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Authors

Singh, Kirit
Batich, Kristen A
Wen, Patrick Y
[et al.](#)

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Designing Clinical Trials for Combination Immunotherapy: A Framework for Glioblastoma



Kirit Singh¹, Kristen A. Batich¹, Patrick Y. Wen², Aaron C. Tan³, Stephen J. Bagley⁴, Michael Lim⁵, Michael Platten⁶, Howard Colman⁷, David M. Ashley¹, Susan M. Chang⁸, Rifaquat Rahman², Evanthia Galanis⁹, Alireza Mansouri¹⁰, Vinay K. Puduvalli¹¹, David A. Reardon², Solmaz Sahebjam¹², John H. Sampson¹, John Simes¹³, Donald A. Berry¹¹, Gelareh Zadeh¹⁴, Tim F. Cloughesy¹⁵, Miness P. Mehta¹⁶, Steven Piantadosi¹⁷, Michael Weller¹⁸, Amy B. Heimberger¹⁹, and Mustafa Khasraw¹

ABSTRACT

Immunotherapy has revolutionized treatment for many hard-to-treat cancers but has yet to produce significant improvement in outcomes for patients with glioblastoma. This reflects the multiple and unique mechanisms of immune evasion and escape in this highly heterogeneous tumor. Glioblastoma engenders profound local and systemic immunosuppression and is remarkably effective at inducing T-cell dysfunction, posing a challenge to any immunotherapy-based approach. To overcome these mechanisms, multiple disparate modes of

immune-oriented therapy will be required. However, designing trials that can evaluate these combinatorial approaches requires careful consideration. In this review, we explore the immunotherapy resistance mechanisms that have been encountered to date and how combinatorial approaches may address these. We also describe the unique aspects of trial design in both preclinical and clinical settings and consider endpoints and markers of response best suited for an intervention involving multiple agents.

Introduction

Patients with glioblastoma survive for 12 to 15 months on average despite treatment with surgery, focal irradiation, alkylating chemotherapy, and tumor treating fields (1–4). Although several immunotherapies are currently under investigation for glioblastoma, none have yet demonstrated a significant survival benefit (5–7). Glioblastoma originates in an immune privileged compartment and is adept at escaping immune surveillance (8). Precision immunotherapy also requires a uniformly expressed tumor-specific antigen (TSA), which remains elusive in highly heterogeneous isocitrate dehydrogenase (IDH) wild-type gliomas or glioblastoma (9). Further, glioblastoma

disrupts immune function both locally and systemically, degrading the ability of immunotherapy to act (10).

Given these significant obstacles, it is clear that a single agent will be insufficient. To unlock the true potential of immunotherapy, combinations with additive and/or synergistic mechanisms of action are required. However, testing these combinations poses unique technical, logistical, and regulatory challenges. In this review, we will explore current opportunities and describe strategies for conducting trials of combination immunotherapy.

Current and Future Combinatorial Strategies

Glioblastoma induces immune dysfunction through multiple mechanisms (10–15). To overcome these, several immunotherapy classes are under investigation, including immune checkpoint blockade (ICB), chimeric antigen receptor T cells (CAR-T), bi-specific T-cell engagers (TCE), tumor antigen vaccination, oncolytic viruses, and immunomodulatory cytokines (16–21). Given the large number of permutations for combination therapy, we must rationalize the available options.

Combinations of multiple immune checkpoint blockade

Single-agent ICB has failed to yield benefit in patients with glioblastoma (22). Given this, studies have been performed using multiple ICB agents, based on effective strategies in other difficult malignancies such as melanoma or advanced renal cell carcinoma (23). A well-described combination in oncology is that of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4), which has been explored in multiple trials (NCT02017717 – Checkmate 143, NCT04145115, NCT03233152, NCT04003649, NCT03422094, NCT02311920, and NCT03707457). However, subsequent work has demonstrated that glioblastoma exhibits cancer lineage-specific resistance to the reversal of T-cell exhaustion, which may reduce the impact of this particular combination (24). Retrospective genomic and transcriptomic analysis of patients who received PD-1

¹Duke University, Durham, North Carolina. ²Dana-Farber Cancer Institute, Boston, Massachusetts. ³National Cancer Centre Singapore, Singapore. ⁴University of Pennsylvania, Philadelphia, Pennsylvania. ⁵Stanford University, Stanford, California. ⁶Medical Faculty Mannheim, MCTN, Heidelberg University and German Cancer Research Center, Heidelberg, Germany. ⁷Huntsman Cancer Institute, Salt Lake City, Utah. ⁸University of California, San Francisco (UCSF), San Francisco, California. ⁹Mayo Clinic Rochester, Rochester, Minnesota. ¹⁰Penn State Cancer Institute, Hershey, Pennsylvania. ¹¹MD Anderson Cancer Center, Houston, Texas. ¹²Moffitt Cancer Center, University of South Florida, Tampa, Florida. ¹³NHMRC Clinical Trials Centre, University of Sydney, NSW, Australia. ¹⁴University of Toronto, Toronto, ON, Canada. ¹⁵University of California Los Angeles, Los Angeles, California. ¹⁶Miami Cancer Institute, Miami, Florida. ¹⁷Brigham and Women's Hospital, Boston, Massachusetts. ¹⁸University Hospital and University of Zurich, Department of Neurology, Zürich, Switzerland. ¹⁹Northwestern University, Chicago, Illinois.

Corresponding Author: Mustafa Khasraw, Duke University Medical Center, Duke University, Duke Cancer Institute, Durham, North Carolina. E-mail: mustafa.khasraw@duke.edu

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inhibitors found that the degree of response to treatment was associated with specific evolutionary pathways resulting in certain molecular and immune expression profiles. This would indicate that only certain subsets of patient may benefit from this form of checkpoint blockade (25). The timing of checkpoint blockade therapies relative to standard-of-care treatment also may play a key role in efficacy. Cloughesy and colleagues report upregulated T-cell and IFN γ gene expression and downregulated cell-cycle gene expression within the tumor when anti-PD-1 therapy was used in the neoadjuvant setting. This effect was however not observed for those patients that received adjuvant therapy alone, suggesting a transient window of opportunity for checkpoint blockade (26). Schalper and colleagues also found positive immune effects associated with neoadjuvant PD-1 blockade, reporting enhanced levels of immune-cell infiltration and greater TCR diversity among tumor-infiltrating lymphocytes (TIL), suggesting that this may be a useful partner with other immunotherapies (27).

Other checkpoint inhibitory molecules highly expressed by TILs in glioblastoma include indoleamine 2,3-dioxygenase (IDO1), T-cell immunoglobulin-mucin-domain containing-3 (TIM-3), and lymphocyte activation gene 3 (LAG3; ref. 28). Phase I clinical trials exploring combination approaches against both PD-1, LAG-3 (NCT02658981), and IDO1 (NCT03707457) are now underway. Despite this, experience of combinatorial PD-1 and IDO1 blockade in the central nervous system (CNS; albeit in metastatic melanoma) have failed to improve outcomes in phase III studies (29). Other combination approaches using IDO1 inhibitors with temozolomide (TMZ; NCT02052648) remain under evaluation in glioblastoma. Although these findings may suggest that combinatorial ICB may still struggle in the CNS, lack of success may actually reflect incomplete checkpoint blockade. Opitz and colleagues have described metabolic pathways in glioma such as activation of the aryl hydrocarbon receptor (AHR) by tryptophan catabolites, which results in enhanced malignancy and immunosuppression (30). Although the AHR pathway was initially associated with IDO1 or the tryptophan-2,3-dioxygenase 2 (TDO2) enzyme, recent work has demonstrated that IL4-induced-1 (IL4I1) is more significantly associated with AHR activity. ICB can induce IDO1 and IL4I1, whereas IDO1 inhibitors previously trialed in combination with ICB do not result in IL4I1 blockade (31). Future combinatorial pairings of ICBs should consider the potential for anti-PD-1 agents to induce metabolic agents which upregulate metabolic pathways of immunosuppression.

Immune checkpoint blockade and T-cell-directed immunotherapy

Intratumoral heterogeneity in glioblastoma poses a significant barrier to antigen-specific immunotherapies such as CAR-T cells or bispecific T-cell engagers. CAR-T cells specific for EGFR variant III (EGFRvIII) have proven ineffective when treating recurrent tumors due to antigen escape (9). The intended selective targeting of cells or spontaneous elimination of target cells at recurrence produces an outgrowth of antigen-negative cells resulting in recurrence (9, 32, 33). A high degree of clonality and, contrary to other cancers, a high mutational burden in glioblastoma has also been associated with resistance to ICB (34, 35). Of note for clinical trials, high tumor mutational burden can be induced by TMZ, which causes defects in DNA mismatch repair genes. One potential approach to overcoming this is by targeting IDH1-R132H, a shared clonal neo-epitope in IDH-mutated gliomas. This uniformly expressed TSA in a subset of patients with glioma has been successfully targeted in recent phase I

trials (NCT02454634), and was found to be both safe and immunogenic (36).

Although prior phase Ib trials (NCT02287428) of neoantigen vaccination in glioblastoma have reported neoepitope-specific systemic immune responses with increased numbers of TILs, these have also been shown to express a profoundly exhausted phenotype (37). Combining ICB with a vaccine strategy targeting a shared clonal neoepitope may therefore work synergistically to overcome ICB resistance while enhancing the neo-epitope immune response. This is supported by preclinical evaluation of multivalent neoantigen vaccines with ICB, which generated greater antitumor responses than monotherapy, even in models with reduced anti-PD-L1 sensitivity (38, 39). Such an approach would therefore be logical to evaluate for other multi-epitope vaccine-based approaches such as that used in the GAPVAC trial (NCT02149225; ref. 40). As mentioned previously, neoadjuvant anti-PD-1 blockade has been associated with enhanced clonal expansion of T cells and greater immune infiltration/TCR diversity (26, 27). This would also likely benefit immunotherapy approaches that rely heavily on T-cell expansion such as vaccination or CAR-T cell therapy. Further, CAR-Ts targeting EGFRvIII have been shown to upregulate expression of PD-L1 within gliomas, contributing to CAR-T cell dysfunction and treatment failure (9). The addition of anti-PD-1 blockade to such approaches may therefore increase both the diversity and potency of the immune response to CAR-T therapy while reducing T-cell exhaustion. This is supported by work by Choi and colleagues who designed a CRISPR-Cas9 modified EGFRvIII CAR-T cell with the endogenous PD-1 receptor knocked out, thereby preventing PD-L1 binding. This CAR-T-EGFRvIII PD-1 construct resulted in prolonged survival in mice bearing EGFRvIII⁺ glioma compared with CAR-T-EGFRvIII cells with an intact PD-1 receptor (41). In this vein, trials are underway evaluating CAR-T cell therapy (NCT04003649) and vaccination (NCT04201873, NCT02529072, NCT02287428) alongside ICB. Newer trial designs are also being deployed such as the AMPLIFY-NEOVAC surgical window-of-opportunity trial (NCT03893903). This will evaluate IDH1R132H vaccination with avelumab (anti-PD-L1) to explore predictive biomarkers for response to ICB in patients with IDH-mutated gliomas.

It is notable that studies such as that performed by Choi and colleagues report prolonged survival with direct intracerebral or intraventricular delivery of CAR-T therapy but that this therapeutic effect is lost with peripheral administration. This finding serves to demonstrate that transiting the blood-brain barrier (BBB) remains a formidable obstacle for many systemically delivered immunotherapies (42). Even in the pathologic glioma state, regions of the BBB likely remain intact, shielding sections of tumor from immunotherapy, which may then act as the focal point for recurrence (43). Although systemic anti-PD-1 therapy has been noted to induce changes in the CNS, it is unclear where this interaction with the immune system occurs and indeed what concentration is necessary to induce an effect at the intracranial tumor site (44). Although one solution may be direct intracranial delivery of agents, this highly invasive approach will not be suitable for all patients and faces significant challenges in achieving equal and persistent drug distribution throughout the tumor (33, 45, 46). Another potential approach may be the use of *ex vivo* activated autologous T cells combined with T-cell engaging therapies. These activated T cells would theoretically adhere to the brain microvascular endothelium and traffic into the brain, carrying their immunotherapy payload on their surface (18, 47). However, this effect has also been associated with neurotoxicity and must therefore

be investigated with caution (48). Accordingly, such an approach is entering phase I safety trials where a hEGFRvIII-CD3 Brain Bi-Specific T Cell Engager (BRiTE) will be evaluated alongside peripheral autologous T-cell infusion (NCT04903795).

Other strategies to enhance the T-cell repertoire and overcome immunosuppression

Combination of immunotherapy with radiotherapy has been demonstrated in melanoma to expand the compartment of effector memory T cells and TILs, while also inducing a more diverse T-cell receptor population when combined with ICB (49). Similar promise has been demonstrated preclinically in glioblastoma, where TIM-3 and PD-1 antibodies combined with radiotherapy achieved long-term survival (50). Other strategies to expand TCR diversity may involve the use of dendritic-cell vaccines, which carry antigen from the tumor to draining lymph nodes, presenting them to effector T cells. Chemokines such as the macrophage inflammatory protein-1 alpha (MIP-1 α , CCL3) may aid in enhancing lymph node chemotaxis of dendritic-cell subsets both to tumor and from tumor to lymph node, resulting in greater diversity of antigen presentation and more potent antigen-specific T-cell responses (51). Dendritic cells may also enhance the polyfunctionality of adoptively transferred T cells targeting tumor-specific antigens in glioblastoma (52).

Enhancing T-cell functionality may be supported by the use of costimulatory agonists such as CD27, 4-1BB, OX40, or CD40, which are now entering early clinical trials (e.g., NCT04547777, NCT02658981, NCT03688178; refs. 53–56). Newer constructs that combine both anti-inhibitory and prostimulatory strategies are under development such as bispecific antibodies targeting both CD27 and PD-L1 (NCT04440943) or TGF β and PD-L1 (57). Novel CAR-T constructs including synNotch and armored CARs with expression of cytokines such as IL12 have also been demonstrated to enhance antitumor efficacy in the context of oncogenic immunosuppression (58, 59). When considering immunosuppression, thought should also be given to the role of dexamethasone, which may induce systemic depletion of memory and naïve CD4/CD8 T cells, reducing the efficacy of immunotherapy (60). In this context, agents that have failed to show efficacy when combined with checkpoint blockade such as bevacizumab (anti-VEGF) may also be worth re-evaluating as an adjunct, specifically for its ability to reduce the need for immunosuppressive corticosteroids (61, 62).

A summary of potential combinatorial approaches is depicted in Fig. 1. However, as described above, enhanced additive synergism between immunotherapies may well extend beyond a bimodal approach. It is reasonable to consider trial designs, which involve three or more elements. This will require flexible trial designs to swiftly identify the optimal combinatorial schedule which are discussed in the following section.

Recommendations for Combinatorial Clinical Trial Design

Population selection

When considering any new combinatorial therapy, a proof-of-principle study is necessary to determine efficacy. Outcome measures in this context usually consist of specific biologic endpoints. However, these studies are typically performed on small numbers of patients with late-stage disease who have received highly variable treatment courses. This can make interpretation of said biologic endpoints difficult. Selecting patients earlier in the disease course with a less heterogeneous and slower growing pathology may make interpretation of biological

markers easier, while also allowing combination immunotherapies sufficient time to synergize and induce maximal biological effect. Particular consideration should also be given to patients with an unmethylated O6-methylguanine-DNA methyltransferase (MGMT) promoter gene, who are less likely to respond to TMZ. In such cases, omission of TMZ entirely would be clinically justifiable and would allow evaluation of new therapies without additional toxicities from TMZ or inducing hypermutation as described previously (63, 64).

Regimen selection

The optimal dosing schedule may be extremely broad when designing trials for two or more agents and is further complicated by the fact that true synergy may exist at nonmaximal doses. Although it is generally not acceptable to reduce doses of standard-of-care agents in combination, an appreciation for the unique pharmacodynamic interplay between combined immunotherapy agents is required. Although this might suggest a need for extensive preclinical testing, newer designs such as phase 0, translational, surgical “window-of-opportunity”, or neoadjuvant trials may offer a route to bypass potentially laborious steps (65, 66).

Phase 0 trials use a microdosing strategy to allow for assessment of potential pharmacodynamic (PD) and pharmacokinetic (PK) properties while minimizing risk. This allows for rapid determination of the biological activity of a potential combination and allows for early termination if said combination does not meet its predefined PD/PK endpoints. Window-of-opportunity studies take an alternative route, using a predefined therapeutic dose and typically aim to define target engagement and/or immune modulatory endpoints. Both trial approaches aim to determine biological and immunomodulatory impact, rather than clinical effect, although window-of-opportunity trials also allow for correlation with eventual patient outcomes. Although these are both useful approaches for evaluating new combinations, perhaps the most sensitive way to determine synergism between two agents is by using neoadjuvant studies in which treatment is administered preoperatively and tumor samples are taken for analysis at time of resection.

Neoadjuvant studies have already been used successfully to determine the effect of immune checkpoint inhibition on the intratumoral T-cell compartment (26). The neoadjuvant approach may also be superior to postsurgical biopsies of tissue, which are prone to sampling error and often have limited tissue availability, resulting in a nonrepresentative immune analysis. A large volume of tissue will allow for not just a determination of raw numbers of TILs but functional activity. Such analyses would be superior to peripheral immune interrogation, which may not be equivalent to events occurring at the tumor site.

Moving beyond phase I

If a combination proves safe and tolerable while also demonstrating evidence of immune response, trials should proceed to phase II trials to assess clinical benefit. However, traditional single-arm phase II trials often use response rates (RR) based on historical controls as the main endpoint, which may be inappropriate for combined immunotherapy. An efficient approach would be to use seamless phase I/II, II/III clinical trials, whereby the protocol specifies when to transition the study from a certain phase (e.g., phase II to III) without the need for a new protocol or regulatory process (67, 68). Similarly, large phase Ib trials and “expansion baskets” of the combination in phase I trials allow for increasing the number of patients enrolled once the recommended phase II dose has been determined. This allows for the phase I study to aid establishing preliminary efficacy in addition to determining the safety of the combination. Basket and platform trial designs using

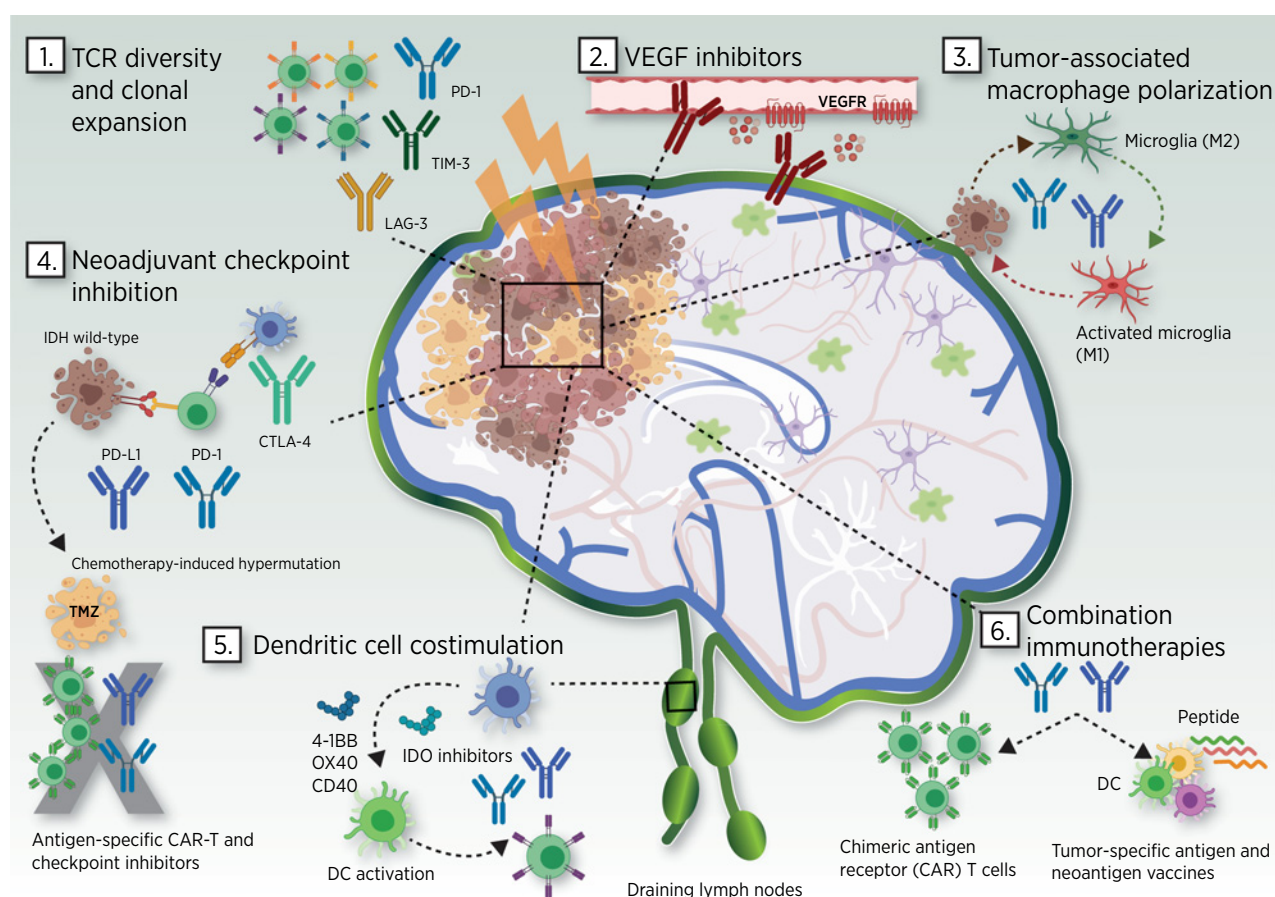


Figure 1.

Combination approaches using checkpoint inhibition and other therapies for glioblastoma. 1. TCR diversity and clonal expansion. Combination radiation therapy and inhibitors of TIL exhaustion and drivers of apoptosis (PD-1, LAG-3, TIM-3) are being studied for synergistic effects on TIL expansion and clonal diversity. 2. VEGF inhibitors. Anti-VEGF therapies such as bevacizumab are being utilized as steroid-sparing agents to harness immunotherapy-related toxicity in the CNS. 3. Tumor-associated macrophage polarization. Glioma cells interact with and maintain a robust population of PD-1-expressing microglia with an anti-inflammatory phenotype (M2). Selective anti-PD-1 blockade on microglia populations is capable of inducing a tumoricidal M1 phenotype (87–89). 4. Neoadjuvant checkpoint inhibition. Treatment-naïve, IDH wild-type glioblastoma upregulates PD-L1 and CTLA-4 offering enhanced sensitivity to immune checkpoint combination approaches. Neoadjuvant checkpoint blockade increases clonal expansion of T cells. Chemotherapy with temozolomide can alter tumor mutational burden resulting in both increased resistance to checkpoint blockade and increased subclone heterogeneity thus limiting the potency of antigen-specific immunotherapies such as CAR-T cells. 5. Dendritic cell costimulation. Costimulatory agonists for 4-1BB, OX40, and CD40 and IDO inhibitors are being evaluated with checkpoint inhibitors to polarize cytotoxic T-cell responses in the tumor microenvironment and within immunosuppressed tumor-draining lymph nodes. 6. Combination immunotherapies. Multivalent neoantigen vaccines and CAR-T cell therapies in combination with checkpoint inhibitors are being evaluated for superior efficacy compared with single modalities even with reduced PD-L1 sensitivity. Abbreviations: CAR, chimeric antigen receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; IDO, indoleamine 2, 3-dioxygenase; LAG-3, lymphocyte activation gene 3 (LAG3); M1 and M2, macrophage pro-inflammatory and anti-inflammatory phenotype; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin-mucin-domain containing-3; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Adapted from an image created with BioRender.com.

master protocols allow for within-basket immune monitoring depending on the approaches being evaluated (e.g., markers of T-cell activation if blocking regulatory T-cell receptors; ref. 69). The use of adaptive designs in this setting allows for adjustment according to evolving data so that poorly performing combinations can be abandoned early, while additional treatment cohorts which test other combinations can be added.

Many basket trials to date (Lung-MAP, NCT02154490; NCI-MATCH, NCT02465060; and My Pathway, NCT02091141) use RR to evaluate targeted therapies, as is typical for traditional single-arm early-phase trials. However, this may not be an appropriate endpoint for the evaluation of immunotherapies, which can yield clinical

benefit without a high RR. Indeed, immunotherapies have been noted to induce imaging changes interpreted as indicative of progression (70). To address this, adaptive designs have been initiated including the INSIGHT adaptive platform trial (NCT02977780), the glioblastoma AGILE phase II/III adaptive platform trial (NCT03970447; refs. 64, 71) in the United States, and the NCT neuro master match umbrella phase I/IIa trial (NCT03158389) in Germany. Overall survival (OS) can be used in these trials as the primary endpoint rather than RR (72, 73). These designs are mostly used to evaluate targeted therapies to circumvent lengthy pauses between trial phases, but their usage still lags behind for immunotherapy-based approaches (74).

Recommendations for Ensuring Safety and Determining Outcomes

In the context of combinatorial immunotherapy, toxicity considerations are complicated by the need to determine the appropriate regimen for commencing two or more agents at once. Multiple agents may act synergistically in both efficacy and toxicity, which could result in potentially fatal complications such as cytokine release syndrome (CRS; ref. 75). Model-assisted designs are a useful tool to assess the prestudy probability of toxicity and can inform dose-escalation decisions using real-time adverse event data (76, 77). These can maximize the number of patients treated at or near the MTD and outperform traditional designs such as 3 + 3 dose escalation, which have yielded inconsistent dose-toxicity or dose-escalation correlations (78, 79). However, although the flexibility and accuracy of model-assisted designs may be of particular use when evaluating immunotherapy combinations, these require sustained biostatistical collaboration, which can be time and resource intensive. A detailed evaluation of the dose-escalation strategy and how best to determine response is therefore vital before commencing clinical trials.

Determining the starting dose

Many combinatorial trials use agents with a known safety profile and where the biologically active dose is known. For that reason, such

trials could begin at the optimal dose rather than using conservative escalations. When neither component is known to be effective or approved for the indicated use, and when neither will be effective alone, a two-arm design comparing combination to a control agent (or placebo) can be considered (shown in Fig. 2). However, although this minimizes exposure of patients to inactive therapies, this design may not demonstrate the inactivity of specific components. When the safety profile or optimal dose is unknown for either component in a combination, the optimal immune response may be an appropriate endpoint for early-phase trials, providing there are no significant toxicities. However, establishing response with multiple agents and titrating each element to maximize efficacy may not be practical.

Measuring effect

Patients in phase I trials are likely to have relapsed or progressed on previous therapies but preliminary signals of activity can still be noted using RR, progression-free survival (PFS), recurrence-free survival, or OS. However, in an advanced-disease population, immunotherapies may not induce a strong immune effect that manifests as a reduction in disease burden. Patients may also not survive long enough to have time to generate the immune response that would provide a clinical benefit. A further complication is that OS can be extended by immunotherapy without radiologic response or pseudo-progression (80). The modified response assessment in neuro-oncology (mRANO) and immune-

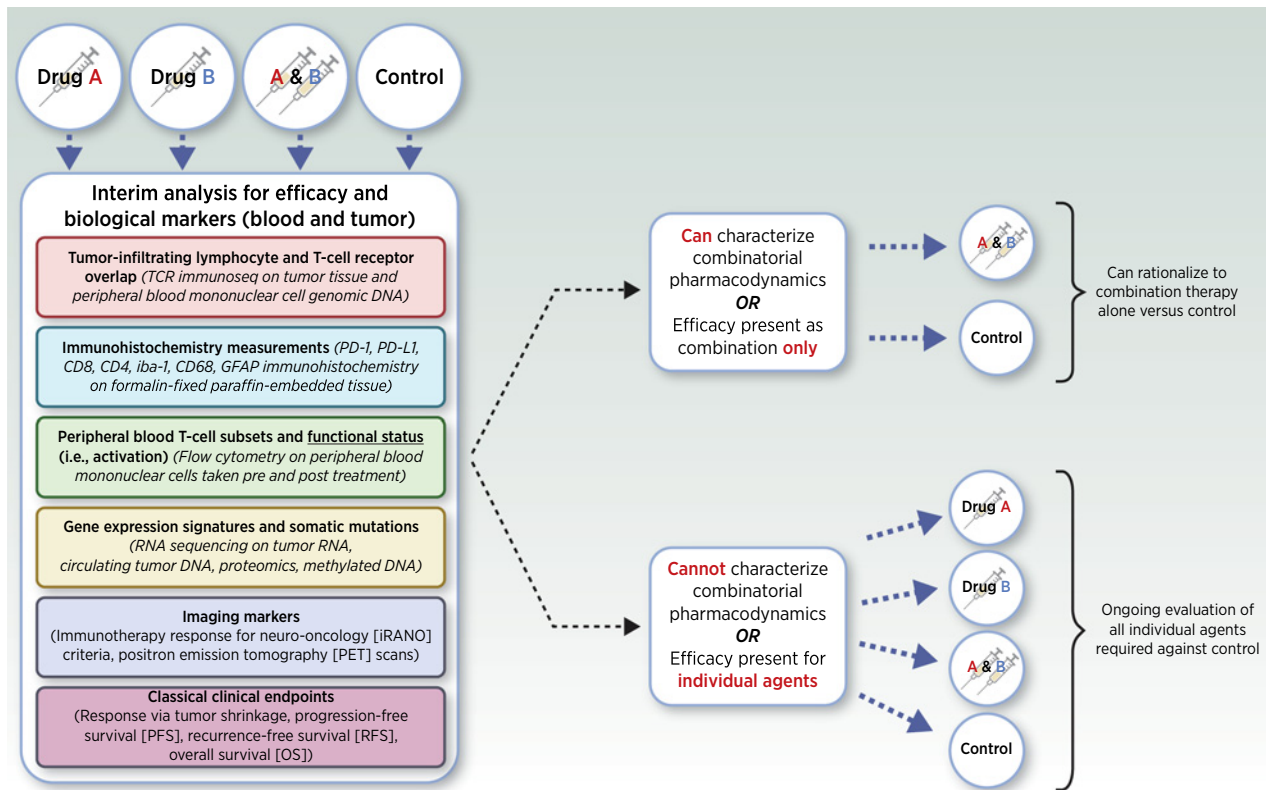


Figure 2.

Rationalizing trial designs for combinatorial immunotherapy. Regulatory approval of combinations of therapeutic agents in medicine usually requires a demonstration of each component's independent contribution. The ability to evaluate pharmacodynamic effects of single agents and/or combinations may help determine whether randomized studies require arms including both single agents and combinations. When neither component is known to be effective or approved for its indicated use, or when neither have efficacy as a single agent by itself, a two-arm design comparing the combination to a control agent (placebo) can be considered. Assessment of immune response must not only quantify the degree of immune activation but also the functional status of the response generated. Adapted from an image created with BioRender.com.

specific RANO (iRANO) aim to standardize determination of response, but their utility in trials using experimental combinations is yet to be validated (70). To determine response more accurately and avoid premature treatment discontinuation, the definition of progression will need to require confirmation on two separate observations or to not consider early progression within a prospectively defined time-interval as per modified RANO (81). In patients who do respond after early progression, PFS should be based on the start of therapy.

Determining potency, predicting clinical effects, and understanding the impact of the manufacturing process on the final drug product and stability are all required for regulatory approval. However, demonstrating these effects in combination therapies poses practical and ethical problems. Evaluation of purity and potency can be difficult if a treatment is composed of a combination of heterogeneous components (e.g., autologous blood or tumor-derived cellular therapies; ref. 82). One approach to overcome this is to use quantitative assessments such as the time to kill 50% of target tumor cells (KT₅₀; ref. 83). Other approaches may involve correlating outcomes with serial immune assays to quantitatively measure the relative immunogenicity of a combination. Statistical modeling using toxicity and antitumor toxicity have also been considered (84, 85). However, many of the techniques used including cytokine release, tetramer, cytotoxicity, and the enzyme-linked immune absorbent spot (ELISpot) assays are often only technically validated in the research laboratory, and the frequency with which they are performed vary widely, leading to variable results (86).

To generate valid and transferable data based on immune assays, harmonization and standardization of techniques is required to establish the expected immune response from known effective immunotherapies, against which new combinations can be evaluated. Current FDA-approved biomarkers of tumor mutational burden (TMB) that are used to predict response to the checkpoint inhibition pembrolizumab in solid tumors, do not have the same predictive value in gliomas (35) and therefore a pathology-specific assay is required.

Conclusion

Although immunotherapy holds significant promise for overcoming the challenges of immune dysfunction and tumoral heterogeneity, it is increasingly apparent that a single agent alone will not suffice. Exploration of combinations of ICB with neo-epitope vaccination strategies in IDH mutant gliomas is one promising approach, but IDH is expressed in a minority of glioblastomas. Further, the cancer lineage-specific ability of glioblastoma to drive T-cell anergy and apoptosis poses a significant obstacle for ICB therapies. Given this, more work is required on alternative strategies such as combinatorial T-cell costimulation or blockade of tumoral metabolic pathways. Evaluating potential combinations in patients who may not benefit from TMZ and are therefore less prone to hypermutation will be helpful to accurately determine biological activity. The timing of administration relative to routine clinical interventions such as steroid administration, radiation therapy, and the aforementioned alkylating chemotherapy, all of which possess varying immuno-modulatory effects, must also be weighed. Neoadjuvant and surgical window of opportunity studies (where tumor tissue can be collected after combinatorial immunotherapy) may offer the most sensitive PD and PK analysis, but other surrogate markers of effect such as KT₅₀ are also useful. Model-assisted trial designs may help assess dosage and schedules for different combinations, but it is important to consider that maximal synergistic effect may not occur at the maximal therapeutic dose. Although combinatorial approaches may unlock

the true potential for immunotherapy in glioblastoma, the lack of success in glioblastoma immunotherapy trials demands a tailor-made combinatorial approach. International collaboration will be necessary to develop trials, which have the scope and recruitment necessary to integrate such biologic complexities into their design (see summary box).

Summary Box and Key Messages

To date, immune-based monotherapies have failed to improve survival of patients with glioblastoma.

Glioblastoma exerts cancer lineage-specific mechanisms of immune escape and can induce profound local and systemic immunosuppression.

Given the lack of efficacy seen when using combinations with anti-PD-1/anti-CTLA-4 to date, cancer lineage-specific checkpoint inhibition (e.g., IDO1, LAG3, TIM3) and costimulatory agonistic targets (e.g., CD40, CD27) are worth exploring.

Timing of combinatorial immunotherapy relative to standard-of-care treatment must be carefully weighed.

TMZ may induce hypermutation and drive heterogeneity, and drive resistance to immunotherapies and their combinations.

Bevacizumab may help to reduce edema and therefore reduce the need for immunosuppressive corticosteroids, and can be used as an adjunct to combination immunotherapy.

Harmonization, standardization of immune technologies, and generation of reference values will help accelerate preclinical and early clinical development in glioblastoma.

Flexible trials such as model-assisted and adaptive designs are required to rapidly assess potential novel combinations.

Surgical window-of-opportunity trials, neoadjuvant trials, and trials with primary biologic (PD, PK) endpoints are recommended as they may help shorten lengthy pre-clinical and often futile clinical investigation.

Authors' Disclosures

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References

- Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol* 2017;18:1373–85.
- Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352:997–1003.
- Wen PY, Weller M, Lee EQ, Alexander BA, Barnholtz-Sloan JS, Barthel FP, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro-oncol* 2020;22:1073–113.
- Stupp R, Taillibert S, Kanner A, Read W, Steinberg DM, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA* 2017;318:2306–16.
- Chuntova P, Chow F, Watchmaker P, Galvez M, Heimberger AB, Newell EW, et al. Unique challenges for glioblastoma immunotherapy: discussions across neuro-oncology and non-neuro-oncology experts in cancer immunology. *Neuro Oncol* 2021;23:356–75.
- Khasraw M, Reardon DA, Weller M, Sampson JH. PD-1 inhibitors: do they have a future in the treatment of glioblastoma? *Clin Cancer Res* 2020;26:5287–96.
- Goswami S, Walle T, Cornish AE, Basu S, Anandhan S, Fernandez I, et al. Immune profiling of human tumors identifies CD73 as a combinatorial target in glioblastoma. *Nat Med* 2020;26:39–46.
- Raper D, Louveau A, Kipnis J. How do meningeal lymphatic vessels drain the CNS? *Trends Neurosci* 2016;39:581–6.
- O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrisette JJD, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med* 2017;9:eaaa0984.
- Chongsathidkiet P, Jackson C, Koyama S, Loebel F, Cui X, Farber SH, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nat Med* 2018;24:1459–68.
- Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nat Neurosci* 2016;19:20–7.
- Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. *Immunol Rev* 2008;222:206–21.
- Mu L, Long Y, Yang C, Jin L, Tao H, Haitao Ge, et al. The IDH1 mutation-induced oncometabolite, 2-hydroxyglutarate, may affect DNA methylation and expression of PD-L1 in gliomas. *Front Mol Neurosci* 2018;11:82.
- Röver LK, Gevensleben H, Dietrich J, Bootz F, Landsberg J, Goltz D, et al. PD-1 (PDCD1) promoter methylation is a prognostic factor in patients with diffuse lower-grade gliomas harboring isocitrate dehydrogenase (IDH) mutations. *EBioMedicine* 2018;28:97–104.
- Lee KS, Lee K, Yun S, Moon S, Park Y, Han JH, et al. Prognostic relevance of programmed cell death ligand 1 expression in glioblastoma. *J Neurooncol* 2018;136:453–61.
- Strohl WR, Naso M. Bispecific T-cell redirection versus chimeric antigen receptor (CAR)-T cells as approaches to kill cancer cells. *Antibodie* 2019;8:41.
- Rosenthal M, Balana C, Linde MEV, Sayehli C, Fiedler WM, Wermke M, et al. Novel anti-EGFRvIII bispecific T cell engager (BiTE) antibody construct in glioblastoma (GBM): Trial in progress of AMG 596 in patients with recurrent or newly diagnosed disease. *J Clin Oncol* 2019;37:TPS2071–TPS.
- Gedeon PC, Schaller TH, Chitneni SK, Choi BD, Kuan CT, Suryadevara CM, et al. A rationally designed fully human EGFRvIII:CD3-targeted bispecific antibody redirects human T cells to treat patient-derived intracerebral malignant glioma. *Clin Cancer Res* 2018;24:3611–31.
- Scott EM, Duffy MR, Freedman JD, Fisher KD, Seymour LW. Solid tumor immunotherapy with T cell engager-armed oncolytic viruses. *Macromol Biosci* 2018;18.
- Wainwright DA, Chang AL, Dey M, Balyasnikova IV, Kim CK, Tobias A, et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. *Clin Cancer Res* 2014;20:5290–301.
- Tritz ZP, Ayasoufi K, Malo C, Himes B, Khadka R, Yokanovich L, et al. Combination immunotherapy of α PD-1 and extended half-life IL-2 clears

- established GL261 gliomas in an MHC class I independent fashion. *J Immunol* 2020;204:169.15-15.
22. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol* 2020;6:1003-10.
 23. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *J Exp Clin Cancer Res* 2019;38:255.
 24. Ott M, Tomaszowski KH, Marisetty A, Kong LY, Wei J, Duna M, et al. Profiling of patients with glioma reveals the dominant immunosuppressive axis is refractory to immune function restoration. *JCI Insight* 2020;5:e134386.
 25. Zhao J, Chen AX, Gartrell RD, Silverman AM, Aparicio L, Chu T, et al. Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. *Nat Med* 2019;25:462-9.
 26. Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat Med* 2019;25:477-86.
 27. Schalper KA, Rodriguez-Ruiz ME, Diez-Valle R, López-Janeiro A, Porcuncula A, Idoate MA, et al. Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. *Nat Med* 2019;25:470-6.
 28. Woroniecka K, Chongsathidkiet P, Rhodin K, Kemeny H, Dechant C, Farber SH, et al. T-cell exhaustion signatures vary with tumor type and are severe in glioblastoma. *Clin Cancer Res* 2018;24:4175-86.
 29. Long GV, Dummer R, Hamid O, Gajewski TF, Caglevic C, Dalle S, et al. Epcadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. *Lancet Oncol* 2019;20:1083-97.
 30. Opitz CA, Litzenburger UM, Sahn F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 2011;478:197-203.
 31. Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, et al. IL4I1 is a metabolic immune checkpoint that activates the AHR and promotes tumor progression. *Cell* 2020;182:1252-70.
 32. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol* 2010;28:4722-9.
 33. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med* 2016;375:2561-9.
 34. Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrsen CL, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature* 2020;580:517-23.
 35. Khasraw M, Walsh KM, Heimberger AB, Ashley DM. What is the burden of proof for tumor mutational burden in gliomas? *Neuro-oncol* 2020;23:17-22.
 36. Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature* 2021;592:463-8.
 37. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 2019;565:234-9.
 38. Liu CJ, Schaettler M, Blaha DT, Bowman-Kirigin JA, Kobayashi DK, Livingstone AJ, et al. Treatment of an aggressive orthotopic murine glioblastoma model with combination checkpoint blockade and a multi-valent neoantigen vaccine. *Neuro-oncol* 2020;22:1276-88.
 39. Woroniecka K, Fecci PE. Immuno-synergy? Neoantigen vaccines and checkpoint blockade in glioblastoma. *Neuro-oncol* 2020;22:1233-4.
 40. Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanović S, Gouttefangeas C, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 2019;565:240-5.
 41. Choi BD, Yu X, Castano AP, Darr H, Henderson DB, Bouffard AA, et al. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. *J Immunother Cancer* 2019;7:304.
 42. Pardridge WM. Blood-brain barrier and delivery of protein and gene therapeutics to brain. *Frontiers in Aging Neuroscience* 2020;11:373.
 43. Sarkaria JN, Hu LS, Parney IF, Pafundi DH, Brinkmann DH, Laack NN, et al. Is the blood-brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. *Neuro-oncol* 2018;20:184-91.
 44. Portnow J, Wang D, Blanchard MS, Tran V, Alizadeh D, Starr R, et al. Systemic anti-PD-1 immunotherapy results in PD-1 blockade on T cells in the cerebrospinal fluid. *JAMA Oncol* 2020;6:1947-51.
 45. Choi BD, Suryadevara CM, Gedeon PC, Herndon JE 2nd, Sanchez-Perez L, Bigner DD, et al. Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. *J Clin Neurosci* 2014;21:189-90.
 46. Brady M, Raghavan R, Sampson J. Determinants of intraparenchymal infusion distributions: modeling and analyses of human glioblastoma trials. *Pharmaceutics* 2020;12:895.
 47. Klinger M, Zugmaier G, Nägele V, Goebeler M-E, Brandl C, Steljes M, et al. Adhesion of T cells to endothelial cells facilitates blinatumomab-associated neurologic adverse events. *Cancer Res* 2020;80:91-101.
 48. Oved JH, Barrett DM, Teachey DT. Cellular therapy: Immune-related complications. *Immunol Rev* 2019;290:114-26.
 49. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015;520:373-7.
 50. Kim JE, Patel MA, Mangraviti A, Kim ES, Theodoros D, Velarde E, et al. Combination therapy with anti-PD-1, anti-TIM-3, and focal radiation results in regression of murine gliomas. *Clin Cancer Res* 2017;23:124-36.
 51. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature* 2015;519:366-9.
 52. Reap EA, Suryadevara CM, Batich KA, Sanchez-Perez L, Archer GE, Schmittling RJ, et al. Dendritic cells enhance polyfunctionality of adoptively transferred T cells that target cytomegalovirus in glioblastoma. *Cancer Res* 2018;78:256-64.
 53. Mardiana S, Solomon BJ, Darcy PK, Beavis PA. Supercharging adoptive T cell therapy to overcome solid tumor-induced immunosuppression. *Sci Transl Med* 2019;11:eaaaw2293.
 54. Belcaid Z, Phallen JA, Zeng J, See AP, Mathios D, Gottschalk C, et al. Focal radiation therapy combined with 4-1BB activation and CTLA-4 blockade yields long-term survival and a protective antigen-specific memory response in a murine glioma model. *PLoS One* 2014;9:e101764.
 55. Do P, Perdue LA, Chyong A, Henry CJ, Porter CC, Dreaden EC. Promoting anti-tumor immunity via bispecific T cell engaging cytokine (biteokine) therapy. *J Immunol* 2020;204:169.22-22.
 56. Riccione KA, He LZ, Fecci PE, Norberg PK, Suryadevara CM, Swartz A, et al. CD27 stimulation unveils the efficacy of linked class I/II peptide vaccines in poorly immunogenic tumors by orchestrating a coordinated CD4/CD8 T cell response. *Oncoimmunology* 2018;7:e1502904.
 57. Khasraw M, Weller M, Lorente D, Kolibaba K, Lee CK, Gedye C, et al. Bintrafusp alfa (M7824) a bifunctional fusion protein targeting TGF- β and PD-L1: results from a phase I expansion cohort in patients with recurrent glioblastoma. *Neuro-Oncology Advances* 2021;3:vdab058.
 58. Choe JH, Watchmaker PB, Simic MS, Gilbert RD, Li AW, Krasnow NA, et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci Transl Med* 2021;13:eabe7378.
 59. Avanzi MP, Yeku O, Li X, Wijewarnasuriya DP, van Leeuwen DG, Cheung K, et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. *Cell Rep* 2018;23:2130-41.
 60. Gustafson MP, Lin Y, New KC, Bulur PA, O'Neill BP, Gastineau DA, et al. Systemic immune suppression in glioblastoma: the interplay between CD14+HLA-DRlo/neg monocytes, tumor factors, and dexamethasone. *Neuro-oncol* 2010;12:631-44.
 61. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med* 2014;370:709-22.
 62. Filley AC, Henriquez M, Dey M. Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. *Oncotarget* 2017;8:91779-94.
 63. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009;10:459-66.

64. Alexander BM, Ba S, Berger MS, Berry DA, Cavenee WK, Chang SM, et al. Adaptive global innovative learning environment for glioblastoma: GBM AGILE. *Clin Cancer Res* 2018;24:737–43.
65. Isaacs J, Tan AC, Hanks BA, Wang X, Owzar K, Herndon JE, et al. Clinical trials with biologic primary endpoints in immuno-oncology: concepts and usage. *Clin Cancer Res* 2021 Jul 26 [Epub ahead of print].
66. Piantadosi S. Translational clinical trials: an entropy-based approach to sample size. *Clinical Trials* 2005;2:182–92.
67. Jiang L, Li R, Yan F, Yap TA, Yuan Y. Shotgun: A Bayesian seamless phase I-II design to accelerate the development of targeted therapies and immunotherapy. *Contemporary Clinical Trials* 2021;104:106338.
68. Stallard N, Todd S. Seamless phase II/III designs. *Stat Methods Med Res* 2011;20: 623–34.
69. Woodcock J, LaVange LM. Master protocols to study multiple therapies, multiple diseases, or both. *N Engl J Med* 2017;377:62–70.
70. Okada H, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. *Lancet Oncol* 2015;16:e534–e42.
71. Alexander BM, Trippa L, Gaffey S, IC A-R, Lee EQ, Rinne ML, et al. Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGHT): a bayesian adaptive platform trial to develop precision medicines for patients with glioblastoma. *JCO Precis Oncol* 2019;3:PO.18.00071.
72. Wick W, Dettmer S, Berberich A, Kessler T, Karapanagiotou-Schenkel I, Wick A, et al. N2M2 (NOA-20) phase I/II trial of molecularly matched targeted therapies plus radiotherapy in patients with newly diagnosed non-MGMT hypermethylated glioblastoma. *Neuro-oncol* 2018;21:95–105.
73. Pfaff E, Kessler T, Balasubramanian GP, Berberich A, Schrimpf D, Wick A, et al. Feasibility of real-time molecular profiling for patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation—the NCT Neuro Master Match (N2M2) pilot study. *Neuro-oncol* 2017; 20:826–37.
74. Prowell TM, Theoret MR, Pazdur R. Seamless oncology-drug development. *N Engl J Med* 2016;374:2001.
75. Murthy H, Iqbal M, Chavez JC, Kharfan-Dabaja MA. Cytokine release syndrome: current perspectives. *Immunotargets Ther* 2019;8:43–52.
76. Thall PF, Millikan RE, Mueller P, Lee SJ. Dose-finding with two agents in phase I oncology trials. *Biometrics* 2003;59:487–96.
77. Yin G, Li Y, Ji Y. Bayesian dose-finding in phase I/II clinical trials using toxicity and efficacy odds ratios. *Biometrics* 2006;62:777–87.
78. Le Tourneau C, Lee JJ, Siu LL. Dose escalation methods in phase I cancer clinical trials. *J Natl Cancer Inst* 2009;101:708–20.
79. Rahma OE, Reuss JE, Giobbie-Hurder A, Shoja ERG, Abu-Shawar O, Mehra P, et al. Early 3+3 trial dose-escalation phase I clinical trial design and suitability for immune checkpoint inhibitors. *Clin Cancer Res* 2021;27:485–91.
80. Wen PY, Chang SM, MJVD B, Vogelbaum MA, Macdonald DR, Lee EQ. Response assessment in neuro-oncology clinical trials. *J Clin Oncol* 2017;35: 2439–49.
81. Ellingson BM, Wen PY, Cloughesy TF. Modified criteria for radiographic response assessment in glioblastoma clinical trials. *Neurotherapeutics* 2017; 14:307–20.
82. Weathers SP, Penas-Prado M, Pei BL, Ling X, Kassab C, Banerjee P, et al. Glioblastoma-mediated immune dysfunction limits CMV-specific T cells and therapeutic responses: results from a phase I/II trial. *Clin Cancer Res* 2020;26: 3565–77.
83. Cerignoli F, Abassi YA, Lamarche BJ, Guenther G, Santa Ana D, Guimet D, et al. In vitro immunotherapy potency assays using real-time cell analysis. *PLoS One* 2018;13:e0193498.
84. Yuan Y, Lee JJ, Hilsenbeck SG. Model-assisted designs for early-phase clinical trials: simplicity meets superiority. *JCO Prec Oncol* 2019;3:PO.19.00032.
85. Yuan Y, Yin G. Bayesian phase I/II adaptively randomized oncology trials with combined drugs. *Ann Appl Stat* 2011;5:924.
86. Macchia I, Urbani F, Proietti E. Immune monitoring in cancer vaccine clinical trials: critical issues of functional flow cytometry-based assays. *Biomed Res Int* 2013;2013:726239.
87. Rao G, Latha K, Ott M, Sabbagh A, Marisetty A, Ling X, et al. Anti-PD-1 induces M1 polarization in the glioma microenvironment and exerts therapeutic efficacy in the absence of CD8 cytotoxic T cells. *Clin Cancer Res* 2020; 26:4699–712.
88. Latchman YE, Liang SC, Wu Y, Chernova T, Sobel RA, Klemm M, et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *PNAS* 2004;101:10691–6.
89. Ren X, Akiyoshi K, Vandenbark AA, Hurn PD, Offner H. Programmed death-1 pathway limits central nervous system inflammation and neurologic deficits in murine experimental stroke. *Stroke* 2011;42:2578–83.