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The addition of PD-1/PD-L1 axis blockade to BRAF and MEK inhibition for advanced melanoma patients harboring *BRAF* mutations: a systematic review and meta-analysis

A adição do bloqueio do eixo PD-1/PD-L1 à inibição de BRAF e MEK para pacientes com melanoma avançado com mutações *BRAF*: uma revisão sistemática e metanálise

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ABSTRACT

Objectives: Immune-checkpoint inhibitors (ICI) and targeted-therapies (TT) have become standard options for *BRAF*-V600 metastatic melanomas (MM). Recently, randomized trials (RCT) addressed the efficacy of combined approaches, with conflicting results. We sought to evaluate efficacy and safety of first-line combination ICI and BRAF/MEK inhibitors (triplets) versus BRAF/MEKi (doublets). **Methods:** We performed a systematic review and meta-analysis of RCT comparing triplet versus doublet published in MEDLINE and EMBASE from 2016-September/2020. We obtained pooled effect estimates through random-effects model assuming p<0.05 as statistically significant. **Results:** Among 1,784 studies, 3 RCT were selected. Triplets demonstrated progression-free survival (PFS) (HR 0.79 – CI 0.68-0.91, *p*=0.001) and overall survival (OS) improvement (HR 0.81 – CI 0.67-0.98, *p*=0.03), with increased rates of grades 3/4 adverse events (AEs), any grade pyrexia, arthralgia, and aminotransferases elevation. AE-discontinuation rates of all drugs remained similar. **Conclusions:** Triplets improved PFS and OS with manageable toxicities. These are preliminary results and mature data are expected.

Keywords: Melanoma, Programmed Cell Death 1 receptor, Proto-oncogene proteins b-raf, MAP kinase signaling system, Immunotherapy.

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RESUMO

Objetivos: Inibidores de checkpoints imunológicos (ICI) e terapias-alvo (TT) tornaram-se opções padrão para melanomas metastáticos (MM) *BRAF*-V600 mutados. Recentemente, ensaios clínicos randomizados (ECR) abordaram a eficácia das abordagens combinadas, com resultados conflitantes. Procuramos avaliar a eficácia/segurança da combinação de ICI e inibidores BRAF/MEK de primeira linha (terapia triple) versus BRAF/MEKi (terapia dupla). **Métodos:** Realizamos uma revisão sistemática e metanálise de ECR comparando terapia tripla versus dupla publicados no MEDLINE e EMBASE de 2016 a setembro/2020. Obtivemos estimativas de efeito agrupado por meio do modelo de efeitos aleatórios assumindo p<0,05 como estatisticamente significativo. **Resultados:** Entre 1.784 estudos, 3 ECR foram selecionados. Os triplets demonstraram melhora na sobrevida livre de progressão (SLP) (HR 0,79-CI 0,68- 0,91, p=0,001) e na sobrevida global (SG) (HR 0,81-CI 0,67-0,98, p=0,03), com maiores taxas de eventos adversos (EAs) graus 3/4, pirexia, artralgia e elevação de aminotransferases de qualquer grau. As taxas de descontinuação de EA de todos os medicamentos permaneceram semelhantes. **Conclusão:** A terapia tripla melhorou a SLP e a SG com toxicidades manejaveis. Estes são resultados preliminares e dados maduros sao esperados. RESUMO Descritores: Melanoma; Imunoterapia; Receptor de morte celular programada 1; Proteínas proto-oncogênicas B-raf; Sistema de sinalização MAP kinase.

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INTRODUCTION

The development of BRAF inhibitors (BRAFi) revolutionized the treatment of *BRAF* V600-mutant metastatic melanoma (MM).[1,2] However, despite the initial advantage in efficacy endpoints when compared to chemotherapy, objective responses with BRAFi monotherapy were usually short-lived, with second primary cutaneous tumors arising as a result of the paradoxical MAP kinase (MAPK) pathway activation. Aiming to overcome these limitations, MEK inhibitors (MEKi) were associated, resulting in statistically significant improvements in objective response rates (ORR), progression-free survival (PFS) and overall survival (OS) in favor of combined BRAF plus MEK inhibition (BRAF/MEKi), when compared to single-agent BRAFi.[3,4,5,6] As an example, in an updated pooled analysis of the COMBI-d and COMBI-v trials, a sustained benefit with the combination of BRAF/MEKi was demonstrated, with nearly 21% and 35% of the patients progression-free and alive at 5 years, respectively. In addition, the small proportion of primary progressors and fast response kinetics have contributed to consolidate BRAF/MEKi in the frontline setting of MM presenting with high volume and symptomatic disease. Nevertheless, most patients still experience disease progression (PD) and ultimately die within this follow-up period, which underscores that the development of novel strategies to enhance disease control is a task of utmost importance.[7]

Similarly, immune checkpoint inhibitors (ICI) directed against the *cytotoxic T-lymphocyte-associated antigen 4* (CTLA4) and programmed death receptor 1 (PD-1), changed the treatment landscape of MM, irrespective of BRAF mutation status.[8,9,10] In the 5-year landmark analysis of the CheckMate 067 randomized study, patients with *BRAF* V600-mutant MM achieved remarkable outcomes when treated with ICI (single agent nivolumab or the combination of ipilimumab and nivolumab) in the first line setting, with 5-year OS rates ranging from 46% to 60% and ORR of 45-58%.[9] Also, data from a post-hoc 5-year analysis of the KEYNOTE-006 trial revealed a 47% ORR and median OS not reached with pembrolizumab monotherapy in BRAF/MEKi-naïve patients, corroborating the good outcomes resulting from ICI in *BRAF*-mutant MM patients and adding more complexity to treatment choice in the frontline setting.[10] Unfortunately, despite the possibility of long-lasting responses in this treatment modality, a significant proportion of patients develop secondary resistance to ICIs, in addition the nearly 24-38% of individuals who present disease progression as best response due to primary resistance mechanisms.[4,5,11-13]

More recently, preclinical data suggesting enhanced anti-tumor immunogenicity with MAPK pathway inhibition have raised expectations of overcoming limitations inherent to the isolated use of either strategies.[14] Among described alterations that could potentially confer better outcomes through the combination of BRAK/MEKi and ICIs are: increased expression of melanoma-associated antigens (e.g. Melan-A, tyrosinase), interferon alfa receptor 1 (IFNAR1) and human leucocyte antigen class I (HLA-I); increased tumor infiltration by CD8+ T-cells – tumor infiltrating lymphocytes (TILs); decreased activity of immunosuppressive cytokines, regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment.[15,16,17,18,19,20] This biological rationale has prompted the development and conduction of prospective clinical trials to address the role of combined regimens comprising ICIs and BRAF/MEKi as first-line therapies for MM patients.

In several phase I trials, triplet regimens demonstrated encouraging antitumor activity, with very few patients experiencing disease progression as best response, along with durable benefits in those achieving disease control.[21-24] For instance, the combination of dabrafenib, trametinib and durvalumab showed 76% ORR, with a 100% disease control rate among 15 patients enrolled in the *BRAF*-mutant cohort of the study [21]; likewise, vemurafenib, cobimetinib and atezolizumab presented 85.3% (29/34 patients) unconfirmed response rate assessed by RECIST 1.1, with nearly 69% of them experiencing ongoing response at the time of data cut-off.[23]

This impressive activity demonstrated in early-phase studies prompted the rapid development of randomized trials addressing the efficacy of triplet combinations, having BRAFi/MEKi as the comparator arms. In both KEYNOTE-022 and COMBI-i studies, the superiority of dabrafenib and trametinib backbone combined with an anti-PD1 (pembrolizumab and spartalizumab, respectively) was assessed against a placebo-controlled dual BRAF/MEKi; similarly, the IMspire150 trial tested the performance of the triplet vemurafenib, combimetinib and atezolizumab over vemurafenib plus cobimetinib without ICI.[25-27] However, these trials have produced conflicting results, and the current indications for triplet regimens remains uncertain.

Hence, we conducted a systematic review and meta-analysis of randomized controlled trials to assess the efficacy and safety of combined BRAF/MEKi and PD-1/PD-L1 axis blockade ("triplet") when compared to BRAF/MEKi alone in MM patients.

METHODS

Search and selection criteria

We performed a systematic review for studies published between 2016 and September/2020 encompassing MEDLINE and EMBASE citation indexes. The review process was conducted according to the PRISMA guidelines.[28] Database search was conducted according to the strategy available in the Supplementary Material (Figure S1). Publications with the following criteria were selected: prospective, randomized, controlled trials (RCT) which directly compared triplet combinations with either anti-PD-1 or anti-PD-L1 agents vs BRAF/MEKi for patients with MM harboring *BRAF*-V600 mutations. We also performed a manual search of reference lists of studies selected for data extraction.

Two investigators independently reviewed each study's title and abstract against prespecified inclusion criteria (CBX and MFSAR), followed by a third blinded reviewer in case of divergence. A qualitative systematic literature review and critical evaluation of the evidence were performed. Articles with OS, PFS, ORR, any grade adverse events (AE), grade 3/4 AEs and information regarding discontinuation of all study drugs owing to AEs were pooled in meta-analyses.

Data extraction

Two investigators (CBX and MFSAR) independently extracted the following data, using standardized collection forms: journal/conference and year of publication, number of patients planned, accrued and included in the analyses, age, median follow-up, toxicity information limited to proportion of specific predefined any grade adverse events (AE), grades 3/4 AEs, serious AEs, discontinuation of all study drugs owing to AEs, as well as OS, PFS and ORR with their respective hazard ratios/odds ratios and confidence intervals.

Methodological Assessment of Quality

Two investigators independently assessed each RCT quality as "low risk" or "high risk" of bias by using predefined quality criteria suggested by Higgins and colleagues, where both study methods and results are evaluated with a quality check-list.[29] Quality criteria include evaluations of the randomization process, concealment of treatment allocation, methods of blinding and handling of dropouts. A resulting global quality score indicates the risk of bias (Supplementary Material - Figure S2).

Outcome Measures

The purpose of this meta-analysis was to investigate the efficacy outcomes of the combination of PD1/PD-L1 axis blockade plus BRAF/MEKi (triplet) versus BRAF/MEKi and to compare the incidence and severity of AEs in patients with MM harboring *BRAF* mutations. The primary endpoint was progression-free survival (PFS). overall survival (OS), progression-free survival (PFS) and objective response rate (ORR). Secondary endpoints included overall survival (OS), objective response rate (ORR), the incidence of selected any grade AEs, grades 3/4 AEs, serious AEs, discontinuation of all study drugs owing to AEs and selected treatment-related AEs.

Analysis and synthesis

Meta-analyses for pooled effect measures were performed using RevMan 5.4 software (Cochrane Collaboration Information Management System). Time-to-event outcomes were compared using Hazard Ratios (HR). Respective 95% confidence intervals (95% CI) were calculated for each estimate and presented in forest plots. The pooled HR, symbolized by a solid diamond at the bottom of the forest plot (the width of which represents the 95% CI) is the best estimate of the true effect size. The meta-analyses were performed using a random-effect model. The heterogeneity between the risk ratios for the same outcome between different studies was assessed using the chi-square-based *Q* statistic (Chi^2), with significance at a p value of less than 0.10 and expressed in I^2 index. If there was evidence of heterogeneity ($I^2 > 40\%$), we performed a subsequent sensitive analysis (Supplementary Material - Figures S3 and S4) to evaluate the source of the heterogeneity based on clinical or methodological factors and possible explanations were investigated and reported.

Results were expressed as HR for time-to-event outcomes or odds ratios (OR) for adverse events, with their respective 95% CIs. A HR or OR < 1.0 favored the triplet treatment group.

RESULTS

Results of Search Strategy

Our search resulted in 1,784 entries; 160 duplicated records were removed upfront. From the remaining 1,624 studies, 350 were assessed for eligibility and three randomized trials met the predefined criteria, totalizing 1,166 randomized patients.[25-27] The search process is summarized in Figure 1.

Characteristics of Eligible Trials

The characteristics of the three eligible studies are summarized in Table 1. One of the trials, a phase II RCT, evaluated the superiority of combination dabrafenib, trametinib and pembrolizumab over combination dabrafenib, trametinib and placebo as the first-line treatment for MM.[25] The second one, a phase III trial, compared the association of dabrafenib, trametinib and spartalizumab versus dabrafenib, trametinib and placebo; this is a three-part study in which only the third part randomized patients to receive triplet or doublet regimens.[27] The third trial tested the superiority of vemurafenib, cobimetinib and atezolizumab over vemurafenib, cobimetinib and placebo. More detailed characteristics of the studies are available at Table 1. We considered all three studies as low-risk of bias RCTs after a thorough examination taking the following criteria into consideration: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other biases.[29]

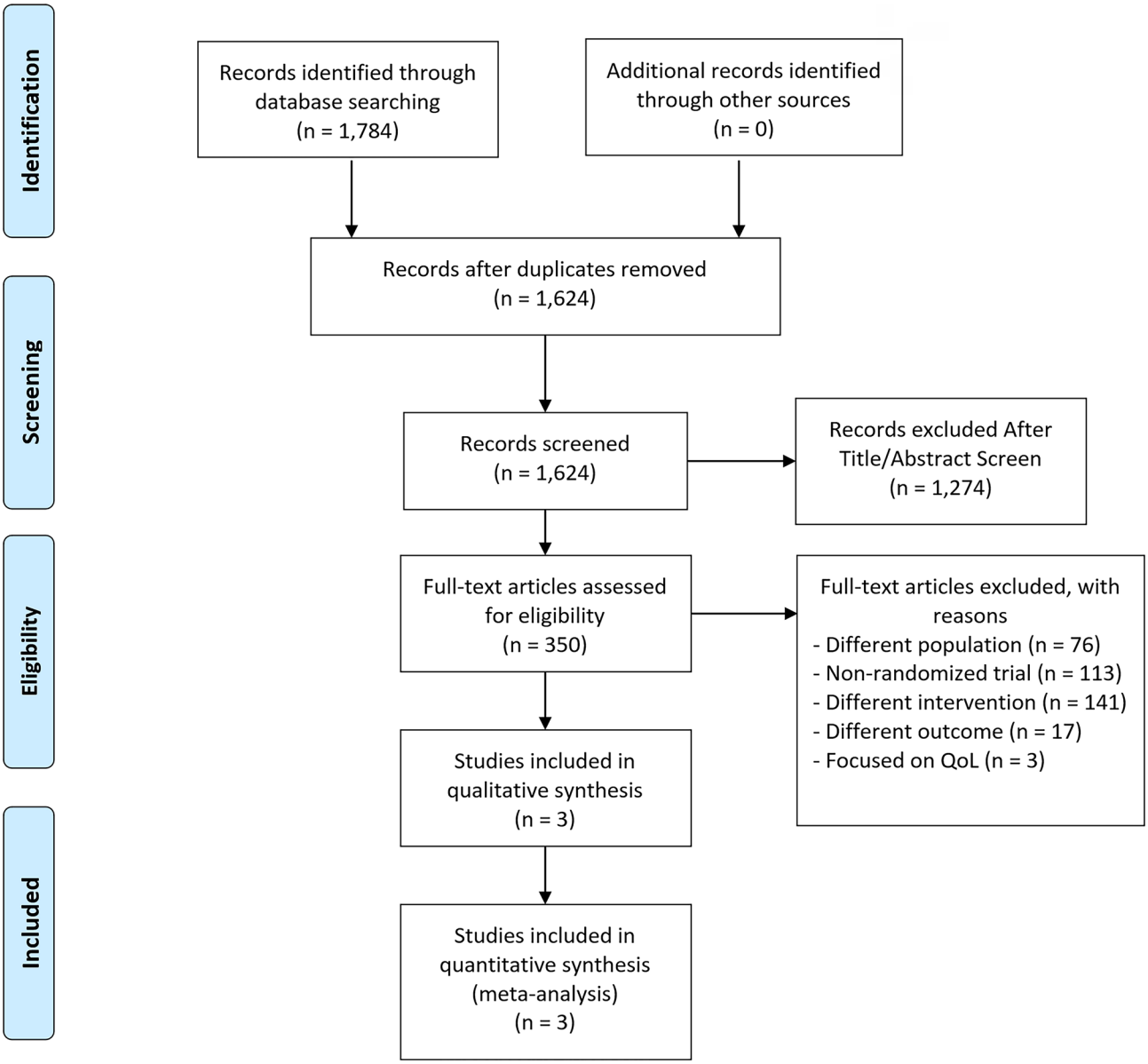

Figure 1. PRISMA flowchart describing the search process.

Table 1. Summarized results of RCT included in the meta-analysis.

Study	KEYNOTE-022	COMBI-i	IMspire 150
N of patients randomized (BRAF+MEKi/Triplet)	60 / 60	265 / 267	258 / 256
Experimental arm	D + T + Pembro	D + T + Sparta	Vem + Cobi + Atezo[a]
Control arm	D + T + Placebo	D + T + Placebo	Vem + Cobi + Placebo[b]
Primary outcome	PFS (investigator)	PFS (investigator)	PFS (investigator)
Risk of bias	Low	Low	Low
mFU (mo) (95%CI)	9.6 (2.7 - 23.4)	27.2 (24.0 - 33.6)	18.9 (10.4 - 23.8)
mPFS (mo) assessed by the investigator (95%CI)	10.3 (7-15.6) / 16.0 (8.6-21.5)	12 (10.2-15.4) / 16.2 (12.7-23.9)	10.6 (9.3-12.7) / 15.1 (11.4-18.4)
mPFS (mo) assessed by independent review committee (95%CI)	N/A	N/A	12.3 (10.8-14.7) / 16.1 (11.3-18.5)
12 mo PFS (%) assessed by the investigator (95%CI)	45.2 (31.9-57.6) / 59.3 (44.9-71.1)	50 / 58	45.1 / 54
24 mo PFS (%) assessed by the investigator	N/A	36 / 44	N/A
ORR (%) assessed by the investigator (95%CI)	71.7 (58.6-82.5) / 63.3 (49.9-75.4)	64.2 (58.1-69.9) / 68.5 (62.6 - 74.1)	65 (58.7-71) / 66.3 (60.1-72.1)
CRR (%) assessed by the investigator	18.3	17.7 / 19.9	17.1 / 15.7
mDOR (mo) assessed by the investigator (95%CI)	12.5 (6-14.1) / 18.7 (10.1-22.1)	20.7 / NR	12.6 (10.5-16.6) / 21.0 (15.1-NE)
mOS (mo) assessed by the investigator (95%CI)	23.4 (17.8-NR) / NR (16.9-NR)	NR (28.3-NR) / NR (30.6-NR)	NR
12 mo OS (%) (95%CI)	72.9 (59.6-82.5) / 79.9 (67.3-88.0)	79 / 84	76 / 77
24 mo OS (%)	N/A	62 / 68	53 / 60

mFU = Median follow up; mPFS = Median progression-free survival; mOS = Median overall survival; ORR = Objective response rate; CRR = Complete response rate; mDOR = Median duration of response; N/A = Not available; D = Dabrafenib 150mg BID; T = Trametinib 2mg OD; Pembro = Pembrolizumab 2mg/kg every 3 weeks; Sparta = Spartalizumab 400mg every 4 weeks; Atezo = Atezolizumab 840mg every 2 weeks; [a]cycle 1 = Vem 960mg BID, Cobi 60mg OD; subsequent cycles = Vem 720mg BID; [b]All cycles = Vem 960mg BID, Cobi 60mg OD.

Progression Free Survival

All RCTs contributed to our analysis of investigator-assessed PFS; PFS was the primary endpoint in the three studies included in this meta-analysis. PFS was defined in two trials as the time from the date of randomization to the date of the first documented and radiologically confirmed PD assessed by the investigator or death from any cause, whichever occurred first.[25],[26] In the part 3 of the COMBI-i study, PFS was defined from the date of the first dose of the assigned regimens to the date of radiological progression assessed by the investigator or death from any cause, whichever occurred first.[27] Frontline triplet demonstrated superior results when compared to dual BRAF/MEK inhibition (HR: 0.79, 95% CI 0.68-0.91, *p*=0.001) (Figure 2). Of note, in KEYNOTE-022 and COMBI-i part 3 trials, despite a numerical difference favoring triplet regimens, no statistically significant advantage was detected.

Overall Survival

Data on OS were available in all selected trials. In all of them, OS was measured from the date of randomization to date of death from any cause. The meta-analysis of reported HR for OS showed that combined BRAF-MEK-ICI resulted in improved OS, as compared to BRAF/MEKi inhibition (HR 0.81, 95CI% 0.67-0.98, *p*=0.03) (Figure 3). Nevertheless, it is important to highlight that, in the COMBI-i study, OS could only be analyzed for statistical significance if the primary endpoint (PFS) was reached, which did not occur until the most recent presentation of the study.

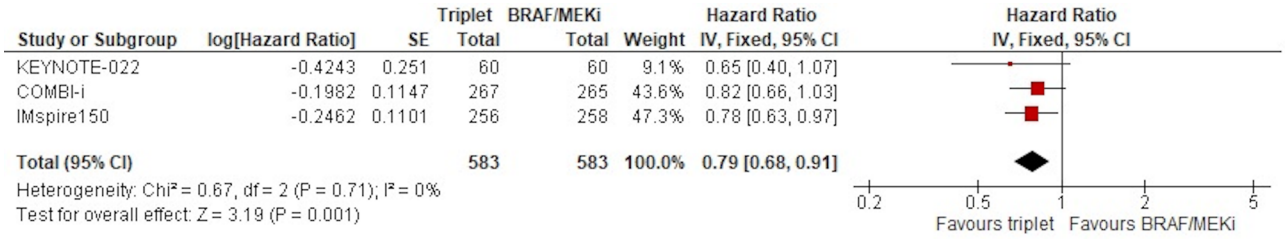

Figure 2. Progression Free Survival. Forest plot depicting statistically significant PFS advantage favoring the triplet.

Objective Response Rate

We identified similar investigator-assessed ORR between the triplet and BRAF/MEKi arms. There were 390 objective responses among 583 patients in the triplet arm (pooled ORR: 66.9%) versus 373 in the BRAF/MEKi arm (pooled ORR: 64%) (OR: 1.14, 95%CI 0.89-1.45, *p*=0.30) (Supplementary Material Figure S5).

Toxicity

We did not observe any differences between treatment strategies regarding the overall incidence of unselected any grade AEs (OR 0.46 95%CI 0.15-1.37, *p*=0.16) (Supplementary Material Figure S6). Although triplet combinations resulted in a higher incidence of grade 3/4 AEs (OR 0.57 95%CI 0.42-0.78, *p*=0.0004) (Figure 4), the proportion of treatment-related AEs prompting all drug discontinuations (OR 0.71 95%CI 0.37-1.37, *p*=0.30) and treatment-related serious AEs (SAEs) (OR 0.55 95%CI 0.27-1.15, p=0.11) were similar (Supplementary Material Figures S7 and S8, respectively). Among 10 frequently reported and selected any grade AEs, pyrexia (OR 0.50 95%CI 0.39-0.65, *p*<0.00001), arthralgia (OR 0.73 95%CI 0.58-0.95, *p*=0.02), elevated aspartate aminotransferase (AST) (OR 0.63 95%CI 0.48-0.83, *p*=0.0010) and elevated alanine aminotransferase (ALT) (OR 0.60 95%CI 0.42-0.85, *p*=0.005) were more frequent in the triplet arm. Regarding other selected any grade AEs, including diarrhea, nausea, elevated blood creatine phosphokinase (CK), fatigue, rash and asthenia, no statistically significant difference was noted. The remaining forest plots are available in Supplementary Material Figures S9-S18.

DISCUSSION

Over the past decade, the treatment landscape for patients with MM has experienced a significant paradigm shift. BRAF/MEKi and ICIs became the cornerstone of *BRAF* V600-mutant MM treatment owing to their proven superiority in all efficacy endpoints over chemotherapeutic regimens[3-5,9,10]; however, these strategies have always been employed separately in routine practice due to the lack of prospective clinical data demonstrating any advantages arising from their combination. As previously mentioned, the rationale supported by a myriad of preclinical studies favoring the combination of BRAF/MEKi and ICIs lead clinical researchers to investigate whether the triplet would provide additional benefits to patients when compared to the already prescribed dual BRAF/MEKi.[18] In the present systematic review and meta-analysis comprising RCT assessing triplet regimens containing anti-PD1/PD-L1 versus BRAF/MEKi, we detected a statistically significant advantage in both PFS and OS favoring the triplet, with an increased rate of grade 3/4 AEs. Nevertheless, we did not identify any differences between triplet and doublet regimens regarding ORR, any grade toxicities, treatment-related AEs prompting all drug discontinuation, SAEs and most specific toxicities.

The influence of MAPK inhibition on tumor microenvironment (TME) has been the subject of diverse preclinical studies throughout the years. The ability of BRAF/MEKi to alter gene expression profiling (GEP) by inducing modifications in the complex interplay between *BRAF*-mutant melanoma and immune cells within 2-4 weeks of treatment, paved the way for a more comprehensive biomarker analysis in early-phase studies.[16,18,30-33] Interestingly, tumors exposed to BRAF/MEKi present early in the course enhanced expression of melanoma-related antigens (MRA), HLA type I, PD-L1, and other co-inhibitory molecules such as TIM-3 and LAG-3; likewise, increased infiltration by both CD8+ TILs and CD4+ T helper cells occurs, along with reduction in Tregs and MDSCs.

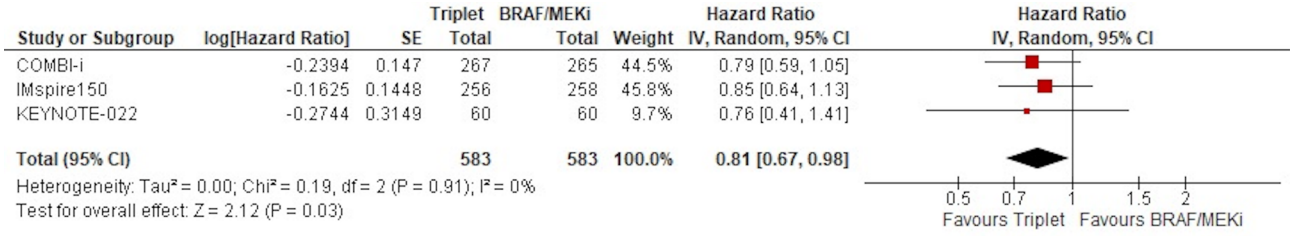

Figure 3. Overall survival. Forest plot depicting statistically significant OS advantage favoring the triplet.

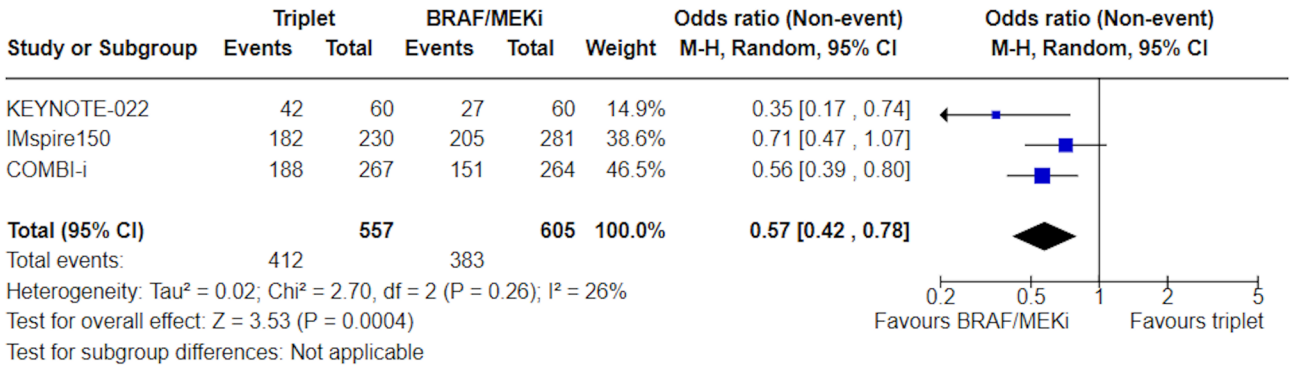

Figure 4. Incidence of grade 3/4 AEs. Forest plot displaying a statistically significant difference favoring BRAF/MEKi.

Noteworthy, MAPK blockade has also been associated with a marked inhibition of the immunosuppressive extracellular adenosine (eADO) signaling pathway in both melanoma cell lines and mouse models driven by reduction in CD73+ cells.[18,34,35] Other findings that corroborate the acquisition of a "hot" TME phenotype in MM under BRAF/MEKi are the higher level of plasma interferon-gama (IFN-g) and increased T-cell inflamed GEP observed after treatment.[18,36] Hu-Lieskovan et al described the superior anti-tumor activity of the triplet dabrafenib,trametinib and anti-PD1 when compared to either modality alone in a mouse model harboring the SM1 cell line, disclosing the attractive potential arising from this combination.[33] Dummer and colleagues recently reported the results of the run-in and biomarker cohorts of the COMBI-i where, despite the reduced number of subjects, 78% achieved objective responses (44% of CRs). Apparently, the most relevant features for a favorable prognosis were high baseline tumor mutational burden (TMB) and T-cell inflamed GEP.[32] Collectively, these encouraging findings point towards the enormous potential of combining ICIs and BRAF/MEKi in MM patients, which has prompted the conduction of several prospective trials.

The phase II KEYNOTE-022 trial evaluated whether the combination of dabrafenib 150mg twice daily (BID), trametinib 2mg once daily (OD) and pembrolizumab 2mg/kg every 3 weeks was superior to the placebo-controlled BRAF/MEKi in treatment-naïve *BRAF* V600-mutant MM patients. Despite presenting numerically higher investigator-assessed PFS favoring the triplet arm (median PFS: 16.0 vs 10.3 months), which was the primary endpoint of the study, the result was not statistically significant (HR 0.66, 95% CI 0.40-1.07, p=0.043 – required p value=0.0025); in addition, the triplet also did not demonstrate OS and ORR advantages (63.3% with the triplet arm versus 71.7% in the placebo-controlled arm). It is worth mentioning though, that imbalances regarding adverse prognostic factors between arms may have influenced the performance of the triplet therapy, since the intervention arm contained 18.4% more M1c patients (81.7% vs 63.3%) and nearly 10% more patients with metastases at more than two sites. According to the authors, these imbalances may have contributed to underestimate the true effect of the triplet despite baseline lactate-dehydrogenase (LDH) stratification.[25] The recently presented results from the first interim analysis of the phase III COMBI-i study also did not confirm any improvements in investigator-assessed PFS with dabrafenib 150mg BID, trametinib 2mg OD and spartalizumab 400mg every 4 weeks over placebo-controlled doublet (median PFS: 16.2 vs 12 months, HR 0.820, 95%CI 0.655-1.027, p=0.042 – required p value=0.024). Due to the statistical planning, OS was not tested for significance at that time. ORR was similar (68.5% vs 64.2%, respectively) and study arms were well-balanced for important covariates. A subgroup analysis suggested that patients with TMB ≥ 10 muts/Mb or ≥ 66mm in the sum of lesion diameters at baseline would benefit the most in terms of PFS. These results deserve careful interpretation until data from the final analysis are available.[27] Conversely, a PFS advantage favoring the combination of vemurafenib 720mg BID, cobimetinib 60mg OD (21 days on-7 days off) and atezolizumab 840mg every 2 weeks over placebo-controlled doublet has recently been reported in the phase III IMspire 150 trial (median PFS: 15.1 vs 10.6 months, HR: 0.78, 95%CI 0.63-0.97, p=0.025). Within a median follow-up period of nearly 18 months, 43.6% and 31.6% of the patients were progression-free in the triplet and placebo-controlled doublet arms, respectively, with no specific subgroups apparently deriving a greater magnitude of benefit. Moreover, duration of response (DOR) was numerically higher in the atezolizumab-containing arm (median DOR 21.0 - 95%CI 15.1-not estimated vs 12.6 months - 95%CI 10.5-16.6). There were neither between-group imbalances, nor differences with regards to OS and ORR in this first interim analysis report.[26] Discordant results between these trials might have occurred due to a relatively short follow-up time (assuming that longer periods would be more suitable to appreciate sustained responses following triplet therapy, especially if the effect size is small), between-arm imbalances (in the case of KEYNOTE-022), differences among study protocols regarding run-in periods with BRAF/MEKi prior to triplets (as with IMspire150), less stringent boundaries for statistical significance in preliminary analyses (IMspire150 versus the others), efficacy differences between targeted therapy backbones and immunotherapy agents.

The tolerability of these triplet regimens has also been a matter of concern and was carefully addressed in prospective studies. We identified increased incidence in any grade pyrexia, arthralgia and elevated aminotransferases, along with unselected G3/4 AEs. However, rates of any grade AEs, SAEs and discontinuation of all study drugs were not different. These data suggest that, despite being apparently associated with higher incidence of G3/4 AEs, the discontinuation of all study drugs due to toxicity in the triplet arm remained similar, maybe owing to more frequent dose adjustments and interruptions, as described in each trial. In the KEYNOTE-022, Ascierto et al reported dose reductions of dabrafenib or trametinib in 25% of the patients in the triplet arm versus 13.3% in those receiving doublets, as well as a 15% difference in dose interruptions in any of these drugs. The rates of treatment discontinuation of at least one drug were 41.7% vs. 21.7% favoring the placebo-controlled arm.[25] In line with the previous findings, Nathan et al presented a 10% lower dose intensity of dabrafenib and trametinib in the spartalizumab-containing arm when compared to the placebo arm; the authors reported dose adjustments or interruptions related to AEs in 235/267 patients (88%) in the triplet arm versus 192/264 patients (72.7%) in the doublet arm, which strongly suggests that modifications in the regimen during the study period may have contributed to keep patients on-treatment.[27] Moreover, in both trials, the rates of treatment related G3/4 AEs and AEs leading to all study drug discontinuation were numerically superior in the triplet arm, ranging 7-10% and 21.4-25% absolute differences, respectively.[25,27]

Conversely, in the IMspire150 trial, minimal differences regarding G3/4 AEs and discontinuation of all study drugs were observed; according to the authors, the high incidence of G3/4 AEs irrespective of treatment arms may have occurred due to the intense routine laboratory work-up, since even in the vemurafenib-cobimetinib-placebo arm rates are approximately 10% higher than those observed in the CoBRIM study.[5],[26] Differently from the KEYNOTE-022 and COMBI-i studies, discontinuation rates of all study drugs were similar (13% vs 16%), which could also be associated with the reduced dose of vemurafenib after the first cycle in the atezolizumab arm.[25,26,27]

In the same way as all the three prospective trials analyzed in the present publication, our analysis has potential limitations. Despite the data suggesting OS and PFS advantages favoring the triplet arm, both COMBI-i and KEYNOTE-022 were formally negative studies; whether this fact represents the absence of benefit in these trials' populations, between-arm imbalances (as exemplified by the KEYNOTE-022) or is a consequence of a more conservative approach to detect statistical significance in interim analyses is unknown. Nonetheless, provided that mature data are pending and trials are ongoing, efficacy results reported in the present meta-analysis merit careful interpretation and should be considered preliminary. Also, considering that objective responses and specific immune-related AEs arising in the setting of ICIs might take longer to be documented, more extensive follow-up periods can provide valuable contributions to better understand how these triplets perform. It is worth mentioning that the theoretical complexity of handling triplets in a real-world setting alongside considerable financial toxicity may pose additional challenges to a wider acceptance of these combinations in the future (especially when dealing with small effect sizes, even if statistically significant). In addition, important clarification regarding enhanced efficacy for patients with unfavorable prognostic features and/or high TMB is not addressed in this study, remaining to be elucidated. Another important point is whether BRAF/MEKi can be considered the most appropriate comparator arm; once there is a rationale supporting enhanced immunogenicity with the addition of TT to CPI, one may advocate that a combination of CPIs (e.g. ipilimumab/nivolumab) would be more adequate as a control arm. Of note, all three RCT adopted investigator-assessed PFS rather than independent-review assessed PFS as primary endpoint, with only IMspire150 disclosing concordance rate of 77% between these methods; though one could argue that independent central review may provide more reliable and objective response data, IMspire150 authors described an even higher proportion of progressors as per investigator assessment, well-balanced across study arms, and unlikely to interfere with the efficacy results of the study. Lastly, since a small number of studies were available for quantitative analysis and the phase III IMspire150 had considerable average weight, it is possible that results favoring the triplet regimen might have suffered from its influence (i.e. higher heterogeneity observed in treatment-related AEs prompting all drug discontinuations, SAEs and diarrhea analyses).[25-27]

While notorious efforts to improve the management of *BRAF* V600-mutant MM patients have been conducted, several other challenges related to the use of triplet therapies persist. The still partially understood safety profile along with the intrinsic financial toxicity arising from these combinations may represent limiting factors for their widespread adoption in case superior performance becomes unquestionable. Either difficulties to better identify the most appropriate candidates (since useful biomarkers are only speculative to date) or the scant therapeutic alternatives to patients progressing under BRAF/MEKi plus PD-1/PD-L1 concurrent blockade are also barriers to have in mind when discussing the implementation of this upcoming strategy. Biomarker-driven identification of specific subgroups of patients who might derive the greatest benefit from this combined approach will be highly necessary to appropriately select candidates if triplet regimens become part of our therapeutic arsenal in the upcoming future. Up to now, *BRAF*-mutant MM remains a defying and deadly disease, for which novels approaches aiming to maximize benefits while ensuring patient's quality of life are warmly welcome.

CONCLUSIONS

This systematic review and meta-analysis suggested superior outcomes with BRAF/MEKi plus anti-PD1/PD-L1 antibodies in comparison to BRAF/MEKi, with improvements in OS and PFS. Despite higher incidences of G3/4 AEs, rates of treatment discontinuations and SAEs were similar. These data contribute to a better comprehension of the management of *BRAF* V600-mutant MM patients. While mature data regarding efficacy and safety of the triplet combination are awaited, results from the present study merit careful interpretation.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

The addition of PD-1/PD-L1 axis blockade to BRAF and MEK inhibition for advanced melanoma patients harboring BRAF mutations: a systematic review and meta-analysis.

EMBASE - 29/September/2020	
No	Query
1	exp melanoma/
2	vemurafenib.ab,ti,kw.
3	dabrafenib.ab,ti,kw.
4	trametinib.ab,ti,kw.
5	cobimetinib.ab,ti,kw.
6	selumetinib.ab,ti,kw.
7	MEK162.ab,ti,kw
8	binimetinib.ab,ti,kw.
9	7 or 8
10	LGX818.ab,ti,kw.
11	encorafenib.ab,ti,kw.
12	10 or 11
13	or/2-6
14	ipilimumab.ab,ti,kw.
15	pembrolizumab.ab,ti,kw.
16	nivolumab.ab,ti,kw.
17	atezolizumab.ab,ti,kw
19	spartalizumab.ab,ti,kw
20	or/14-18
21	9 or 12 or 13 or 20
22	1 and 21
23	("clinical trial" or "clinical trial, phase i" or "clinical trial, phase ii" or clinical trial, phase iii or clinical trial, phase iv or controlled clinical trial or "randomized controlled trial").pt. or double-blind method/ or clinical trials as topic/ or clinical trials, phase i as topic/ or clinical trials, phase ii as topic/ or clinical trials, phase iii as topic/ or clinical trials, phase iv as topic/ or controlled clinical trials as topic/ or randomized controlled trials as topic/ or early termination of clinical trials as topic/ or ((randomi?ed adj7 trial*) or (controlled adj3 trial*) or (clinical adj2 trial*) or ((single or doubl* or tripl* or treb*) and (blind* or mask*))).ti,ab,kw.
24	22 and 23
25	limit 24 to yr="2016-current"

Figure S1 - Structured search used to perform the systematic review.

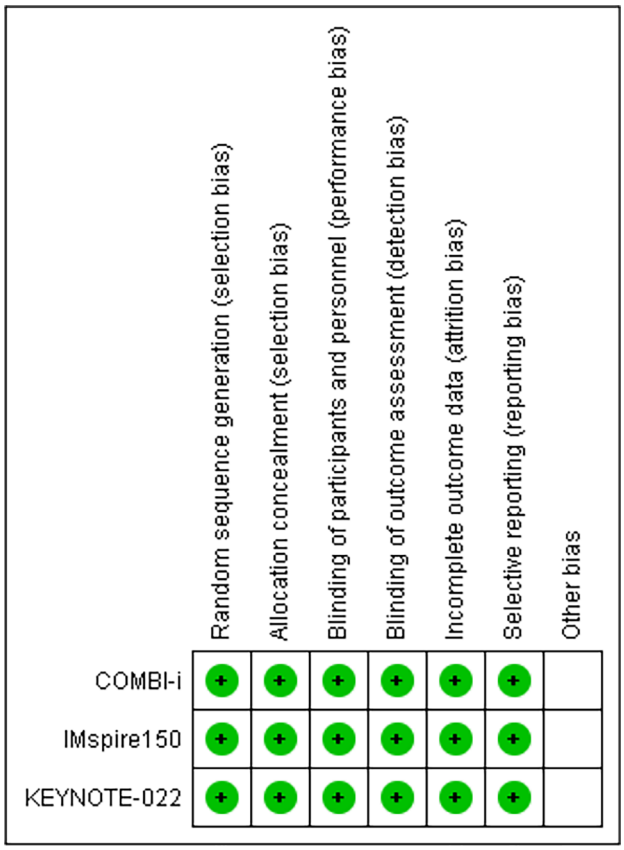

Figure S2 - Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

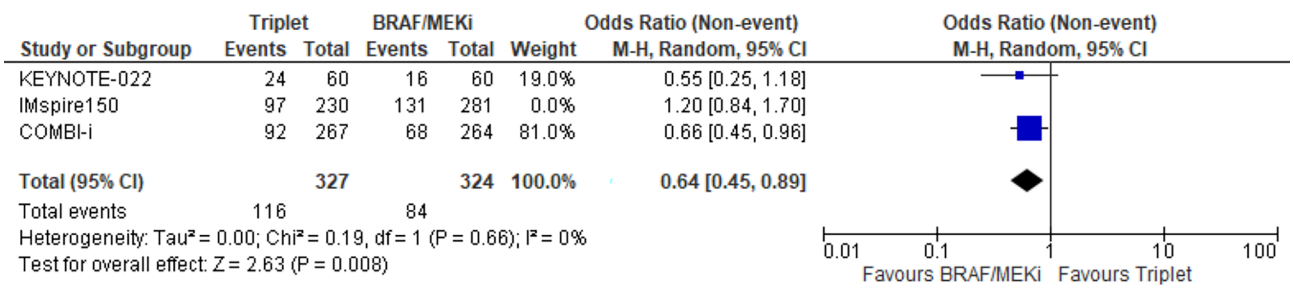

Figure S3 - Forest plot showing a sensitivity analysis performed to assess the source of high heterogeneity for "any grade diarrhea". The exclusion of I Mspire150 considerably reduced I^2.

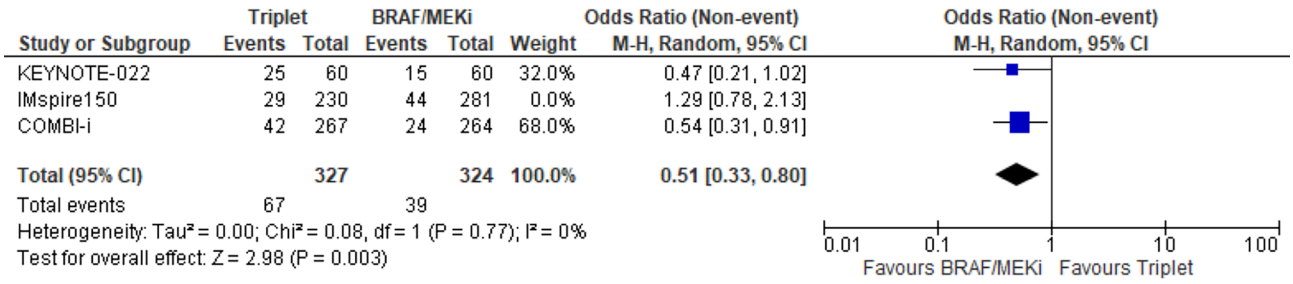

Figure S4 - Forest plot showing a sensitivity analysis performed to assess the source of high heterogeneity for AEs prompting all-drug discontinuations. The exclusion of IMspire150 data considerably reduced I2.

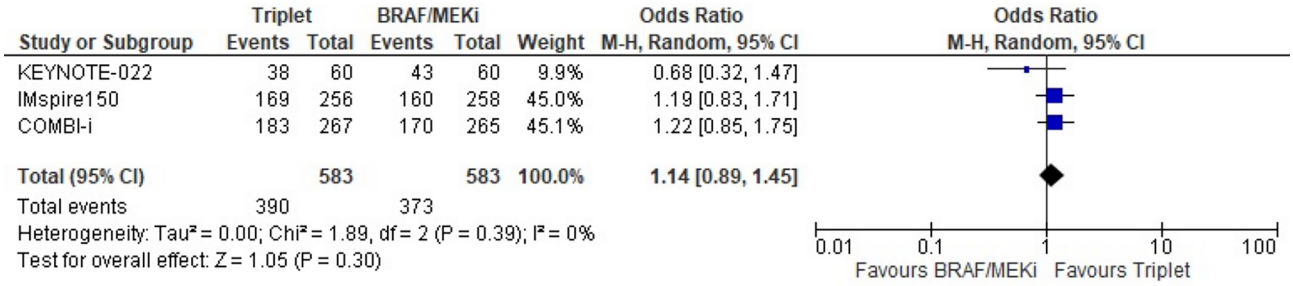

Figure S5 - Objective Response Rate. Forest plot depicting similar ORR between arms.

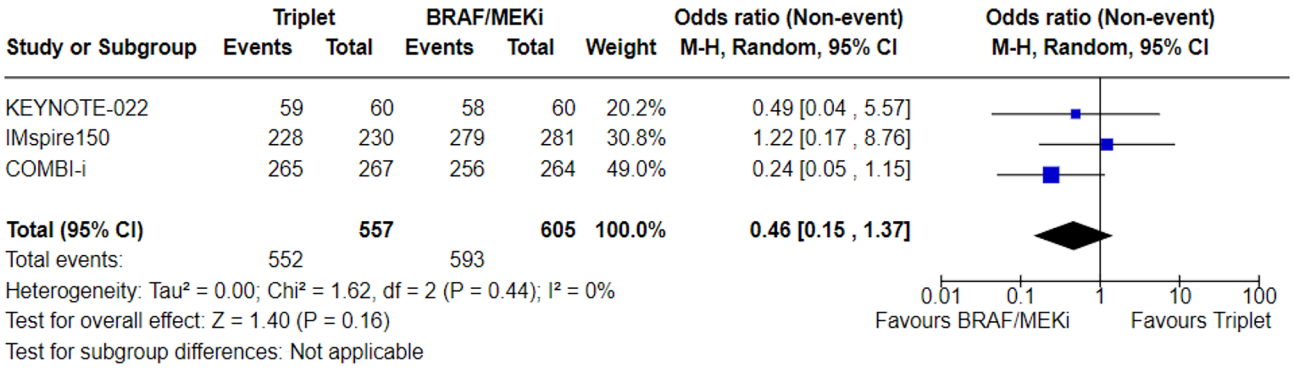

Figure S6 - Forest plot depicting all grade AEs

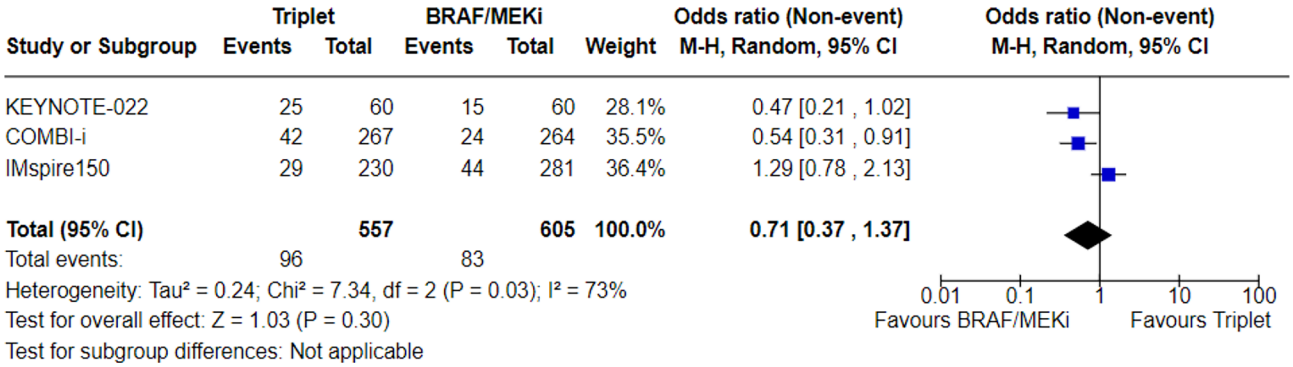

Figure S7 - Incidence of treatment-related AEs prompting all-drug discontinuations. Forest plot displaying the lack of a statistically significant difference.

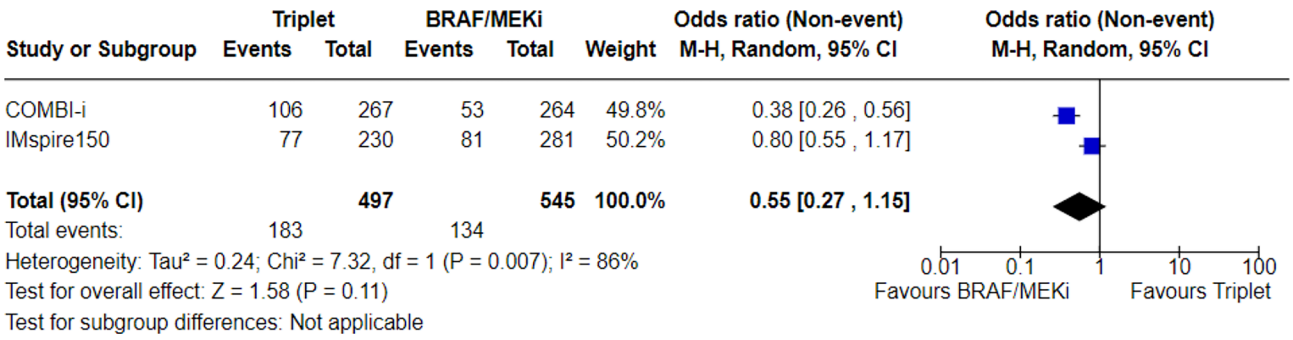

Figure S8 - Forest plot showing treatment-related serious AEs.

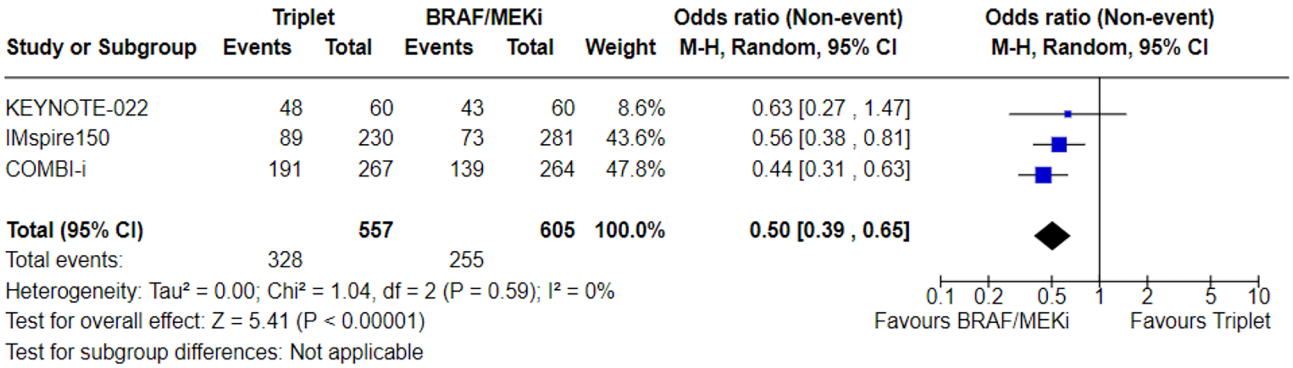

Figure S9 - Forest plot showing any grade pyrexia.

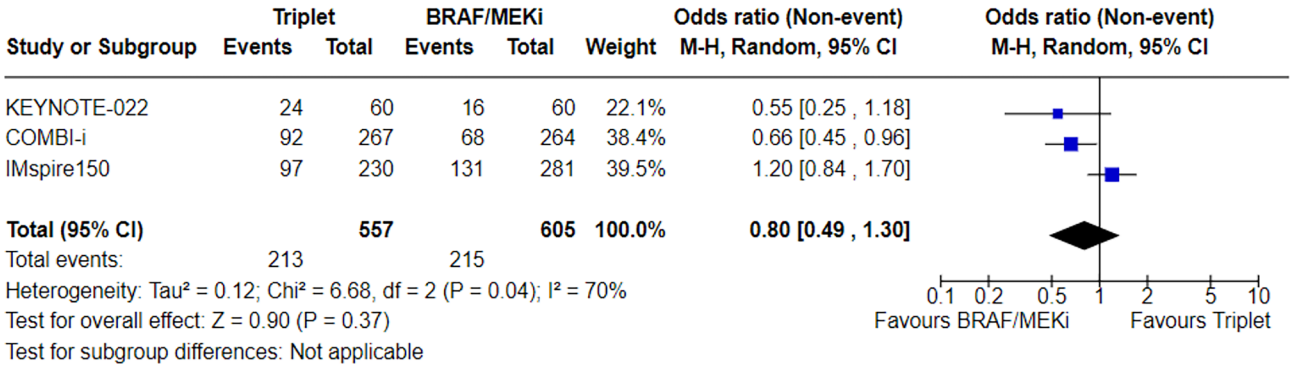

Figure S10 - Forest plot showing any grade diarrhea.

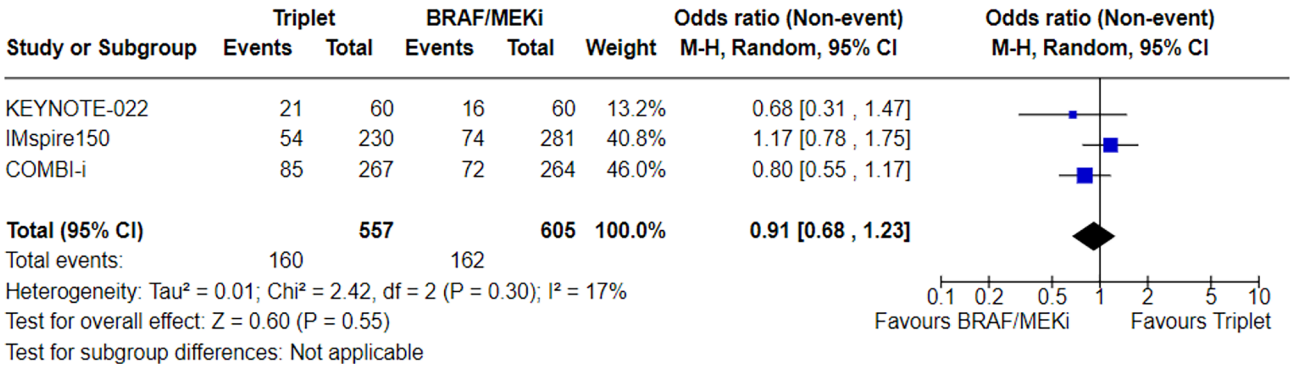

Figure S11 - Forest plot showing any grade nausea.

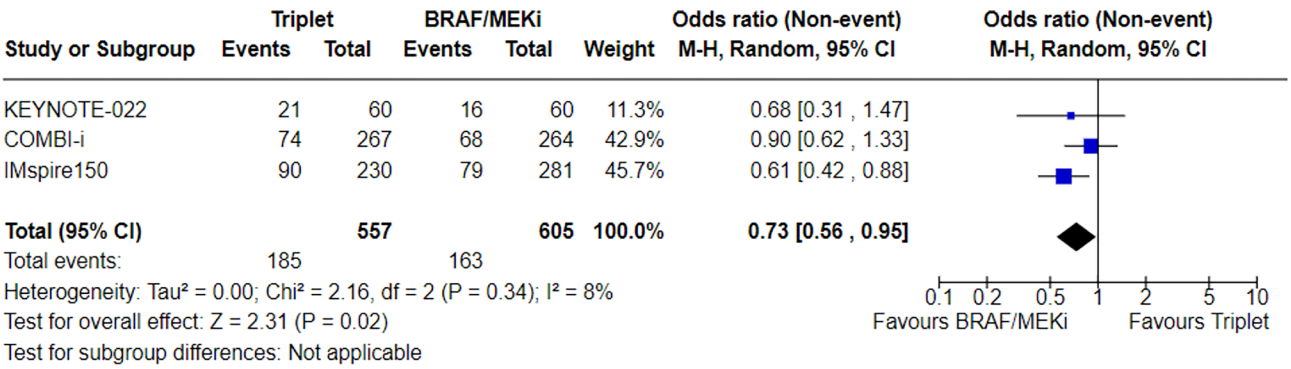

Figure S12 - Forest plot showing any grade arthralgia.

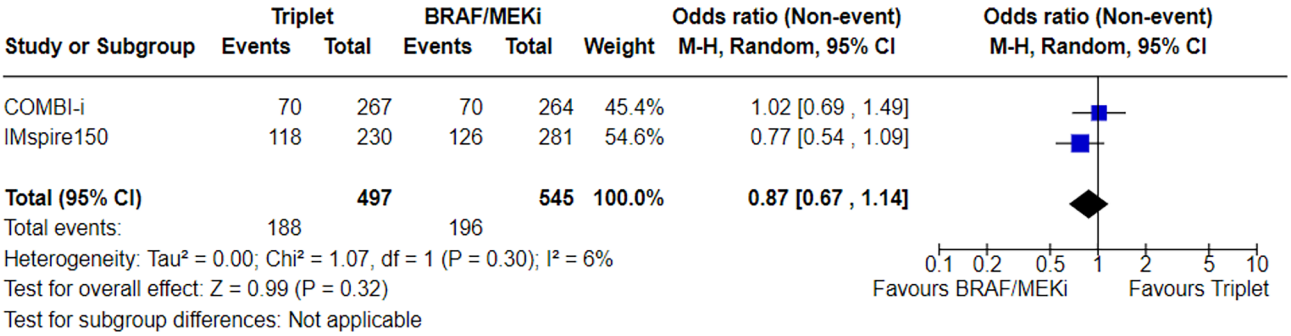

Figure S13 - Forest plot showing any grade creatinine phosphokinase increase.

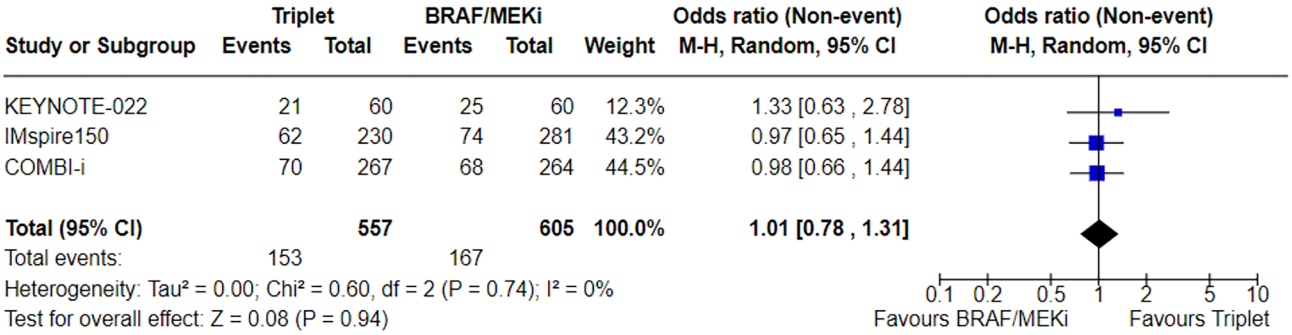

Figure S14 - Forest plot showing any grade fatigue.

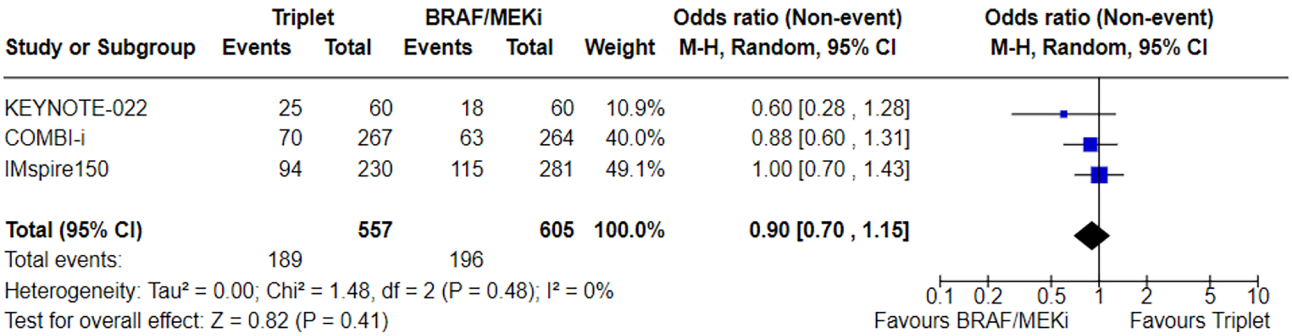

Figure S15 - Forest plot showing any grade rash.

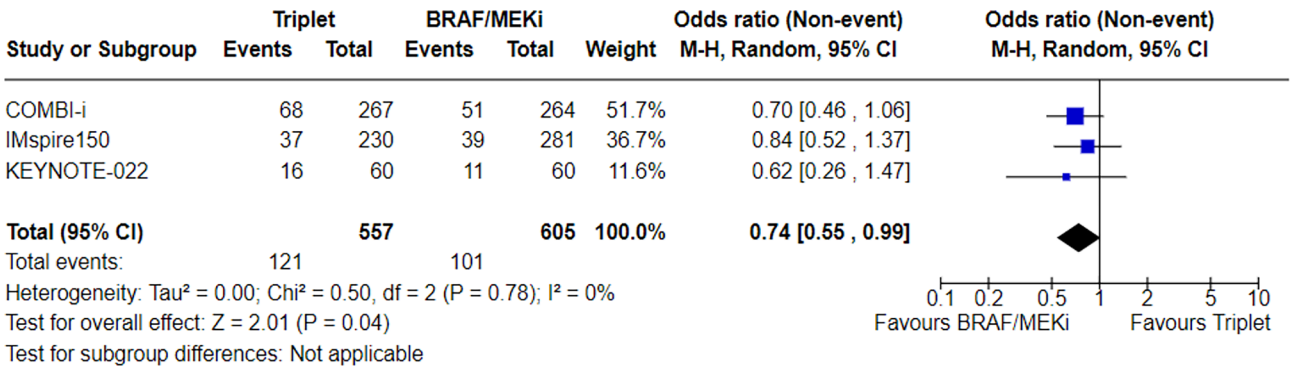

Figure S16 - Forest plot showing any grade asthenia.

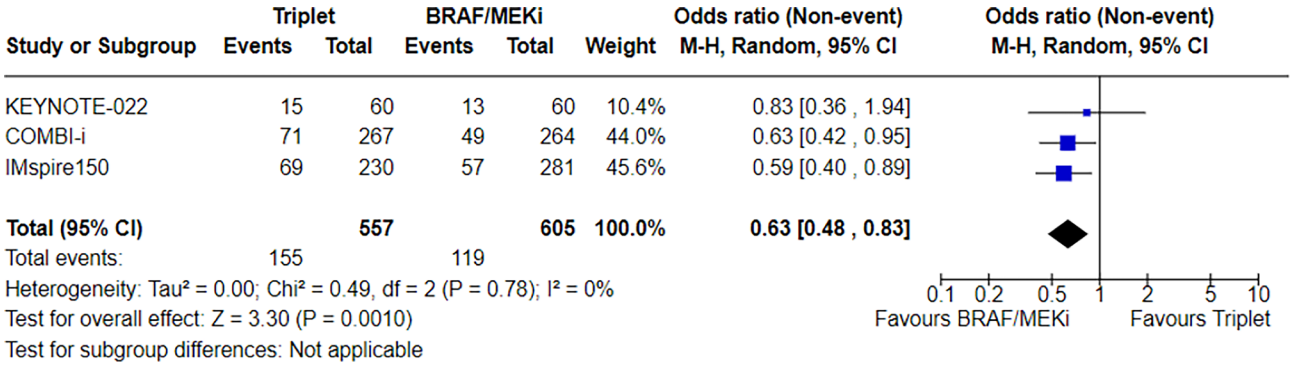

Figure S17 - Forest plot showing any grade AST elevation.

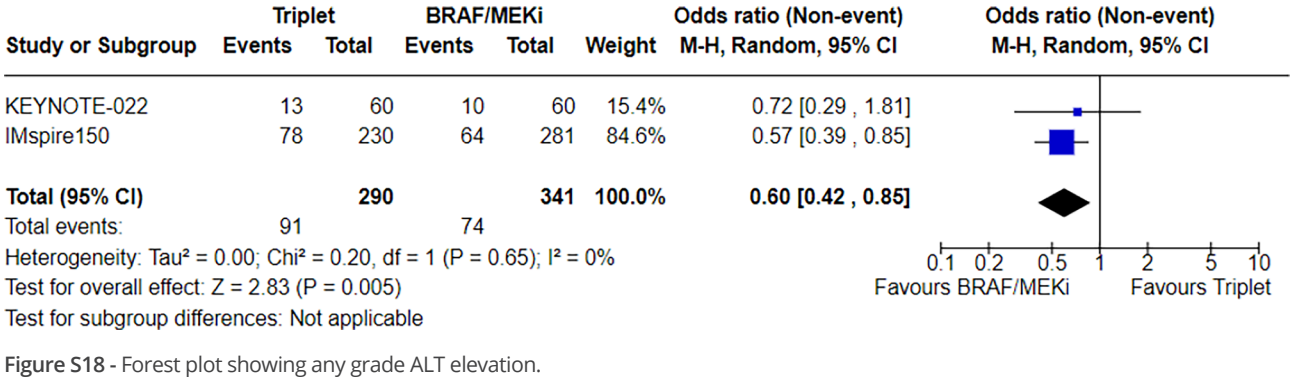

Figure S18 - Forest plot showing any grade ALT elevation.