Supplementary File 1. A systematic review of the use of adjuvanted trivalent inactivated vaccine in the elderly

The *objective* of the present review was to provide an up-to-date summary of the immunogenicity, effectiveness and safety of the adjuvanted trivalent influenza vaccine (aTIV) in the elderly population.

Methods

Study outcomes and endpoints. The study objective was conceptualized by means of the PICOS (patients, intervention, comparator, outcomes and study design) tool and was formulated as follows:

- *P*: subjects aged ≥65 years (henceforth referred to as "elderly");
- *I*: active immunization with aTIV;
- *C*: any other seasonal influenza vaccine or non-vaccination/non-influenza vaccine;
- *O*: various (see below);
- *S*: randomised controlled trails (RCTs); cohort and case-control observational studies and their variations; previously published systematic reviews and/or meta-analyses.

A variety of study outcomes were considered; the outcomes were conceptually divided into five groups:

- Absolute (i.e. versus non-vaccination or non-influenza vaccine) immunogenicity of aTIV;
- Relative (i.e. versus any active comparator) immunogenicity of aTIV;
- Absolute (i.e. versus non-vaccination or non-influenza vaccine) efficacy and effectiveness of aTIV;
- Relative (i.e. versus active comparator) efficacy and effectiveness of aTIV;
- Frequency of adverse events following immunization with aTIV.

Relative measures considered only the influenza vaccines currently available in Italy, i.e. unadjuvanted trivalent (TIV) and quadrivalent (QIV) influenza vaccines.[1]

Previously published systematic reviews and/or meta-analyses and primary research were summarized separately. Any serological assay [e.g. haemagglutination inhibition (HI), neutralisation (NT) and single-radial haemolysis (SRH)][2] was potentially eligible for the immunogenicity outcome assessment. The following endpoints relative to absolute immunogenicity were potentially eligible: mean fold increase (MFI, defined as average ratio in geometric mean titres (GMTs) between post-dose and pre-dose values), seroconversion rate [SCR, defined as proportion of vaccinees with ≥4-fold increase in HI titres following vaccination, or a post-vaccination HI titre ≥40 in seronegative subjects (HI titre <10)] and seroprotection rate (SPR, defined as proportion of vaccinees with a post-vaccination HI titre ≥40).[3] The MFIs, SCRs and SPRs extracted were compared against the criteria for seasonal influenza vaccines issued by the Committee for Medicinal Products for Human Use (CHMP)[3]. Briefly, in the elderly, MFI, SCR and SPR should be >2, >30% and >60%, respectively.

Analogously, relative immunogenicity was calculated as the geometric mean ratio (GMR, defined as ratio of GMTs following a dose of aTIV and active comparator), ΔSCR (defined as difference in SCRs between aTIV and active comparator) and ΔSPR (defined as difference in SPRs between aTIV and active comparator).

Studies reporting immunogenicity endpoints at different times following vaccination were described separately. The immune response versus homologous (vaccine-like) and heterovariant/drifted A(H1N1), A(H3N2) and B strains were also described separately.

With regard to effectiveness, the following endpoints were eligible:[4]

- Laboratory-confirmed influenza (diagnosed by polymerase chain reaction or culture);
- Influenza-like illness;
- Hospitalisation for influenza and/or pneumonia;
- Hospitalisation for any respiratory disease;
- Hospitalisation for acute cardio- or cerebrovascular events;
- Mortality due to influenza and/or pneumonia;
- Mortality due to any respiratory disease;
- Mortality due to acute cardio- or cerebrovascular events;
- All-cause mortality.

Adverse events comprised both local (pain, erythema, induration) and systemic (fever ≥38°C, chills, malaise, myalgia, arthralgia, headache) events.

Search strategy. A comprehensive search strategy was constructed on the basis of Jefferson et al.[4] and the National Advisory Committee on Immunization (NACI)[5] and is reported in Table S1. The search was performed in OVID by using the following databases:

- Embase;
- Ovid MEDLINE® Daily and Ovid MEDLINE®;
- EBM Reviews – Cochrane Database of Systematic Reviews;
- EBM Reviews – Database of Abstracts of Reviews of Effects;
- EBM Reviews – Cochrane Central Register of Controlled Trials;
- EBM Reviews – Cochrane Methodology Register;
- EBM Reviews – Health Technology Assessment;
- EBM Reviews – NHS Economic Evaluation Database.

Given that the first clinical trials of aTIV were conducted in the early 1990's,[6] the search was limited to the period starting from 01/01/1990 and was initially conducted on 7/12/2016 by DA. No other restrictions were applied. The search was then updated on 5/01/2017 and 12/10/2018.

The so-called "grey literature" and unpublished reports were searched in Google and Google Scholar by using a simpler search string (i.e. "MF59" OR "MF 59" OR "Fluad"). Finally, a standard manual reference check of the studies included was performed.

Study selection and extraction. Following the removal of duplicates, records with clearly irrelevant titles and/or abstracts were removed. The remaining set underwent full text analysis, in which a number of inclusion and exclusion criteria were applied. The inclusion criteria were formulated in accordance with the PICOS tool and are described earlier in the text. The following exclusion criteria were applied:

- Case reports, narrative reviews and redundant publications;
- Animal studies;
- Number of vaccinees with aTIV <10;

- Modelling studies with no original data;
- Studies investigating immunogenicity, effectiveness or safety of the paediatric aTIV formulation, aTIV formulations not commercially available (e.g. with different amounts of antigen and/or adjuvant), pandemic MF59®-adjuvanted monovalent vaccine, other adjuvanted (e.g. historically available virosomal vaccine) and quadrivalent MF59®-adjuvanted vaccine;
- Studies without separate data for subjects of different ages (e.g. elderly and non-elderly adults pooled together) or vaccinated with different vaccines (e.g. aTIV and TIV recipients pooled together).

However, regarding this last exclusion criterion, if >90% of the study participants belonged to a group of interest, the study was included.

Data were extracted to an *ad hoc* spreadsheet by two investigators, each working independently (DP and DA). The following information was extracted:

- Authors and year of publication;
- Study design (see above);
- Country and hemisphere;
- Age of vaccinees;
- Sample size;
- Institutionalisation (community-dwelling, institutionalised elderly, mixed);
- Comparators used;
- Outcomes of interest (see above);
- Other potentially relevant information.

If data were not readily extractable (i.e. data were presented by means of charts without data labels), they were extracted by means of the WebPlotDigitizer software.[7]

Results

Study selection process and characteristics of the papers included. The initial search identified a total of 2,388 papers; of these, 46 met the inclusion criteria. Another seven records were found through manual search. An updated search identified five further papers. In sum, a total of 58 records[8-67] were included in the present report. The whole process of study selection is depicted in Fig. S1.

Nine papers[8-16] were classified as previously published systematic reviews and/or meta-analyses. Moreover, a briefing document submitted by the Sponsor to the Food and Drug Administration (FDA) Advisory Committee[17] was equated to the former category, as it contained unpublished data. The remaining 48 papers[18-65] were classified as primary experimental or observational research; their main characteristics are reported in Table S2.

Overview of previously published systematic reviews. Martin[8] reported that aTIV met all the CHMP criteria regarding MFI for all three strains 28 days after vaccination [2.75, 6.68, 3.47 for A(H1N1), A(H3N2) and B, respectively] and for two out of three strains on day 180 post-vaccination (1.68, 3.24, 2.03 for A(H1N1), A(H3N2) and B, respectively), indicating a sufficient persistence of antibody titres. Camilloni et al.[14] systematically collected data on immunogenicity toward both homologous and heterovariant strains in subjects vaccinated with aTIV or intradermal split vaccine. Specifically, they ascertained that, in elderly subjects vaccinated with aTIV, the values of MFI, SCR and SPR fulfilled the

CHMP criteria. Moreover, the authors concluded that aTIV was generally associated with higher immunogenicity than non-adjuvanted vaccines in the elderly. Concerning GMR parameters, five studies compared aTIV with non-adjuvanted vaccines.[8-10,12,17] aTIV proved significantly superior in 100% of cases with regard to the A(H3N2) and B strains, and in 50% of cases with regard to the A(H1N1) strains. In the meta-analysis of 16 first-dose RCTs[17], aTIV was associated with significantly higher GMTs than TIV [GMRs of 1.15 (95% CI: 1.01–1.31), 1.30 (95% CI: 1.18–1.44), 1.23 (95% CI: 1.15–1.31) for A(H1N1), A(H3N2) and B, respectively]. Analogously, more aTIV recipients seroconverted [ΔSCR of 9.5% (95% CI: 5.2–13.9%), 10.5% (95% CI: 6.6–14.5%), 12.7% (95% CI: 8.6–16.8%) for A(H1N1), A(H3N2) and B, respectively]. Similar and statistically significant results were also reported for what concerns heterovariant strains, regardless of (sub)type.[17]

aTIV has also been found to be more immunogenic in elderly subjects with at least one underlying chronic disease than in healthy elderly subjects.[10] The meta-analysis by Podda[9] reported higher GMR values after re-vaccination (2nd and 3rd doses).

On clinical follow-up during the various influenza seasons,[1] aTIV proved to be more effective than conventional vaccines in reducing mortality {Relative Risk [RR] 0.39 (95% CI: 0.17–0.91)}. Moreover, in subjects vaccinated with aTIV, lower rates of hospitalisation for all causes [RR 0.87 (95% CI: 0.52–1.44)] and for cardiac [RR 0.72 (95% CI: 0.24–2.13)] and pulmonary diseases [RR 0.42 (95% CI: 0.04–4.60)] were observed, though the differences did not reach statistical significance.

The absolute and relative effectiveness of aTIV against various outcomes related to influenza has recently been analysed by Domnich et al.[15] Their analysis of 11 case-control and cohort studies (546,015 persons-season, 52.3% of whom vaccinated with aTIV) revealed that the absolute vaccine effectiveness (adjusted) was above 50%, regardless of the outcome considered. In particular, their pooled estimate was 60% (95% CI: -1–84%, *P*=0.054) and 51% (95% CI: 39–61%, *P*=<0.05) for laboratory-confermed influenza and hospitalization for pneumonia/influenza, respectively. Moreover, they concluded that aTIV was generally seen to display greater effectiveness in the field than traditional non-adjuvanted vaccines.[15]

With regard to unsolicited adverse events, the primary source of information was constituted by the integrated analysis of 64 clinical studies conducted by Pellegrini et al.[11] The adverse events reported in elderly subjects were significantly less frequent in those vaccinated with aTIV [adjusted RR: 0.73 (95% CI: 0.66–0.81)]; specifically, during the study period, the authors observed a 42% reduction in cardiovascular events [adjusted RR: 0.58 (95% CI: 0.47– 0.73)], a 27% reduction in relapses of chronic diseases [adjusted RR: 0.73 (95% CI: 0.59–0.91)] and a 30% reduction in mortality [adjusted RR: 0.70 (95% CI: 0.54–0.91)]. A 9% reduction in hospitalizations was also recorded in subjects vaccinated with aTIV, though this did not reach statistical significance [adjusted RR: 0.91 (95% CI: 0.81–1.02)]. Finally, another noteworthy finding was the low frequency of adverse events of autoimmune origin, which occurred in 0.71 per 1,000 vaccinees in the aTIV group and in 0.67 per 1,000 in those vaccinated with the traditional vaccines. In general, it emerged that aTIV was, on average, more reactogenic than non-adjuvanted vaccines. Indeed, the above-mentioned integrated analysis by Pellegrini et al.[11] showed that elderly subjects vaccinated with aTIV (*N*=4,109) had a higher risk of suffering unsolicited adverse events, whether local [weighted RR: 1.74 (95% CI: 1.57–1.94)] or systemic [weighted RR: 1.29 (95% CI: 1.10–1.52)]. The same trend was also observed in the report submitted to the FDA[17] [local events: adjusted RR of 1.85 (95% CI: 1.73–1.98); systemic events: adjusted RR of 1.23 (95% CI: 1.15–1.32)]. By contrast, the meta-analysis conducted by Beyer et al.[12] found a higher RR (aTIV vs subunit, split or virosomal vaccines)

with regard to injection-site pain [2.12 (95% CI: 1.65–2.71)] but not headache [1.07 (95% CI: 0.87–1.31)]. Ruiz-Aragon et al.[13] reported that 7 of 11 (63.6%) and 2 of 9 (28.6%) of the studies included in their systematic review had found higher odds ratios (ORs) in subjects vaccinated with aTIV than in those vaccinated with other types of vaccine (including split, subunit and virosomal vaccines) in local and systemic adverse events. Of the seven above-mentioned systematic reviews, five [1-3,6,8] reported the frequencies of the solicited adverse events preselected in the present systematic review. The results of these five studies were similar. Furthermore, it should be mentioned that the same pattern of local and systemic adverse reactions was observed after re-vaccination (2^{nd}/3^{rd} doses) both in subjects vaccinated with aTIV and in those who had received traditional vaccines.[9]

In terms of severity, the FDA report[17] revealed a good level of safety of aTIV, in that about 75% of solicited adverse events were mild and the frequency of severe events was very low. The results of the pooled analysis indicated that the frequency of severe adverse events was similar in subjects vaccinated with aTIV (6.7%, 384/5,754) and in those who received TIV (7.0%, 366/5,198), the RR being 0.95 (95% CI: 0.82–1.09). The most frequent severe adverse events were: pneumonia (aTIV: 0.7%; TIV: 0.8%), congestive heart failure (aTIV: 0.3%; TIV: 0.5%), acute myocardial infarction (aTIV: 0.2%; TIV: 0.3%), acute cerebrovascular events (aTIV: 0.2%; TIV: 0.3%), chronic obstructive pulmonary disease (aTIV: 0.2%; TIV: 0.3%), hypertension (aTIV: 0.2%; TIV: 0.2%), osteoarthritis (aTIV: 0.2%; TIV: 0.2%) and atrial fibrillation (aTIV: 0.1%; TIV: 0.2%).[17]

Finally, the most recent systematic review and meta-analysis by Baay et al.[16] confirmed the previous systematic evidence. Specifically, no difference emerged between aTIV and control groups with regard to serious adverse events [RR 1.00 (95% CI: 0.84–1.18)] and unsolicited adverse events [RR 1.00 (95% CI: 0.90–1.12)]. The rate of solicited adverse events in the aTIV group was significantly higher only with regard to pain [RR 1.67 (95% CI: 1.29–2.15)], but not redness [RR 1.17 (95% CI: 0.82–1.66)], swelling [RR 1.57 (95% CI: 0.82–3.01)], fatigue [RR 1.01 (95% CI: 0.85–1.20)], fever [RR 1.41 (95% CI: 0.83–2.40)], headache [RR 0.93 (95% CI: 0.76–1.13)] or myalgia [RR 1.23 (95% CI: 0.91–1.67)].

Absolute immunogenicity in primary research studies. aTIV was generally highly immunogenic against vaccine-like strains (Table S3). Specifically, the MFI in HI was >2 in 94%, 100% and 88% of studies against A(H1N1), A(H3N2) and B strains, respectively. SCRs satisfied the CHMP criterion in 85%, 100% and 74% of studies against A(H1N1), A(H3N2) and B strains, respectively. Similarly, SPRs were >60% in 85%, 100% and 74% of studies against A(H1N1), A(H3N2) and B strains, respectively.

Heterovariant immune responses were assessed in several studies (Tables S4–S6); most of the heterovariant strains tested belonged to A(H3N2) strains. The absolute immune response measured in both HI and NT was generally high and satisfied the CHMP criteria in most instances.

Various studies have also evaluated antibody persistence among aTIV vaccinees up to one year following immunisation. In particular, it has been demonstrated[18] that 360 days after a dose of aTIV, MFI was still >2 for all the vaccine-like strains, being 2.2, 2.4 and 2.5 for A(H1N1), A(H3N2) and B, respectively. Scheifele et al.[53] established that SPRs against A(H1N1) and A(H3N2) were still >60% six months after vaccination. Analogous results were obtained by Seo et al.[55] and Levin et al.[59] six and three months, respectively, after a dose of aTIV.

aTIV has been found to be highly immunogenic in subjects with concomitant chronic diseases,[49] statin users,[52] those affected by chronic obstructive pulmonary disease on steroid treatment (both local and systemic)[29] and those with chronic kidney disease undergoing haemodialysis.[62]

The co-administration of aTIV with either 13-valent pneumococcal conjugate vaccine (PCV13)[64] or 23-valent pneumococcal polysaccharide vaccine (PPV23)[61] does not interfere significantly with the immune response.

Relative immunogenicity in primary research studies. Compared with TIVs, aTIV is generally more immunogenic towards vaccine-like strains, independently of (sub)type and serological outcome. Indeed, as shown in Table S7, most studies found a better performance of aTIV than TIV. Similarly, aTIV tended to be more immunogenic than TIVs against heterovariant strains (Tables S8–S10) although most of these belonged to A(H3N2). Interestingly, Ansaldi et al.[41] noted that the advantage of aTIV over TIV increased on increasing the degree of mismatch, as measured by both antigenic and genetic distances between vaccine and circulating strains. It has been also reported[18,49,53] that aTIV may determine longer-lasting antibody persistence than TIV.

No studies directly comparing aTIV and QIV were found.

Absolute effectiveness in primary research studies. aTIV was generally highly effective (43–94%) against different influenza-related outcomes, including laboratory-confirmed influenza (Table S11). Its absolute effectiveness was generally significantly (P<0.05) different from zero, independently of setting and influenza season.

Relative effectiveness in primary research studies. Table S12 shows the relative immunogenicity of aTIV versus TIV and QIV. In all studies, aTIV proved to be more effective (P<0.05) than both TIVs and QIV. Moreover, Rizzo et al.[63] showed that, in the 2014/15 influenza season, aTIV was significantly more effective than split TIV against A(H1N1) (68.2% vs 23.3%) and B influenza.

Safety of aTIV in primary research studies. Fig. S2 reports the relative frequency of local and systemic adverse events observed in single studies. Local events, such as pain, erythema and induration, were the most frequent; most of these, however, were mild.

A large phase IV study (N=13,721, 67% immunised with aTIV) demonstrated that only 0.3% of aTIV recipients and 0.4% of TIV recipients suffered an adverse event requiring some treatment.[21] Villa et al.[43] reported a comparable very low frequency of unsolicited adverse events in elderly subjects immunized with aTIV (>88,000 doses) and subunit TIV. In that study, the frequency of adverse events of special interest (including anaphylaxis, autoimmune hepatitis, Bell's palsy, convulsions, demyelinating disorders, encephalitis, Guillain-Barré syndrome, immune thrombocytopenic purpura, vasculitis) were comparable in aTIV and TIV recipients.

aTIV can be safely co-administered (in different arms) with either PCV13[64] or PPV23.[61]

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Fig S1. Study selection process

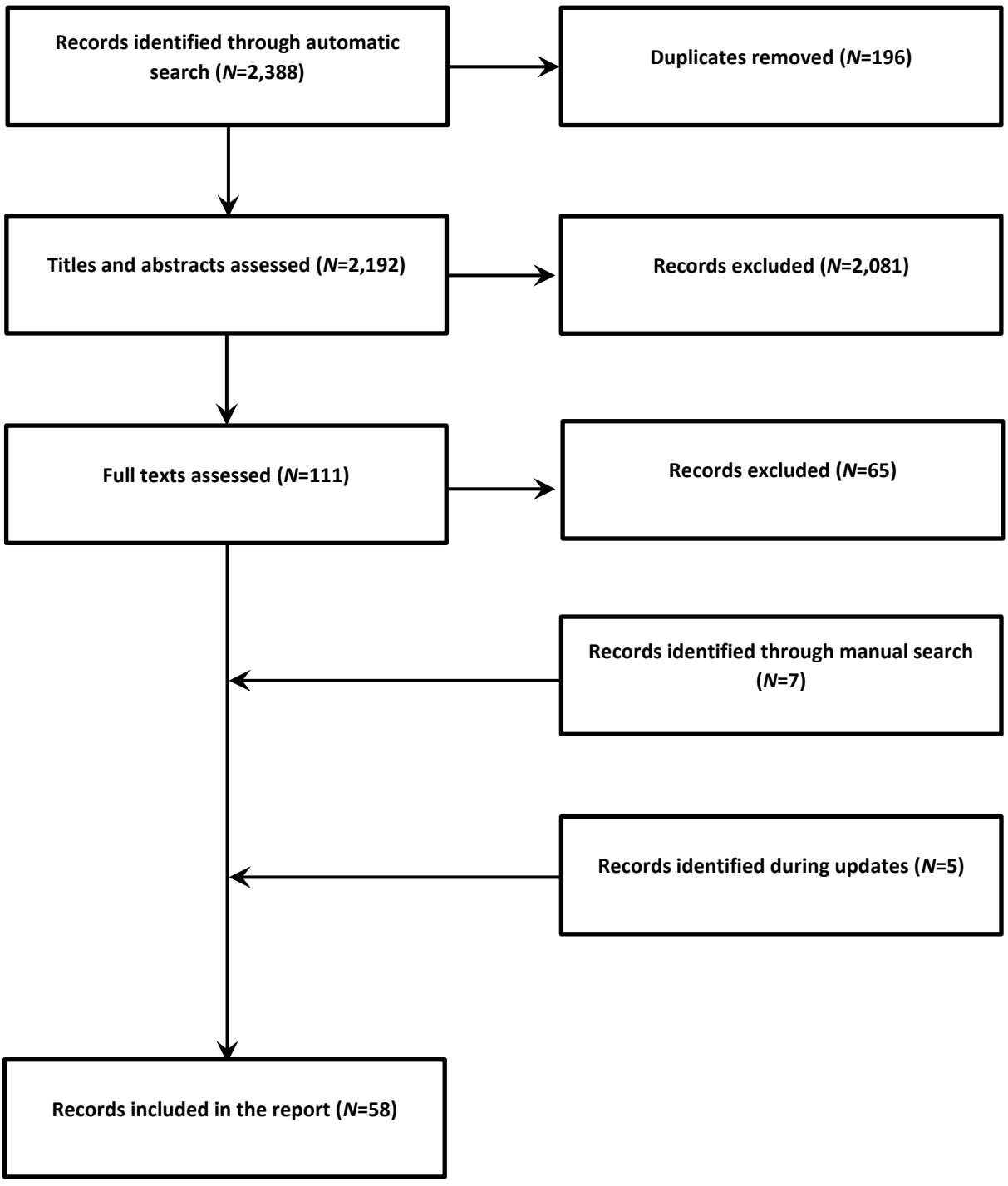
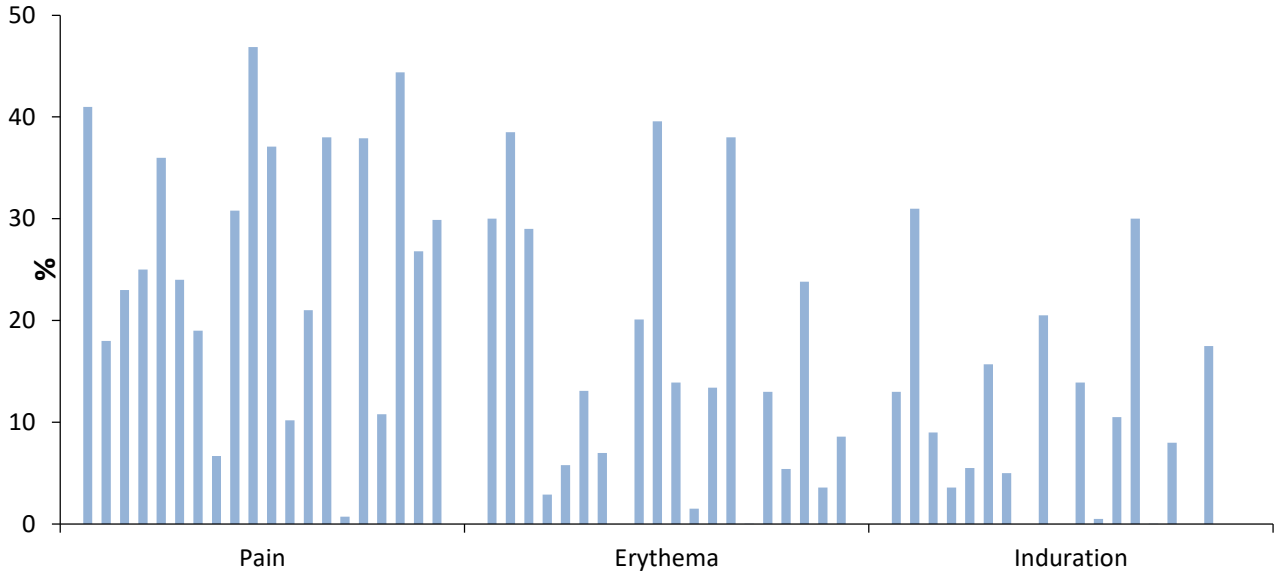


Fig S2. Frequency (%) of local (A) and systemic (B) adverse events following immunisation with aTIV



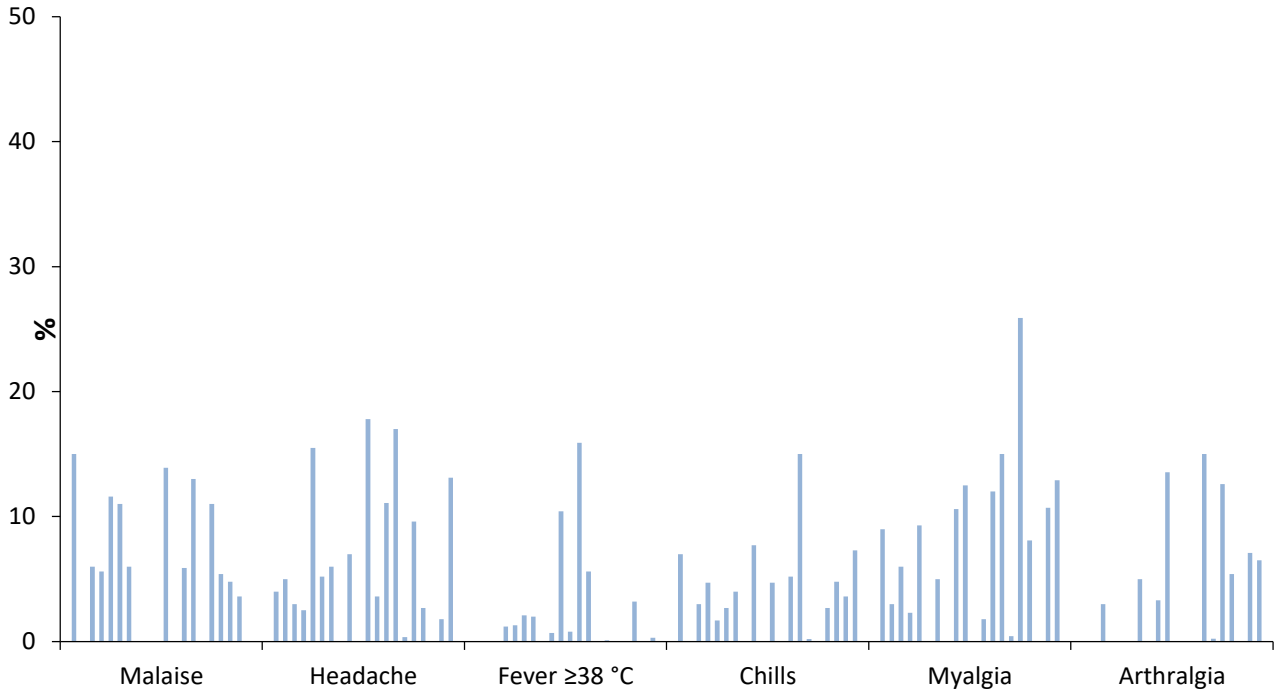


Table S1. The search strategy adopted

#	Syntax
1	(fluad* or MF59* or MF 59*).mp
2	exp Influenza Vaccines/ or influenza vaccin*.mp. or ((influenza or flu*) adj5 (vaccin* or immuni* or innoculat*)).mp.
3	influenza.mp. or exp Influenza, Human/
4	exp Vaccines/ or vaccin*.mp. or exp Viral Vaccines/ or immuni*.mp. or Vaccines, Subunit/ or Vaccines, Synthetic/
5	3 and 4
6	exp Adjuvants, Immunologic/ or adjuvant*.mp. or squalene*.mp. or Polysorbate*.mp. or Emulsion*.mp.
7	(2 or 5) and 6
8	1 or 7
9	exp Adult/
10	Men/
11	Women/
12	Retirement/
13	((old* or age*) adj3 (people* or person* or adult* or women* or men* or citizen* or residen*)).tw.
14	(pension* or retire* or adult* or aged or elderly or senior* or geriatric*).tw.
15	long-term care/ or nursing care/ or palliative care/
16	homes for the aged/ or nursing homes/
17	nursing home*.tw.
18	or/9-17
19	8 and 18
20	limit 19 to yr="1990-Current"
21	remove duplicates from 20

Table S2. Characteristics of the primary research studies included

Study design	Country (influenza season)	Study population	Vaccines used	Outcome(s) reported	N enrolled subjects, per vaccine	Ref.
Phase II, prospective, observer-blind, parallel-group, randomised, single-centre	Italy (1992/93, 1993/94, 1994/95)	Elderly aged ≥65 years	1. aTIV 2. suTIV	Local and systemic AEs; GMTs, SCRs, SPRs (HI titre ≥128) for homologous strains; GMTs for heterovariant strains.	Season 1: 46/46; Season 2: 39/35; Season 3: 35/32	18
Randomised, observer-blind, controlled, single-centre	Italy (1993/94, 1994/95, 1995/96)	Elderly aged ≥65 years	1. aTIV 2. suTIV	Local and systemic AEs; GMTs, GMRs, SCRs, SPRs (HI titre ≥128) for homologous strains; GMTs for a heterovariant strain; effect of pre-vaccination titres on antibody response	Season 1: 106/105; Season 2: 80/73; Season 3: 62/55	19
Phase II, prospective, double-blind, parallel-group, randomised, multicentre	Italy (1994/95)	Elderly aged ≥65 years	1. aTIV 2. suTIV	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	204/104	20
Phase IV, multicentre, randomised	Italy (1997/98)	Elderly aged ≥65 years	1. aTIV 2. suTIV	Frequency of AEs; mortality and hospitalisation rates (indirect comparison with general population)	9,171/4,550	21
Randomised, double-blind, single-centre	Italy (1998/99)	Institutionalised subjects aged 56–99 years[a]	1. aTIV 2. vTIV	Local and systemic reactions; GMTs, SCRs, SPRs for homologous strains	81/82	22
Prospective, observer-blind, randomised, multicentre	Italy (1998/99)	Institutionalised elderly aged >64 years	1. aTIV 2. wvTIV 3. vTIV	Local and systemic AEs; GMTs, SCRs, SPRs for homologous strains; incidence of ILI and laboratory-confirmed influenza	207/213/215	23
Randomised, double-blind, multicentre	Italy (1998/99)	Institutionalised elderly aged ≥65 years	1. aTIV 2. vTIV 3. spTIV	Local and systemic reactions; GMTs, MFIs, SCRs, SPRs for homologous strains	100/100/100	24
Phase IV, open, randomised	Italy (1998/99)	Elderly aged ≥65 years	1. aTIV 2. spTIV	Local and systemic AEs; GMTs, MFIs, SCRs, SPRs for homologous strains	1,074/1,076	25
Extension study[24] on immunogenicity (re-analysis) to compare homologous and heterovariant	Italy (1998/99)[b]	Institutionalised elderly aged ≥65 years	1. aTIV 2. vTIV 3. spTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains; determinants of seroconversion	72/39/88	26

responses				towards heterovariant strains		
Observational (prospective)	Italy (1998/99)	Institutionalised elderly aged 23–100 years[c]	1. aTIV 2. suTIV	VE against ILI	1,487/1,478	27
Randomised, double-blind	Italy (2001/02)	Elderly aged >64 years (unprotected)	1. aTIV 2. spTIV	GMTs, SCRs, SPRs, determinants of seroconversion towards homologous strains	A(H1N1): 85/80 A(H3N2): 113/100 B: 143/127	28
Secondary immunogenicity study to assess the impact of steroids in the aTIV immune response	Germany (2001/02)	Subjects aged 60–89 years with COPD	1. aTIV	Local and systemic AEs; GMTs, SCRs, SPRs for homologous strains	162	29
Open, multicentre, randomised, parallel-group	Germany (2002/03)	Elderly aged ≥60 years	1. aTIV 2. spTIV 3. vTIV	Local and systemic AEs; GMTs, mean log titres, Δ mean log titres, MFIs, SCRs, SPRs for homologous strains	275/273/272	30
Randomised, controlled	Italy (2002/03)	Institutionalised elderly aged ≥65 years	1. aTIV 2. suTIV	Local and systemic AEs; GMTs, MFIs, SCRs, SPRs for homologous strains	96/99	31
Case-control	Spain (2002/03)	Elderly aged ≥65 years	1. aTIV	VE against emergency hospitalisations for pneumonia	486 [total 815 (290 cases, 525 controls)]	32
Immunogenicity study to assess the heterovariant immune response	Unclear (2003/04)	Elderly aged ≥61 years	1. aTIV 2. suTIV 3. spTIV	GMTs, SPRs for homologous and heterovariant A(H3N2) strains	60/29/30	33
Immunogenicity study to assess the immune response towards two B lineages	Italy (2003/04)	Elderly	1. aTIV	GMTs, MFIs, SPRs for homologous and heterovariant B strains	91	34
Randomised, observer-blind, 3-arm, parallel-group, multicentre	Germany, Sweden, Lithuania, Bulgaria (2004/05)	Elderly aged ≥61 years	1. aTIV 2. vTIV 3. suTIV	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	130/127/129	35
Randomised, controlled, immunogenicity study to assess the heterovariant immune response towards an A(H3N2) drifted strain	Italy (2004/05)	Elderly aged ≥65 years	1. aTIV 2. suTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains	25/25	36
Randomised, placebo-controlled, double-blind, cross-over	Italy (2004/05)	Subjects aged ≥18 years[d] in oral anticoagulant therapy	1. aTIV	GMTs, MFIs, SPRs, SCRs for homologous strains; clinical and coagulation-	104	37

				related variables		
Immunogenicity study to assess the homologous and heterovariant A(H3N2) immune response	Italy (2004/05)	Institutionalised elderly aged ≥60 years	1. aTIV	GMTs, MFIs, SPRs, SCRs for homologous and heterovariant strains; laboratory-confirmed influenza	67	38
Case-control	Spain (2004/05)	Elderly aged ≥65 years	1. aTIV	VE against hospitalisations for acute cardio- and cerebrovascular events and pneumonia	971 [total 1,301 (476 cases, 825 controls)]	39
Phase I (followed by phase II/III), open, randomised, observer-blind, controlled	China (2005/06)	Elderly aged ≥60 years	Phase I: 1. aTIV; Phase II/III: 1. aTIV 2. suTIV	Phase I: serious AEs; Phase II/III: Local and systemic AEs; GMTs, MFIs, SCRs, SPRs for homologous strains	Phase I: 25; Phase II/III: 400/200	40
Randomised, controlled, immunogenicity study to assess the heterovariant immune response	Italy (2005/06)	Elderly aged ≥65 years	1. aTIV 2. suTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains; correlation between immunogenicity endpoints and genetic antigenic distances (between vaccine and wild strains)	25/25	41
Observational (prospective)	Italy (2006/07, 2007/2008, 2008/2009)	Elderly aged ≥65 years	1. aTIV 2. suTIV	VE against hospitalisations for pneumonia and influenza; several safety outcomes	84,665/79,589	42, 43
Phase III, multicentre, randomised, controlled, open, parallel-group	France, Belgium (2007/08)	Elderly aged ≥65 years	1. aTIV 2. idTIV	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	397/398	44
Immunogenicity and effectiveness study in a mismatched season	Italy (2007/08)	Institutionalised elderly aged ≥61 years	1. aTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains; laboratory-confirmed influenza	67	45
Multicentre, randomised, observer-blind, dose-finding	Poland, Germany, Belgium (2008/09)	Elderly aged >65 years	1. aTIV 2. suTIV 3. Several vaccine candidates (*N*=8)	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs; magnitude and functional profile of CD4+ response	47/44/359	46
Multicentre, randomised, factorial design, observer-blind,	Poland, Germany, Belgium (2008/09)	Elderly aged >65 years	1. aTIV 2. suTIV 3. Several vaccine	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs	47/44/359	47

dose-finding[e]			candidates			
Randomised, open, single-centre	South Korea (2009/10)	Elderly aged ≥65 years	1. aTIV 2. spTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains	47/48	48
Phase III, randomised, observer-blind, multicentre	Colombia, Panama, Philippines, United States (2010/11)	Elderly aged ≥65 years	1. aTIV 2. suTIV	Lot-to-lot consistency; GMTs, MFIs, SCRs, SPRs, GMRs, ΔSCRs for homologous and heterovariant strains; efficacy; local and systemic AEs	3,552/3,552	49
Case-case comparison, case-control	Spain (2010/11)	Subjects aged ≥18 years[fg]	1. aTIV 2. vTIV 3. suTIV	VE against laboratory-confirmed influenza	113[c]/NA/NA	50
Case-control	Italy (2010/11)	Elderly aged ≥65 years	1. aTIV 2. idTIV 3. vTIV	VE against hospitalisations for pneumonia and influenza	88/42/105 [total 374 (187 cases, 187 controls)]	51
Post-hoc analysis[49] to assess the impact of the use of statins on the immune response	Colombia, Panama, Philippines, United States (2010/11)	Elderly aged ≥65 years (statin users)	1. aTIV 2. suTIV	GMTs, GMRs for homologous strains	3,479/3,482	52
Prospective, randomised, controlled, evaluator-blinded, parallel-group	Canada (2011/12)	Elderly aged ≥65 years	1. aTIV 2. suTIV 3. idTIV	Local and systemic AEs; GMTs, MFIs, SCRs, SPRs for homologous strains	306/310/306	53
Randomised, multicentre	Italy (2011/12)	Institutionalised elderly aged ≥64 years	1. aTIV 2. idTIV	GMTs, MFIs, SCRs, SPRs for homologous and heterovariant strains	40/40	54
Multicentre, randomised, controlled, parallel-group	South Korea (2011/12)	Elderly aged ≥65 years	1. aTIV 2. suTIV 3. idTIV	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	118/118/118	55
Immunogenicity study to assess the heterovariant immune response	Italy (2011/12)	Institutionalised elderly aged ≥65 years	1. aTIV 2. idTIV	GMTs, MFIs, SPRs, SCRs for homologous and heterovariant A(H3N2) strains	52/40[h]	56
Prospective case-control	Canada (2011/12)	Institutionalised and community-dwelling elderly aged ≥ 65 years	1. aTIV 2. spTIV	VE against laboratory-confirmed influenza	165/62	57
Case-control	Italy (2010/11, 2011/12)[i]	Elderly aged ≥65 years	1. aTIV 2. spTIV	VE against hospitalisations for pneumonia and influenza	1,592/1,516	58
Phase I, open, randomised, multicentre	Germany, Belgium (2012/13)	Elderly aged ≥65 years	1. aTIV 2. vTIV 3. idTIV 4. Candidate vTIVs	Local and systemic AEs; GMRs, MFIs, SCRs, SPRs for homologous strains	63/63/60/184	59

Observational	Italy (2012/13)	Institutionalised elderly aged ≥60 years	1. aTIV 2. spTIV 3. idTIV	GMTs, MFIs, SCRs, SPRs for homologous strains	137/26/89	60
Single-centre, open, randomised	South Korea (2013/14)	Elderly aged ≥65 years	1. aTIV 2. aTIV + PPSV23 in different arms 3. aTIV + PPSV23 in the same arm 4. PPSV23	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	56/56/56/56	61
Multicentre, open, randomised	South Korea (2013/14)	Subjects undergoing haemodialysis[j]	1. aTIV 2. suTIV	GMTs, MFIs, SCRs, SPRs for homologous strains	91/88	62
Test negative case-control	Italy (2014/15)	All[k]	1. aTIV 2. spTIV	VE against laboratory-confirmed influenza	Total 1,193 (599 cases, 594 controls)	63
Multicentre, open, randomised	South Korea (2014/15)	Elderly aged ≥60 years	1. aTIV + PCV13 2. PCV13 3. aTIV	Local and systemic AEs; GMTs, MFIs, GMRs, SCRs, SPRs for homologous strains	391/413/390	64
Observational	United States (2017/18)	Elderly aged ≥65 years (Medicare beneficiaries)	1. ccQIV 2. ebQIV 3. hdTIV 4. aTIV 5. TIV	VE against influenza hospital encounters and outpatient visits	653,099/1,844,745/8,449,508/1,465,747/1,007,082	65

Notes:

AE, adverse event; aTIV, adjuvanted trivalent influenza vaccine; ccQIV, cell culture-derived quadrivalent influenza vaccine; COPD, chronic obstructive pulmonary disease; ebQIV, egg-based quadrivalent influenza vaccine; GMR, geometric mean ratio; GMT, geometric mean titre; hdTIV, high-dose trivalent influenza vaccine; HI, haemagglutination-inhibition; idTIV, intradermal trivalent influenza vaccine; ILI, influenza-like illness; MFI, mean fold increase; PCV13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent polysaccharide pneumococcal vaccine; SCR, seroconversion rate; SPR, seroprotection rate; spTIV, split trivalent influenza vaccine; suTIV subunit trivalent influenza vaccine; TIV, trivalent influenza vaccine; VE, vaccine effectiveness; vTIV, virosomal trivalent influenza vaccine; wvTIV, whole-virion trivalent influenza vaccine; [a]Mean age was 84.5 years; [b]Serum samples were from the influenza season 1998/99, while the heterovariant immune response was assessed towards strains recommended for inclusion in the 2006/07 season (northern hemisphere); [c]Subjects aged <65 years accounted for only 3.7% of the total sample; [d]Mean age (standard deviation) of the two groups was 70.8 (8.3) and 71.8 (10.1) years, respectively; [e]The study population was the same as that of Della Cioppa et al.[47]; [f]Subjects immunized with aTIV were all ≥ 65 years; [g]Data provided by the corresponding author Joan Puig-Barberà; [h]aTIV-specific data were available for a total of 11 subjects; [i]aTIV and spTIV were used in 2011/12 and 2010/11 seasons, respectively; [j]Most subjects were immunized with aTIV, and aTIV-/elderly-specific data were readily available; [k]aTIV could be administered only to the elderly; estimates adjusted for the age-class were also available.

Table S3. Absolute immunogenicity of aTIV towards vaccine-like strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by (sub)type and serological outcome

A(H1N1)			A(H3N2)			B			Ref
MFI	SCR, %	SPR, %	MFI	SCR, %	SPR, %	MFI	SCR, %	SPR, %	
5.0	70	22[a]	7.9	70	83[a]	7.7	89	63[a]	18
2.3	28	87[a]	6.0	74	92[a]	2.6	36	74[a]	18[b]
1.8	20	77[a]	2.5	37	51[a]	2.1	20	43[a]	18[b]
2.6	32	88[a]	8.1	83	3[a]	3.7	52	71[a]	19
1.9	20	88[c]	3.3	52	51[c]	2.6	35	54[c]	20
7.1	91	100	3.6	85	83	3.1	59	98	22
7.1	76	98	5.8	68	100	2.8	34	85	23
8.8	85	100	5.0	93	80	3.6	63	98	24
13.7	76	83	5.4	61	94	2.6	22	38	25
8.7	94	100	5.5	76	78	3.8	67	100	26
–	92	99	–	83	90	–	70	88	28
4.0	56	64	2.1	68	84	3.3	82	93	29[d]
3.2	56	66	2.5	80	90	3.8	89	93	29[e]
4.1	65	73	2.4	60	80	2.8	86	93	29[f]
11.1	70	90	11.3	70	88	15.6	80	95	30
3.9	55	96	4.7	61	99	5.3	70	97	31
–	–	–	4.6	–	98	–	–	–	33
–	–	–	–	–	–	1.8	–	81	34
11.9	69	84	17.6	83	100	16.2	81	94	35
–	–	–	4.6	68	100	–	–	–	36
3.2	47	92	7.5	74	100	8.9	81	96	37[g]
2.7	33	92	7.7	82	100	5.5	68	96	37[h]
2.5	28	76	6.9	73	96	5.0	67	79	38
2.7	83	100	26.9	85	88	4.6	33	36	40
–	–	–	8.9	76	100	–	–	–	41
9.0	73	88	4.9	50	98	2.4	22	57	44
5.2	60	72	4.7	53	87	2.4	27	58	45
7.1	62	95	15.0	87	96	4.1	49	66	46
4.1	55	92	9.1	75	92	4.0	40	55	48
13.0	77	91	10.0	74	99	4.8	47	64	49
4.2	50	91	3.4	45	88	1.6	17	99	53
3.7	50	73	3.3	48	88	1.6	10	75	54
4.4	54	85	3.4	45	89	1.6	7	24	55
–	–	–	4.5	64	82	–	–	–	56
5.4	51	94	6.2	70	100	3.6	32	38	59
2.1	23	40	2.4	30	71	2.6	33	64	60
3.0	45	82	2.3	34	98	2.0	13	77	61
4.1	52	71	3.2	48	95	2.4	33	81	62
4.5	59	92	3.8	50	99	2.1	23	72	64

Notes:

MFI, mean fold increase; SCR, seroconversion rate; SPR, seroprotection rate; [a]HI titre ≥128; [b]Revaccination studies; [c]HI titre ≥160; [d]COPD patients without steroid treatment; [e]COPD patients on inhaled steroid treatment; [f]COPD patients in systemic steroid treatment; [g]Vaccine followed by placebo; [h]Placebo followed by vaccine.

Table S4. Absolute immunogenicity of aTIV towards heterovariant A(H1N1) strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by serological outcome[a]

MFI	SCR, %	SPR, %	Ref
5.3	–	–	18
6.1	68	88	26
3.2	39	57	45
1.1	6	15	48
1.9	13	32	48
2.2	26	79	48

Notes:

MFI, mean fold increase; SCR, seroconversion rate; SPR, seroprotection rate; [a]Each row represents a heterovariant/drifted strain.

Table S5. Absolute immunogenicity of aTIV towards heterovariant A(H3N2) strains in haemagglutination-inhibition (HI) and neutralisation assays (NT) assays 3-4 weeks after vaccination, by serological outcome[a]

Haemagglutination-inhibition assay			Neutralisation assay			Ref
MFI	SCR, %	SPR, %	MFI	SCR, %	SPR, %	
7.9	–	–	–	–	–	18
3.1	42	79	–	–	–	26
6.6	–	98	–	–	–	33
4.3	60	100	5.0			36
2.7	48	80	3.6			36
3.0	44	64	5.0			36
4.1	55	90	–	–	–	38
5.3	56	68	4.2	52	100	41
16.9	84	88	3.3	52	64	41
10.8	64	92	4.0	48	100	41
20.5	84	100	5.1	56	100	41
10.3	76	100	8.2	80	96	41
12.8	80	100	2.1	44	96	41
20.0	80	100	6.6	56	88	41
14.7	84	100	11.2	72	100	41
13.6	84	100	1.7	32	100	41
12.1	76	100	5.8	52	100	41
3.0	68	100	3.4	48	52	41
14.7	80	96	4.1	56	72	41
4.2	68	96	4.7	56	56	41
16.2	72	96	4.4	56	72	41
4.3	45	66	–	–	–	45
4.3	57	94	–	–	–	48
5.8	–	96	–	–	–	49
5.0	–	99	–	–	–	49
2.1	28	70	–	–	–	54
2.3	25	48	–	–	–	54
2.5	38	68	–	–	–	54
2.3	25	83	–	–	–	54
3.1	45	64	–	–	–	56
2.0	27	45	–	–	–	56
1.7	9	9	–	–	–	56
2.0	36	27	–	–	–	56
2.4	27	64	–	–	–	56

Notes:

MFI, mean fold increase; SCR, seroconversion rate; SPR, seroprotection rate; [a]Each row represents a heterovariant/drifted strain.

Table S6. Absolute immunogenicity of aTIV towards heterovariant B strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by serological outcome[a]

MFI	SCR, %	SPR, %	Ref
9.1	–	–	18
2.0	25	69	26
1.9	–	87	34
1.7	–	97	34
1.7	–	53	34
1.9	24	67	45
5.8	53	76	49

Notes:

MFI, mean fold increase; SCR, seroconversion rate; SPR, seroprotection rate; [a]Each row represents a heterovariant/drifted strain.

Table S7. Relative immunogenicity of aTIV vs TIV towards vaccine-like strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by (sub)type and serological outcome

A(H1N1)			A(H3N2)			B			Ref
GMR	ΔSCR, %	ΔSPR, %	GMR	ΔSCR, %	ΔSPR, %	GMR	ΔSCR, %	ΔSPR, %	
1.5	20	5	1.3	-2	22	1.6	22	22	18
1.6	5	10	1.5	14	18	1.3	10	14	18[a]
1.5	11	30	1.4	14	13	1.3	11	9	18[a]
1.4	10	8	2.0	21	25	1.6	21	28	19
1.1	9	3	1.9	23	17	1.5	8	19	20
1.3	13	0	1.0	14	-1	1.2	12	-2	24
1.8	12	12	1.2	0	4	1.3	8	9	25
1.1	9	1	1.2	14	-2	1.2	12	2	26
0.9	-1	0	1.4	26	25	1.3	20	14	28
0.7	-5	-4	0.9	2	-2	1.3	2	4	30
1.4	13	0	1.5	20	0	0.9	24	-1	31
–	–	–	1.5	–	2	–	–	–	33[b]
–	–	–	1.9	–	2	–	–	–	33[c]
0.8	-9	-5	1.2	7	1	1.0	5	5	35
–	–	–	2.7	40	4	–	–	–	36
–	3	0	–	19	16	–	8	7	40
–	–	–	2.6	4	0	–	–	–	41
2.4	32	18	1.8	17	3	1.6	19	17	46
1.0	10	0	1.7	8	6	1.4	1	7	48
1.4	9	7	1.6	13	2	1.2	5	6	49
1.4	13	13	1.4	20	-5	1.1	6	-1	53
1.4	15	13	1.9	19	18	1.4	5	6	55
2.0	11	21	1.5	11	5	1.0	-6	3	60
1.5	27	14	1.5	19	2	1.2	26	-5	62

Notes:

GMR, geometric mean ratio; SCR, difference in seroconversion rates (aTIV–TIV); SPR, difference in seroprotection rates (aTIV–TIV); [a]Revaccination studies; [b]Subunit TIV; [c]Split TIV.

Table S8. Relative immunogenicity of aTIV vs TIV towards heterovariant A(H1N1) strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by serological outcome[a]

GMR	ΔSCR, %	ΔSPR, %	Ref
2.0	–	–	18
2.0	25	5	26
2.4	36	19	26
1.0	2	-2	48
0.7	-8	-14	48
1.0	-10	-3	48

Notes:

GMR, geometric mean ratio; SCR, difference in seroconversion rates (aTIV–TIV); SPR, difference in seroprotection rates (aTIV–TIV); [a]Each row represents a heterovariant/drifted strain.

Table S9. Relative immunogenicity of aTIV vs TIV towards heterovariant A(H3N2) strains in haemagglutination-inhibition (HI) and neutralisation assays (NT) assays 3-4 weeks after vaccination, by serological outcome[a]

Haemagglutination-inhibition assay			Neutralisation assay			Ref
MFI	SCR, %	SPR, %	MFI	SCR, %	SPR, %	
1.7	–	–	–	–	–	18
1.6	–	–	–	–	–	19
1.7	19	23	–	–	–	26
1.6	14	1	–	–	–	26
1.5	–	22	–	–	–	33
2.2	–	18	–	–	–	33
2.1	36	0	1.6	–	–	36
2.3	40	52	1.3	–	–	36
1.7	24	44	1.7	–	–	36
2.4	24	8	2.1	20	0	41
6.4	52	56	1.8	28	28	41
4.5	32	4	1.0	-4	0	41
2.8	28	0	3.0	8	12	41
3.9	20	8	2.3	4	16	41
1.8	4	0	1.8	-8	-4	41
3.5	4	0	1.8	-20	4	41
2.6	8	0	2.5	12	0	41
2.2	12	8	1.2	0	0	41
3.4	20	8	2.0	12	8	41
1.1	20	8	1.5	28	28	41
6.1	60	68	2.1	28	40	41
3.1	64	64	2.0	44	36	41
7.0	60	72	2.2	36	52	41
1.3	14	4	–	–	–	48
1.5	11	2	–	–	–	49
1.4	12	1	–	–	–	49
0.7	-8	-13	–	–	–	54
0.9	8	-25	–	–	–	54
0.9	13	-20	–	–	–	54
0.8	5	-8	–	–	–	54

Notes:

MFI, mean fold increase; SCR, seroconversion rate; SPR, seroprotection rate; [a]Each row represents a heterovariant/drifted strain.

Table S10. Relative immunogenicity of aTIV vs TIV towards heterovariant B strains in haemagglutination-inhibition (HI) assay 3-4 weeks after vaccination, by serological outcome[a]

GMR	ΔSCR, %	ΔSPR, %	Ref
1.9	–	–	18
1.3	17.3	23	26
1.1	-1.1	1	26
1.1	4	4	49

Notes:

GMR, geometric mean ratio; SCR, difference in seroconversion rates (aTIV–TIV); SPR, difference in seroprotection rates (aTIV–TIV); [a]Each row represents a heterovariant/drifted strain.

Table S11. Absolute effectiveness of aTIV, by setting and influenza-related outcome

Setting	Influenza-related outcome	Vaccine effectiveness, %		Ref
		Estimate	95% CI	
Institutionalised elderly	Influenza-like illness	94	47–100	27
Community-dwelling elderly	Emergency admissions for pneumonia	48	20–66	32
Community-dwelling elderly	Hospitalisations for acute coronary syndrome	87	35–97	39
Community-dwelling elderly	Hospitalisations for cerebrovascular accidents	93	52–99	39
Community-dwelling elderly	Hospitalisations for pneumonia	69	29–86	39
Community-dwelling elderly	Hospitalisations for laboratory-confirmed influenza	43	-111–85	50
Community-dwelling elderly	Hospitalisations for pneumonia or influenza	88	-39–99	51
Community-dwelling elderly	Laboratory-confirmed influenza	72	2–93	57
Mixed	Laboratory-confirmed influenza	58	5–82	57
Community-dwelling elderly	Hospitalisations for pneumonia or influenza	49	30–60	58

Table S12. Relative effectiveness of aTIV versus unadjuvanted vaccines, by setting and influenza-related outcome

Comparator	Setting	Influenza-related outcome	Vaccine effectiveness, %		Ref
			Estimate	95% CI	
TIV	Institutionalised elderly	Influenza-like illness	34	18–47	27
TIV	Community-dwelling elderly	Hospitalisations for pneumonia or influenza	25	2–43	42
TIV	Mixed	Laboratory-confirmed influenza	63	4–86	57
QIV	Community-dwelling elderly	Influenza hospital encounters	3.3	–[a]	65

Notes:

QIV, quadrivalent influenza vaccine; TIV, trivalent influenza vaccine; [a]The 95% CI does not include zero.