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From Famine to Feast: Developing Early Phase Combination Immunotherapy Trials Wisely

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Abstract

Not until the turn of this century has immunotherapy become a fundamental component of cancer treatment. While monotherapy with immune modulators such as immune checkpoint inhibitors provides a subset of patients with durable clinical benefit and possible cure, combination therapy offers the potential for anti-tumor activity in a greater number of patients. The field of immunology has provided us with a plethora of potential molecules and pathways to target. This abundance makes it impractical to empirically test all possible combinations efficiently. We recommend that potential immunotherapy combinations be chosen based on sound rationale and available data to address the mechanisms of primary and acquired immune resistance. Novel trial designs may increase the proportion of patients receiving potentially efficacious treatments and, at the same time, better define the balance of clinical activity and safety. We believe that implementing a strategic approach in the early development of immunotherapy combinations will expedite the delivery of more effective therapies with improved safety and durable outcomes.

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INTRODUCTION

The hypothesis that the immune system can be manipulated to fight cancer was made over a century ago. Despite significant advances in the scientific insights of antitumor immunity, repeated prior therapeutic attempts - largely aimed at immune stimulation via cancer vaccines - have met limited success. Recently, anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and anti-programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) monoclonal antibodies targeting immune inhibitory pathways referred to as checkpoints, have demonstrated durable responses in multiple tumor types including melanoma (1, 2), renal cell carcinoma (RCC) (3), non-small cell lung cancer (NSCLC) (4), bladder cancer (5), Hodgkin's lymphoma (6), gastric cancer (7), head and neck squamous cell carcinoma (8), and microsatellite unstable colon cancer (9); these results have led to a growing number of regulatory indications.

Single agent activity is limited to a minority of patients and emerging long-term follow-up data in melanoma indicate that a substantial proportion of patients previously responding to immune checkpoint inhibitor therapy develop resistance (10–12). Evidence-based combinations may lead to therapeutic synergies to overcome resistance. The enhanced efficacy of dual CTLA-4 and PD-1 blockade in melanoma (13–15) is an example. Multiple new agents targeting various immune processes are entering clinical development. Examples include other immune checkpoint inhibitors, co-stimulatory agonists, oncolytic viruses, vaccines and adoptive cell therapy (Table 1), the broad potential of immunotherapies is being explored in novel combinations and in combination with conventional therapies.

A fundamental challenge for the immuno-oncology field is the rational selection of agents from a vast number of possible combinations while contending with escalating financial costs and limited resources. The current clinical trial framework will need to be modernized to support the successful development and implementation of immunotherapy combinations into standard clinical care. Key components to consider include new approaches to optimize dose determination and operational efficiency, the incorporation of clinically fitting endpoints, and the integration of biomarker assessment to guide patient selection. This paper builds upon previous recommendations by the Clinical Trial Design Task Force (CTD) of the National Cancer Institute (NCI) Investigational Drug Steering Committee (IDSC) on combination phase I trials (16) and complements other papers in the *CCR Focus* series, outlining guidance on the design and conduct of immunotherapy clinical trials. The complex challenges of and recommendations for combination immunotherapy development are discussed here, with an emphasis on early phase trials (Table 2).

MECHANISMS OF RESISTANCE TO IMMUNOTHERAPY AND THE RATIONALE FOR COMBINATIONS

Characterization of the human tumor micro-environment (TME), in particular its molecular features and the presence of lymphocytic infiltration, has led to the identification of distinct immunophenotypes (Figures 1 and 2) (17). These include a T cell-infiltrated phenotype with a broad chemokine profile and type I interferon signature and a non-T cell-infiltrated phenotype that lacks inflammatory signals for recruitment of T cells (18). The predominant

mechanism of immunosuppression in the T cell-infiltrated or so-called ‘inflammed’ TME is postulated to be upregulation of inhibitory pathways rendering T cells dysfunctional, providing a rationale for targeting co-inhibitory molecules (see quadrant A in Figures 1 and 2). In fact, correlative data indicate that an underlying immune-active TME characterized by the presence of CD8+ T cells may be a pre-condition for response to immune checkpoint inhibitors (19, 20). Disabling a singular pathway may be insufficient, however, and may trigger compensatory mechanisms leading to resistance (21). These may be circumvented by inhibition of additional immune checkpoints or modulation of T cell co-stimulatory molecules, which upon engagement function to promote T cell activity. This hypothesis is validated by the demonstration of synergistic activity of multi-checkpoint blockade in non-clinical studies (22, 23). In some cases, interference with antigen presentation (B in Figures 1 and 2) is the primary barrier to T cell response and may be therapeutically targeted by strategies to enhance antigen-presenting cell function. Conversely, patients with non-T cell infiltrated or ‘non-inflammed’ phenotypes are unlikely to respond to immunomodulatory agents alone (C and D in Figure 1 and 2). Such patients will likely require more intensified combination therapies to induce and promote tumor T cell infiltration or recognition of tumor antigens, through modalities such as adoptive cell therapy, inflammatory cytokines, immune stimulatory agents and vaccines. Conventional therapies, such as chemotherapy, radiotherapy, and molecularly-targeted agents also have a role in priming the immune response by causing tumor death-related antigen presentation in addition to other immunomodulatory roles, with synergism found in combination with immunotherapies in multiple nonclinical studies (24–29). Another emerging combinatorial approach involves epigenetic therapies with *in vivo* demonstration of synergy; growing evidence indicates that epigenetic reprogramming may suppress immune-related genes and/or tumor-specific antigens (30). An alternative pragmatic classification model stratifies the TME into four types based on the presence or absence of tumor-infiltrating lymphocytes and PD-L1 expression (31, 32). However, caveats include the lack of standardized methodology and sampling challenges in light of intratumoral heterogeneity and the adaptive and dynamic nature of immune resistance. Furthermore, relevant variables such as tumoral stromal and molecular factors, and other immune cell populations are not characterized.

Arguably the greatest challenge in the immuno-oncology field is primary and acquired resistance to therapy. While stratification of the TME provides a context for understanding anti-tumor immunity and guidance for treatment selection, more translational studies are essential for dissecting the molecular complexities of immune resistance. Interestingly, two recent genomic profiling studies linked acquired resistance to anti-PD-1 therapy and primary resistance to CTLA-4 blockade to defects in the pathways regulating interferon receptor signalling (33, 34). Additionally, tumor-intrinsic active β-catenin signalling has been identified as one potential mechanism of T cell exclusion (35). Another study in melanoma patients found innately anti-PD-1 therapy-resistant tumors display upregulation of genes involved in mesenchymal transition, cell adhesion, and angiogenesis, suggesting that these biological processes and their effects on the TME may impede anti-tumor immunity (36). Lastly, pharmacological factors including drug exposure and clearance, receptor occupancy and tumor penetrance; patient-intrinsic factors such as age, gender and body weight; and other cellular processes may also affect treatment and should be considered. Figures 1 and 2

illustrate examples of tumor immune escape mechanisms and a number of suggested combinatorial strategies to target these.

LESSONS LEARNED FROM PRIOR IMMUNOTHERAPY COMBINATIONS

Immunotherapy-immunotherapy combinations

The combination of the anti-CTLA-4 antibody, ipilimumab, and the anti-PD-1 antibody, nivolumab, is the most clinically studied immunotherapy doublet thus far (13–15, 37, 38), and the key lessons learnt are detailed in Table 3. In summary, the combination achieved enhanced activity characterized by earlier and deeper antitumor responses for a greater proportion of patients with melanoma, compared with monotherapy. Early survival data in the randomized phase III study in melanoma found that the combination significantly improved overall survival compared with ipilimumab (hazard ratio, 0.55; $P<0.0001$). At the present time with a minimum follow-up of 28 months, median overall survival has not been reached for the combination and nivolumab alone arms. In any case, the study is not powered for a statistical comparison between these two arms (39). Despite considerable activity, substantial treatment-related toxicities may challenge the clinical application of combination CTLA-4 and PD-1 blockade. Interestingly, the candidate dose regimen selected has differed among trials in melanoma, NSCLC, and RCC, primarily due to a determination in early studies that non-melanoma populations did not tolerate the regimen containing a higher ipilimumab dose (13, 37, 38). Differential dosing highlights potential tumor-specific differences in immune checkpoint inhibitor tolerability and efficacy, and the importance of thorough dose exploration studies. Pharmacological analyses have shown that treatment efficacy and toxicity is likely to be dose-dependent for ipilimumab while anti-PD-1 agents demonstrated relatively flat exposure-efficacy relationships (40–42). Moreover, despite regulatory approvals in Europe and North America, the optimal combination dose regimen in melanoma is still not clear and is the subject of an ongoing trial (NCT02714218). The challenges of varying dosing strategies of immune checkpoint inhibitors are further discussed by Baik and colleagues (43).

Another critical challenge in immuno-oncology is patient selection. Although antitumor activity was enriched in the PD-L1 positive population in the landmark phase III study in melanoma, incremental progression-free survival (PFS) gains compared with single-agent nivolumab was greater in the PD-L1 negative population in subgroup analysis, suggesting PD-L1 may have a role in selecting patients who require doublet therapy (15). Nevertheless, the therapeutic success of pairing ipilimumab and nivolumab has spurred the ongoing investigation of similar combinations in various tumor types (44), including the combination of anti-PD-L1 antibody, durvalumab and anti-CTLA-4 antibody, tremelimumab which has demonstrated encouraging results in NSCLC in a phase Ib study (45). Additionally, numerous novel immunotherapy combinations are in various stages of development, often with CTLA-4 or PD-1/PD-L1 inhibitors as backbones, and many are exhibiting promising early activity and safety profiles. Examples include idoleamine-2,3-dioxygenase 1 (IDO-1) inhibitor with ipilimumab (46) in melanoma and the anti-PD-1 antibody, pembrolizumab (47) in selected tumor types, and intratumoral injections of oncolytic virus, Talimogene laherparepvec (T-VEC) in combination with pembrolizumab in melanoma (48). In fact,

currently there are over 800 clinical trials testing approximately 20 anti-PD-1/PD-L1/PD-L2 agents alone and in combinations for numerous indications (49).

Immunotherapy in combination with chemotherapy, radiotherapy and molecularly-targeted agents

A phase II study in advanced NSCLC showed that the addition of ipilimumab to platinum-based chemotherapy in a 'phased' fashion (cycle 3 to cycle 6) modestly improved median immune-related PFS (50). In another phase II study in non-squamous NSCLC, pembrolizumab combined with chemotherapy demonstrated improved response rate and PFS, compared with chemotherapy alone, with acceptable tolerability (51).

Pre-clinical and clinical data suggest that radiotherapy is a promising modality for combinatorial immunotherapy strategies. In addition to debulking tumor and releasing tumor antigens, radiotherapy has well-established immunomodulatory effects which may counteract mechanisms of resistance such as poor-immunogenicity and T-cell exclusion, and elicit systemic abscopal effects (52). This was aptly demonstrated in a proof-of-principle trial in advanced solid tumor patients, where the combination of radiotherapy to a single metastatic lesion with granulocyte-macrophage colony stimulating factor produced objective abscopal responses in 27% of patients (53). To date, clinical trials employing radiotherapy and CTLA-4 blockade have not conclusively shown clear benefit, including a negative phase III trial of ipilimumab versus placebo after radiotherapy in prostate cancer, but do confirm the safety of the combination (26, 54, 55). Trials combining radiotherapy with PD-1/PD-L1 checkpoint blockade are underway. A recent secondary analysis of the KEYNOTE-001 trial suggests that patients treated with PD-1 blockade who had received prior radiotherapy experienced an improved median progression-free survival (4.4 versus 2.1 months, p=0.019) and median overall survival (10.7 versus 5.3 months, p=0.026) (56), but these findings should be interpreted cautiously and require confirmation in prospective randomized trials. Immune-stimulatory agents such as interleukin-2 (57) in melanoma and RCC and a Toll-like receptor agonist (58) in low grade B cell lymphoma have demonstrated promising early results in combination with radiotherapy.

Early phase data of anti-PD-1/PD-L1 agents combined with standard-of-care molecularly-targeted agents in multiple tumor types appear to be well tolerated, although efficacy outcomes are largely pending (59–61). Recently, novel combinations of anti-PD-1/PD-L1 therapy in early phase trials with the MEK inhibitor cobimetinib in colorectal cancer (62); immunomodulatory agent lenolidamide in relapsed/refractory multiple myeloma (63); and antiandrogen enzalutamide in enzalutamide-resistant prostate cancer (64) have resulted in compelling efficacy despite expectedly limited monotherapy activity. The finding in the last example complements correlative data demonstrating PD-L1 upregulation in enzalutamide-resistant prostate cancer cells (65), suggesting that the molecular features within the TME may evolve in response to treatment pressures, and hence by inference, sensitivity to immunotherapy may fluctuate at different stages of the disease process. Genomic factors such as possible underlying mismatch repair defects, defects in DNA proofreading due to loss of function of DNA polymerase epsilon (66) or *BRCA2* mutations may have also influenced the antitumor activity seen in this subgroup and remain to be explored.

Despite these early sources of enthusiasm, important caveats remain when combining immunotherapy with conventional therapy. Firstly, toxicities may be potentiated and unanticipated, as evidenced by the first clinical experiences combining anti-CTLA-4 antibodies with the BRAF inhibitor vemurafenib and the vascular endothelial growth factor receptor (VEGFR) inhibitor sunitinib where hepatoxicity and rapid-onset acute renal failure, respectively, led to trial closures (29, 67, 68). Interestingly, a number of reports of patients receiving vemurafenib following treatment with anti-CTLA-4 and anti-PD-1 antibodies suggest sequential therapies may also lead to severe cutaneous and systemic adverse events (69–71). Although there is currently limited understanding of the pharmacological and immune-mediated mechanisms underlying these toxicities, these findings emphasize the need to demonstrate safety of new combinations in the clinical trial setting, even for agents with regulatory approval and non-overlapping toxicity profiles. The adequate washout periods in the case of sequential treatments are also not known and clinicians need to monitor vigilantly for potential augmented toxicities. Secondly, timing and sequencing of treatments are likely to have an impact on efficacy. Emerging evidence suggests that the immune responses induced by molecularly-targeted agents may be early and transient, and low CD8+ T cell density is seen at treatment progression (72). In the case of chemotherapy and radiation, the immunomodulatory effects are complex and some of these effects may be suppressive (73). Additionally, there is considerable variability in both modalities in terms of treatment type and quality, dose and fractionation, and schedule and timing. Limited mechanistic data are available to guide how to best combine these treatments and immunotherapy in light of these variables. Thus, caution must be exercised as the consequence of compromising the efficacy of established treatments is substantial, particularly in curative settings, and every effort should be made to elucidate individual and combined immunomodulatory and pharmacological effects.

GOALS AND CHALLENGES OF IMMUNOTHERAPY COMBINATIONS

Scientific challenges: prioritizing evidence-based combinations

Current combination selection is largely empiric, based on availability and a supposition of complementary and non-redundant mechanisms of action. As more targets and therapies are discovered, prioritizing the most promising combinations and rational sequencing of therapies will be crucial. Combinations must be designed to address clinical and biological challenges and should provide a significant advantage over monotherapy by deactivating mechanisms of immune escape, or substantially augmenting responses, while maintaining acceptable tolerability. Given these objectives, industry collaborations should be strongly encouraged to avoid duplication of efforts and investigational pipelines, in order to minimize cost, redundant resource utilization and regulatory pressures. Goals and recommendations for early phase combination immunotherapy trials are summarized in Table 2, beginning with a strong scientific hypothesis supported by nonclinical or clinical data.

Currently, there are substantial limitations to nonclinical studies, including suboptimal reproducibility (74, 75), publication bias and insufficiently characterized combination index (defined as a quantitative measure of combination drug effects) (76). In immunotherapy research, an additional barrier arises from inherent differences in immune systems across

species and tumor antigen repertoire, leading to poor recapitulation of host immune effects, as highlighted by unforeseen severe immune-mediated toxicities in the first-in-human (FIH) study of a CD28 agonist (77). Active efforts are being made to improve the reliability of nonclinical models to better simulate clinical complexities (78), including the development of alternative translational models such as various immunocompetent allograft mouse models and 'humanized' mouse models in which murine immune-related genes or proteins are replaced with human equivalents (79). Another example is the use of companion canines that develop spontaneous tumors in the setting of an intact immune system (27). The advantages of canine models include large population size and tumor and immune system characteristics that are more akin to that of humans compared with rodent models (80).

Limitations notwithstanding, nonclinical studies are an excellent platform for mechanistic and exploratory studies and have helped to guide the selection of current immunotherapy combinations. Recommended aims of nonclinical studies to consider when designing experimental conditions are detailed in Table 2. Additionally, considering the limited predictive capacity of nonclinical studies, initial small proof-of-principle clinical studies with high efficacy bars may also be appropriate to select combinations to take forward.

Patient selection considerations and biomarkers

An imperative for immunotherapy combinations is to focus on populations with unmet needs, particularly those who are unlikely to derive benefit from monotherapy. However, currently there is no precise method of biomarker-driven patient selection. PD-L1 expression is the most mature biomarker for anti-PD-1/PD-L1 therapy and several companion diagnostic PD-L1 assays have been approved by the US Food and Drug Administration (FDA). It enriches for responders in some but not consistently in all disease indications, and a negative result cannot reliably predict non-response (3, 15, 81). Additional drawbacks include variability in assay techniques and quantitative cut-offs (81). The initial results of a collaborative project evaluating the analytical comparability of the four PD-L1 companion assays used in NSCLC, found that while three assays demonstrated similar tumor cell PD-L1 expression, inter-observer discrepancy was high for immune cell PD-L1 expression. Notably, the use of alternative assays would lead to discordance in PD-L1 positivity and the treatment-determining threshold in 37% of cases (82).

To refine personalized treatment selection, intensive efforts have been invested in biomarker discovery for immunotherapies and these approaches are likely to be complementary to PD-L1 expression. For example, the aforementioned stratification of the TME based on PD-L1 status and lymphocytic infiltration has been described to guide treatment options (32, 83). Although several groups found tumor mutational burden and neoantigen load were positively associated with immune checkpoint inhibitor response, there was overlap in the range of mutations and neoantigens between the responders and nonresponders (84, 85). A phase II study demonstrated the utility of DNA mismatch repair status as a predictor of response to PD-1 blockade (9). Mismatch repair-deficiency results in microsatellites and far greater numbers of mutation-associated neoantigens which is thought to be the basis of increased immune infiltrates and improved immunotherapy response in these tumors, compared with mismatch repair-proficient tumors (9). Other promising emerging biomarkers

include gene expression patterns and signatures. Elevated baseline expression of immune-related genes, including T helper type-1 and interferon-gamma pathway-related genes is associated with favorable response to immunotherapies in multiple tumor types (8, 20, 86, 87). Conversely, analyses from The Cancer Genome Atlas in 13 tumor histologies showed that increased Wnt/β-catenin pathway signalling correlates with absence of T cell gene expression and may mediate both primary and acquired resistance to immunotherapy (35, 88).

It is foreseeable that in the near future, novel techniques such as immune monitoring, tumor antigen profiling, T cell receptor sequencing and gene expression signatures at multiple treatment time points can provide integrated multidimensional and dynamic data on a patient's immune milieu, offering hope for individualized treatment selection (89). The challenges and future directions of immune biomarkers are discussed by Mehnert and colleagues in this *CCR Focus* series (90). At present, in the absence of validated biomarkers, one approach for combination trials may be to restrict eligibility to salvage settings for patients who are or likely to be monotherapy-refractory. One example is a randomized phase II study assessing the efficacy of ipilimumab versus ipilimumab and nivolumab in anti-PD-1 therapy-refractory patients (NCT02731729).

Dose selection and the need for innovative trial designs

Traditional rule-based designs, such as the classic '3+3' design, that use toxicity-driven dose escalation to define the maximum tolerated dose (MTD) and assume a linear dose-efficacy-toxicity relationship, are unlikely to be adequate for immunotherapies. Immune-driven effects are difficult to predict and depend on a myriad of poorly understood factors beyond drug dose and exposure. Well-tolerated agents may also achieve the desired target effect without producing significant detectable toxicity. In fact, in many immune checkpoint inhibitor phase I trials, MTDs were not reached with few dose-limiting toxicity (DLT) events (91). Immune-related adverse events (IrAEs) may also be delayed, and will not be sufficiently captured by the DLT observational period (generally the first cycle of treatment). Moreover, combination agents introduce further challenges including pharmacodynamic (PD) and pharmacokinetic (PK) interactions, potentially overlapping or additive toxicity profiles, and multiple possible combinations of MTDs and schedules. These complexities necessitate novel integrated approaches in trial design.

Model-based designs and Bayesian methods were developed to provide more precise estimates of the recommended phase II dose (RP2D), by building on a pre-study *a priori* dose-toxicity curve, then using accumulating data during the trial to update the curve and inform prospective dose escalation decisions. Features of contemporary designs can be extended to include other clinically relevant endpoints such as efficacy, pharmacology parameters and long term tolerability (92). Parallel PK/PD assessments help to define pharmacologic properties for each agent and in combination, and may inform dose determination. For example, pharmacological data (in this case, the dose sufficient to maintain target drug levels) assisted in determining the dose of the anti-PD-L1 antibody, atezolizumab in a phase I study when MTD was not reached (93).

Although 3+3 designs have been used in the majority of past phase I trials owing to their operational simplicity, model-based, Bayesian and hybrid approaches are increasingly adopted as the therapeutic landscape and statistical capabilities evolve (92, 94). The modified toxicity probability interval design, which couples a rule-based dose-finding scheme with guidance by Bayesian posterior estimates (95), is employed in numerous ongoing immunotherapy combination trials. To delineate the degree of additive toxicity by a combination regimen, a randomized Bayesian phase I design has been proposed in which dose determination is based on the difference of probability of DLTs between the control (single agent) arm and the combination arm (96). Furthermore, multiple statistical designs have been described using both toxicity and efficacy endpoints, and may be well-suited to immunotherapies combination trials to assess for early activity (92, 97). One such example is the parallel phase I/II zone design which utilizes rule-based dose escalation and subsequent Bayesian adaptive randomization to maximize the number of patients treated with the most effective dose combinations (97).

The choice of dose escalation design should be individualized, taking into consideration non-clinical and single agent pharmacology data, desired trial outcomes and aims, target patient population characteristics and the intended drug development plan. In combination trials, where a wide range of dose pairings are possible, a pragmatic approach may be to identify an effective dose range or a number of admissible schedules for further evaluation in subsequent expansion or phase II studies (98). Although regimen selection is preferred prior to the registration study, post-approval dose optimization may be necessary. The aforementioned trial of ipilimumab and nivolumab is an example (NCT02714218). Additionally, the duration of anti-PD-1 therapy sufficient to trigger durable immune responses is currently undefined and is the theme of ongoing investigation (NCT02821013).

Aside from dose escalation trials, population pharmacological modelling correlating exposure and other PK data with toxicity, efficacy and other multifactorial endpoints may have a complementary role in supporting dose selection and may further characterize the target therapeutic window (40–42). Indeed, flat-dosing of nivolumab and pembrolizumab were found to be comparable to weight-based dosing in population PK/PD analyses, leading to FDA approval of flat dosing for a number of indications (99). Moreover, in the case of pembrolizumab, findings from translational PK/PD murine modelling and human simulations were applied to select a minimum effective dose to guide ongoing clinical evaluation (100).

Measures of success: assessing outcomes

As discussed by Anagnostou and colleagues in this series, the determination of clinically meaningful efficacy endpoints in immunotherapy trials is contentious, owing to atypical immune response patterns (101–103). Delayed anti-tumor effect can lead to late separation of survival curves in randomized trials, affecting study duration and statistical power in detecting differences in the overall treatment effect (104). Sustained stable disease in the absence of tumor shrinkage can also be seen in a subset of patients and is associated with improved survival (105). Conversely, concurrent PD-1 and CTLA-4 inhibition is associated with deep and early tumor responses, with complete response rates approaching 20% in the

first line treatment of melanoma (14, 15). Thus, endpoint selection needs to appropriately capture the expected biology of the agent(s) under investigation based on the mechanisms of action and disease setting; in therapeutic combinations, this is likely to be driven by the most active agent. For agents with delayed or cytostatic activity, disease control rate, PFS or overall survival may be preferred, although the latter may be confounded by subsequent treatments. For combinations anticipated to have substantial activity, response-based endpoints such as complete response rate, durable response rate, or composite measures encapsulating both depth and duration of response are likely reliable measures of early efficacy and surrogates for long-term survival. As previously recommended by the IDSC, randomized combination phase II trials are preferred to single-arm studies to firmly establish efficacy (106). Furthermore, early phase trials should incorporate comprehensive PD assessments and biomarkers to correlate clinical outcomes with mechanistic biological effects.

Importantly, an acceptable balance between toxicity and efficacy is fundamental to the success and clinical utility of drug therapies, and is of particular concern when agents are combined. Immune-mediated tissue injuries are wide-ranging and variable in presentation and time of onset (107). A systematic review found substantial heterogeneity in the completeness and quality of irAE reporting across immune checkpoint inhibitor trials (108). A further consideration in clinical trials of novel combinations is that the causality attribution of adverse events may be problematic, particularly in the absence of monotherapy comparator treatment arms. Additionally, health-related quality of life (HRQoL) is a key consideration and a goal of anticancer care alongside lengthening life. In particular, chronic low grade toxicities can significantly affect patient wellbeing, but are generally under-reported in clinical trials and not captured as DLTs. Encouragingly, HRQoL is increasingly assessed in late phase immunotherapy trials using existing instruments, with improvements shown compared with standard-of-care therapies (109, 110). To more precisely evaluate the kinetics and clinical impact of immune-driven toxicities, standardized reporting of irAEs and patient-reported HRQoL - ideally utilizing tools developed for immunotherapies - should be routinely incorporated into combination clinical trials, in both palliative and adjuvant settings.

Improving drug development efficiency: the tension between speed and safety

The unprecedented success of immune checkpoint inhibitors has generated tremendous enthusiasm to expedite the development of new immunotherapeutics and combinations. In response, clinical trial designs are evolving from the conventional sequential phase I–II–III model, characterized by lengthy timelines and high failure rates (111), to approaches such as seamless phase I/II and phase I/III trials, and the use of large cohort expansions in FIH trials (112–114). Early phase trials using novel designs can be geared toward answering more complex objectives and emerging hypotheses beyond dose-finding, such as preliminary efficacy and biomarker evaluation. For example, multi-cohort FIH trials of novel agents using a common anti-PD-1/PD-L1 backbone under a master protocol can rapidly screen for the most effective combinations for further investigation. Additionally, expansion cohorts are increasingly utilized as an early enrichment strategy, often to estimate efficacy in disease-specific or biomarker-specific groups and in exceptional cases, have supported accelerated

approval (113, 114). However, to mitigate the risks of these streamlined approaches, maintain quality control and safeguard patient interests, clearly defined objectives, flexible statistical designs, pre-determined futility rules and scheduled independent oversight by external data and safety monitors are required. To avoid the immense financial and human costs of negative large late-phase trials, it must be stressed that abbreviated development pathways should be restricted to agents showing substantial activity and foreseeable advantages over standard therapies.

Recognizing the need for therapeutic combinations and in an endeavour to promote industry collaboration, the FDA has provided guidance on the co-development of unmarketed drugs, with an emphasis on frequent interactions with the FDA during the investigational and marketing process. Furthermore, FDA-directed expedited programs, such as breakthrough designation, provide intensive regulatory support that can work in concert with accelerated clinical development strategies outlined above (113). Lastly, cumbersome processes can hinder trial conduct and accrual (115), and efforts should be directed to reform the existing clinical trials system to improve operational efficiency, as recommended by reports from the NCI and the Institute of Medicine (116, 117).

CONCLUSIONS

CTLA-4 and PD-1/PD-L1-based immunotherapies have produced durable responses and even long-term survival in patients with advanced cancer, pioneering the concept of the ‘clinical cure’. Owing to their success and accumulating scientific knowledge in tumor immunology, novel therapies and combinations are rapidly entering development, with unprecedented therapeutic potential to transform cancer care. However, the conventional nonclinical and clinical framework predominantly designed for cytotoxic drug development may not be adequate for immunotherapies and these shortcomings are amplified in the combination setting. A coordinated effort from industry, regulatory authorities, and the scientific and medical communities is required to meet these challenges and much progress has already been made. An immediate goal in the field is the rational selection of combinations based on mechanistic evidence or robust biological rationale to overcome intrinsic and acquired immune resistance, particularly to anti-PD-1/PD-L1 agents. Novel trial designs and statistical methodologies tailored to immunotherapy characteristics should be applied to investigate these combinations effectively and in an efficient manner. As biomarker-based techniques mature, they will help to lend longitudinal insight into tumor-immune interactions, identify predictors of response, and refine patient selection with the eventual goal of personalized medicine.

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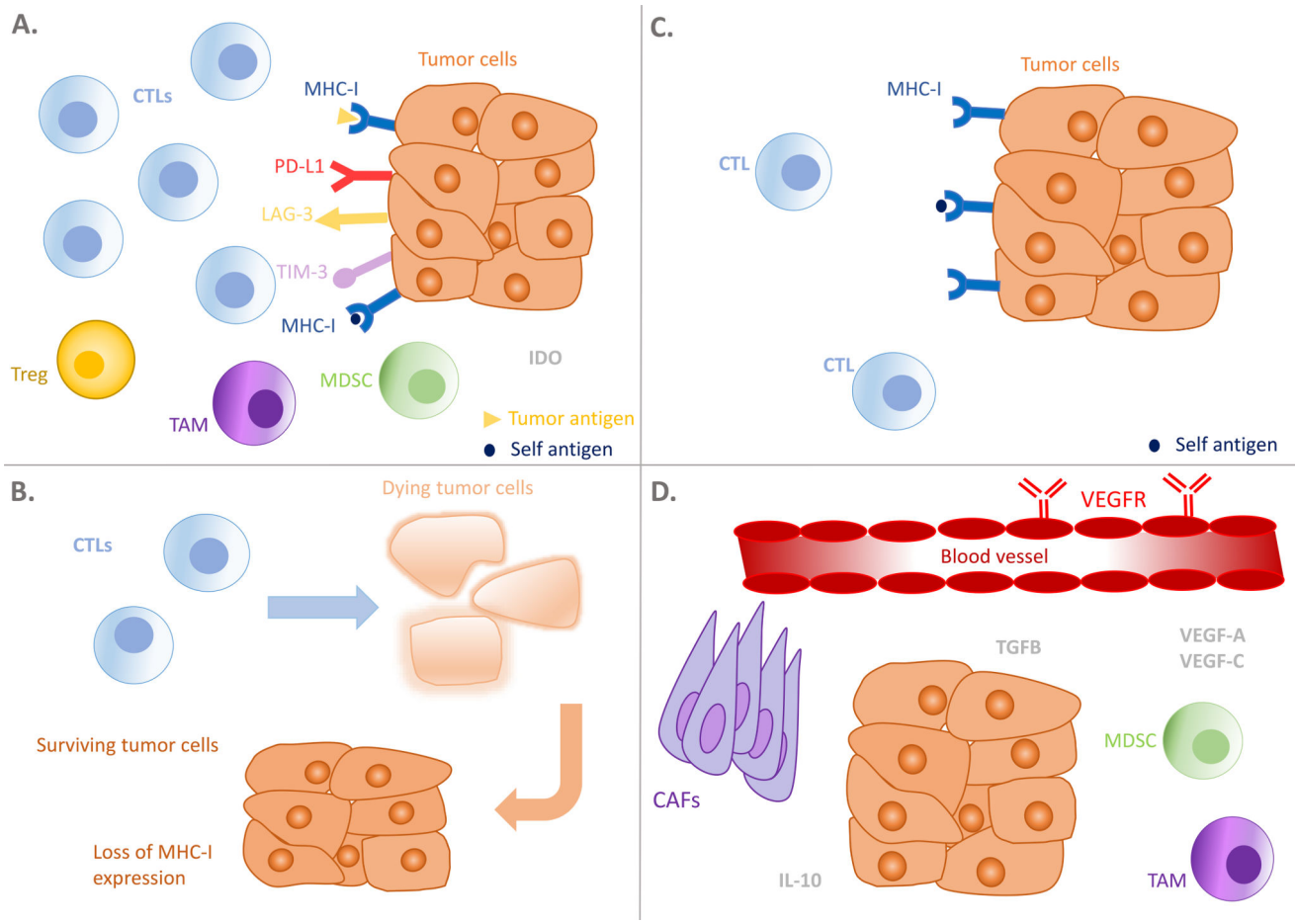

Figure 1. Schematic representation of examples of mechanisms of resistance

CTL, cytotoxic T lymphocyte; Treg, T-regulatory cell; TAM, tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; MHC-I, major histocompatibility complex-I; PD-L1, programmed death ligand 1; LAG-3, lymphocyte-activation gene 3; TGFβ, transforming growth factor-β; vascular endothelial growth factor receptor VEGFR; VEGF, vascular endothelial growth factor; CCL2, chemokine ligand 2; CAFs, cancer-associated fibroblasts; IL-10, interleukin 10.

The tumor microenvironment (TME) in A is T cell-rich, however T cells have been rendered dysfunctional by upregulated co-inhibitory pathways and/or immunosuppressive cells and metabolites. In B, another frequent mechanism of immune evasion is demonstrated, that is, the loss or downregulation of MHC-I expression, thereby affecting antigen presentation and recognition by T cells. The TME in C is characterized by poor immunogenicity and expression of tumor antigens, leading to minimal chemokine expression and T cell infiltration. Lack of co-stimulation may also leave the T cells present to be anergic or unresponsive. D shows a number of processes tumor exploit to prevent T cell recruitment, including adverse stromal factors, secretion of suppressive soluble factors (e.g. TGF-β and II-10) and dysfunctional tumor vasculature, which is turn is maintained by proangiogenic growth factors such as VEGF and fibroblast growth factor, and immunosuppressive myeloid cells such as MDSCs and TAMs.

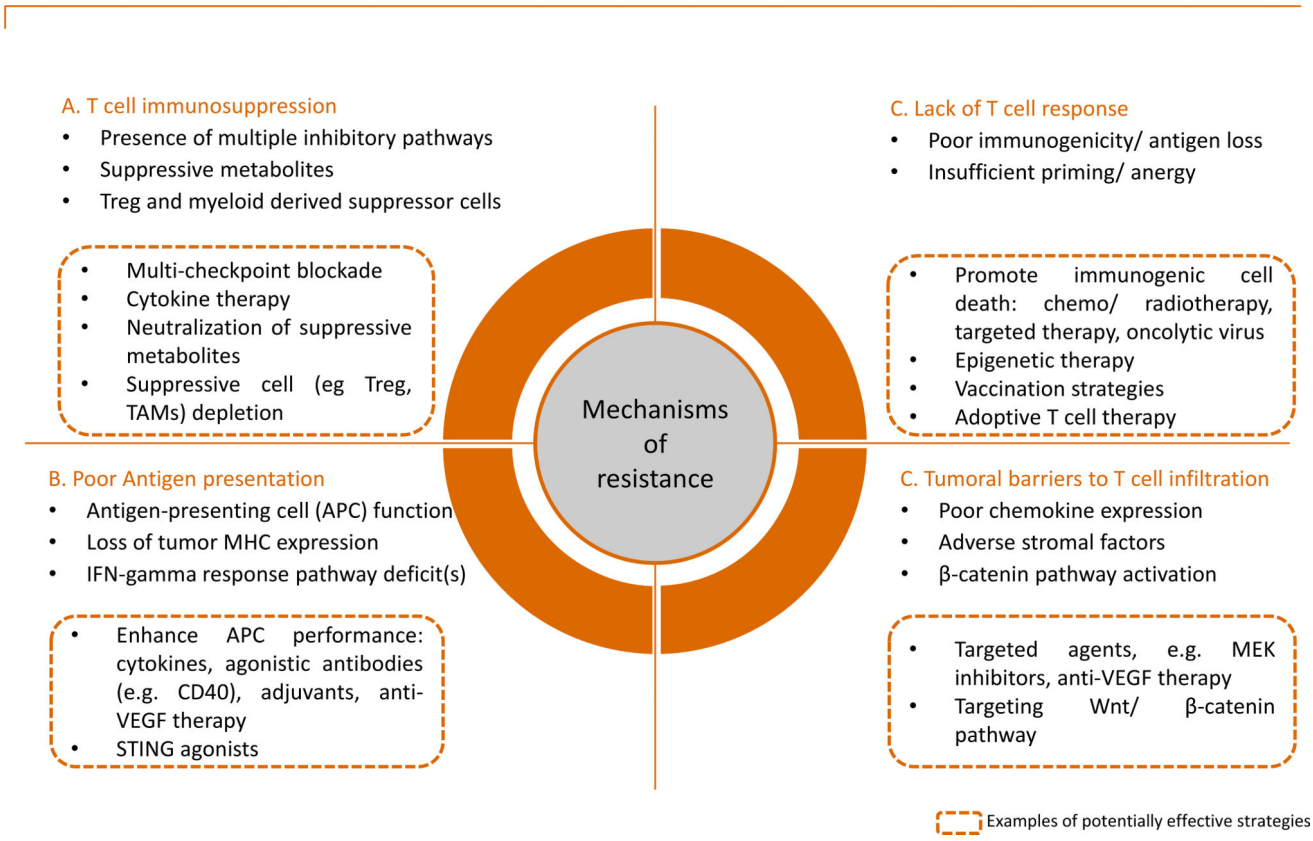

Figure 2. Potential mechanisms of resistance to immunotherapy and examples of therapeutic strategies

APC, antigen-presenting cell; IFNγ, interferon gamma; Treg, regulatory T cells; TAMs, tumor-associated macrophages; MEK, mitogen-activated protein kinase kinase; VEGF, vascular endothelial growth factor; MHC, major histocompatibility complex; STING, stimulator of interferon genes.

Table 1

Immunotherapeutic agents in current development

Co-inhibitory molecules (targets of immune checkpoint inhibitors)	**Co-stimulatory molecules (targets of immune-stimulatory agonists)**
- CTLA-4 - PD-1 - PD-L1 - LAG3 - TIM3 - BTLA - TIGIT - VISTA - KIR	- OX40 (CD134) - GITR - CD137 - CD40 - ICOS - 4-1BB
Vaccines	Adoptive T cell therapy
- Tumor antigen-based vaccines - Dendritic cell-based vaccines	- Tumor-infiltrating lymphocytes (TILS) - Chimeric antigen receptors (CAR) - T cell receptor (TCR) transduction - Natural killer (NK) cells
Immunosuppressive soluble factors	Cytokines
- IDO-1 - Adenosine	- IL-1 - IL-5 - IL-7 - IL-15 - IL-21
Oncolytic virus	T regulatory cell depletion therapy
- T-VEC	- Cytotoxic chemotherapy - Anti-CD25
Bispecific T cell engaging antibody-based technologies	Endogenous adjuvants
- Blinatumomab (CD3/CD19 construct) - IMCgp100 (TCR/anti-CD3 T cell redirector)	- Stimulator of interferon genes (STING) agonists - Toll like receptor (TLR) agonists

CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; PD-1, programmed death 1; PD-L1, programmed death ligand 1; LAG3, lymphocyte activation gene 3; TIM3, T cell immunoglobulin (Ig)-3; BTLA, B and T lymphocyte attenuator; TIGIT, T cell immunoglobulin and ITIM domain; VISTA, V-domain Ig-containing suppressor of T cell activation; KIR, killer IgG-like receptor; GITR, Glucocorticoid-induced tumour necrosis factor receptor; ICOS, Inducible T cell COStimulator; IDO-1, idoleamine-2,3-dioxygenase 1; IL, interleukin; T-VEC, Talimogene laherparepvec; TCR, T cell receptor.

Table 2

Summary of recommendations

Nonclinical studies
- Demonstrate proof-of-principle: target engagement and activity, synergistic or additive effect at tolerable and achievable doses. - Characterize PK and PD profile of individual drugs and the combination. - Identify optimal concentrations of each drug to inform clinical dose selection. - Explore potential biomarkers which can later be refined clinically. - Set pre-determined benchmarks defining success prior to considering clinical testing. - Develop validated immuno-competent animal models. - Develop models of putative mechanisms of resistance. - Use multiple models where practicable.
Early phase trials: combination selection and overall goals
- Focus on populations with unmet need, e.g. PD-1 blockade-refractory patients. - Sound biological rationale is a prerequisite for starting clinical development. - Combination therapy should offer significant therapeutic advantage over monotherapy with manageable toxicity. - Set clearly-defined clinical development plan from the outset and pre-determined decision rules with criteria to define success or failure. - Combine best-in-class agents. - Industry and academic collaboration vital to minimize duplication of investigational studies and resources.
Early phase clinical trial design
- Trial design features including method of dose escalation and endpoints, should be carefully considered based on nonclinical or single agent clinical data, tumor and patient characteristics, and objectives. - Novel trial designs, including model-based designs, should be strongly considered. - Parallel biomarker development studies encouraged to assess mechanisms of resistance and response, PK/PD endpoints and to identify predictive biomarkers. - Trial designs aimed at accelerating the development process such as seamless designs with expansion cohorts in phase I may be appropriate for highly efficacious agents and ideally should be implemented in concert with FDA expedited programs to protect patient safety and purpose-fit efforts (112,113).

PD-1, programmed death 1; PK, pharmacokinetic; PD, pharmacodynamic.

Table 3

Lessons learned from the ipilimumab and nivolumab combination

1 Improved activity is seen with the combination in melanoma	- Objective response rate >50% (single agent response rates: ipi 11–19%, nivo 44%). Early and deep responses seen, 10–22% complete response rate (13–15). - PFS not reached in the phase II study and approaching 12 months in the phase III study in melanoma (ipi 2.9m, nivo 6.9m) (14,15). Of note, this study is not powered for a statistical comparison between the combination and nivo arms. - Early survival data from the phase III study showed improved survival compared with ipi (HR, 0.55; p<0.0001) (39).
2 Substantial toxicity associated with the combination	- 55% grade 3 or 4 treatment-related AE rate in melanoma (ipi 27% and nivo 16%). - 37% discontinuation rate due to toxicities (ipi 15% and nivo 8%). - AEs are qualitatively similar compared with monotherapy experience and are reversible in the majority of cases. - 83% of patients required immune modulatory agents to manage toxicities (15).
3 PD-L1 enriches for response, but incremental benefit above monotherapy may be greater if PD-L1 negative	- In melanoma, responses were seen irrespective of baseline PD-L1 expression (72% in PD-L1 positive patients compared with 55% in PD-L1 negative patients). - Incremental PFS gains compared with single agent nivolumab was greater in the PD-L1 negative population in subgroup analysis (PD-L1 positive treated with combination vs nivo: median PFS 14m vs 14m; PD-L1 negative: 11.2m vs 5.3m) (15).

4 Optimal dose and schedule vary depending on tumor type	- Recommended dose regimens differ in melanoma, NSCLC and RCC studies (13–15,37,38).
	  - Melanoma: ipi 3mg/kg and nivo 1mg/kg Q3W for 4 doses followed by nivo maintenance therapy.
	  - NSCLC: the above dose regimen was not well tolerated. Additionally, clinical activity was suboptimal with lower doses of nivo (below 1mg/kg). Nivo 3mg/kg Q2W and ipi 1mg/kg Q6W chosen for further development.
	  - RCC: nivo 3mg/kg and ipi 1mg/kg demonstrated an improved toxicity profile compared with nivo 1mg/kg and ipi 3mg/kg.
	- In general, greater toxicity is seen with higher doses of anti-CTLA-4 agents.
	  - Exposure-response analysis of ipi: higher doses produce greater trough concentrations which were associated with higher rates of irAEs, greater tumor responses and longer survival (40).
	  - Analyses for anti-PD-1 agents: relatively flat dose-efficacy relationships (41). One study of nivo found melanoma and RCC patients reached plateaus in efficacy at lower exposures (≥1mg/kg) compared with NSCLC patients(≥3mg/kg) (42).

5 Sequential dosing with a short break may not be more tolerable than concurrent therapy	- Randomized phase II study investigating planned switch from 12 weeks of nivo to 12 weeks of ipi compared with the reverse sequence:
	  - Nivo followed by ipi with a 2-week break in between was more efficacious and more toxic. Response and severe AE rates were similar to concurrent therapy (15,118).
	  - Caveats: the study was underpowered and there were imbalances in baseline patient prognostic factors.
	  - Pharmacological properties such as prolonged PD-1 receptor occupancy by nivo leading to overlapping exposure to both may have contributed to this finding. (118).

Ipi, ipilimumab; nivo, nivolumab; m, months; HR, hazard ratio; AE, adverse event; PD-L1, programmed death ligand 1; vs, versus; PD-1, programmed death 1; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; QXW, every X weeks; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; irAEs, immune-related adverse events.