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Immunogenicity and safety of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults: a systematic review and meta-analysis

Settore scientifico disciplinare: MEDS-24/B

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INTRODUCTION

Within the current healthcare and regulatory framework, there is an increasing demand for high-quality evidence to support drug development, health technology assessments, access decisions, product differentiation, and clinical decision-making. This requirement is particularly relevant for influenza, a highly variable infectious disease that necessitates continuous collection and evaluation of data on safety, efficacy, and effectiveness. The efficacy of influenza vaccines is primarily established through randomized clinical trials (RCTs), while their effectiveness is assessed using studies conducted under real-world conditions. Both sources of evidence are complementary and essential to fully understand vaccine performance. RCTs remain the cornerstone of early clinical development, providing rigorous data on dosing, safety, and efficacy under controlled conditions. Their design minimizes bias and ensures internal validity. However, the strict inclusion criteria and controlled settings of RCTs often limit their generalizability. In contrast, real-world data (RWD)—routinely collected after-market authorization—allow for the assessment of how vaccines perform in everyday clinical practice, across diverse populations and healthcare systems. Such data are critical for the continuous monitoring of vaccine effectiveness and for post-marketing safety surveillance. One of the major limitations of RCTs relates to the study population. Groups at higher risk of influenza complications—such as young children, individuals with chronic diseases, patients receiving multiple concomitant treatments, and pregnant or lactating women—are often excluded from traditional trials. As a result, evidence derived exclusively from RCTs may not accurately reflect vaccine performance in these

populations. Therefore, the integration of real-world evidence (RWE) has become essential to generate a more comprehensive understanding of a product's benefit–risk profile across the entire target population. RWE complements data from RCTs and supports regulatory, clinical, and public health decision-making. In this context, the PhD program ReWorldEvi – "Real-world evidence role in the development of drugs" focuses on the integration of RWE in drug development and evaluation. As part of this program, a systematic review and meta-analysis were conducted to assess the available evidence on the MF59-adjuvanted seasonal influenza vaccine in the non-elderly population. Historically, this vaccine has been primarily used in older adults, where it demonstrated superior effectiveness compared with standard non-adjuvanted influenza vaccines. However, in 2023, the age indication for the MF59-adjuvanted vaccine was expanded to include individuals aged 50 years and older, offering new opportunities for immunization and public health protection. Notably, when the vaccine was first approved in Italy in 1997, it was indicated for individuals aged 12 years and above. The revised indication provides a timely opportunity to re-examine and synthesize nearly three decades of evidence on the vaccine's safety and effectiveness in younger age groups. The findings from this systematic review and meta-analysis aim to provide a rigorous synthesis of the existing evidence base, offering valuable insights into the role of the MF59-adjuvanted seasonal influenza vaccine in the non-elderly population. Ultimately, this research contributes to the broader understanding of how real-world evidence can inform vaccine policy, optimize immunization strategies, and support regulatory decisions in the evolving field of influenza prevention

1. Background

Trivalent (aTIV) and quadrivalent (aQIV) MF59-adjuvanted seasonal influenza vaccines have been developed to enhance the immune response to vaccination and potentially

improve vaccine effectiveness. Until recently, aTIV and aQIV were licensed for adults aged ≥65 years. In the last 20 years, multiple studies have demonstrated the advantages of aTIV/aQIV over non-adjuvanted standard-dose formulations [1,2]. For instance, two systematic reviews of studies in older adults have shown that the use of aTIV induces higher immune response towards homologous vaccine-like [3,4], and heterologous influenza virus strains [4], and generally improved effectiveness against several influenza-related outcomes [5,6]. These advantages are attributable to the MF59 adjuvant, which exercises its immunostimulatory effect in several ways. Briefly, MF59 allows the recruitment of key immune cells to the injection site to make antigen uptake and transport to the lymph nodes more efficient. It then promotes T-cell activation and improves B-cell expansion, which leads to a greater number and diversity of antibodies [7,8].

Historically, most of the research on the burden of influenza focused on older adults, since they are the primary target group for annual vaccination [9]. Indeed, most severe complications, hospitalizations, and deaths related to influenza occur in the elderly population [10,11]. However, influenza attack rates are typically inversely related to age. The cumulative incidence of influenza in non-elderly adults is estimated to be about twice that of older adults (8.9% vs. 3.9%) in the United States (US) [12]. Younger adults comprise most of the workforce, and thus play a crucial role in socioeconomic welfare [13]. Up to 88% of the economic burden of influenza in non-elderly adults is attributable to indirect costs, mostly related to absenteeism and loss of productivity [9]. Nonetheless, the efficacy of the available influenza vaccines in non-elderly adults is suboptimal: a Cochrane meta-analysis estimated the efficacy of inactivated vaccines in healthy adults to be 59% [14].

In November 2023, the European Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion on the extension of the age indication for aQIV from ≥65 to ≥50 years [15]. Many may be unaware that the age indication for aTIV was ≥12 years when it was first approved in Italy in 1997. It was particularly indicated for older adults who are

subject to immunosenescence, and individuals with immunosuppressive conditions [16]. In the subsequent few years, this age indication was changed to ≥65 years.

In Italy, aQIV and high-dose non-adjuvanted vaccines are preferentially recommended to older adults aged ≥65 years [17]. This is in line with the recommendations in other countries, such as the United Kingdom [18], US [19], and Australia [20]. As of February 2024, no specific recommendations on the use of aQIV in adults aged 50–64 years have been made in Italy or other European countries. Given the historical availability of aTIV in Italy for people aged ≥12 years [16], we anticipated that studies of aTIV in younger adults had been conducted. The objective of this review was to systematically collect and evaluate available experimental and observational data on the immunogenicity, efficacy, effectiveness and safety of aTIV/aQIV in non-elderly adults.

2. Methods

2.1. Review protocol and reporting standards

A protocol for this review was prospectively registered with the international, prospective register of systematic reviews (PROSPERO; ID: CRD42024512472) and no amendments were made afterwards. This review conforms to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [21] **(Table S1)**

2.2. Eligibility criteria

The eligibility criteria were formulated using the PICO (population, intervention, comparison, outcome) framework [22]. The population of interest was working-age adults aged <65 years regardless of the presence of underlying health conditions and other characteristics. The intervention consisted of a single dose of the authorized formulations aTIV or aQIV. Formulations of the historically available aTIV and currently available aQIV are almost

identical except for the inclusion of an additional B strain in aQIV. According to the aQIV summary of product characteristics [23], data on aTIV are also relevant for aQIV because both vaccines are manufactured using the same process, and have overlapping compositions. Furthermore, following the likely extinction of the B/Yamagata lineage during the Coronavirus disease 2019 (COVID-19) pandemic, the World Health Organization (WHO) has recently recommended the removal of the B/Yamagata component, and thus a return to trivalent vaccine formulations [24]. For these reasons, we treated aTIV and aQIV interchangeably. For the comparison, both non-active (placebo, non-influenza vaccines or non-vaccination) and active (any type of non-adjuvanted trivalent [TIV] or quadrivalent [QIV] influenza vaccines) comparators were considered. The study outcomes included several endpoints related to the domains of immunogenicity, efficacy, reactogenicity, and safety (detailed in section 2.3). We planned to assess effectiveness of aTIV/aQIV, but no studies were identified. For all outcomes, experimental and observational studies of any design were eligible.

The following were set as exclusion criteria: (i) reviews, modeling studies, and other secondary publications without original data; (ii) non-authorized experimental formulations of aTIV/aQIV (e.g. different amount of the antigen or MF59); (iii) pandemic monovalent formulations of the MF59-adjuvanted vaccines; (iv) vaccines adjuvanted with non-MF59 adjuvants (e.g. AS03, virosomes); (v) mixed study population of elderly and non-elderly adults with no separate data on the latter. With regards to the last criterion, we included studies if the majority of participants were <65 years.

2.3. Study outcomes

Our primary endpoints were humoral immunogenicity outcomes including different statistical parameters related to antibody titers measured in the hemagglutination-inhibition (HAI) assay. The HAI titer ≥1:40 is a universally recognized correlate of protection [25]. For the

absolute humoral immune response of aTIV/aQIV (i.e. with no respect to comparators), seroconversion rates (SCRs) and seroprotection rates (SPRs) were primary endpoints. SCR was defined as the proportion of vaccinees with at least four-fold increase in HAI titers from before to after vaccination, while SPR was defined as the proportion of vaccinees reaching the HAI titer ≥1:40 post-vaccination [25,26]. According to the Center for Biologics Evaluation and Research (CBER) criteria for the accelerated approval of inactivated influenza vaccines, the lower bounds of the two-sided 95% confidence intervals (CIs) of SCRs and SPRs in adults aged <65 years should be at least 40% and 70%, respectively [27]. Additionally, as some studies suggested that the HAI titer ≥1:40 may be insufficient [26,28] and some early studies on aTIV in older adults used a more conservative HAI threshold of ≥1:160 [29], this latter definition of SPR was also considered and tested in a sensitivity analysis.

For the relative humoral immune response (i.e. compared with non-adjuvanted vaccines), our primary endpoints were differences in SCRs (defined as $SCR_{aTIV/aQIV} - SCR_{Comparator}$), SPRs ($SPR_{aTIV/aQIV} - SPR_{Comparator}$), and post-vaccination geometric mean titer (GMT) ratios (GMTRs; defined as $GMT_{aTIV/aQIV} : GMT_{Comparator}$).

All serological parameters were analyzed by influenza vaccine-like strains, including A(H1N1), A(H3N2), and B for trivalent formulations, and A(H1N1), A(H3N2), and B/Victoria and B/Yamagata for quadrivalent formulations. Immune response towards heterologous or drifted strains was also considered. Serological parameters measured at approximately 1 month post-vaccination were of primary interest. Secondary endpoints were immunogenicity assessments performed at later time periods (up to 12 months post-vaccination), which may indicate the duration of vaccine-induced protection [30].

Additional endpoints included humoral immunogenicity measured in other serological tests (e.g. neutralization, single radial hemolysis assays, enzyme-linked immunosorbent assay [ELISA]) and cell-mediated immune response measured with any technique [25].

Efficacy was defined as reduction in the risk of influenza in individuals immunized with aTIV/aQIV, compared with placebo/non-influenza vaccines (absolute efficacy) and any available non-adjuvanted vaccine (relative efficacy), as estimated from randomized controlled trials (RCTs) [31].

For evaluation of reactogenicity and safety, we considered the frequency of both solicited (i.e. actively collected, pre-specified list of events, usually collected through diaries within the first week post-vaccination) and unsolicited (any events other than solicited events that are typically collected for the entire study duration) adverse events (AEs) recorded through RCTs. For injection site-solicited reactions, any local event, pain, erythema, induration, and ecchymosis were pre-specified. For systemic events, any systemic event, fever (≥38°C), chills, myalgia, arthralgia, headache, malaise, nausea, and rash were of interest. Within unsolicited events, frequency of serious AEs (SAEs) and SAEs judged to be vaccine-related were considered. SAEs were defined as events that resulted in death, caused persistent or significant disability or incapacity, were life-threatening or required hospitalization [32].

2.4. Search strategy

The search was first conducted in MEDLINE (via Ovid), Biological Abstracts (via Ovid), Web of Science and Cochrane Library. In all searches, no restrictions (e.g. language or year of publication) were applied. Additionally, the ClinicalTrials.gov prospective registry was searched for completed studies. The last automatic search was performed on 16 February 2024. We used a combination of medical subject headings (MeSH) and text-wide terms. The database-specific search scripts are reported in **Table S2**.

A manual search was then performed. First, a standard backward cross-reference check of the included studies was conducted. We then performed a forward citation search by using Google Scholar (https://scholar.google.com/). Finally, vaccine manufacturers were asked to

suggest other relevant studies; in particular those that are not yet published and/or have only been presented at conferences.

2.5. Study selection

Search outputs from each citation database were pooled in a single spreadsheet and duplicates were removed. Titles and abstracts were then screened against the inclusion and exclusion criteria, and clearly irrelevant records were excluded. Full texts of the remaining records were located and assessed for their eligibility. The list of studies identified through the automatic search was eventually finalized by adding those located through the manual search. Study selection was performed by two authors, AD and CST, each working independently. Eventual conflicts were solved by consensus.

2.6. Data extraction and abstraction

Relevant data were extracted from the full text and/or associated supplementary materials into an *ad hoc* spreadsheet. The following data items were extracted: (i) citation record; (ii) study location; (iii) influenza season; (iv) study design; (v) study population and main population characteristics (age and co-morbidities); (vi) sample size per study arm; (vii) outcome domains evaluated in the study; (viii) vaccine and test strains used; (ix) point estimates for the outcomes of interest with any available dispersion measures; (x) funding source; (xii) other potentially relevant information.

Data of interest that were not readily extractable from the primary publication record were handled as follows. The main publication source was firstly cross-checked against other sources (e.g. results posted on the clinical trial registry). For dichotomous outcomes (SCR, SPR, frequency of AEs) missing numerators or denominators were imputed arithmetically from the available percentages. Results presented only in graphical-form data were imputed using the WebPlotDigitizer v.4.6 online web application

(https://automeris.io/WebPlotDigitizer). Data extraction was performed by AD and cross-checked by CST.

2.7. Risk of bias

The risk of bias (RoB) in randomized studies was appraised by using the revised Cochrane RoB 2 tool [33]. For non-randomized trails, the RoB in non-randomized studies of interventions (ROBINS-I) tool [34] was used. The RoB tools were applied separately by AD and CST and disagreements were solved by consensus.

2.8. Data synthesis

A qualitative synthesis was provided first by visualizing tabulated data and forest plots. For the quantitative synthesis of single proportions related to the absolute immunogenicity and safety parameters of aTIV/aQIV, a proportional meta-analysis with double arcsine transformation to stabilize variances [35] was performed. For binary outcomes of the relative immunogenicity and safety parameters of aTIV/aQIV vs. non-adjuvanted vaccines, a binary meta-analysis using the Mantel-Haenszel's method was performed. The summary effect measures with 95% Clopper-Pearson exact CIs were risk difference for SCRs (ΔSCR) and SPRs (ΔSPR) [27] and risk ratio (RR) for AEs [36]. We also planned *a priori* to conduct a pooled analysis on log-transformed GMTs for aTIV/aQIV vs. TIV/QIV, as well as absolute and relative aTIV/aQIV efficacy/effectiveness.

In all pooled analyses, both fixed-effects (FE) and random-effects (RE) models were applied. RE models are an appropriate choice, as it captures heterogeneity between studies [37], which is expected to be high especially for the proportional meta-analysis [38]. However, when the number of included studies (k) is small, the estimated heterogeneity may be biased [39] and a FE model may be considered [37]. Heterogeneity was measured by means of τ^2 and I^2 statistics. In all analyses, the 95% prediction intervals (PIs) were also computed.

A subgroup analysis by the presence of immunosuppressive conditions was conducted. A univariable meta-regression analysis was conducted to explore the influence of study characteristics on the outcomes of interest. This analysis was performed only for analyses with k ≥10 [40]. Publication bias was assessed by visualizing funnel plots. This was evaluated only for meta-analyses with k ≥10 [40].

We performed three sensitivity analyses. The first concerned the operational definitions of SPR, which were described in section 2.3. In the second, we excluded studies with overlapping study populations (i.e. studies that also enrolled elderly participants). Thirdly, the trim-and-fill procedure [41] was implemented to estimate the number of potentially unavailable studies and to adjust pooled estimates for publication bias.

Quantitative synthesis was performed in R environment (R Foundation for Statistical Computing; Vienna, Austria) using the packages “meta” v. 7.0-0 and “metaphor” v. 4.4-0.

3. Results

3.1. Characteristics of the studies included

The automatic literature search resulted in 2,880 records. Following de-duplication, 2,064 titles and abstracts were screened, and 34 records were judged potentially eligible. Of 34 full texts evaluated, 22 [42–63] met the inclusion criteria and were retained. Twelve publications were excluded with reasons **(Table S3)**. Two additional citations [64,65] were found by manual search. The twenty-four records that were included corresponded to 18 vaccination cohorts. RCTs by Baldo et al. [50] and Kumar et al. [54] had extensions [51,55], in which sera were reanalyzed for the purpose of cross-reactive immune response. Similarly, a research group reported results of HAI testing towards different homologous and heterologous strains in two publications [47,64]. Fenoglio et al. [49] reported additional results on a subset of participants from an RCT report by Durando et al. [48], and the results

of the exploratory BIOVAXSAFE trial were published in three different records [56–58]. A flowchart of the study selection process is depicted in **Figure 1**.

The main characteristics of the included studies are summarized in **Table 1**. Briefly, the available studies were conducted between 1995/1996 and 2020/2021 northern hemisphere influenza seasons, and 50% (9 of 18) came from Italy [43,44,46–48,50,52,53,65]. Sample size was in the range of 17–2,044 participants (median 98); cumulatively the trials enrolled 4,628 participants, of which 50.2% received one dose of aTIV/aQIV. Most (72%; 13/18) studies [42,43,45,46,48,50,54,56,59,60,62,63,65] were randomized and controlled in design. A trial by Iorio et al. [44] was controlled, but the allocation was systematic, while the remaining four studies [47,52,53,61] reported results of single-arm trials. The study population of eight studies [42,47,50,52,56,61,63,65] was composed of immunocompetent adults aged <65 years with or without co-morbidities. Conversely, the remaining 10 studies evaluated the immunogenicity, efficacy, and/or safety of aTIV in immunocompromised cohorts, including HIV-seropositive individuals [44,46,48,53], solid organ [43,45,54,62] and hematopoietic stem [60] cell transplant recipients, and those with end-stage renal disease [59]. Although the majority of study populations were composed of working-age adults, four of the studies on transplant recipients [43,54,60,62] also included elderly individuals. The trivalent formulation was used in all studies [42–62,64,65] except one [63], which used aQIV. Finally, nine trials were publicly funded [46,47,50,52,53,54,59,60,62], and eight studies were sponsored by the vaccine manufacturer [42,44,45,48,56,61,63,65], while the funding source was not disclosed in one study [43].

3.2. Risk of bias

Among randomized studies **(Table S4)**, seven [50,54,56,59,62,63,65] were judged as low RoB, while the remaining six were rated as high RoB [43,45,46] or some concerns for bias due to randomization [42,48,60].

Among five non-randomized studies **(Table S5),** only one trial by Kazmin et al. [61] was judged as low RoB in all dimensions. In the other four studies [44,47,52,53], there was a moderate-to-high RoB due to confounding and participant selection issues.

3.3. Immunogenicity towards vaccine-like strains

Findings on the HAI response against homologous vaccine-like strains approximately 3–4 weeks after vaccination were reported in 17 publications [42,44–48,50,52,54,56,59–65]. As shown by the extracted raw data **(Table S6)**, strain-specific SCRs and SPRs following one dose of aTIV/aQIV exceeded 40% and 70%, respectively, in the large majority of trials. For relative immunogenicity parameters, aTIV/aQIV was generally more immunogenic than non-adjuvanted counterparts. However, statistically significant differences were observed only in some, generally larger studies. In these latter trials, the advantage of aTIV/aQIV over TIV/QIV was more pronounced for type A strains. For GMTRs (aTIV/aQIV vs. TIV/QIV), use of aTIV/aQIV was associated with higher GMTs (GMTR >1.00) in 39 out of 43 (91%) estimates extracted, where the relative advantage of aTIV/aQIV varied from 6% to 250%. However, only 20 estimates were statistically significant (p<0.05) **(Table S6)**.

Depending on the vaccine strain and immunological parameter, pooled analysis could be performed on 14–17 vaccine cohorts. RE and FE pooled estimates were generally similar in terms of the effect size, but RE estimates were less precise. As expected, prevalence estimates of the absolute SCRs and SPRs showed high heterogeneity (≥82%) **(Table 2, Figures S1–S6).** Pooled RE estimates for SCRs were 56.5%, 62.8% and 53.5% towards A(H1N1), A(H3N2) and B vaccine-like strains, respectively, and all lower bounds of the related 95% CIs exceeded 40%. The corresponding pooled RE estimates for SPRs (HAI titer ≥1:40) were 89.7%, 90.7% and 80.8%, and lower bounds of their 95% CIs were ≥70%. As one RCT [42] defined SPR using a more conservative HAI titer threshold of ≥1:160, a sensitivity analysis was conducted by also including this study. No significant changes

occurred and the RE estimates were 90.7%, 90.9%, and 82.7% towards A(H1N1), A(H3N2) and B vaccine-like strains, respectively **(Table S7)**.

Meta-analytical estimates for the relative immunogenicity parameters were generally associated with less heterogeneity **(Table 2, Figures S7–S12)**. For the A(H1N1) strain, 8.8% (95% CI: 3.7%, 14.0%; I^2=49.1%) more aTIV/aQIV than TIV/QIV recipients seroconverted, while the difference in SPRs was 3.8% (95% CI: 1.0%, 6.6%; I^2=44.3%). The advantage of aTIV/aQIV was more pronounced for the A(H3N2) subtype, where RE ΔSCR and ΔSPR were 13.1% (95% CI: 6.7%, 19.6%; I^2=69.9%) and 8.6% (95% CI: 4.0%, 13.2%; I^2=74.3%), respectively. For the B vaccine-like strains, aTIV/aQIV determined higher SCRs (11.7%; 95% CI: 7.2%, 16.2%; I^2=36.3%) and SPRs (5.1%; 95% CI: 1.6%, 8.6%; I^2=44.3%) than TIV/QIV **(Table 2)**.

In a pre-specified subgroup analysis, immunosuppression status was a significant determinant of most pooled estimates. As shown in **Table 3**, compared with trials on general adult populations, individuals with immunosuppressive conditions showed significantly lower SCRs and SPRs. For instance, the RE estimates for SCRs towards A(H1N1) among individuals with and without immunosuppression were 47.1% and 65.0%, respectively. However, when considering relative immunogenicity parameters, the advantage of aTIV/aQIV was more pronounced in immunocompromised populations. For example, ΔSCR aTIV/aQIV vs. TIV/QIV towards A(H1N1) were 13.1% and 5.4% in adults with and without immunosuppression, respectively **(Table 3)**.

Visual inspection of funnel plots **(Figures S13–S18)** suggested some evidence of publication bias, especially for ΔSCR and ΔSPR towards A(H3N2). In a sensitivity analysis with the trim-and-fill method, the number of hypothetically missing studies ranged from zero to six. With these imputed studies, RE estimates for the difference in SCRs were 8.8% (95% CI: 3.7%, 14.0%), 3.3% (95% CI: -4.9%, 11.5%), and 7.4% (95% CI: 2.3%, 12.5%) towards A(H1N1), A(H3N2), and B, respectively. The corresponding ΔSPR parameters were 1.4%

(95% CI: -2.8%, 5.6%), 1.7% (95% CI: -4.8%, 8.2%), and 2.1% (95% CI: -2.5%, 6.8%), respectively. When three studies [54,60,62] with partially overlapping populations were excluded in another sensitivity analysis **(Table S8)**, no major changes occurred.

In an exploratory meta-regression analysis to investigate study-level determinants of ΔSCRs and ΔSPRs for aTIV/aQIV vs. TIV/QIV **(Table S9),** only one statistically significant association emerged. Particularly, studies on immunocompromised populations showed, on average, greater (p = 0.030) ΔSCRs towards A(H3N2). No significant associations for other variables, including below the median sample size, industry sponsorship, RoB, and enrollment of overlapping population groups, were found.

As several studies did not report dispersion parameters for the GMT point estimates, their imputation was judged unfeasible (as it could favor positive results). Meta-analysis of GMTs for aTIV/aQIV vs. TIV/QIV was therefore abandoned.

3.4. Immunogenicity towards heterologous strains

HAI immune response towards heterologous strains was assessed in six publications [47,51,52,55,64,65] **(Table S10)**. Three of these studies [47,52,64] were small single-arm trials. Camilloni et al. [47] analyzed post-vaccination SPRs in adults vaccinated with the 2002/2003 aTIV formulation that contained a B strain belonging to the Victoria lineage (B/Hong Kong/330/2001). Post-vaccination sera were tested against three different heterologous B strains, one of which belonged to the same Victoria lineage (B/Malaysia/2506/2004) and the other two belonged to the Yamagata lineage (B/Sichuan/379/1999 and B/Shanghai/361/2002). One month after vaccination with aTIV, a high level of response was observed for the same-lineage strain and one cross-lineage strain B/Sichuan/379/1999 with 92.3% and 88.5% of individuals achieving HAI titers ≥1:40, respectively. Conversely, the derived SPR towards another cross-lineage strain B/Shanghai/361/2002 was relatively low (38.5%). Iorio et al [64] analyzed humoral

immunogenicity in adults vaccinated with aTIV formulations containing seasonal (pre-2009) A(H1N1) strains (formulations 2003/2004 [64] and 2007/2008 [52]) towards an A(H1N1)pdm09-like strain. SCRs and SPRs were 12.5–19.2% and 12.5–30.7% **(Table S10)**. Three RCTs [51,55,65] compared heterologous immune response of aTIV vs. TIV. The former was usually associated with higher immune response **(Table S10)**. In the pooled analysis of these studies **(Table S11)**, aTIV was associated with significantly higher cross-clade SCRs (FE: 10.7% [95% CI: 3.2%, 18.2%); RE: 10.6% [95% CI: 3.2%, 18.0%); I^2=0%] and SPRs (FE: 10.5% [95% CI: 4.9%, 16.1%); RE: 10.2% [95% CI: 0.5%, 19.9%); I^2=55.6%] towards A(H3N2) strains. For the A(H1N1) heterologous strains, aTIV was associated with higher SPRs (FE: 10.0% [95% CI: 0.7%, 19.3%); RE: 9.0% [95% CI: 0.1%, 17.9%); I^2=0%], but not SCRs (FE: 8.3% [95% CI: -1.2%, 17.8%); RE: 0.2% [95% CI: -26.2%, 26.2%); I^2=83.4%]. The difference between aTIV and TIV was not significant for the cross-lineage B response. In all available studies, aTIV determined higher GMTs (by 3–90%) for all heterologous strains; 50% (4/8) of these estimates were statistically significant. Meta-analysis of the differences in GMTs was not performed, as no studies reported dispersion parameters.

3.5. Persistence of antibodies

In seven studies [42,46,48,56,59,62,63] reporting immunogenicity findings at longer post-vaccination periods (mostly 6 months), 43–100% of aTIV/aQIV users were still seroprotected. Although SPRs were generally higher in aTIV/aQIV than TIV/QIV users, the reported differences were usually not statistically significant **(Table S12)**.

In the pooled analysis of absolute SPRs at 6 months **(Table S13)**, the RE estimates (I^2>96%) were 82.0% (95% CI: 63.4%, 95.3%), 80.5% (95% CI: 63.0%, 93.6%), and 65.4% (95% CI: 47.4%, 81.5%) towards A(H1N1), A(H3N2), and B strains, respectively. When compared with TIV/QIV, aTIV/aQIV was associated with marginally higher SPRs at six months towards

A(H1N1) (FE: 4.1% [95% CI: 2.1%, 6.2%]; RE: 6.9% [95% CI: -0.2%, 14.0%]; I^2=65.7%) and A(H3N2) (FE: 3.5% [95% CI: 1.0%, 6.1%]; RE: 4.2% [95% CI: -0.3%, 8.6%]; I^2=0%) but not influenza B (FE: 1.2% [95% CI: -1.8%, 4.2%]; RE: 3.3% [95% CI: -4.2%, 10.8%]; I^2=43.0%).

3.6. Cell-mediated immunogenicity

Findings on cell-mediated immunity were reported in eight publications [48,49,52,53,56–58,61]. As expected, these trials used different assays and methods and therefore were summarized narratively. In the RCT by Durando et al. [48], double-positive CD3+CD4+ T-cell proliferation was assessed 1 and 3 months after a dose of aTIV or TIV was administered to HIV-1-seronegative and HIV-1-seropositive adults. In HIV-1-seronegative adults, both vaccines induced a measurable increase in memory T lymphocytes at both time points. However, the difference between the two vaccine arms was not statistically significant. By contrast, 1 month after vaccination, there was a clear advantage (p = 0.0002) of aTIV in HIV-1-seropositive adults. A subsequent subset study [49], compared production of interleukins (IL) 23 (IL-23) and IL-6 in response to stimulus with hemagglutinin. Both IL-6 and IL-23 syntheses increased ($p \leq 0.001$) upon vaccination with aTIV but not TIV. This increase was seen in both HIV-1 negative and positive cohorts. Conversely, Fabbiani et al. [53] reported that following one dose of aTIV, the production of cytokines increased significantly only in HIV-negative individuals. Among HIV-positive individuals, higher HIV viral load was significantly associated with reduced post-vaccination IL-10 levels.

A small single-arm study by Iorio et al. [52] showed that aTIV induced antigen-specific activation of T-cell responses, which were measured through quantification of double-positive CD69+CD3+ or CD69+CD8+ lymphocytes. A significant increase was shown for all vaccine-like strains and a heterologous A(H1N1)pdm09-like strain (aTIV contained a pre-2009 seasonal H1N1 strain). A similar increase was also seen in the interferon-γ enzyme-linked immunospot assay. The authors also reported no correlation between cellular and

humoral immunogenicity of aTIV [52]. In the BIOVACSAFE study [56–58] the frequency of H1N1-specific IL-21 producing T follicular helper cells persisted at higher levels in aTIV recipients compared with adults vaccinated with TIV [56]. However, both TIV and aTIV did not result in significant changes in frequencies and phenotypes of resting and activated regulatory T-cells [57]. aTIV induces significantly high early activation of interferon- and innate-cell-related genes, which are indispensable for control of viral infections and immune regulation [58]. Finally, vaccination with aTIV in adults was shown to induce a persistent transcriptional signature of innate immunity [61].

3.7. Immune response measured in ELISA

Two studies [43,53] reported results of aTIV-induced immunogenicity measured by commercially available ELISA kits. These data should be interpreted cautiously since this assay is not recommended by regulatory bodies [27] and may be associated with inaccurate results [66]. Magnani et al. [43] reported that, among heart transplant recipients, both aTIV and TIV determined a significant rise in both IgM and IgG towards both influenza A and B. However, there was no difference between the two vaccines. A single-arm study by Fabbiani et al. [53] showed a significant increase in IgM and IgG in both HIV-positive and HIV-negative adults. Notably, the post-vaccination fold-rise in IgM was higher in HIV-positive than in HIV-negative individuals (4.35 vs. 1.14).

3.8. Efficacy

Efficacy against RT-PCR-confirmed influenza was a secondary outcome in an RCT that enrolled solid-organ transplant recipients [62]. Participants were randomized on a 1:1:1 ratio to receive QIV, aTIV or high dose non-adjuvanted TIV (hdTIV). The incidence of influenza in the study arms was similar: 5.6% (11/198), 5.4% (11/205) and 6.7% (13/195) of participants in QIV, aTIV and hdTIV groups, respectively, were diagnosed with laboratory-

confirmed influenza. Therefore, the relative efficacy of aTIV vs. QIV and aTIV vs hdTIV was not significant, being 3.4% (95% CI: -117.7%, 57.4%) and 19.5% (95% CI: -75.3%, 63.0%), respectively. When restricted to the active surveillance RT-PCR (i.e., subjects self-collected five nasopharyngeal swabs at weeks 2, 4, 6, 8 and 10 after the start of the influenza season), the incidence of influenza was 5.1% (10/198), 3.4% (7/205) and 4.6% (9/195) in QIV, aTIV and hdTIV arms, respectively. In this analysis, the relative efficacy of aTIV vs QIV and aTIV vs hdTIV was 32.4% (95% CI: -74.1%, 73.7%) and 26.0% (95% CI: -94.8%, 71.9%), respectively. However, it should be stressed that this RCT was not powered to find differences in the efficacy estimates, as its primary outcome was humoral immune response.

3.9. Safety, reactogenicity and tolerability

At least one safety aspect of interest was reported in 11 trials [42,45,46,48,50,54,60–63,65]. Compared with non-adjuvanted vaccines, aTIV/aQIV was associated with an increased risk of solicited reactions, especially those at the injection site **(Table S14)**. However, the overwhelming majority of the reactogenic events were mild-to-moderate and self-limiting.

In the pooled RE analysis, any local and systematic reactions were reported by 49.2% and 31.8% of adults vaccinated with aTIV/aQIV. The most common injection site reactions were pain (51.0%) and induration (13.1%), while malaise (19.9%), headache (19.4%), and myalgia (15.3%) were the most common systemic reactions. Other solicited reactions were less frequent (<10**%**) **(Table 4, Figures S19–S31)**. Pooled analysis for rash was not performed, as no cases (0%) were reported in two studies [42,50].

In the pooled analysis of relative (vs. TIV/QIV) reactogenicity, aTIV/aQIV users showed increased RRs of any local (FE RR: 1.69; I^2=0%) and systemic (RE RR: 1.35; I^2=68.7%) reactions, pain (RE RR: 1.93; I^2=69.4%), induration (FE RR: 1.82; I^2=16.1%), fever (FE RR: 2.24; I^2=27.9%), myalgia (RE RR: 1.94; I^2=53.1%), arthralgia (FE RR: 1.49; I^2=0%),

headache (FE RR: 1.15; I^2=0%), malaise (FE RR: 1.59; I^2=0%), and nausea (FE RR: 1.59; I^2=0%) **(Table 4, Figures S32–S44)**.

As for unsolicited events, SAEs were infrequent (FE: 1.6% [95% CI: 0.9%, 2.4%]; RE: 0.8% [95% CI: 0.0%, 2.3%]; I^2=62.0%) **(Figure S45)** and most of them were unrelated to study vaccines. There was no difference in SAE reporting between aTIV/aQIV and TIV/QIV arms (FE RR: 1.02 [95% CI: 0.64, 1.63]; RE: 1.01 [95% CI: 0.63, 1.62]; I^2=0%) **(Figure S46)**.

4. Discussion

This review systematically appraised available experimental evidence on the performance of aTIV/aQIV in non-elderly adults. We showed that aTIV/aQIV is immunogenic and satisfies the currently available regulatory criteria for the absolute immune response [27]. Some evidence suggests that aTIV/aQIV is also immunogenic against antigenically dissimilar strains, which may be indicative of a certain level of cross-protection. Compared with non-adjuvanted standard-dose vaccines, aTIV/aQIV may be more immunogenic, although the magnitude of effect size depends on immunological parameter, vaccine strain and presence immunosuppressive conditions. Despite some increase in transient reactogenic events, aTIV/aQIV has an acceptable safety profile and the incidence of SAEs is low and comparable to that observed with non-adjuvanted vaccines. However, data on efficacy of aTIV/aQIV are scant and inconclusive. In view of its recent expansion in terms of age indication, which changed from ≥65 to ≥50 years [15], aTIV/aQIV is a valuable option for the prevention of seasonal influenza in adults aged 50–64 years, especially those who are immunocompromised. However, the current evidence does not allow inference of any preference of aTIV/aQIV over non-adjuvanted vaccines.

From its first Italian registration in 1997, experimental and observation research on aTIV/aQIV has been mostly focused on older adults [1–6]. It is therefore worth comparing our findings with those reported by the latest immunogenicity meta-analysis by Nicolay et

al. [4], which is based on 39 studies involving 27,116 individuals aged ≥65 years. In their study, at 1 month post-vaccination, the absolute difference in SCRs for aTIV vs. TIV towards vaccine-like strains was 9.5% (95% CI: 5.2%, 13.9%), 10.5% (95% CI: 6.6%, 14.5%), and 12.7% (95% CI: 8.6%, 16.8%) against A(H1N1), A(H3N2) and B strains, respectively. In our study, the corresponding differences established in RE models were comparable: 8.8% (95% CI: 3.7%, 14.0%), 13.1% (95% CI: 6.7%, 19.6%) and 11.7% (95% CI: 7.2%, 16.2%), respectively. Regarding differences in SPRs, similarly to our findings, Nicolay et al. [4] reported smaller but still significant effect sizes (A[H1N1]: 2.4% [95% CI: 0.8%, 4.0%]; [(H3N2]: 2.7% [95% CI: 0.9%, 4.5%]; B: 4.5% [95% CI: 1.8%, 7.1%]). Collectively, these data indicate that aTIV/aQIV is more immunogenic than non-adjuvanted standard-dose vaccines in both elderly and non-elderly adults.

We also demonstrated that, compared with general adult cohorts, the advantage of aTIV over TIV was greater in immunocompromised individuals. This superiority was particularly pronounced for the A(H3N2) subtype, where the absolute difference in SCRs reached approximately 20%. An early meta-analysis (13 studies) by Banzhoff et al. [3] compared the immunogenicity of aTIV and TIV in older adults with or without underlying chronic conditions. In healthy adults, GMTRs for aTIV vs. TIV were 1.10, 1.18 and 1.17 towards A(H1N1), A(H3N2) and B strains, respectively. The corresponding GMTRs in adults with co-morbidities were higher: 1.17, 1.43 and 1.37, respectively. When the presence of co-morbidities was added as a covariate, their model showed a significant interaction effect ($p = 0.004$) for A(H3N2), while this latter was not significant for A(H1N1) ($p = 0.41$) and B ($p = 0.065$) strains. The most probable explanation for this finding is poor immunogenicity of standard influenza vaccines in individuals with immunodeficiencies [26,67] and therefore an extra effort is needed to achieve protective titers in this target group [68]. Additionally, limited evidence suggests [48,49] that compared with TIV, aTIV enhances some components of cell-mediated immunity only in adults with immunodeficiency. Altogether,

these data suggest that individuals with underlying immunosuppressive conditions may benefit more from aTIV/aQIV and this population group may be considered a primary target for the recent aQIV availability for adults aged 50–64 years. In accordance with these findings, the most recent edition (December 2025) of the Calendario per la vita [69] —a comprehensive consensus document formulated by a multidisciplinary panel of immunization experts representing five leading scientific societies—advocates for the consideration of administering adjuvanted trivalent (aTIV) or adjuvanted quadrivalent (aQIV) influenza vaccines to individuals aged 50 to 60 years who have chronic medical conditions that result in impaired immune function. This recommendation is grounded in accumulating evidence demonstrating that such individuals are at an increased risk of developing severe influenza-related complications. The use of adjuvanted vaccines has been shown to elicit a more robust and sustained immunogenic response in populations with compromised immunity compared to conventional influenza vaccines. Therefore, targeted immunization strategies employing aTIV or aQIV may offer enhanced protection and reduce morbidity and mortality in this vulnerable subgroup.

Our third major finding is that aTIV is statistically superior to TIV in terms of cross-clade immunogenicity towards the A(H3N2) subtype: the absolute difference in both SCRs and SPRs was approximately 10%. This result is fully in line with several previous studies on heterologous immune response induced by aTIV in the elderly [4,70–72]. In older adults, aTIV has been also shown to be more effective than QIV in preventing hospitalizations due to influenza A(H3N2) [73]. Mechanistically, non-adjuvanted vaccines mostly recognize epitopes of the more conserved stem region of hemagglutinin. Conversely, MF59 induces epitope spreading from hemagglutinin stem to hemagglutinin globular head, which is much more variable and subjected to immune pressure generating antibody escape variants [74]. Indeed, the A(H3N2) subtype has the highest mutation rate [75] and vaccine effectiveness

against this virus is comparatively low in all age groups [76]. However, in view of the absence of efficacy/effectiveness data in non-elderly adults and considering that HAI is an imperfect correlate of protection [25,26], it remains unclear whether the observed immunogenicity advantage of aTIV can translate into a better protection against influenza A(H3N2) in non-elderly adults.

The available data on antibody persistence are limited and less conclusive. While at 6 months, the period which roughly corresponds to the entire influenza season, most individuals vaccinated with aTIV were still seroprotected, the advantage of aTIV/aQIV over TIV/QIV diminished and most pooled estimates were not statistically significant. Similar immunogenicity results have been established in a large pivotal RCT of aTIV vs. TIV in the elderly [77]. In that trial, GMTRs for aTIV vs. TIV were maximum at 1 month post-vaccination and then approached 1.0 (i.e. no difference between the two vaccines) at later time periods.

In adults, the use of aTIV/aQIV may be judged safe, as most AEs are reactogenic in nature, and transient and mild-to-moderate in intensity, while SAEs are uncommon. However, aTIV/aQIV was generally more reactogenic compared to TIV/QIV, especially when considering local injection site reactions. The observed effect sizes (RRs of 1.69 and 1.35 for any local and any systemic events, respectively) were similar to those previously reported. For example, the latest meta-analysis by O Murchu [36], which predominantly included studies on older adults, has reported pooled RRs of 1.90 (95% CI: 1.50, 2.39) and 1.18 (95% CI: 1.02, 1.38) for any local and any systemic reactions, respectively. Being immunostimulants, all adjuvants usually increase reactogenicity compared with inactivated formulations without adjuvant [78,79]. On the contrary, the incidence of unsolicited SAEs, most of which were unrelated to study vaccines, was similar between aTIV/aQIV and TIV/QIV. This is, again, in line with the previous pooled analyses on older adults [80, 81].

We identified some important study limitations. At review level, we were unable to identify unpublished RCTs that had been likely conducted. Considering that the first aTIV licensure

in Italy was for individuals aged ≥12 years [16], other adult-specific data could exist. Second, the RoB of some trials published before 2010 could be inaccurate, as these studies were not drafted according to the available reporting guidelines. Indeed, following development of the consolidated standards of reporting trials, these were not homogeneously adopted by journals, the academic community and clinical trial researchers [82,83]. Here, we adopted a conservative approach and rated these early RCTs at high RoB and therefore our ratings may be biased. Finally, we decided against pooling the continuous variable of relative GMTs of aTIV/aQIV vs. TIV/QIV, as several studies did not report dispersion measures. We noted that the 95% CIs were more likely reported in case of statistically significant results and by more recently published trials. Omission of studies with no dispersion measures would undoubtedly favor aTIV/aQIV, while the imputation of standard deviations was judged unfeasible owing to a significant number of GMTs without 95% CIs. In any case, our approach is conservative as the overwhelming majority of GMTR point estimates favored aTIV/aQIV. At present, efficacy/effectiveness data are unavailable. However, generate, collect and examine evidence regarding aTIV/aQIV administration in non-elderly populations remains critical for informed evaluation. For instance, as reported in the review wrote by Marchi et al. [84], the burden of influenza in subjects 50–64 age-group is relevant, particularly considering that in this age cohort the subjects are still workers. Provide a more appropriate and effective vaccination in this category will reduce, not only influenza burden on health care system but even on production and economic system. Furthermore, individuals aged 50–64 years had a 3-times higher rate of hospitalization and a 9-fold higher mortality rate attributable to influenza than those aged 18–49-years [85]. These findings indicate that this cohort could exhibit greater frailty compared to the younger group, suggesting that it maybe deserves a different and more effective vaccine. Notably, examining the cost associated to influenza-related hospitalization, adopting a more efficient and effective vaccine means to generate a saving in term of cost and represents a valuable strategy with a view to the efficiency of resource allocation. [86, 87]

The major limitation of the current body of evidence is the small sample sizes of most studies. Indeed, one half of the included trials enrolled <100 participants. These studies likely contributed to the substantial heterogeneity in some meta-analyses. The second important limitation is that data on the absolute and relative efficacy and effectiveness of aTIV/aQIV were very limited or lacking altogether. Considering the recent European authorization of aQIV for adults aged 50–64 [15] years, comparative vaccine effectiveness studies in this population group are warranted.

5. Conclusion

In conclusion, in this study based on 24 publications and 4,628 individuals, we showed that aTIV/aQIV does not raise safety concerns in adults and meets the regulatory criteria for absolute immunogenicity. aTIV/aQIV is generally more immunogenic than non-adjuvanted standard-dose vaccines towards both homologous and heterologous strains. The magnitude of this benefit, however, depends on vaccine component and characteristics of vaccinees, being higher for the A(H3N2) subtype and in adults with immunosuppressive conditions.

These results needed to be associated and reinforced by evidence on efficacy and/or effectiveness. Understanding the value of providing adjuvanted vaccine, or any other enhanced vaccines authorized in subjects <65 years old to a broader population, instead than limiting it administration to elderly population, could be an opportunity that deserve to be considered. Particularly, considering that one of the main reasons for administering enhanced vaccines is immunosenescence, a phenomenon related to the progressive decline in immune system functionality with age, the timing of its onset remains a topic of heated debate in the scientific community.

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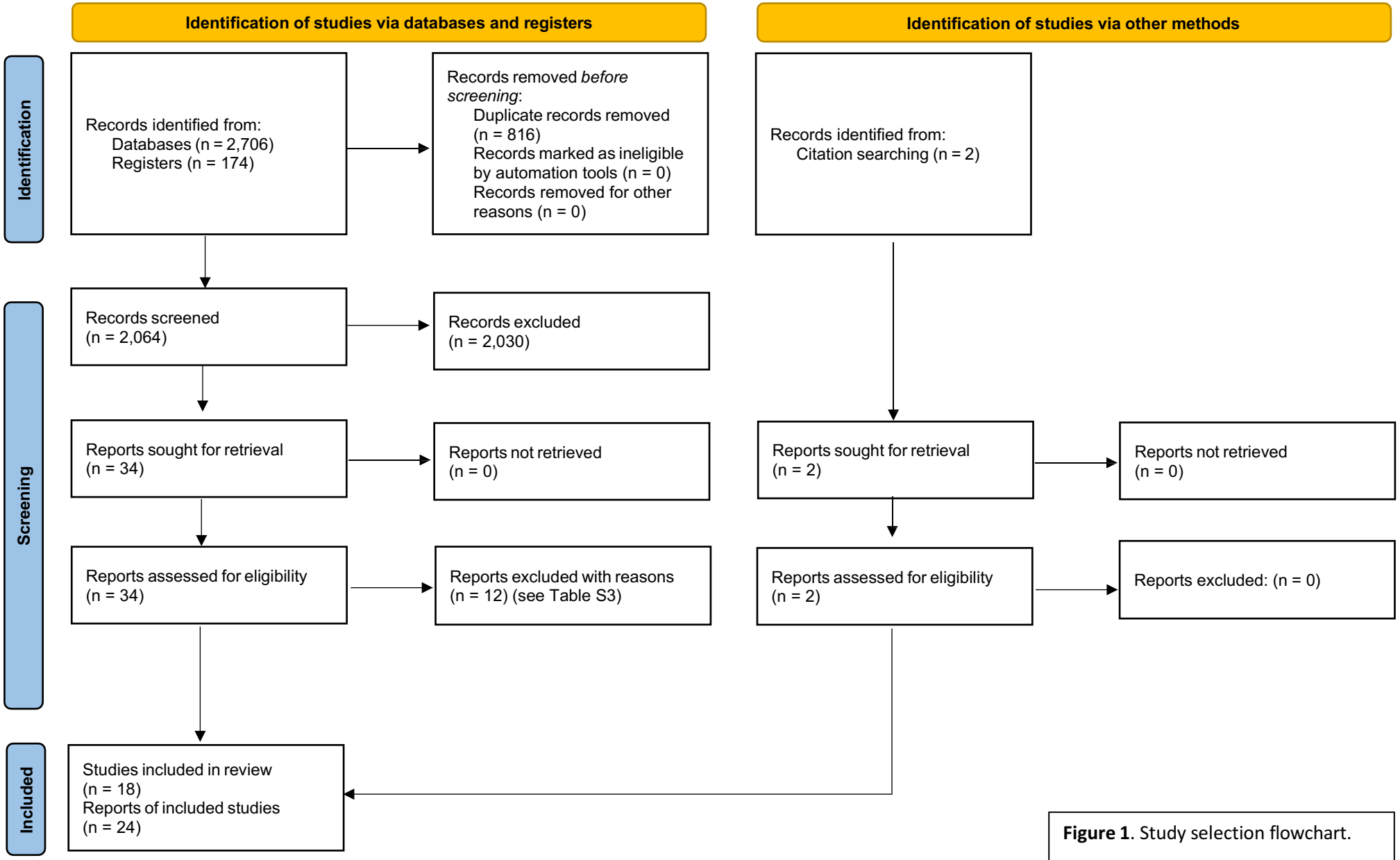


Figure 1. Study selection flowchart.

Table 1. Characteristics of the studies included.

Study [Ref]	Study design	Outcomes of interest reported	Country (season)	Study population	Adjuvanted vaccine (*N*)	Comparator (*N*)
Frey 2003 [42]	Randomized, observer-blind, controlled, multicenter	HAI (vaccine-like strains), safety	United States (1995/1996)	18–64 years (healthy)	aTIV (150)	TIV (151)
Magnani 2005 [43]	Randomized, open-label, controlled, single-center	ELISA	Italy (1999/2000)	Heart transplant recipients (mean age 55 years)	aTIV (21)	TIV (21), no vaccination (16)
Lorio 2003 [44]	Systematic allocation, controlled, single-center	HAI (vaccine-like strains)	Italy (2000/2001)	Non-elderly adults (HIV-1-seropositive)	aTIV (44)	TIV (40)
Pollok 2004 [45]	Randomized, observer-blind, controlled, multicenter	HAI (vaccine-like strains), safety	Germany (2000/2001)	18–64 years (renal transplant recipients)	aTIV (60)	TIV (56)
Gabutti 2005 [46]	Randomized, open-label, controlled, multicenter	HAI (vaccine-like strains), safety	Italy (2002/2003)	18–65 years (HIV-1-seropositive)	aTIV (18)	TIV (19)
Camilloni 2009 [47], Iorio 2011 [64]	Non-randomized (single-arm), single-center	HAI (vaccine-like and heterologous strains)	Italy (2003/2004)	32–46 years (healthy)	aTIV (26)	None
Durando 2008 [48], Fenoglio 2011 [49]	Randomized, open-label, controlled, multicenter	HAI (vaccine-like strains), cell-mediated immunity, safety	Italy (2005/2006)	18–65 years (37.1% positive for HIV-1)	aTIV (127)	TIV (129)
Baldo 2007 [50,51]	Randomized, double-blind, controlled, single-center	HAI (vaccine-like and heterologous strains), safety	Italy (2005/2006)	18–60 years (with comorbidities)	aTIV (128)	TIV (128)
Baldo 2012 [65]	Phase III, randomized, controlled, observer-blind, single-center	HAI (vaccine-like and heterologous strains), safety	Italy (2006/2007)	18–60 years (with comorbidities)	aTIV (180)	TIV (179)
Lorio 2012 [52]	Non-randomized (single-arm), single-center	HAI (vaccine-like and heterologous strains), cell-mediated immunity	Italy (2007/2008)	25–56 years (healthy)	aTIV (17)	None

Fabbiani 2013 [53]	Non-randomized (single-arm), single-center	ELISA, cell-mediated immunity	Italy (2010/2011)	Adults [median (IQR) age 45 (34–53) years] (73.0% positive for HIV)	aTIV (111)	None
Kumar 2016 [54] and 2017 [55]	Randomized, double-blind, controlled, single-center	HAI (vaccine-like and heterologous strains), safety	Canada (2012/2013)	Adult kidney transplant recipients (83% were 18–64 years)	aTIV (34)	TIV (34)
Spensieri 2016 [56], de Wolf 2017 [57], Weiner 2019 [58]	Exploratory, randomized, partially blinded (vaccinee and laboratory staff), controlled, single-center	HAI (vaccine-like strains), cell-mediated immunity	United Kingdom (2012/2013)	18–45 years (healthy)	aTIV (21)	TIV (21), placebo (8)
Noh 2016 [59]	Randomized, open-label, controlled, multicenter	HAI (vaccine-like strains)	Korea (2013/2014)	19–64 years (patients with chronic kidney disease and hemodialysis)	aTIV (67)	TIV (58)
Natori 2017 [60]	Randomized, blinded, controlled, single-center	HAI (vaccine-like strains), safety	Canada (2015/2016)	Adult allogeneic hematopoietic stem cell transplant recipients (median age 53.5 years)	aTIV (35)	TIV (38)
Kazmin 2023 [61]	Phase II, single-arm, open-label, single center	HAI (vaccine-like strains), cell-mediated immunity, safety	United Kingdom (2015/2016)	25–40 years (healthy)	aTIV (31)	None
Mombelli 2024 [62]	Randomized, double-blind, controlled, superiority multicenter	HAI (vaccine-like strains), efficacy, safety	Switzerland, Spain (2018/2019, 2019/2020)	Adult solid organ transplant recipients [median (IQR) age 57 (45–64) years]	aTIV (209)	QIV (204), hdTIV (203)

Poder 2023 [63]	Randomized, observer-blind, controlled, multicenter	HAI (vaccine-like strains), safety	Estonia, Germany, United States (2021/2022)	50–64 years (89.6% healthy)	aQIV (1,027)	QIV (1,017)

aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; ELISA, enzyme-linked immunosorbent assay; HAI, hemagglutination inhibition assay; hdTIV, high-dose trivalent seasonal influenza vaccine; HIV, human immunodeficiency virus; IQR, interquartile range; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; TIV, trivalent non-adjuvanted seasonal influenza vaccine.

Table 2. Meta-analysis of absolute and relative seroconversion and seroprotection rates towards vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by vaccine strain.

Parameter	Vaccine-like strain	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
SCR	A(H1N1)	17	94.7	67.1 (65.0, 69.1)	56.5 (48.7, 64.1)
	A(H3N2)	16	82.2	63.6 (61.5, 65.8)	62.8 (55.6, 69.8)
	B	16	92.3	48.6 (46.4, 50.8)	53.5 (44.1, 62.7)
SPR	A(H1N1)	15	94.1	97.2 (96.3, 98.0)	89.7 (82.9, 95.1)
	A(H3N2)	15	92.8	94.3 (93.1, 95.3)	90.7 (84.3, 95.7)
	B	16	95.2	86.9 (85.3, 88.4)	80.8 (72.0, 88.4)
ΔSCR	A(H1N1)	14	49.1	7.1 (4.4, 9.9)	8.8 (3.7, 14.0)
	A(H3N2)	14	69.9	8.1 (5.1, 11.0)	13.1 (6.7, 19.6)
	B	14	36.3	8.5 (5.6, 11.5)	11.7 (7.2, 16.2)
ΔSPR	A(H1N1)	14	52.5	3.3 (1.8, 4.8)	3.8 (1.0, 6.6)
	A(H3N2)	14	74.3	5.3 (3.5, 7.1)	8.6 (4.0, 13.2)
	B	14	44.3	4.6 (2.6, 6.7)	5.1 (1.6, 8.6)

FE, fixed effects; k, number of studies; RE, random effects; SCR, seroconversion rate; SPR, seroprotection rate; ΔSCR: difference in seroconversion rates between individuals immunized with adjuvanted vs non-adjuvanted influenza vaccines; ΔSPR: difference in seroprotection rates between individuals immunized with adjuvanted vs non-adjuvanted influenza vaccines.

Table 3. Meta-analysis of absolute and relative seroconversion and seroprotection rates towards vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults: A subgroup analysis by the presence of immunosuppressive conditions.

Parameter	Vaccine-like strain	Immunosuppression	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
SCR	A(H1N1)	No	9	89.8	74.6 (72.4, 76.8)	65.0 (57.6, 72.0)
		Yes	8	85.6	40.2 (35.7, 44.7)	47.1 (35.6, 58.9)
	A(H3N2)	No	8	83.5	66.7 (64.2, 69.0)	70.3 (61.1, 78.7)
		Yes	8	57.4	53.5 (48.9, 58.0)	54.4 (46.4, 62.4)
	B	No	8	93.2	51.9 (49.4, 54.5)	60.2 (49.6, 70.3)
		Yes	8	89.2	38.1 (33.7, 42.6)	46.5 (32.4, 61.0)
SPR	A(H1N1)	No	7	92.1	99.5 (98.9, 99.9)	96.3 (90.0, 99.8)
		Yes	8	66.4	83.4 (79.8, 86.7)	82.1 (74.3, 88.9)
	A(H3N2)	No	7	90.7	96.9 (95.8, 97.9)	94.6 (87.5, 99.1)
		Yes	8	86.8	82.8 (79.2, 86.2)	86.4 (76.1, 94.4)
	B	No	8	95.1	91.1 (89.4, 92.6)	83.5 (71.3, 92.9)
		Yes	8	91.3	70.9 (66.7, 75.0)	78.1 (64.4, 89.4)
ΔSCR	A(H1N1)	No	6	34.3	5.4 (2.2, 8.5)	5.4 (1.3, 9.5)
		Yes	8	41.9	12.6 (7.0, 18.3)	13.1 (4.6, 21.5)
	A(H3N2)	No	6	65.7	4.7 (1.3, 8.1)	7.5 (-0.2, 15.1)
		Yes	8	31.2	18.8 (12.8, 24.8)	19.7 (12.3, 27.1)
	B	No	6	34.2	6.5 (3.0, 9.9)	8.4 (2.8, 14.0)
		Yes	8	0.0	15.0 (9.7, 20.4)	15.4 (10.1, 20.7)
ΔSPR	A(H1N1)	No	6	63.6	2.5 (1.3, 3.7)	3.4 (0.1, 6.8)
		Yes	8	33.5	6.1 (1.1, 11.0)	5.0 (0.2, 9.7)
	A(H3N2)	No	6	80.4	3.4 (1.7, 5.0)	6.6 (-0.1, 13.4)
		Yes	8	26.8	11.6 (6.4, 16.9)	10.4 (5.8, 14.9)
	B	No	6	64.3	3.9 (1.8, 5.9)	5.8 (0.3, 11.3)
		Yes	8	2.0	7.2 (1.7, 12.7)	5.8 (0.7, 10.9)

FE, fixed effects; k, number of studies; RE, random effects; SCR, seroconversion rate; SPR, seroprotection rate (hemagglutination inhibition titer ≥1:40); ΔSCR: difference in seroconversion rates between individuals immunized with adjuvanted vs. non-adjuvanted influenza vaccines; ΔSPR: difference in seroprotection rates (hemagglutination inhibition titer ≥1:40) between individuals immunized with adjuvanted vs. non-adjuvanted influenza vaccines.

Table 4. Meta-analysis of absolute and relative solicited reactogenicity during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by adverse reaction.

Adverse reaction		k	I^2, %	FE model	RE model
Absolute frequency of adverse reactions in adults vaccinated with aTIV/aQIV, % (95% CI)					
Local	Any	4	18.5	49.2 (46.5, 51.9)	49.2 (46.5, 51.9)
	Pain	9	95.6	49.9 (47.7, 52.2)	51.0 (35.6, 66.4)
	Erythema	7	78.8	8.7 (7.4, 10.0)	9.8 (6.3, 14.0)
	Induration	6	85.9	10.3 (8.8, 11.8)	13.1 (8.5, 18.6)
	Ecchymosis	4	89.2	1.2 (0.7, 1.9)	2.7 (0.5, 6.3)
Systemic	Any	4	91.6	43.2 (40.5, 45.9)	31.8 (14.4, 52.3)
	Fever	10	89.6	2.7 (1.9, 3.6)	3.5 (0.6, 8.0)
	Chills	4	0.0	6.6 (5.3, 7.9)	6.6 (5.3, 7.9)
	Myalgia	7	82.9	14.5 (12.9, 16.1)	15.3 (10.7, 20.5)
	Arthralgia	6	91.6	11.4 (9.9, 12.9)	9.0 (3.8, 15.9)
	Headache	6	78.7	20.6 (18.8, 22.5)	19.4 (14.3, 25.1)
	Malaise	4	87.6	19.7 (16.6, 23.1)	19.9 (11.4, 30.2)
	Nausea	4	63.4	6.1 (4.9, 7.4)	5.1 (3.0, 7.7)
Relative risk (RR) of adverse reactions in adults vaccinated with aTIV/aQIV vs. TIV/QIV, RR (95% CI)					
Local	Any	4	0.0	1.69 (1.53, 1.86)	1.68 (1.52, 1.86)
	Pain	8	69.4	1.80 (1.66, 1.95)	1.93 (1.60, 2.32)
	Erythema	7	70.1	1.59 (1.26, 2.01)	1.49 (0.91, 2.42)
	Induration	6	16.1	1.82 (1.44, 2.30)	1.75 (1.30, 2.35)
	Ecchymosis	4	0.0	1.08 (0.63, 1.85)	1.06 (0.61, 1.83)
Systemic	Any	4	68.7	1.21 (1.10, 1.33)	1.35 (1.01, 1.81)
	Fever	8	27.9	2.24 (1.54, 3.28)	2.07 (1.14, 3.78)
	Chills	4	17.2	1.21 (0.91, 1.61)	1.18 (0.88, 1.57)
	Myalgia	7	53.1	1.89 (1.57, 2.29)	1.94 (1.37, 2.75)
	Arthralgia	6	0.0	1.49 (1.22, 1.81)	1.47 (1.21, 1.79)
	Headache	6	0.0	1.15 (1.01, 1.31)	1.15 (1.00, 1.31)
	Malaise	4	0.0	1.59 (1.23, 2.07)	1.60 (1.23, 2.08)
	Nausea	4	0.0	1.59 (1.16, 2.18)	1.59 (1.16, 2.19)

aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; FE, fixed effects; k, number of studies; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; RE, random effects; RR, relative risk; TIV, trivalent non-adjuvanted seasonal influenza vaccine.

Figure S1. Forest plot of absolute seroconversion rates towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

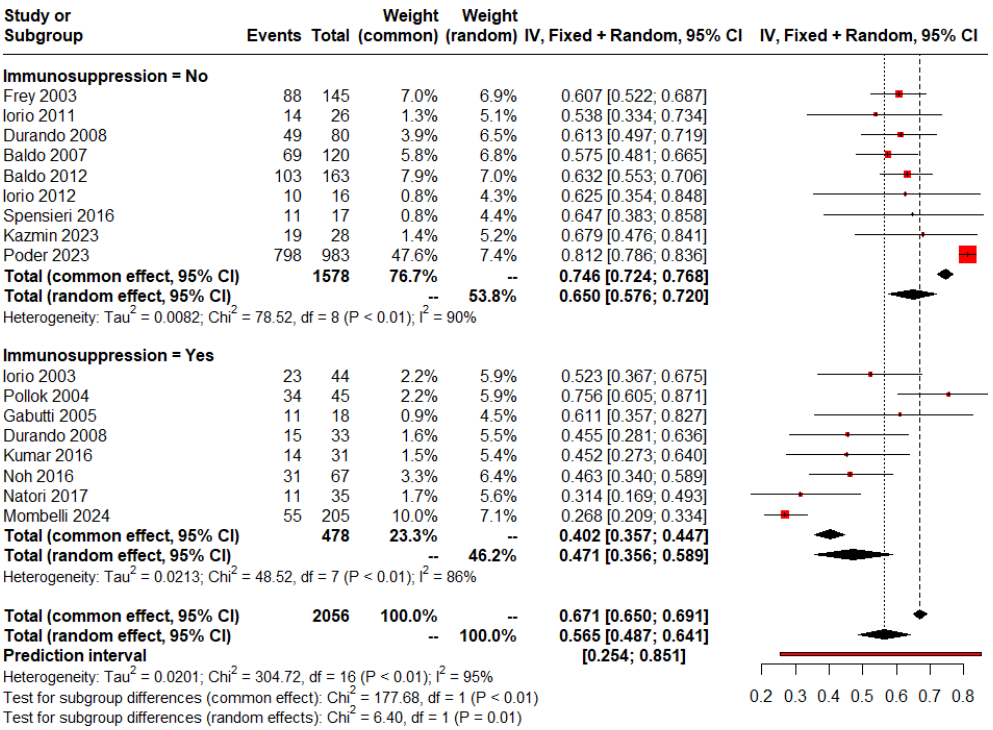


Figure S2. Forest plot of absolute seroconversion rates towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

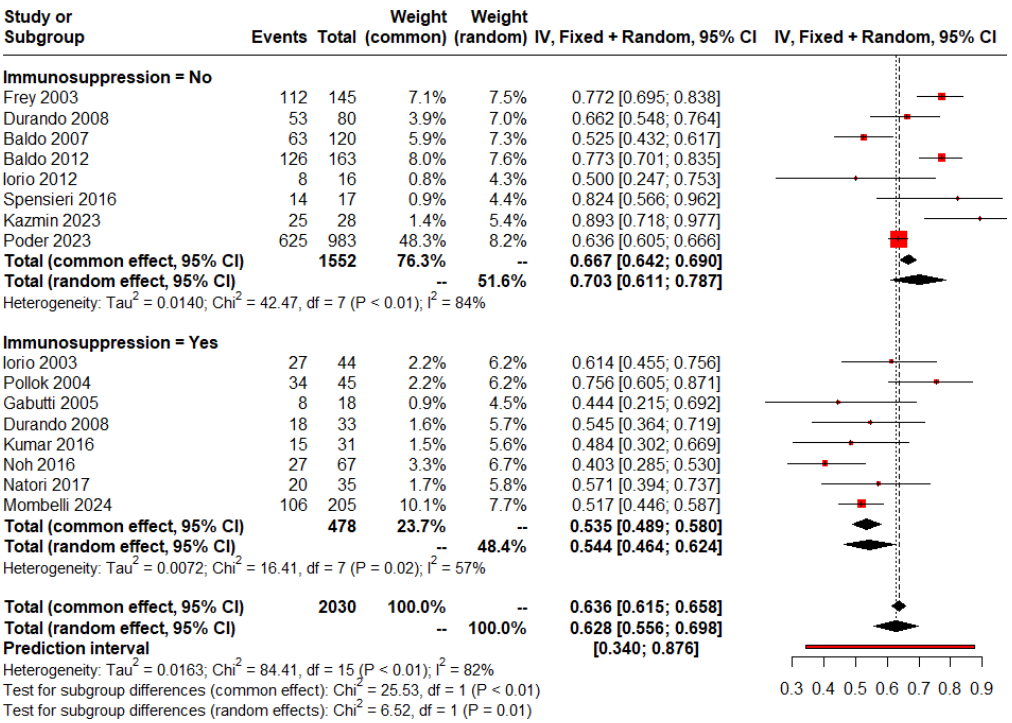


Figure S3. Forest plot of absolute seroconversion rates towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

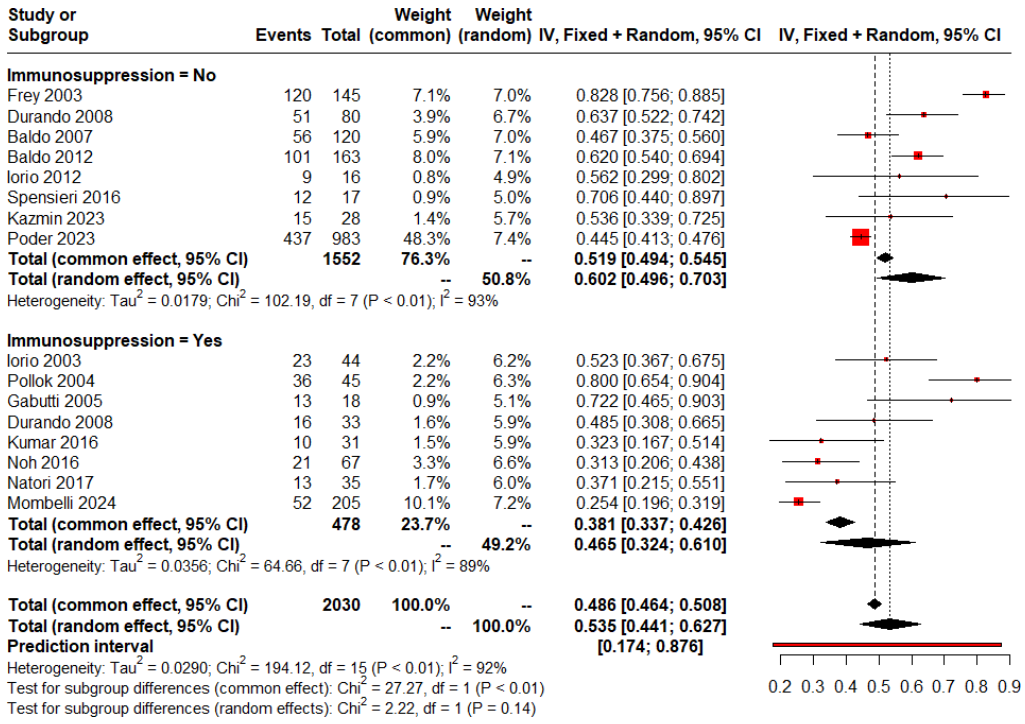


Figure S4. Forest plot of absolute seroprotection rates (hemagglutination inhibition titer ≥1:40) towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

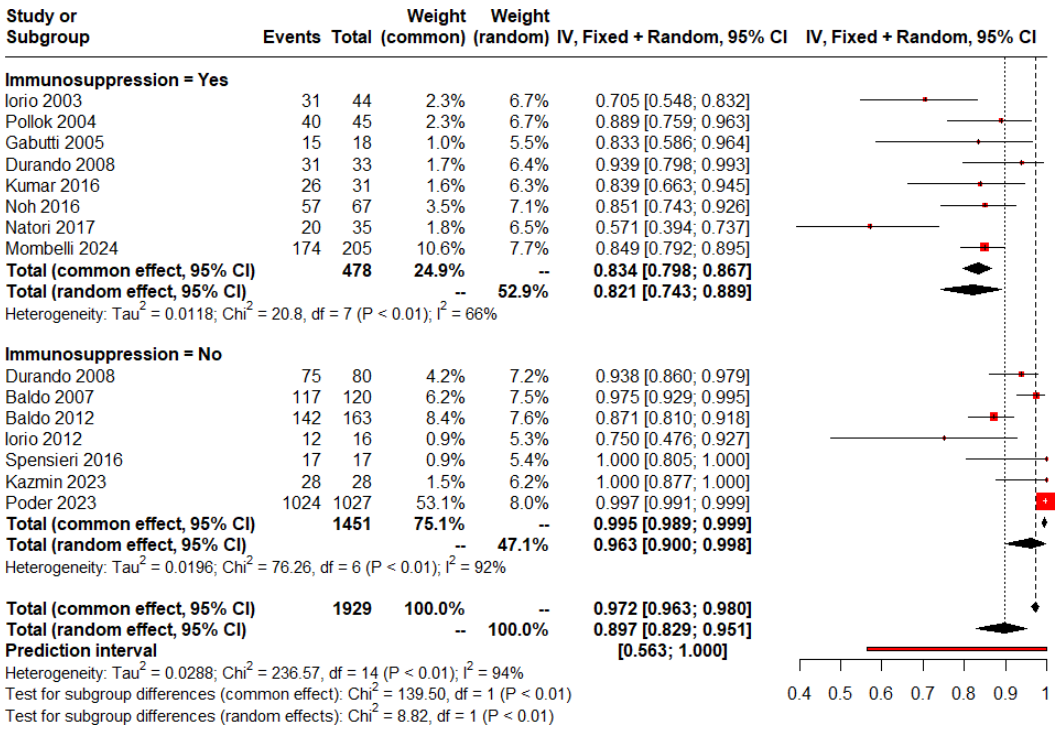


Figure S5. Forest plot of absolute seroprotection rates (hemagglutination inhibition titer ≥1:40) towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

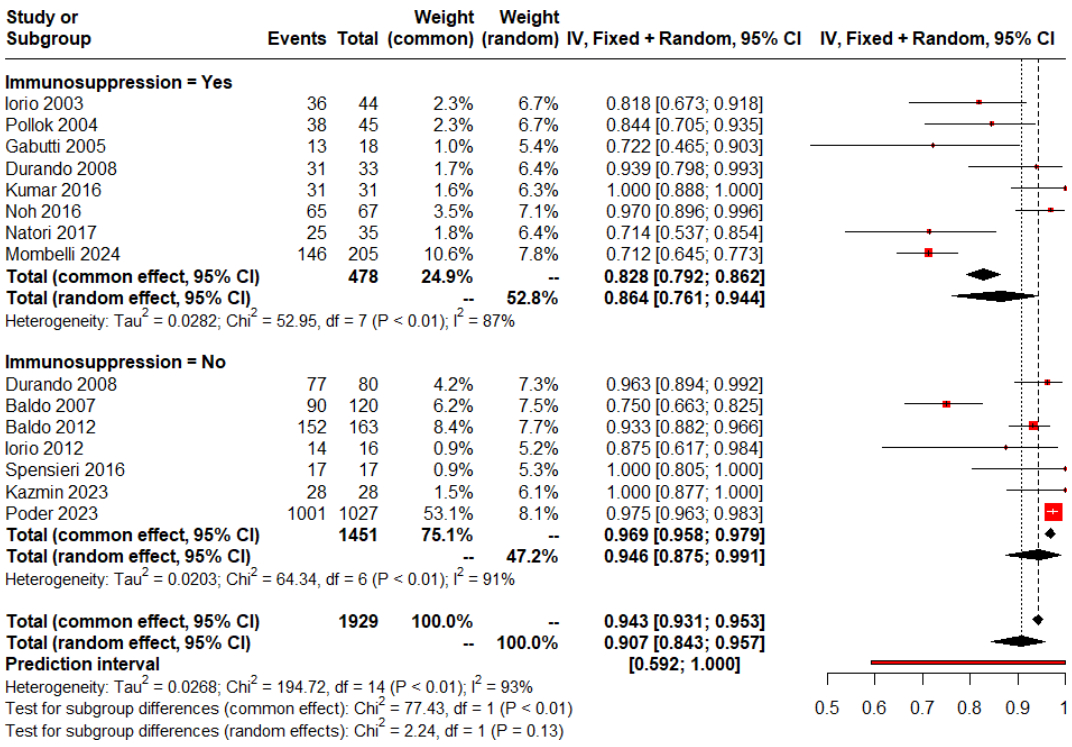


Figure S6. Forest plot of absolute seroprotection rates (hemagglutination inhibition titer ≥1:40) towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults, by immunosuppression status.

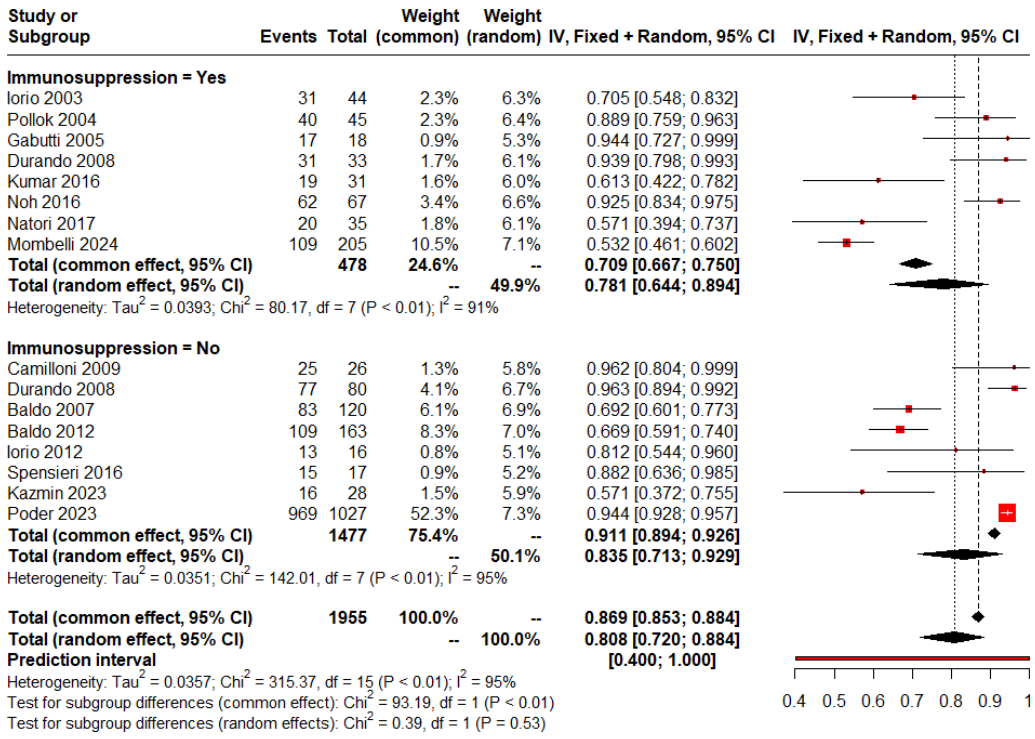


Figure S7. Forest plot of difference in seroconversion rates towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

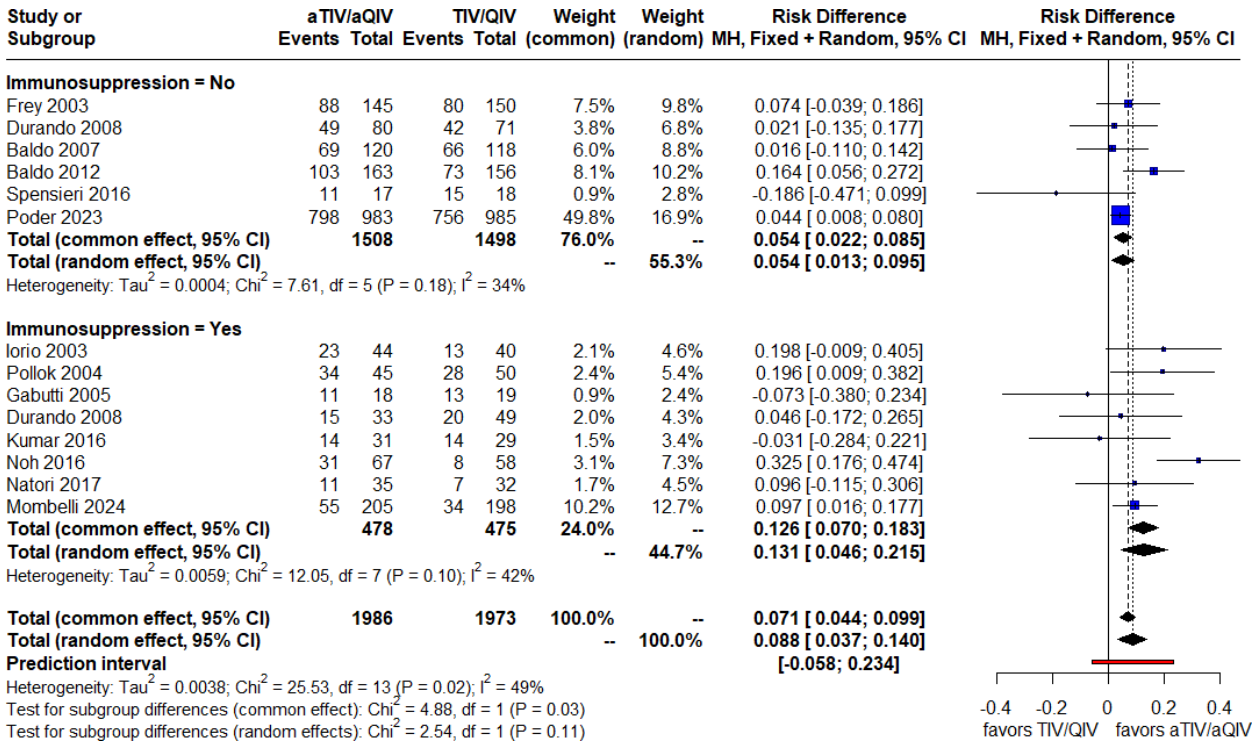


Figure S8. Forest plot of difference in seroconversion rates towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

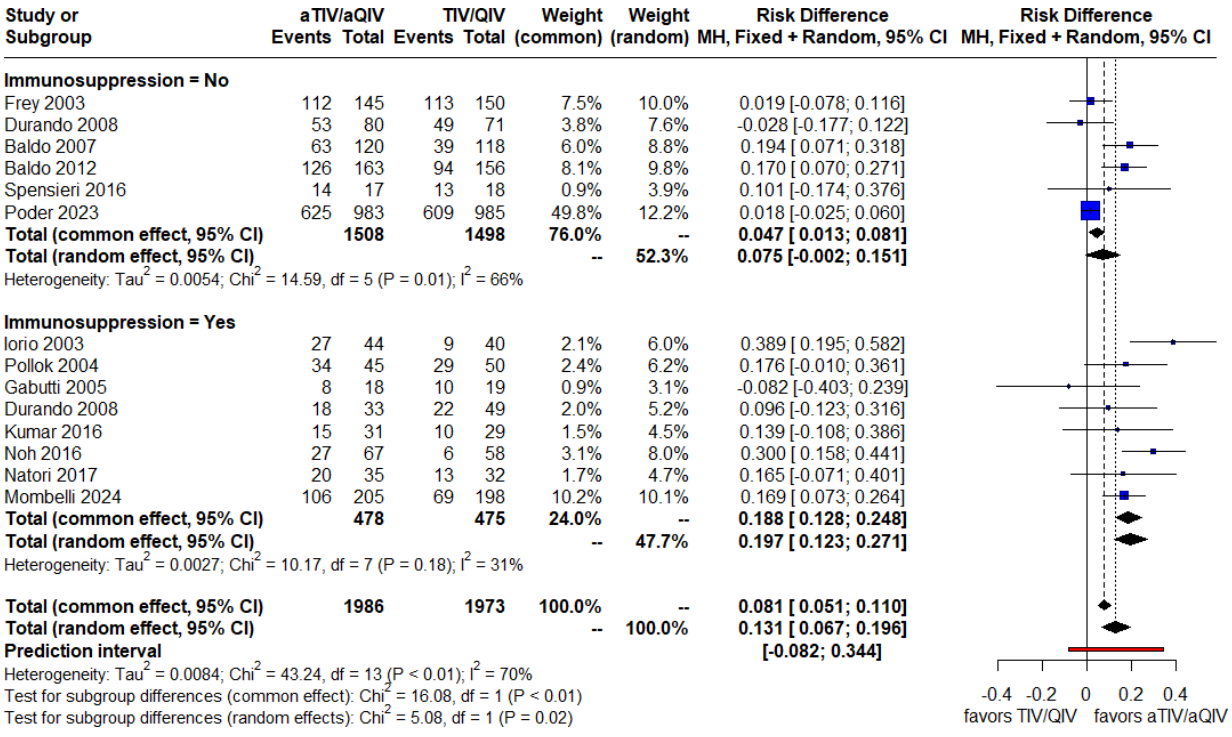


Figure S9. Forest plot of difference in seroconversion rates towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

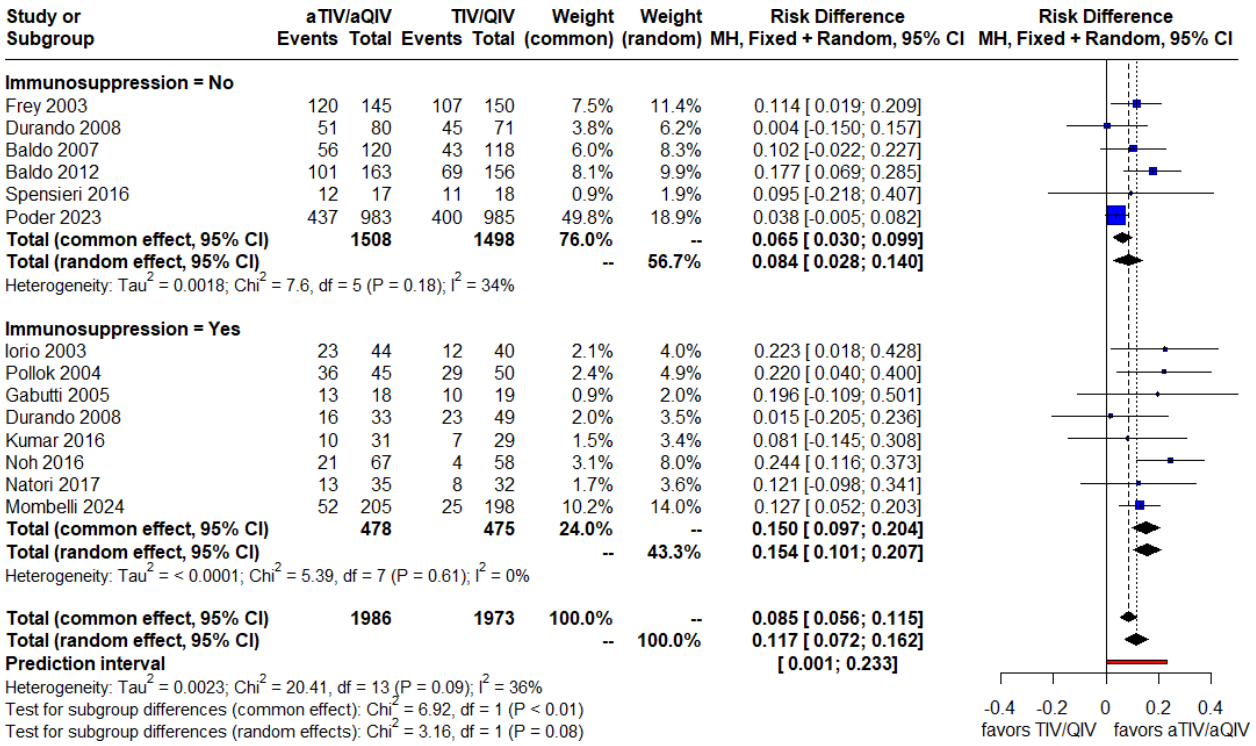


Figure S10. Forest plot of difference in seroprotection rates towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

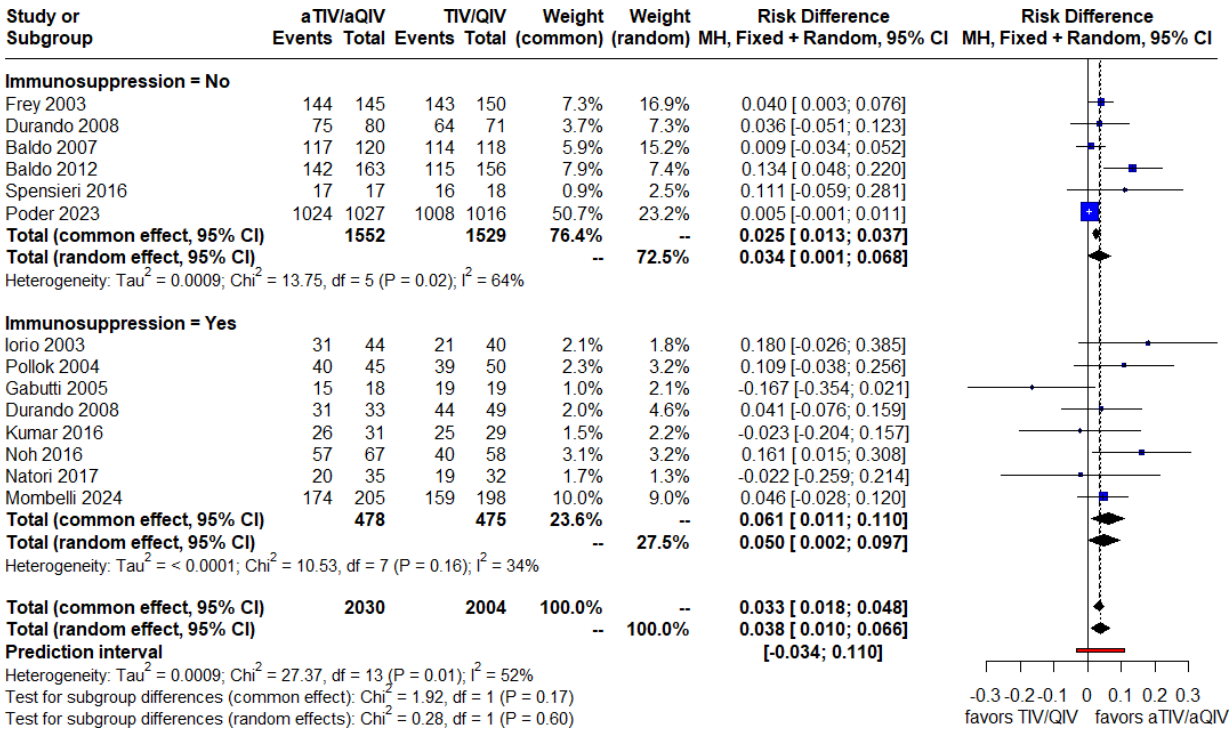


Figure S11. Forest plot of difference in seroprotection rates towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

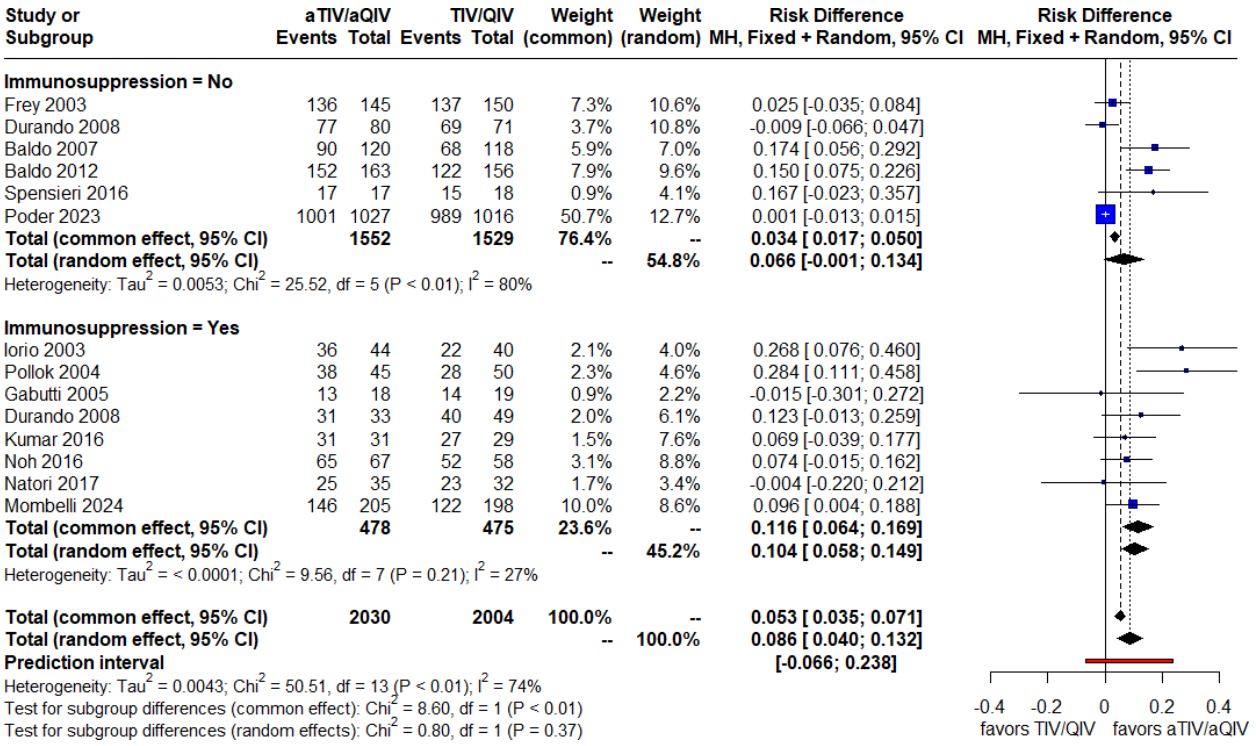


Figure S12. Forest plot of difference in seroprotection rates towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by immunosuppression status.

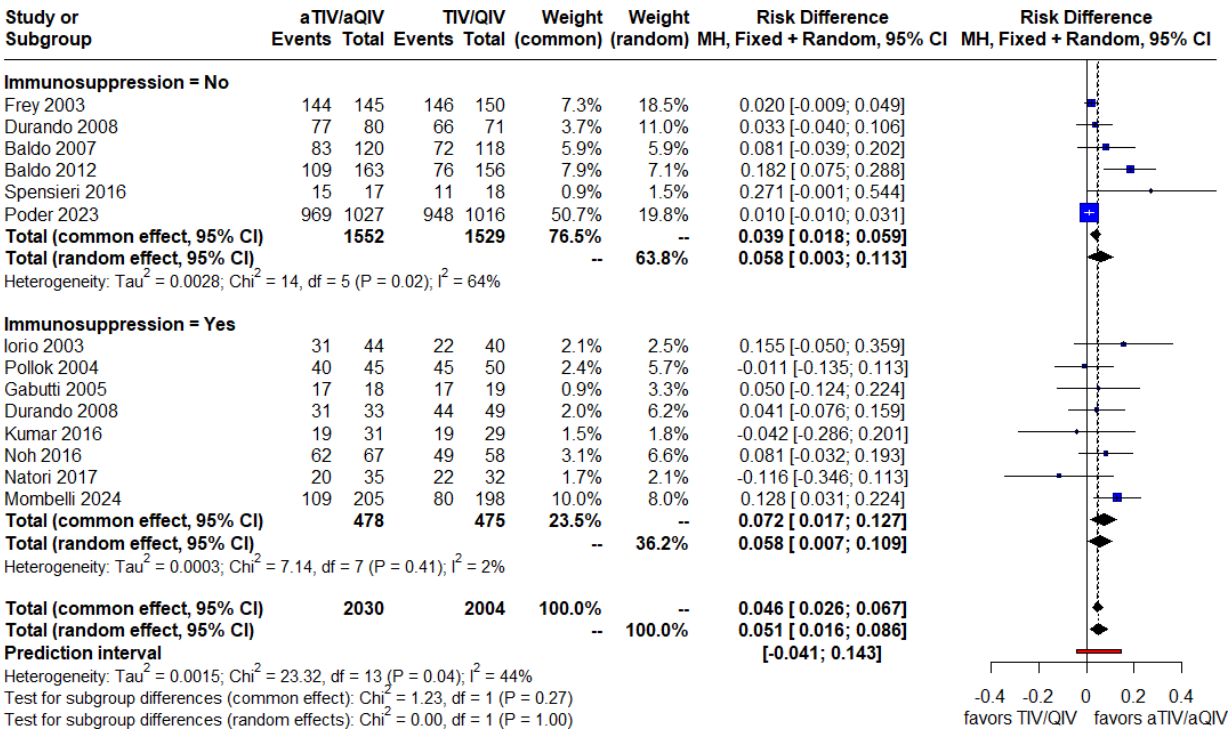
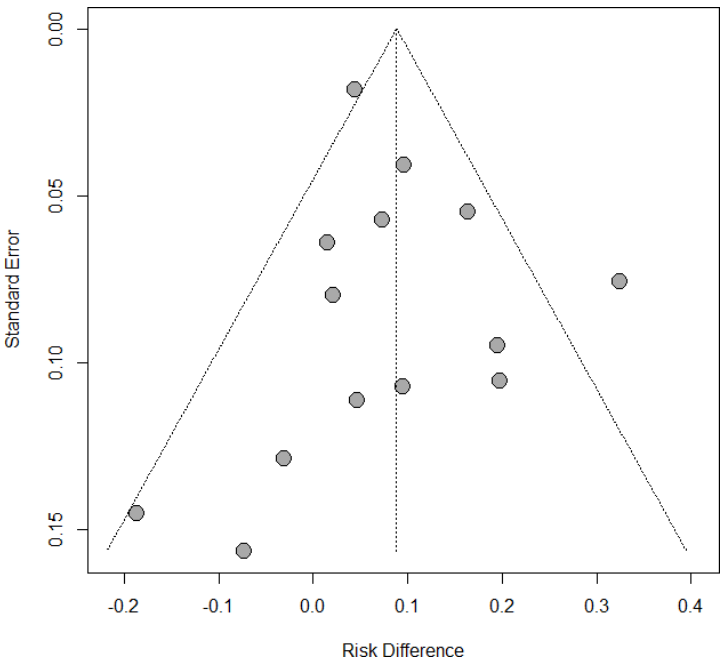
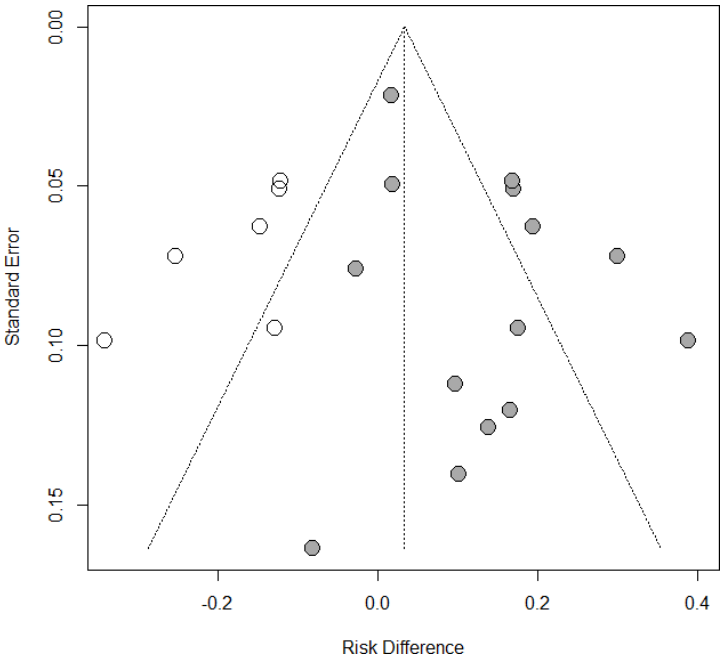


Figure S13. Funnel plot of difference in seroconversion rates towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



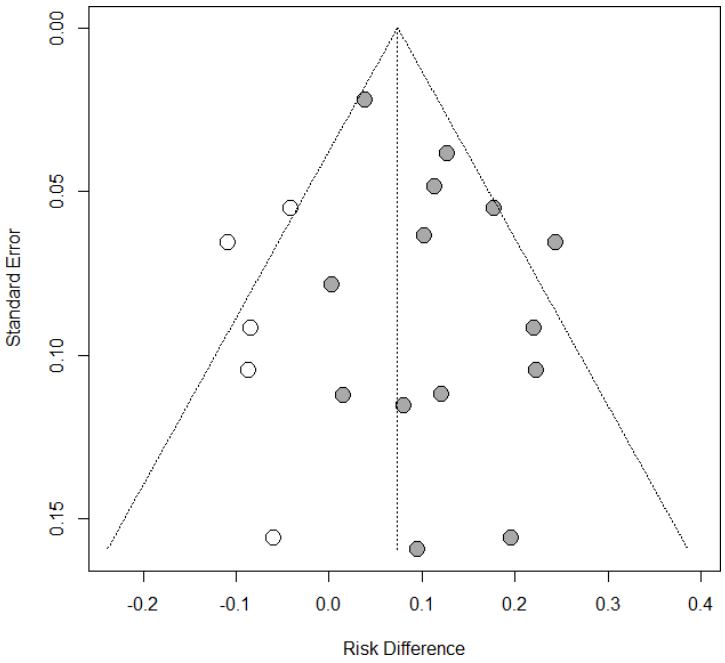
Observed studies Imputed studies Adjusted random-effects estimate: 8.8% (95% CI: 3.7%, 14.0%)

Figure S14. Funnel plot of difference in seroconversion rates towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



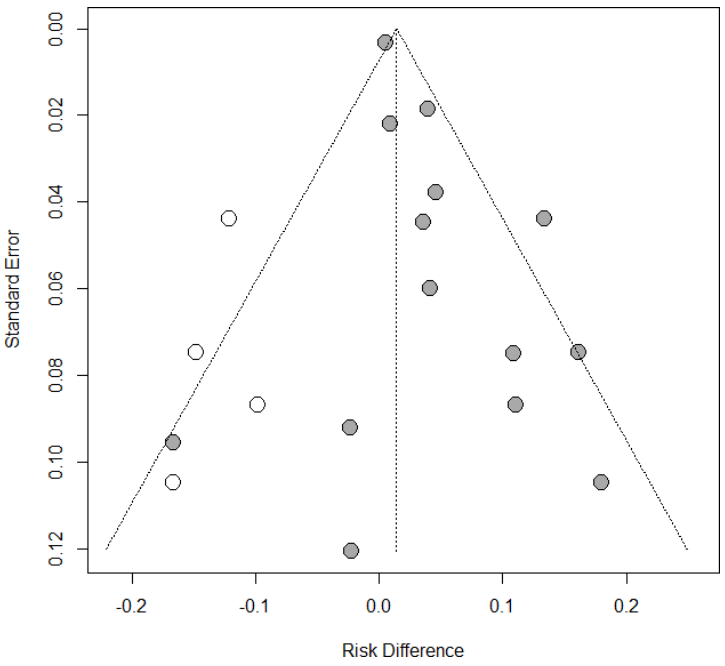
Observed studies Imputed studies Adjusted random-effects estimate: 3.3% (95% CI: -4.9%, 11.5%)

Figure S15. Funnel plot of difference in seroconversion rates towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



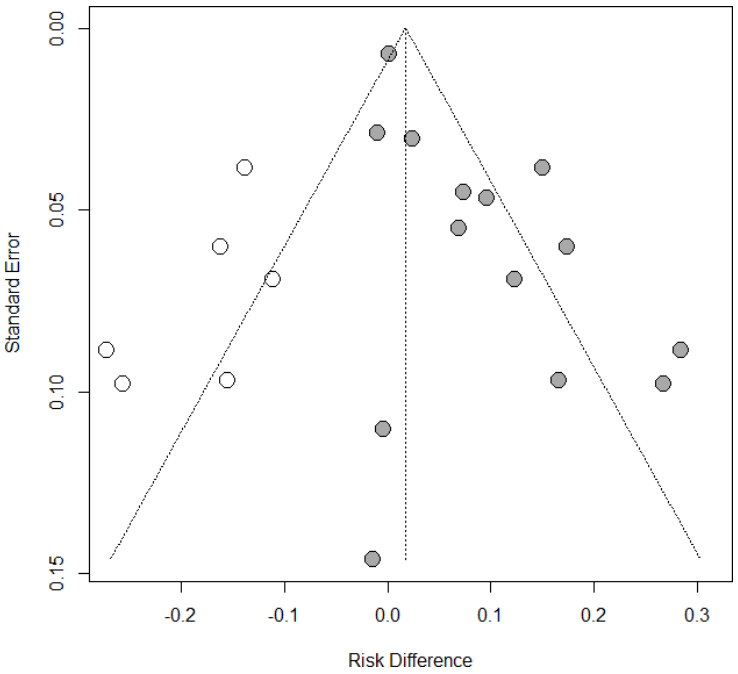
Observed studies Imputed studies Adjusted random-effects estimate: 7.4% (95% CI: 2.3%, 12.5%)

Figure S16. Funnel plot of difference in seroprotection rates towards vaccine-like A(H1N1) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



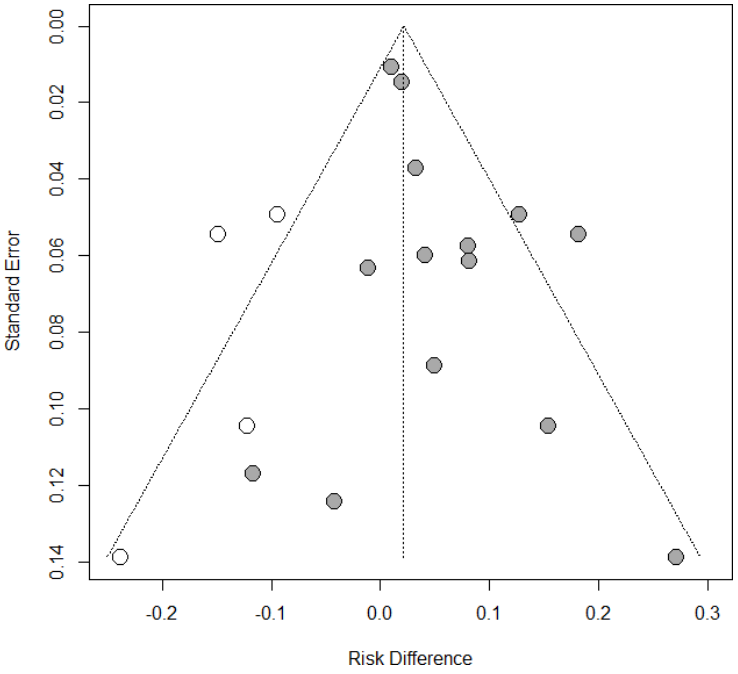
Observed studies Imputed studies Adjusted random-effects estimate: 1.4% (95% CI: -2.8%, 5.6%)

Figure S17. Funnel plot of difference in seroprotection rates towards vaccine-like A(H3N2) strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



Observed studies Imputed studies Adjusted random-effects estimate: 1.7% (95% CI: -4.8%, 8.2%)

Figure S18. Funnel plot of difference in seroprotection rates towards vaccine-like B strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.



Observed studies Imputed studies Adjusted random-effects estimate: 2.1% (95% CI: -2.5%, 6.8%)

Figure S19. Frequency of any solicited local/injection site reaction during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

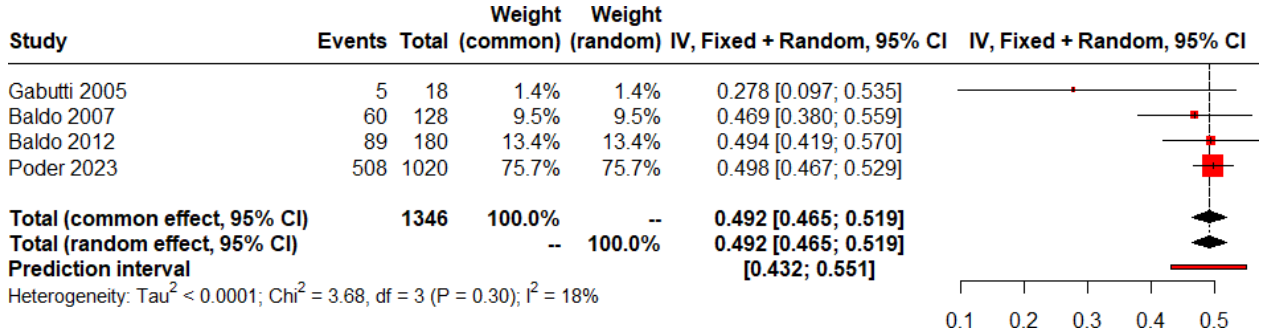


Figure S20. Frequency of solicited injection site pain during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

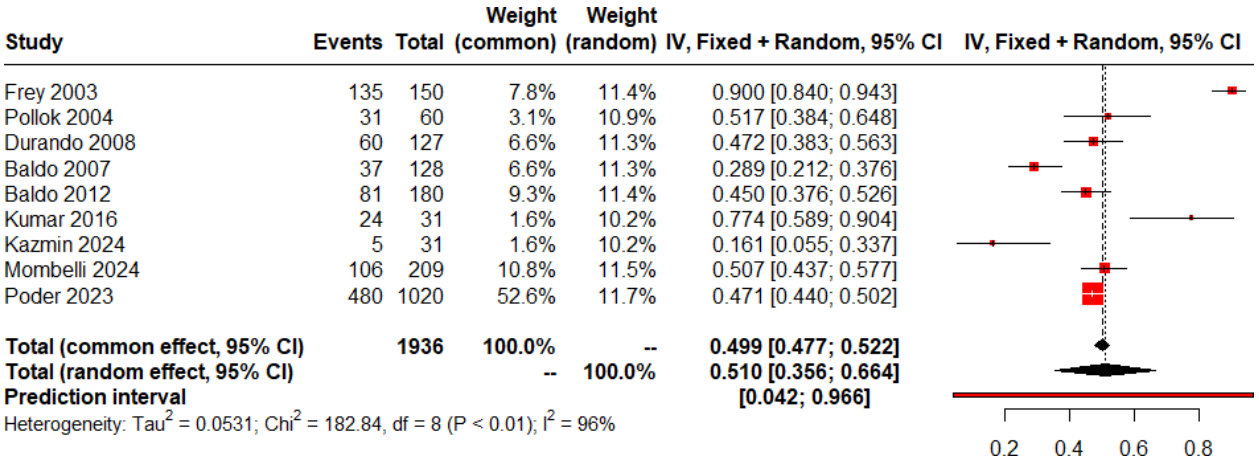


Figure S21. Frequency of solicited injection site erythema during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

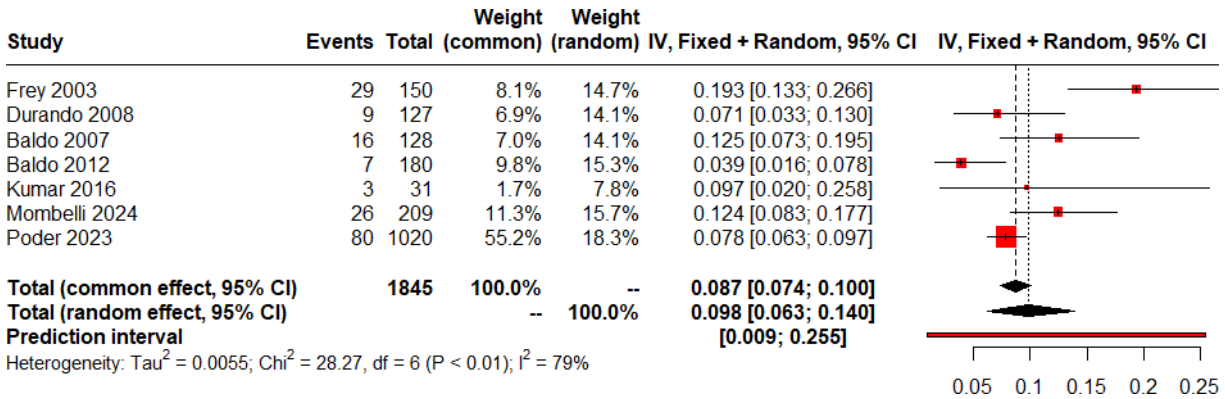


Figure S22. Frequency of solicited injection site induration during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

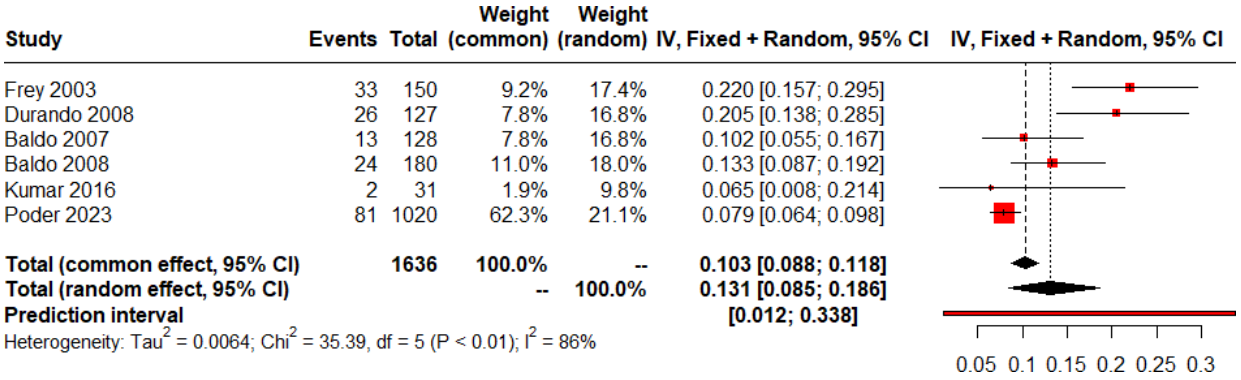


Figure S23. Frequency of solicited injection site ecchymosis during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

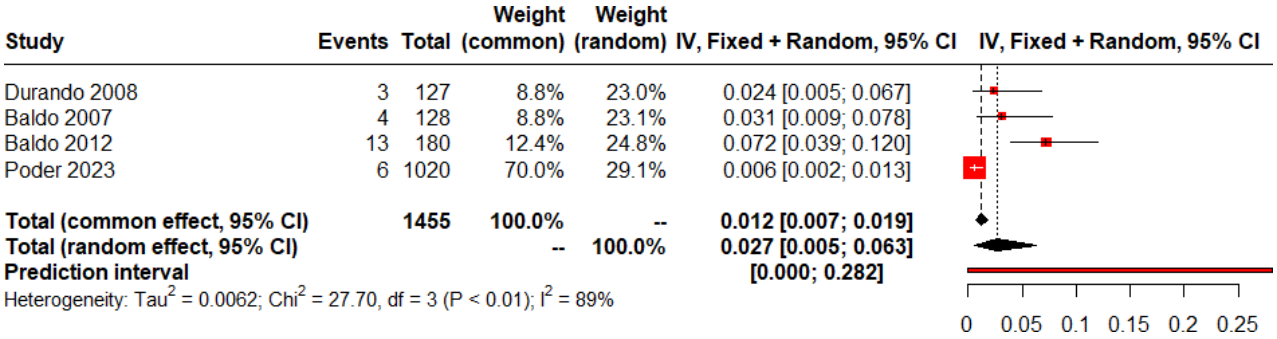


Figure S24. Frequency of any solicited systemic reaction during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

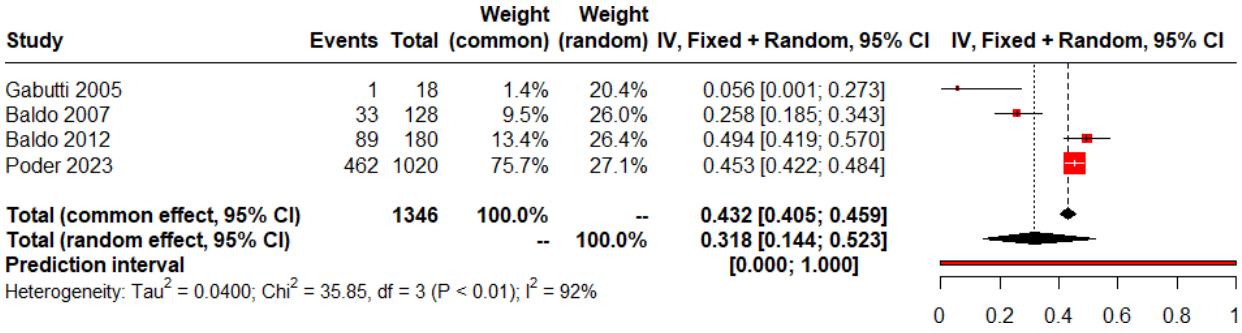


Figure S25. Frequency of solicited fever during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

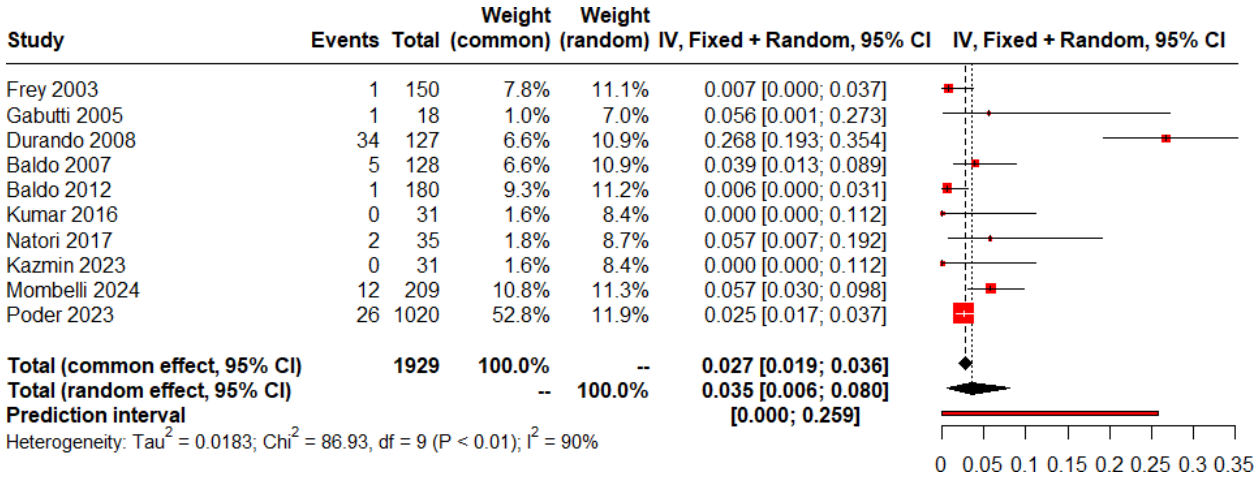


Figure S26. Frequency of solicited chills during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

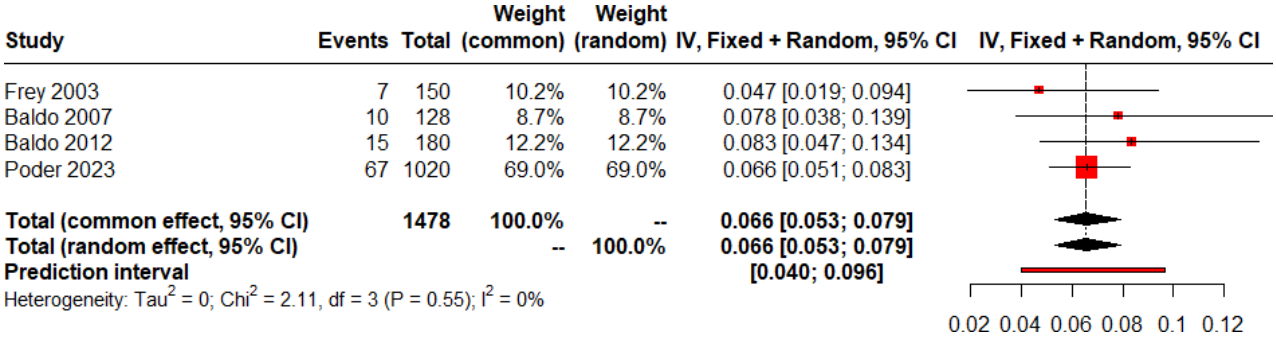


Figure S27. Frequency of solicited myalgia during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

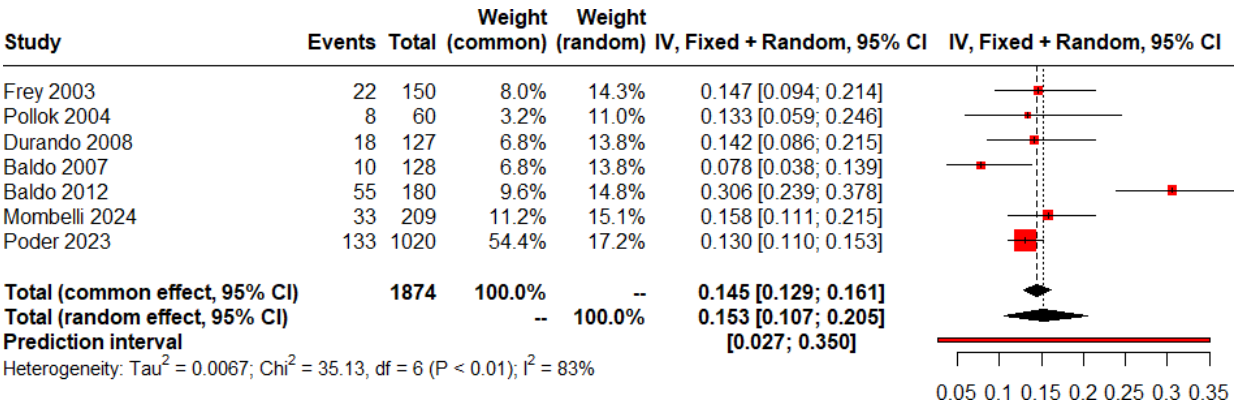


Figure S28. Frequency of solicited arthralgia during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

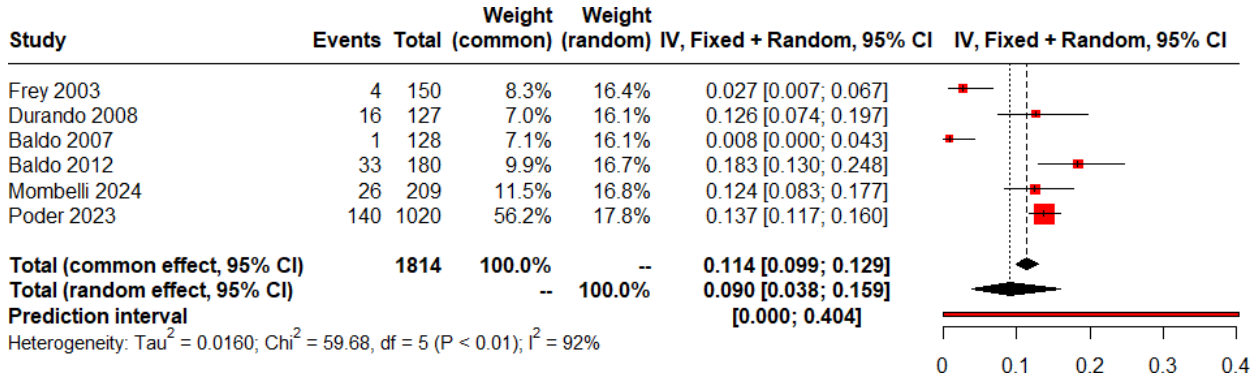


Figure S29. Frequency of solicited headache during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

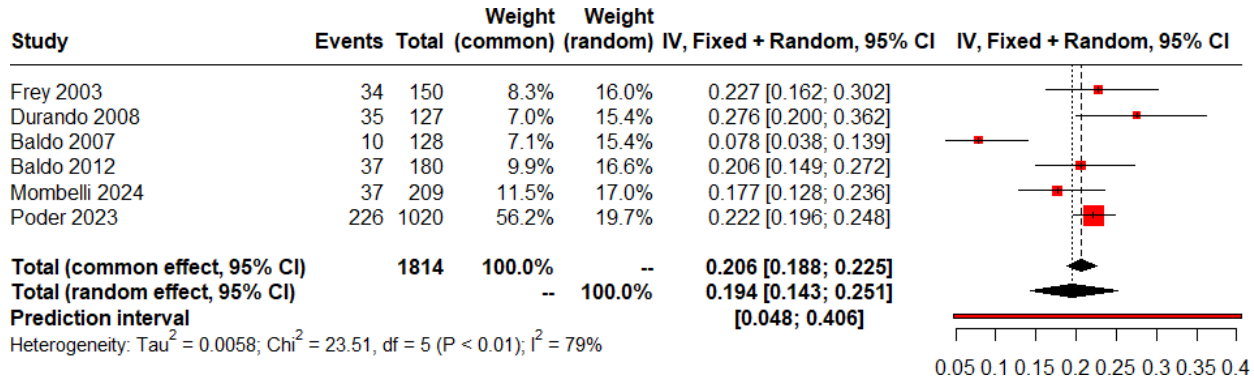


Figure S30. Frequency of solicited malaise during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

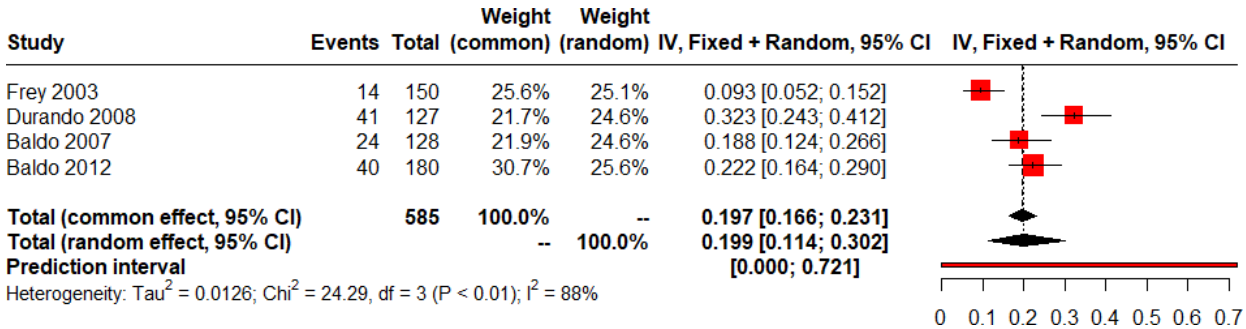


Figure S31. Frequency of solicited nausea during the first week after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

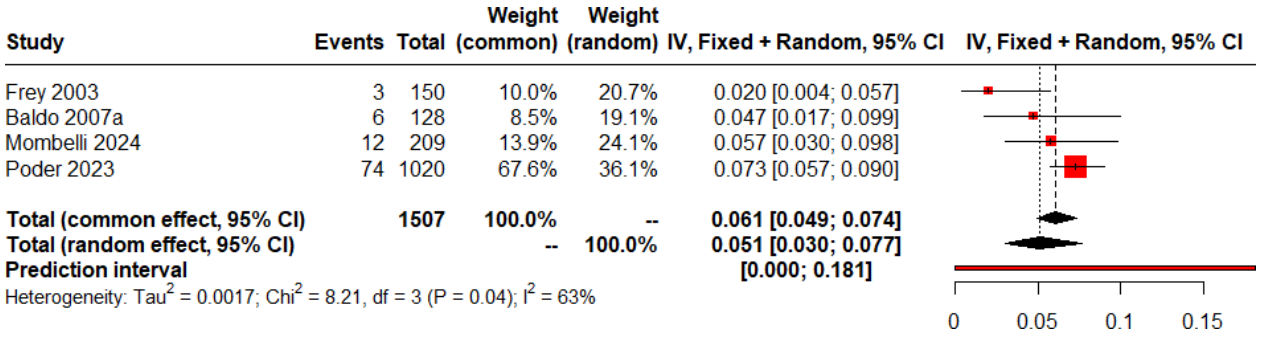


Figure S32. Relative risk of any solicited local/injection site reaction during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

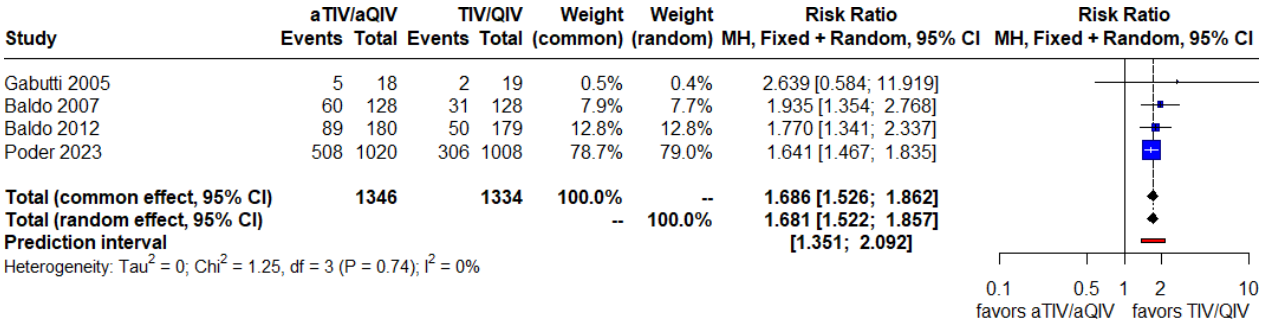


Figure S33. Relative risk of solicited injection site pain during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

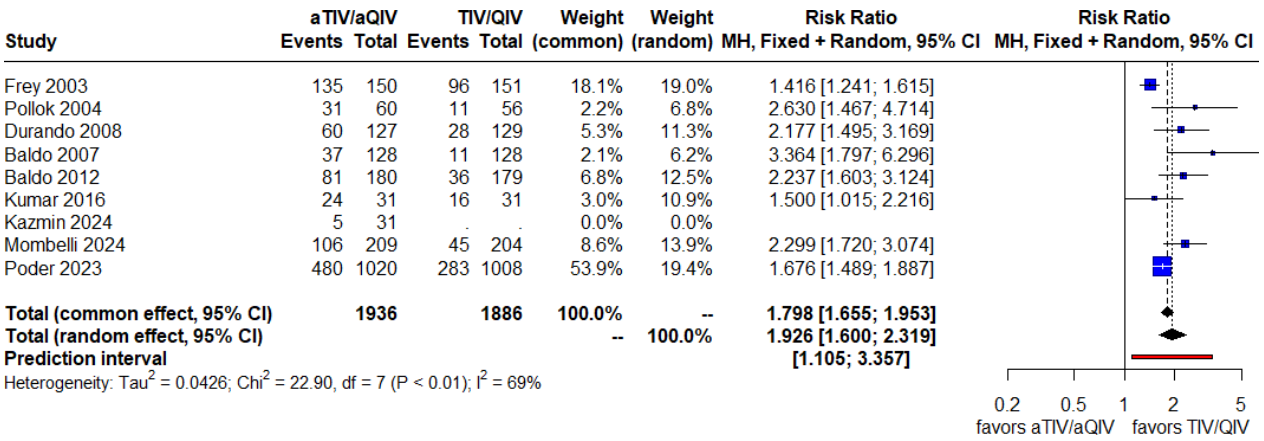


Figure S34. Relative risk of solicited injection site erythema during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

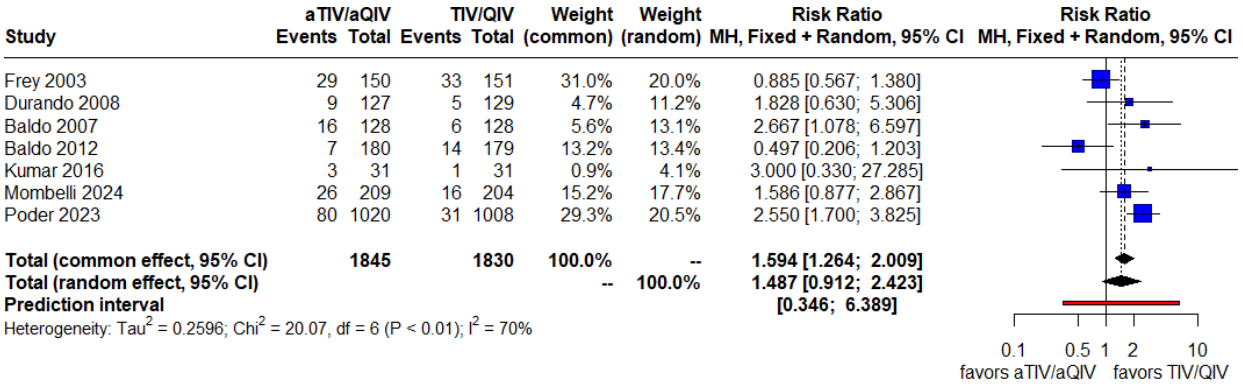


Figure S35. Relative risk of solicited injection site induration during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

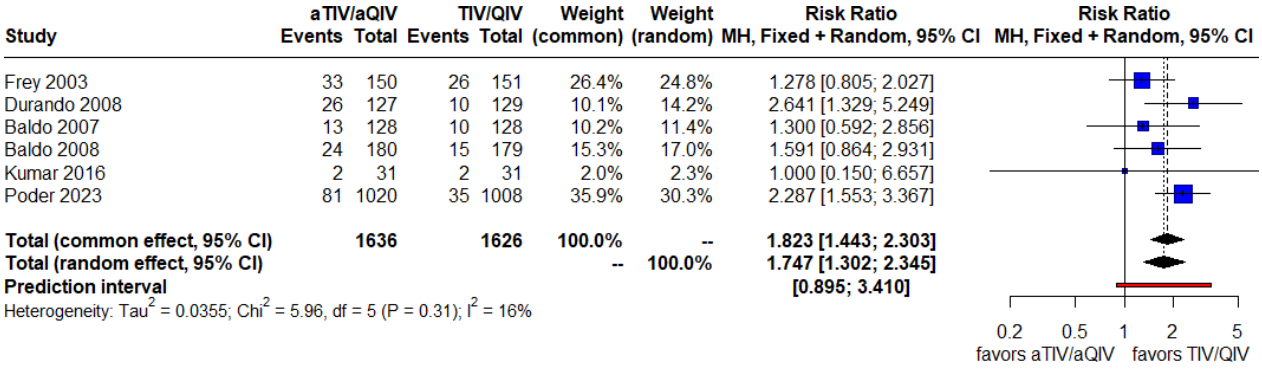


Figure S36. Relative risk of solicited injection site ecchymosis during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

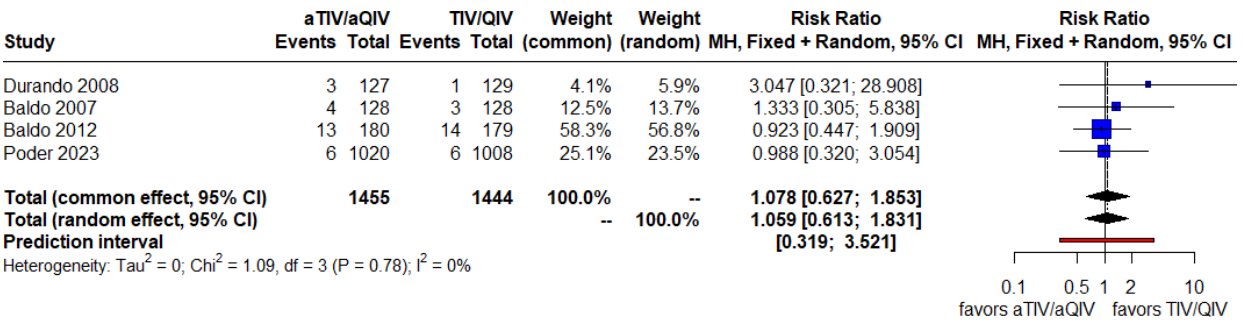


Figure S37. Relative risk of any solicited systemic reaction during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

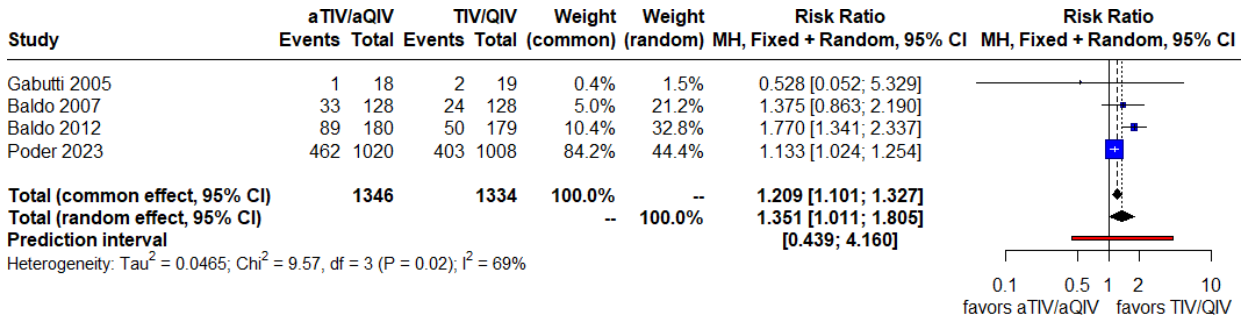


Figure S38. Relative risk of solicited fever during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

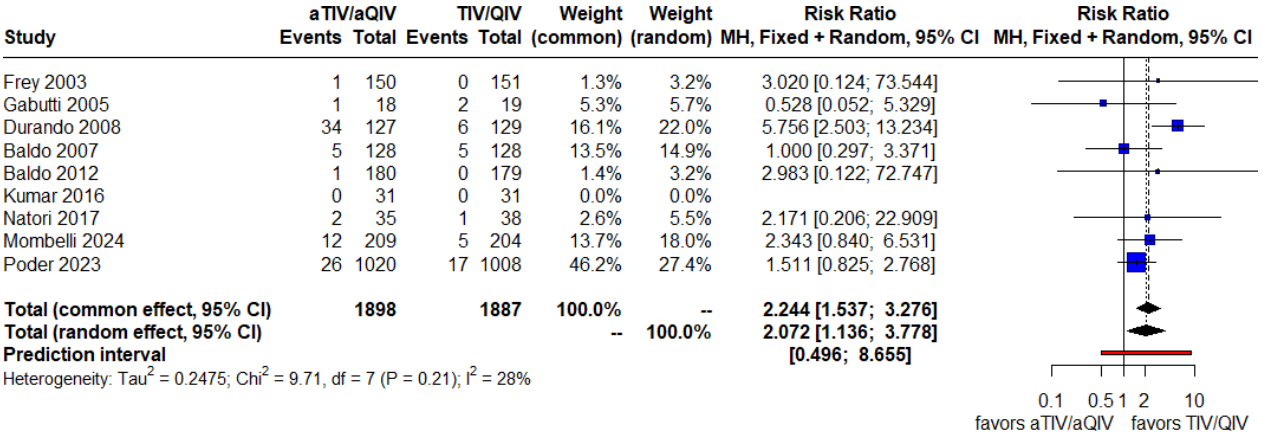


Figure S39. Relative risk of solicited chills during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

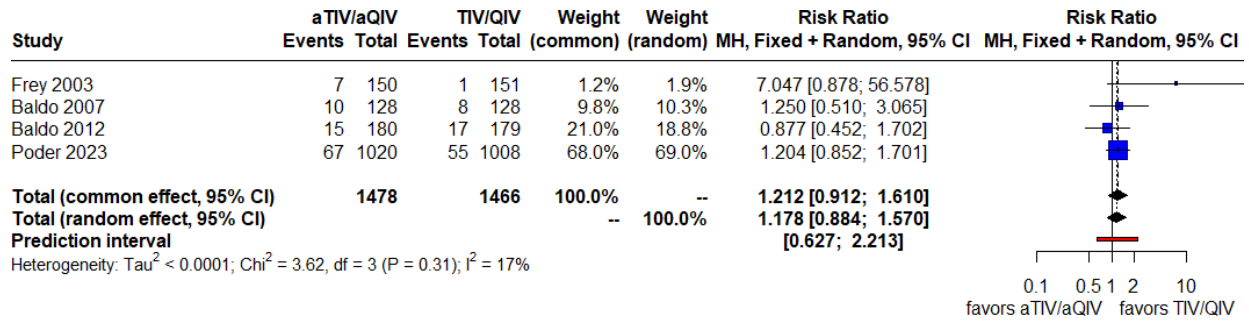


Figure S40. Relative risk of solicited myalgia during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

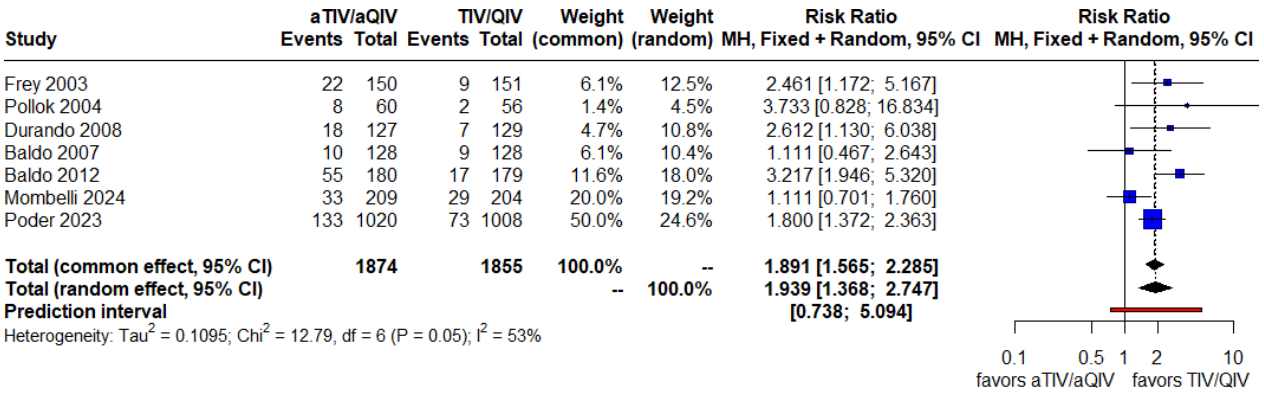


Figure S41. Relative risk of solicited arthralgia during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

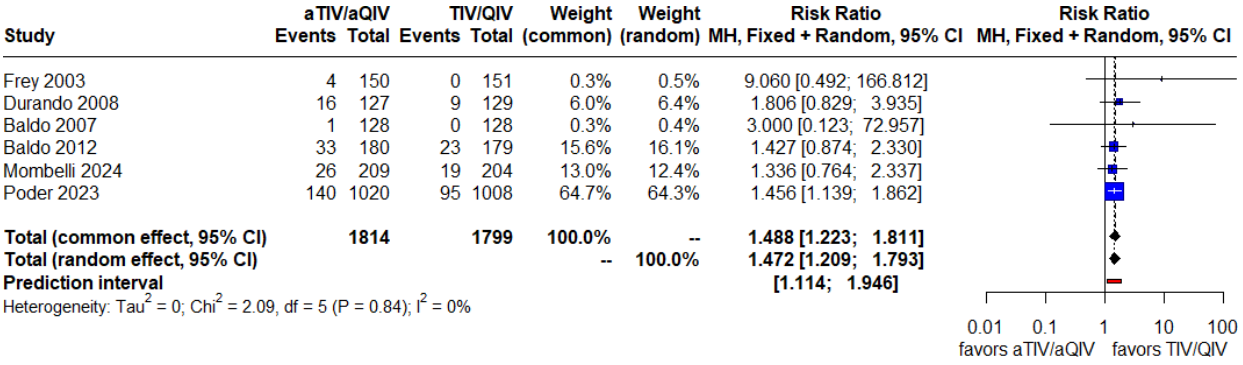


Figure S42. Relative risk of solicited headache during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

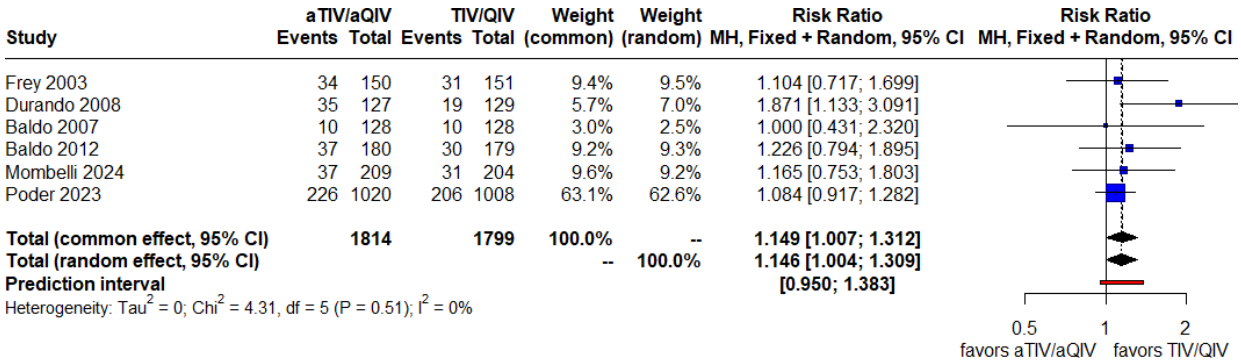


Figure S43. Relative risk of solicited malaise during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

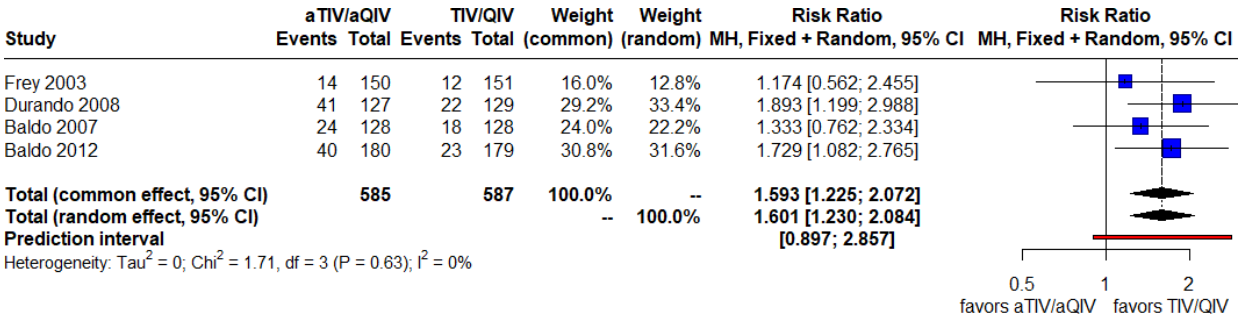


Figure S44. Relative risk of solicited nausea during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

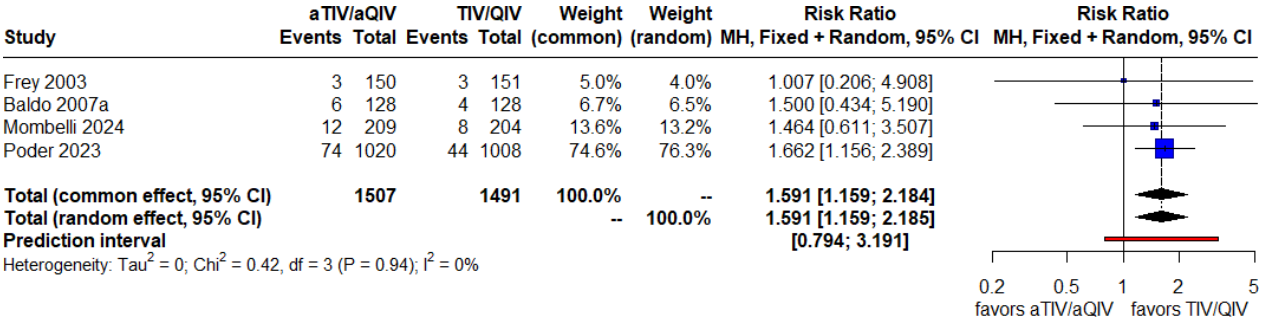


Figure S45. Frequency of serious adverse events during after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults.

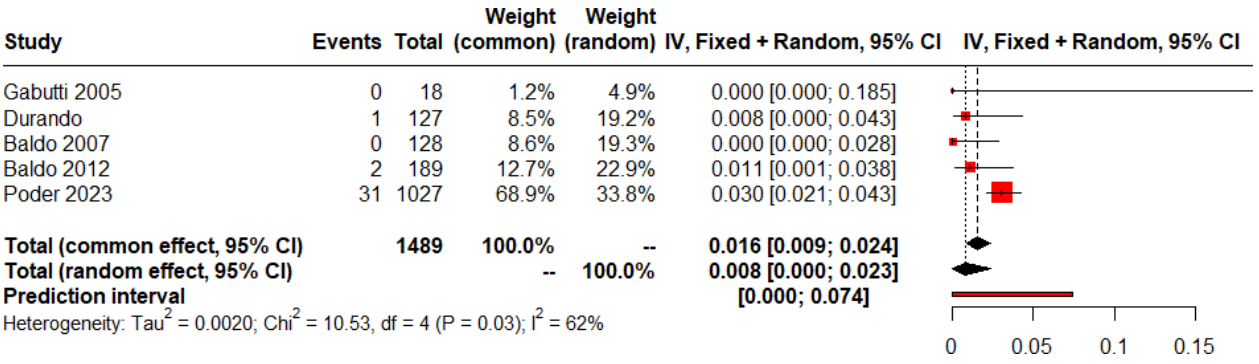


Figure S46. Relative risk of serious adverse events after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults.

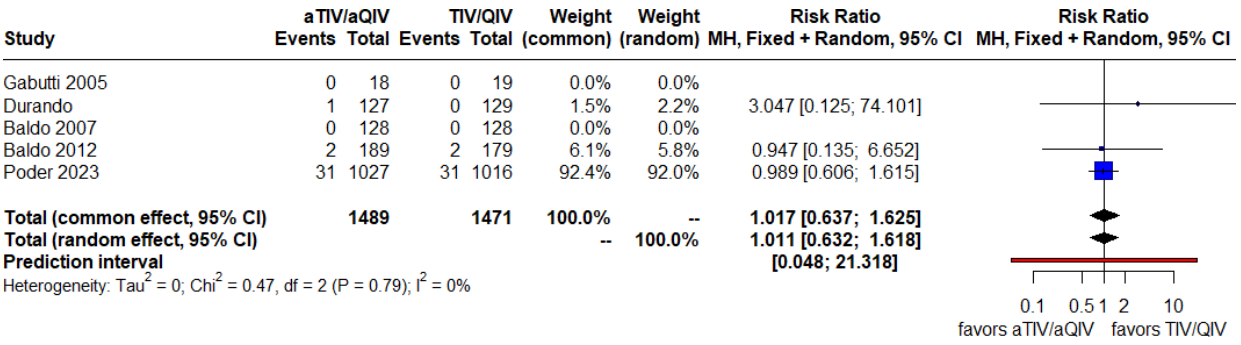


Table S1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) checklist.

Section and Topic	Item #	Checklist item	Section (§) where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction (1–3)
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction (4)
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Methods/Eligibility criteria (1–2)
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Methods/Search strategy (1–2)
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	**Table S2**
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Methods/Study selection (1)
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Methods/Data extraction and abstraction (1–2)
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Methods/Study outcomes (1–2, 4–6)
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Methods/Study outcomes (3)
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many revi ewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Methods/Risk of bias (1)
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Methods/Data synthesis (1)
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Methods/Eligibility criteria (2)
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Methods/Data extraction and abstraction (2)
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Methods/Data synthesis (1)
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Methods/Data synthesis (2)
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Methods/Data synthesis (3)
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Methods/Data synthesis (5)

Section and Topic	Item #	Checklist item	Section (§) where item is reported
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Methods/Data synthesis (4, 5)
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Results/Characteristics of the studies included (1), Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Table S3
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Results/Risk of bias (1–2), Tables S4–S5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Tables S6, S10, S12, S14
Results of syntheses	20a	For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies.	Across Results
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Tables 2–4, S11, S13; Figures S1–12, S19–S46,
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Tables 3 and S9; Figures S1–S12
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Tables S7–S8; Figures S13–S18
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Figures S13–18
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion (1–6)
	23b	Discuss any limitations of the evidence included in the review.	Discussion (8)
	23c	Discuss any limitations of the review processes used.	Discussion (7)
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion (1, 9)
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Methods/ Review protocol and reporting standards (1)
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Methods/ Review protocol and reporting standards (1)
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Methods/ Review protocol and reporting standards (1)
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Funding

Section and Topic	Item #	Checklist item	Section (§) where item is reported
Competing interests	26	Declare any competing interests of review authors.	Conflicts of interest
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Data availability statement; Supplementary materials

S2 Table. Algorithm for the research performed on 16 February 2024, by citation database.

Database	Script	Records retrieved
MEDLINE and Biological Abstracts via Ovid	1. (fluad* or MF59* or MF 59*).mp.	1431
	2. exp Influenza Vaccines/ or influenza vaccin*.mp. or ((influenza or flu*) adj5 (vaccin* or immuni* or innoculat*)).mp.	59139
	3. influenza.mp. or exp Influenza, Human/	191433
	4. exp Vaccines/ or vaccin*.mp. or exp Viral Vaccines/ or immuni*.mp. or Vaccines, Subunit/ or Vaccines, Synthetic/	1201645
	5. 3 and 4	76270
	6. exp Adjuvants, Immunologic/ or adjuvant*.mp. or squalene*.mp. or Polysorbate*.mp. or Emulsion*.mp.	537308
	7. (2 or 5) and 6	7281
	8. 1 or 7	7919
	9. exp Adult/	9410260
	10. exp Middle Aged/	4744043
	11. exp Young Adult/	1021927
	12. ((working age* or middle age*) adj3 (people* or person* or adult* or women* or men*)).tw.	705992
	13. or/9-12	9421561
	14. 8 and 13	**1549**
Web of Science	1. TS=(fluad* or MF59*)	1001
	2. TS=((influenza vaccin*) OR ((infuenza* or flu*) near/5 (vaccin* or immuni* or innoculat*)))	51467
	3. TS=influenza*	155112
	4. TS=(vaccin* or immuni*)	793783
	5. #4 AND #3	61421
	6. #5 OR #2	64362
	7. TS=(adjuvant* or squalene* or polysorbate* or emuls*)	372181
	8. #7 AND #6	5406
	9. #8 OR #1	5731
	10. TS=(working age* adult or young adult* or middle age* adult* or working age* women* or young women* or middle age* women* or working age* men* or young men* or middle age* men* or working age* people* or young people* or middle age* people*)	718653
	11. #9 AND #10	**261**
Cochrane Library	1. fluad* or MF59* or MF 59*	1872
	2. MeSH descriptor: [Influenza Vaccines] explode all trees	2104
	3. MeSH descriptor: [Influenza, Human] explode all trees	3545
	4. MeSH descriptor: [Vaccines] in all MeSH products	17306
	5. #3 AND #4	2322
	6. #2 OR #5	2963

	7. MeSH descriptor: [Adjuvants, Immunologic] explode all trees	2800
	8. #6 AND #7	312
	9. #1 OR 8	2069
	10. MeSH descriptor: [Adult] explode all trees	611867
	11. MeSH descriptor: [Middle Aged] explode all trees	404324
	12. MeSH descriptor: [Young Adult] explode all trees	95088
	13. #10 OR #11 OR #12	611867
	14. #9 AND #13	**896**
ClinicalTrials.gov	1. MF59 OR ADJUVANTED | Completed Studies | Influenza | Adult	**174**

Table S3. List of excluded studies with reasons.

Study	Reason for exclusion
Menegon 1999	Ineligible population (predominantly older adults)
Amendola 2001	Ineligible vaccine (virosomal)
Baldo 2006	Ineligible population (older adults)
Del Giudice 2006	Ineligible outcomes
Iorio 2006	Ineligible population (predominantly older adults)
Werba 2008	Ineligible population and outcomes
Herbinger 2014	Experimental formulation of the MF59-adjuvanted influenza vaccine
Kumar 2014	Redundant record (conference abstract of the included full article)
Fernández-Ruiz 2015	Ineligible outcomes; no specific data on the MF59-adjuvanted seasonal influenza vaccine
Pérez-Romero 2015	Ineligible vaccine (pandemic formulation)
Caso 2016	Ineligible outcomes
Sánchez de Prada 2020	Ineligible vaccine and population (MF59-adjuvanted seasonal influenza vaccine used only in older adults)

References:

Amendola A, Boschini A, Colzani D, Anselmi G, Oltolina A, Zucconi R, et al. Influenza vaccination of HIV-1-positive and HIV-1-negative former intravenous drug users. J Med Virol. 2001;65(4):644-8.

Baldo V, Baldovin T, Floreani A, Minuzzo M, Trivello R. Response to influenza vaccine in people with non-protective HI antibody titers. Eur J Epidemiol. 2006;21(11):843-5.

Caso F, Ramonda R, Del Puente A, Darda MA, Cantarini L, Peluso R, et al. Influenza vaccine with adjuvant on disease activity in psoriatic arthritis patients under anti-TNF-α therapy. Clin Exp Rheumatol. 2016;34(3):507-12.

Del Giudice G, Fragapane E, Bugarini R, Hora M, Henriksson T, Palla E, et al. Vaccines with the MF59 adjuvant do not stimulate antibody responses against squalene. Clin Vaccine Immunol. 2006;13(9):1010-3.

Fernández-Ruiz M, Lumbreras C, Arrazola MP, López-Medrano F, Andrés A, Morales JM, et al. Impact of squalene-based adjuvanted influenza vaccination on graft outcome in kidney transplant recipients. Transpl Infect Dis. 2015;17(2):314-21.

Herbinger KH, von Sonnenburg F, Nothdurft HD, Perona P, Borkowski A, Fragapane E, et al. A phase II study of an investigational tetravalent influenza vaccine formulation combining MF59®: adjuvanted, pre-pandemic, A/H5N1 vaccine and trivalent seasonal influenza vaccine in healthy adults. Hum Vaccin Immunother. 2014;10(1):92-9.

Iorio AM, Camilloni B, Basileo M, Guercini F, Conti S, Ferrante F, et al. Influenza vaccination in patients on long-term anticoagulant therapy. Vaccine. 2006;24(44-46):6624-8.

Kumar, D, Campbell P, Hidalgo L, Hoschler K, Al-Dabbagh M, Humar A. Randomized trial of a MF-59 adjuvanted influenza vaccine in kidney transplant recipients. Abstract# D2380. Transplantation 2014;98:767-8.

Menegon T, Baldo V, Bonello C, Dalla Costa D, Di Tommaso A, Trivello R. Influenza vaccines: antibody responses to split virus and MF59-adjuvanted subunit virus in an adult population. Eur J Epidemiol. 1999;15(6):573-6.

Pérez-Romero P, Bulnes-Ramos A, Torre-Cisneros J, Gavaldá J, Aydillo TA, Moreno A, et al. Influenza vaccination during the first 6 months after solid organ transplantation is efficacious and safe. Clin Microbiol Infect. 2015;21(11):1040.e11-8.

Sánchez de Prada L, Sanz Muñoz I, Castrodeza Sanz J, Ortiz de Lejarazu Leonardo R, Eiros Bouza JM. Adjuvanted influenza vaccines elicits higher antibody responses against the A(H3N2) subtype than non-adjuvanted vaccines. Vaccines (Basel). 2020;8(4):704.

Werba JP, Veglia F, Amato M, Baldassarre D, Massironi P, Meroni PL, et al. Patients with a history of stable or unstable coronary heart disease have different acute phase responses to an inflammatory stimulus. Atherosclerosis. 2008;196(2):835-40.

Table S4. Risk of bias (RoB) assessment of the selected randomized trials.

Study [Ref]	D1	D2	D3	D4	D5	Overall
Frey 2003 [42]	SC	Low	Low	Low	Low	SC
Magnani 2005 [43]	High	Low	Low	High	Low	High
Pollok 2004 [45]	SC	Low	High	Low	SC	High
Gabutti 2005 [46]	High	Low	Low	Low	Low	High
Durando 2008 [48]	SC	Low	Low	Low	Low	SC
Baldo 2007 [50]	Low	Low	Low	Low	Low	Low
Baldo 2012 [65]	Low	Low	Low	Low	Low	Low
Kumar 2016 [54]	Low	Low	Low	Low	Low	Low
Spensieri 2016 [56]	Low	Low	Low	Low	Low	Low
Noh 2016 [59]	Low	Low	Low	Low	Low	Low
Natori 2017 [60]	SC	Low	SC	Low	Low	SC
Mombelli 2024 [62]	Low	Low	Low	Low	Low	Low
Poder 2003 [63]	Low	Low	Low	Low	Low	Low

SC, some concerns.
D1: Bias due to randomization.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing data.
D4: Bias due to outcome measurement.
D5: Bias due to selection of reported result.

Table S5. Risk of bias (RoB) assessment of the selected non-randomized trials.

Study [Ref]	D1	D2	D3	D4	D5	D6	D7	Overall
Iorio 2003 [44]	Moderate	Moderate	Low	Low	Low	Low	Low	Moderate
Camilloni 2009 [47]	High	High	Low	Low	Low	Low	Low	High
Iorio 2012 [52]	High	High	Low	Low	Low	Low	Low	High
Fabbiani 2013 [53]	High	High	Low	Low	Low	High	Low	High
Kazmin 2023 [61]	Low	Low	Low	Low	Low	Low	Low	Low

D1: Bias due to confounding.
D2: Bias due to selection of participants.
D3: Bias in classification of interventions.
D4: Bias due to deviations from intended interventions.
D5: Bias due to missing data.
D6: Bias in measurement of outcomes.
D7: Bias due to selection of reported result.

Table S6. Extracted data on the comparison of seroconversion and seroprotection rates and geometric mean titer ratios against vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by strain, presence of immunosuppressive conditions and serological parameter.

Virus	Immuno-suppression	Seroconversion rate, % (n/N)			Seroprotection rate, % (n/N)			GMTR aTIV/aQIV vs TIV/QIV (95% CI or P)	Ref
		aTIV/aQIV	TIV/QIV	P	aTIV/aQIV	TIV/QIV	P		
A(H1N1)	No	61 (88/145)	53 (80/150)	ns	99 (144/145)[a]	95 (143/150)[a]	ns	1.12 (ns)	[42]
		53.8 (14/26)	NA	NA	96.2 (25/26)[b]	NA	NA	NA	[64]
		57.5 (69/120)	55.9 (66/118)	ns	97.5 (117/120)[b]	96.6 (114/118)[b]	ns	1.06 (0.59; 1.53)[c]	[50]
		63 (103/163)	47 (73/156)	<0.01	87 (142/163)[b]	74 (115/156)[b]	<0.01	1.96 (<0.001)	[65]
		62.5 (10/16)	NA	NA	75.0 (12/16)[b]	NA	NA	NA	[52]
		64.7 (11/17)	83.3 (15/18)	ns	100 (17/17)[b]	88.9 (16/18)[b]	ns	2.46 (ns)	[56]
		61 (49/80)	59 (42/71)	ns	94 (75/80)[b]	90 (64/71)[b]	ns	2.01 (0.005)	[48]
		67.9 (19/28)	NA	NA	100 (28/28)	NA	NA	NA	[61]
		81.2 (798/983)	76.8 (756/985)	<0.05	99.7 (1024/1027)[b]	99.2 (1008/1016)[b]	ns	1.24 (1.14; 1.34)	[63]
	Yes	76 (34/45)	55 (28/50)	0.046	89 (40/45)[b]	78 (39/50)[b]	ns	1.4 (0.7; 2.7)	[45]
		52 (23/44)	33 (13/40)	ns	70 (31/44)[b]	53 (21/40)[b]	ns	1.76 (<0.05)	[44]
		61 (11/18)	68 (13/19)	ns	83 (15/18)[b]	100 (19/19)[b]	ns	0.42 (ns)	[46]
		45 (15/33)	41 (20/49)	ns	94 (31/33)[b]	90 (44/49)[b]	ns	2.36 (0.003)	[48]
		45.2 (14/31)	48.3 (14/29)	0.81	83.9 (26/31)[b]	86.2 (25/29)[b]	0.80	1.63 (0.34)	[54]
		46.3 (31/67)	13.8 (8/58)	<0.01	85.1 (57/67)[b]	69.0 (40/58)[b]	0.05	2.23 (<0.01)	[59]
		31.4 (11/35)	21.9 (7/32)	0.38	57.1 (20/35)[b]	59.4 (19/32)[b]	0.85	1.11 (0.65)	[60]
		27 (55/205)	17 (34/198)	<0.05	85 (174/205)[b]	80 (159/198)[b]	ns	1.42 (0.005)	[62]
A(H3N2)	No	77 (112/145)	75 (113/150)	ns	94 (136/145)[a]	91 (137/150)[a]	ns	1.22 (ns)[c]	[42]
		52.5 (63/120)	33.1 (39/118)	0.02	75.0 (90/120)[b]	57.6 (68/118)[b]	0.002	2.11 (1.65; 2.54)	[50]
		77 (126/163)	60 (94/156)	<0.01	93 (152/163)[b]	78 (122/156)[b]	<0.001	2.21 (<0.001)	[65]
		50.0 (8/16)	NA	NA	87.5 (14/16)[b]	NA	NA	NA	[52]
		82.4 (14/17)	72.2 (13/18)	ns	100 (17/17)[b]	83.3 (15/18)[b]	ns	2.62 (ns)	[56]
		66 (53/80)	69 (49/71)	ns	96 (77/80)[b]	97 (69/71)[b]	ns	1.24 (ns)	[48]
		89.3 (25/28)	NA	NA	100 (28/28)[b]	NA	NA	NA	[61]
		63.6 (625/983)	61.8 (609/985)	ns	97.5 (1001/1027)[b]	97.3 (989/1016)[b]	ns	1.10 (1.002; 1.21)	[63]
	Yes	76 (34/45)	23 (29/50)	ns	84 (38/45)[b]	56 (28/50)[b]	0.003	3.5 (1.4; 8.7)	[45]
		61 (27/44)	23 (9/40)	<0.01	82 (36/44)[b]	55 (22/40)[b]	<0.05	3.03 (<0.01)	[44]
		44 (8/18)	53 (10/19)	ns	72 (13/18)[b]	74 (14/19)[b]	ns	0.68 (ns)	[46]
		54 (18/33)	45 (22/49)	ns	94 (31/33)[b]	83 (40/49)[b]	ns	1.79 (ns)	[48]
		48.4 (15/31)	34.5 (10/29)	0.28	100 (31/31)[b]	93.1 (27/29)[b]	0.23	1.13 (0.96)	[54]
		40.3 (27/67)	10.3 (6/58)	<0.01	97.0 (65/67)[b]	89.7 (52/58)[b]	0.14	1.62 (0.01)	[59]
		57.1 (20/35)	40.6 (13/32)	0.18	71.4 (25/35)[b]	71.9 (23/32)[b]	0.97	1.34 (0.27)	[60]

		52 (106/205)	35 (69/198)	<0.001	71 (146/205)[b]	62 (122/198)[b]	<0.05	1.57 (0.002)	[62]
B/Victoria	No	NA	NA	NA	96.1 (25/26)[b]	NA	NA	NA	[47]
		62 (101/163)	44 (69/156)	<0.01	67 (109/163)[b]	49 (76/156)[b]	<0.001	1.73 (<0.01)	[65]
		56.3 (9/16)	NA	NA	81.2 (13/16)[b]	NA	NA	NA	[52]
		44.5 (437/983)	40.6 (400/985)	ns	94.4 (969/1027)[b]	93.3 (948/1016)[b]	ns	1.00 (0.93; 1.07)	[63]
	Yes	72 (13/18)	53 (10/19)	ns	94 (17/18)[b]	89 (17/19)[b]	ns	0.63 (ns)	[46]
		25 (52/205)	13 (25/198)	<0.01	53 (109/205)[b]	40 (80/198)[b]	<0.05	1.30 (0.033)	[62]
B/Yamagata	No	83 (120/145)	71 (107/150)	≤0.01	99 (144/145)[a]	97 (146/150)[a]	ns	1.16 (ns)	[42]
		46.7 (56/120)	36.4 (43/118)	ns	69.2 (83/120)[b]	61.0 (72/118)[b]	ns	1.50 (1.07; 1.92)	[50]
		64 (51/80)	63 (45/71)	ns	96 (77/80)[b]	93 (66/71)[b]	ns	1.79 (0.023)	[48]
		70.6 (12/17)	61.1 (11/18)	ns	88.2 (15/17)[b]	61.1 (11/18)[b]	ns	2.59 (ns)	[56]
		53.6 (15/28)	NA	NA	57.1 (16/28)[b]	NA	NA	NA	[61]
		43.4 (427/983)	41.0 (404/985)	ns	95.9 (985/1027)[b]	94.6 (961/1016)[b]	ns	1.06 (0.99; 1.13)	[63]
	Yes	52 (23/44)	30 (12/40)	ns	70 (31/44)[b]	55 (22/40)[b]	ns	1.96 (<0.05)	[44]
		80 (36/45)	58 (29/50)	0.021	89 (40/45)[b]	90 (45/50)[b]	ns	1.2 (0.6; 2.7)	[45]
		48 (16/33)	47 (23/49)	ns	94 (31/33)[b]	90 (44/49)[b]	ns	1.57 (0.003)	[48]
		32.3 (10/31)	24.1 (7/29)	0.49	61.3 (19/31)[b]	65.5 (19/29)[b]	0.73	1.48 (0.33)	[54]
		31.3 (21/67)	6.9 (4/58)	<0.01	92.5 (62/67)[b]	84.5 (49/58)[b]	0.15	1.26 (0.18)	[59]
		37.1 (13/35)	25.0 (8/32)	0.29	57.1 (20/35)[b]	68.8 (22/32)[b]	0.32	1.24 (0.97)	[60]

[a] Hemagglutination inhibition assay titer ≥1:160; [b] Hemagglutination inhibition assay titer ≥1:40.

aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; TIV, trivalent non-adjuvanted seasonal influenza vaccine; GMTR, geometric mean titer ratio; ns, non-significant at P<0.05; NA, not available.

Table S7. Absolute seroprotection rates towards vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted seasonal influenza vaccine in non-elderly adults: A sensitivity analysis by including any hemagglutination inhibition titer threshold (both ≥ 1:40 and ≥ 1:160).

Vaccine-like strain	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
A(H1N1)	16	93.8	97.5 (96.6, 98.2)	90.7 (84.9, 95.8)
A(H3N2)	16	92.3	94.2 (93.1, 95.3)	90.9 (85.1, 95.5)
B	17	95.5	88.2 (86.7, 89.6)	82.7 (73.8, 90.1)

Table S8. Meta-analysis of absolute and relative seroconversion and seroprotection rates towards vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines: A sensitivity analysis by excluding studies with some elderly participants.

Parameter	Vaccine-like strain	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
SCR	A(H1N1)	14	89.1	72.5 (70.4, 74.7)	62.0 (55.4, 68.3)
	A(H3N2)	13	82.2	65.4 (63.1, 67.6)	65.2 (56.9, 73.1)
	B	13	91.0	52.1 (49.7, 54.4)	58.7 (49.6, 67.6)
SPR	A(H1N1)	12	92.5	98.7 (97.9, 99.3)	92.4 (86.1, 97.1)
	A(H3N2)	12	87.9	96.2 (95.1, 97.2)	92.3 (86.5, 96.7)
	B	13	92.2	90.8 (89.2, 92.2)	85.6 (77.8, 92.0)
ΔSCR	A(H1N1)	11	59.0	7.0 (4.0, 9.9)	9.2 (2.4, 16.0)
	A(H3N2)	11	74.4	6.8 (3.6, 9.9)	12.6 (4.6, 20.6)
	B	11	48.4	8.0 (4.7, 11.2)	12.0 (6.4, 17.6)
ΔSPR	A(H1N1)	11	61.8	3.4 (2.0, 4.8)	4.5 (0.9, 8.0)
	A(H3N2)	11	78.5	4.9 (3.2, 6.6)	9.6 (3.7, 15.4)
	B	11	41.7	4.2 (2.2, 6.2)	4.8 (1.3, 8.2)

FE, fixed effects; RE, random effects; SCR, seroconversion rate; SPR, seroprotection rate; ΔSCR: difference in seroconversion rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines; ΔSPR: difference in seroprotection rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines.

Table S9. Univariable meta-regression analysis to investigate sources of heterogeneity in relative seroconversion and seroprotection rates towards vaccine-like strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines.

Virus-like strain	Outcome	Predictor	Coefficient	Standard error	*P*
A(H1N1)	ΔSCR	Sample size <100	-0.072	0.071	0.31
		Low risk of bias	0.002	0.058	0.97
		Industry sponsorship	-0.017	0.057	0.77
		Overlapping population	-0.019	0.070	0.79
		Immunosuppressed population	0.079	0.048	0.10
	ΔSPR	Sample size <100	-0.026	0.049	0.60
		Low risk of bias	0.005	0.033	0.88
		Industry sponsorship	0.034	0.035	0.32
		Overlapping population	-0.019	0.047	0.69
		Immunosuppressed population	0.016	0.032	0.48
A(H3N2)	ΔSCR	Sample size <100	0.056	0.079	0.48
		Low risk of bias	0.049	0.068	0.47
		Industry sponsorship	-0.081	0.063	0.19
		Overlapping population	0.036	0.086	0.67
		Immunosuppressed population	0.121	0.056	0.030
	ΔSPR	Sample size <100	0.024	0.059	0.41
		Low risk of bias	0.013	0.050	0.79
		Industry sponsorship	0.007	0.051	0.90
		Overlapping population	-0.026	0.063	0.68
		Immunosuppressed population	0.049	0.046	0.29
B	ΔSCR	Sample size <100	0.034	0.065	0.60
		Low risk of bias	0.001	0.050	0.99
		Industry sponsorship	-0.047	0.046	0.30
		Overlapping population	0.000	0.059	0.99
		Immunosuppressed population	0.075	0.042	0.071
	ΔSPR	Sample size <100	0.007	0.056	0.91
		Low risk of bias	0.054	0.038	0.15
		Industry sponsorship	-0.029	0.037	0.44
		Overlapping population	0.007	0.056	0.91
		Immunosuppressed population	0.003	0.039	0.95

SCR, seroconversion rate; SPR, seroprotection rate; ΔSCR: difference in seroconversion rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines; ΔSPR: difference in seroprotection rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines.

Table S10. Extracted data on the comparison of seroconversion and seroprotection rates and geometric mean titer ratios against heterologous strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by strain, presence of immunosuppressive conditions and serological parameter.

Vaccine strain (subtype/lineage)	Heterologous strain (subtype/lineage)	Seroconversion rate, % (*n/N*)			Seroprotection rate, % (*n/N*) [a]			GMTR aTIV/aQIV vs. TIV/QIV (*p*)	Ref
		aTIV	TIV	*p*	aTIV/aQIV	TIV/QIV	*p*		
A/New Caledonia/20/1999 (H1N1)	A/Italy/05/2009 (H1N1pdm09)	19.2 (5/26)	NA	NA	30.7 (8/26)	NA	NA	NA	[64]
A/Solomon Islands/3/2006 (H1N1)	A/Italy/05/2009 (H1N1pdm09)	12.5 (2/16)	NA	NA	12.5 (2/16)	NA	NA	NA	[52]
A/New Caledonia/20/1999 (H1N1)	A/Solomon Islands/3/2006 (H1N1)	48 (78/163)	35 (55/156)	0.018	67 (109/163)	56 (87/156)	ns	1.62 (0.013)	[65]
A/California/7/2009 (H1N1pdm09)	A/New Caledonia/20/1999 (H1N1)	9.7 (3/31)	24.1 (7/29)	0.18	90.3 (28/31)	86.2 (25/29)	0.62	1.33 (0.48)	[55]
A/New York/55/2004 (H3N2)	A/Wisconsin/67/2005 (H3N2)	67.5 (81/120)	50.8 (60/118)	0.008	79.2 (95/120)	61.0 (72/118)	0.002	1.58 (0.001)	[51]
A/Wisconsin/67/2005 (H3N2)	A/California/7/2004 (H3N2)	74 (121/163)	66 (103/156)	ns	96 (156/163)	91 (142/156)	ns	1.90 (<0.001)	[65]
A/Victoria/361/ 2011 (H3N2)	A/Texas/50/2012 (H3N2)	41.9 (13/31)	41.4 (12/29)	0.97	83.9 (26/31)	72.4 (21/29)	0.28	1.24 (0.71)	[55]
B/Jiangsu/10/2003 (B/Yamagata)	B/Malaysia/2506/2004 (B/Victoria)	10.0 (12/120)	9.3 (11/118)	ns	39.2 (47/120)	42.4 (50/118)	ns	1.03 (ns)	[51]
B/Malaysia/2506/2004 (B/Victoria)	B/Shanghai/361/2002 (B/Yamagata)	40 (65/163)	35 (55/156)	ns	62 (101/163)	53 (83/156)	ns	1.45 (0.022)	[65]
B/Wisconsin/1/2010 (B/Yamagata)	B/Brisbane/60/2008 (B/Victoria)	16.1 (5/31)	10.3 (3/29)	0.71	41.9 (13/31)	34.5 (10/29)	0.55	1.05 (0.98)	[55]
B/Hong Kong/330/2001 (B/Victoria)	B/Malaysia/2506/2004 (B/Victoria)	NA	NA	NA	92.3 (24/26)	NA	NA	NA	[47]
B/Hong Kong/330/2001 (B/Victoria)	B/Sichuan/379/1999 (B/Yamagata)	NA	NA	NA	88.5 (23/26)	NA	NA	NA	[47]

B/Hong Kong/330/2001 (B/Victoria)	B/Shanghai/361/2002 (B/Yamagata)	NA	NA	NA	38.5 (10/26)	NA	NA	NA	[47]

[a]Hemagglutination inhibition titer ≥ 1:40.
aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; TIV, trivalent non-adjuvanted seasonal influenza vaccine; GMTR, geometric mean titer ratio; ns, non-significant at P<0.05; NA, not available.

Table S11. Meta-analysis of relative seroconversion and seroprotection rates towards heterologous strains 3–4 weeks after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by heterologous strain.

Parameter	Heterologous strain	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
ΔSCR	A(H1N1)	2	83.4	8.3 (-1.2, 17.8)	0.2 (-26.2, 26.2)
	A(H3N2)	3	0.0	10.7 (3.2, 18.2)	10.6 (3.2, 18.0)
	B	3	0.0	3.2 (-3.2, 9.6)	2.4 (-3.3, 8.2)
ΔSPR	A(H1N1)	2	0.0	10.0 (0.7, 19.3)	9.0 (0.1, 17.9)
	A(H3N2)	3	55.6	10.5 (4.9, 16.1)	10.2 (0.5, 19.9)
	B	3	4.8	4.0 (-0.4, 11.8)	3.9 (-5.4, 13.2)

FE, fixed effects; RE, random effects; ΔSCR: difference in seroconversion rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines; ΔSPR: difference in seroprotection rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines.

Table S12. Extracted data on the comparison of seroprotection rates and geometric mean titer ratios towards vaccine-like strains 3–9 months after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by strain, immunosuppression status and time post-vaccination.

Virus	Time, months	Immunosuppression	Seroprotection rate, % (*n/N*)			GMTR aTIV/aQIV vs. TIV/QIV (95% CI or *p*)	Ref
			aTIV/aQIV	TIV/QIV	*p*		
A(H1N1)	3	No	93 (69/74)[a]	91 (60/66)[a]	ns	1.81 (NR)	[48]
	3	Yes	90 (28/31)[a]	81 (39/48)[a]	ns	1.72 (NR)	[48]
	6	No	73 (NR)[b]	57 (NR)[b]	0.025	NR	[42]
	6	Yes	50 (9/18)[a]	74 (14/19)[a]	ns	NR	[46]
	6	No	100 (14/14)	71.4 (10/14)	ns	2.56 (<0.05)	[56]
	6	Yes	65.5 (38/58)	57.7 (30/52)	0.40	1.55 (0.08)	[59]
	6	Yes	80 (153/192)	73 (140/191)	ns	1.43 (0.005)	[62]
	6	No	98.2 (1009/1027)	96.3 (978/1016)	<0.05	1.15 (1.06; 1.25)	[63]
	9	No	NR	NR	NR	1.12 (1.02; 1.23)	[63]
A(H3N2)	3	No	95 (70/74)[a]	98 (65/66)[a]	ns	0.78 (NR)	[48]
	3	Yes	90 (28/31)[a]	83 (40/48)[a]	ns	1.13 (NR)	[48]
	6	No	NR[b]	NR[b]	ns	NR	[42]
	6	Yes	55 (10/18)[a]	47 (9/19)[a]	ns	NR	[46]
	6	No	92.9 (13/14)	71.4 (10/14)	ns	2.56 (ns)	[56]
	6	Yes	89.7 (52/58)	84.6 (44/52)	0.43	1.24 (0.31)	[59]
	6	Yes	60 (116/192)	52 (99/191)	ns	1.16 (0.26)	[62]
	6	No	92.0 (945/1027)	89.9 (913/1016)	ns	1.05 (0.97; 1.14)	[63]
	9	No	NR	NR	NR	1.06 (0.98; 1.14)	[63]
B/Victoria	6	Yes	50 (9/18)[a]	58 (11/19)[a]	ns	NR	[46]
	6	Yes	43 (83/192)	32 (61/191)	<0.05	1.21 (0.10)	[62]
	6	No	83.4 (857/1027)	84.3 (856/1016)	ns	0.97 (0.91; 1.04)	[63]
	9	No	NR	NR	NR	0.98 (0.92; 1.03)	[63]
B/Yamagata	3	No	95 (70/74)[a]	94 (62/66)[a]	ns	1.26 (NR)[c]	[48]
	3	Yes	90 (28/31)[a]	90 (43/48)[a]	ns	0.79 (NR)[c]	[48]
	6	No	NR[b]	NR[b]	ns	NR	[42]
	6	No	64.3 (9/14)	42.9 (6/14)	ns	2.62 (<0.05)	[56]
	6	Yes	77.6 (45/58)	76.9 (40/52)	0.93	1.18 (0.32)[c]	[59]
	6	No	83.9 (862/1027)	84.0 (853/1016)	ns	0.99 (0.93; 1.05)[d]	[63]
	9	No	NR	NR	NR	0.98 (0.93; 1.03)[d]	[63]

[a] HAI titer ≥1:40; [b] HAI titer ≥1:160.

aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; TIV, trivalent non-adjuvanted seasonal influenza vaccine; GMTR, geometric mean titer ratio; ns, non-significant at $p < 0.05$; NR, not reported.

Table S13. Meta-analysis of absolute and relative seroprotection rates towards vaccine-like strains six months after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by strain and time post-vaccination.

Parameter	Vaccine-like strain	k	I^2, %	FE model, % (95% CI)	RE model, % (95% CI)
SPR	A(H1N1)	6	97.5	95.1 (93.8, 96.2)	82.0 (63.4, 95.3)
	A(H3N2)	5	96.4	89.1 (87.2, 90.8)	80.5 (63.0, 93.6)
	B	5	96.9	78.3 (75.9, 80.6)	65.4 (47.4, 81.5)
ΔSPR	A(H1N1)	6	65.7	4.1 (2.1, 6.2)	6.9 (-0.2, 14.0)
	A(H3N2)	5	0.0	3.5 (1.0, 6.1)	4.2 (-0.3, 8.6)
	B	5	43.0	1.2 (-1.8, 4.2)	3.3 (-4.2, 10.8)

FE, fixed effects; RE, random effects; SPR, seroprotection rate; ΔSPR: difference in seroprotection rates between subjects immunized with adjuvanted vs non-adjuvanted influenza vaccines.

Table S14. Extracted data on the comparison of local/injection site and systemic solicited adverse reactions during the first week after one dose of the MF59-adjuvanted or non-adjuvanted seasonal influenza vaccines in non-elderly adults, by adverse reaction.

Adverse reaction		**Study [Ref]**							
		Frey 2003 [42][a]		**Pollok 2004 [45][a]**		**Gabutti 2005 [46][b]**		**Durando 2008 [48][b]**	
		aTIV (*N*=150)	**TIV (*N*=151)**	**aTIV (*N*=60)**	**TIV (*N*=56)**	**aTIV (*N*=18)**	**TIV (*N*=19)**	**aTIV (*N*=127)**	**TIV (*N*=129)**
Local	Any	NA	NA	NA	NA	27.8 (5)	10.5 (2)	NA	NA
	Pain	90.0 (135)	63.6 (96)	52 (31)	20 (11)	NA	NA	47.2 (60)	21.7 (28)
	Erythema	19.3 (29)	21.9 (33)	NA	NA	NA	NA	7.1 (9)	3.9 (5)
	Induration	22.0 (33)	17.2 (26)	NA	NA	NA	NA	20.5 (26)	7.8 (10)
	Ecchymosis	NA	NA	NA	NA	NA	NA	2.4 (3)	0.8 (1)
Systemic	Any	NA	NA	NA	NA	5.6 (1)	10.5 (2)	NA	NA
	Fever	0.7 (1)	0 (0)	NA	NA	5.6 (1)	10.5 (2)	26.8 (34)	4.7 (6)
	Chills	4.7 (7)	0.7 (1)	NA	NA	NA	NA	NA	NA
	Myalgia	14.7 (22)	6.0 (9)	14 (8)	4 (2)	NA	NA	14.2 (18)	5.4 (7)
	Arthralgia	2.7 (4)	0 (0)	NA	NA	NA	NA	12.6 (16)	7.0 (9)
	Headache	22.7 (34)	20.5 (31)	NA	NA	NA	NA	27.6 (35)	14.7 (19)
	Malaise	9.3 (14)	7.9 (12)	NA	NA	NA	NA	32.3 (41)	17.1 (22)
	Nausea	2.0 (3)	2.0 (3)	NA	NA	NA	NA	NA	NA
	Rash	0 (0)	0.7 (1)	NA	NA	NA	NA	NA	NA
Adverse reaction		**Study [Ref]**							
		Baldo 2007 [50][a]		**Baldo 2012 [65][a]**		**Kumar 2016 [54][a]**		**Natori 2017 [60][a]**	
		aTIV (*N*=128)	**TIV (*N*=128)**	**aTIV (*N*=180)**	**TIV (*N*=179)**	**aTIV (*N*=31)**	**TIV (*N*=31)**	**aTIV (*N*=35)**	**TIV (*N*=38)**
Local	Any	46.9 (60)	24.2 (31)	49 (89)	28 (50)	NA	NA	NA	NA
	Pain	28.9 (37)	8.6 (11)	45 (81)	20 (36)	77.4 (24)	51.6 (16)	NA	NA
	Erythema	12.5 (16)	4.7 (6)	4 (7)	8 (14)	9.7 (3)	3.2 (1)	NA	NA
	Induration	10.2 (13)	7.8 (10)	13 (24)	8 (15)	6.5 (2)	6.5 (2)	NA	NA
	Ecchymosis	3.1 (4)	2.3 (3)	7 (13)	8 (14)	NA	NA	NA	NA
Systemic	Any	25.8 (33)	18.8 (24)	49 (89)	28 (50)	NA	NA	NA	NA
	Fever	3.9 (5)	3.9 (5)	1 (1)	0 (0)	0 (0)	0 (0)	5.7 (2)	2.6 (1)
	Chills	7.8 (10)	6.3 (8)	8 (15)	9 (17)	NA	NA	NA	NA
	Myalgia	7.8 (10)	7.0 (9)	31 (55)	9 (17)	NA	NA	NA	NA
	Arthralgia	0.8 (1)	0 (0)	18 (33)	13 (23)	NA	NA	NA	NA
	Headache	7.8 (10)	7.8 (10)	21 (37)	17 (30)	NA	NA	NA	NA
	Malaise	18.8 (24)	14.1 (18)	22 (40)	13 (23)	NA	NA	NA	NA
	Nausea	4.7 (6)	3.1 (4)	NA	NA	NA	NA	NA	NA
	Rash	0 (0)	0 (0)	NA	NA	NA	NA	NA	NA
Adverse reaction									

		Kazmin 2023 [61][c]	Mombeli 2024 [62][a]			Poder 2023 [63][a]	
		aTIV (*N*=31)	aTIV (*N*=209)	QIV (*N*=204)	hdTIV (*N*=203)	aQIV (*N*=1,020)	QIV (*N*=1,008)
Local	Any	NA	NA	NA	NA	49.8 (508)	30.4 (306)
	Pain	16 (5)	51 (106)	22 (45)	41 (84)	47.1 (480)	28.1 (283)
	Erythema	NA	12 (26)	8 (16)	11 (23)	7.8 (80)	3.1 (31)
	Induration	NA	NA	NA	NA	7.9 (81)	3.5 (35)
	Ecchymosis	NA	NA	NA	NA	0.6 (6)	0.6 (6)
Systemic	Any	NA	NA	NA	NA	45.3 (462)	40.0 (403)
	Fever	0 (0)	6 (12)	2 (5)	7 (15)	2.5 (26)	1.7 (17)
	Chills	NA	NA	NA	NA	6.6 (67)	5.5 (55)
	Myalgia	NA	16 (33)	14 (29)	19 (38)	13.0 (133)	7.2 (73)
	Arthralgia	NA	12 (26)	9 (19)	10 (21)	13.7 (140)	9.4 (95)
	Headache	NA	18 (37)	15 (31)	25 (50)	22.2 (226)	20.4 (206)
	Malaise	NA	NA	NA	NA	NA	NA
	Nausea	NA	6 (12)	4 (8)	11 (22)	7.3 (74)	4.4 (44)
	Rash	NA	NA	NA	NA	NA	NA

[a]Solicited adverse reactions were collected during the first seven days post-vaccination; [b]Solicited adverse reactions were collected during the first four days post-vaccination; [c]Solicited adverse reactions were collected during the first three days post-vaccination;
aQIV, quadrivalent MF59-adjuvanted seasonal influenza vaccine; aTIV, trivalent MF59-adjuvanted seasonal influenza vaccine; hdTIV, high-dose seasonal influenza vaccine; QIV, quadrivalent non-adjuvanted seasonal influenza vaccine; TIV, trivalent non-adjuvanted seasonal influenza vaccine; NA, not available.