wei J. Combining oncolytic viruses with cancer immunotherapy: establishing a new generation of cancer treatment. Frontiers in immunology. 2020 Apr 28;11:683.
116. Gatenby RA, Brown JS. Integrating evolutionary dynamics into cancer therapy. Nature reviews Clinical oncology. 2020 Nov;17(11):675-86.
117. Jonklaas J, Bianco AC, Cappola AR, Celi FS, Fliers E, Heuer H, McAninch EA, Moeller LC, Nygaard B, Sawka AM, Watt T. Evidence-based use of levothyroxine/liothyronine combinations in treating hypothyroidism: a consensus document. European thyroid journal. 2021 Mar 1;10(1):10-38.
118. Ghany MG, Buti M, Lampertico P, Lee HM. Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in chronic hepatitis B and D: Report from the 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference. Hepatology. 2023 Nov 1;78(5):1654-73.
119. Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nature reviews Clinical oncology. 2020 Dec;17(12):725-41.
120. Seliger B, Massa C. Immune therapy resistance and immune escape of tumors. Cancers. 2021 Feb 1;13(3):551.
121. Liu Z, Zhang Y, Shi C, Zhou X, Xu K, Jiao D, Sun Z, Han X. A novel immune classification reveals distinct immune escape mechanism and genomic alterations: implications for immunotherapy in hepatocellular carcinoma. Journal of Translational Medicine. 2021 Dec;19:1-7.
122. Ai L, Xu A, Xu J. Roles of PD-1/PD-L1 pathway: signaling, cancer, and beyond. Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy. 2020:33-59.
123. Rumolo A. Isolation, expansion and characterization of tumor infiltrating lymphocytes derived from metastatic colorectal carcinoma patients for adoptive immunotherapy. 2023.
124. Goebeler ME, Bargou RC. T cell-engaging therapies—BiTEs and beyond. Nature Reviews Clinical Oncology. 2020 Jul;17(7):418-34.
125. Faghfuri E. Recent advances in personalized cancer immunotherapy with immune checkpoint inhibitors, T cells and vaccines. Personalized medicine. 2024 Jan;21(1):45-57.
126. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, Thakare RP, Banday S, Mishra AK, Das G, Malonia SK. Next-generation sequencing technology: current trends and advancements. Biology. 2023 Jul 13;12(7):997.