Healthy elderly Singaporeans show no age-related humoral hyporesponsiveness nor diminished plasmablast generation in response to influenza vaccine

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Abstract: Improving influenza vaccine efficacy is a priority to reduce the burden of influenza-associated morbidity and mortality. By careful selection of individuals based on health we show sustained response to influenza vaccination in older adults. Sustaining health in aging could be an important player in maintaining immune responses to influenza vaccination.

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Keywords: Aging, Influenza vaccination, Stratification, Co-morbidity

Influenza vaccination responses in older adults
Influenza infection is associated with considerable morbidity annually and the elderly are amongst those at highest risk of serious outcomes [1–3]. Annual influenza vaccination is a strategy endorsed by the World Health Organization [4], and is most effective when the vaccine strains closely match the circulating influenza viruses [5]. However, the antibody response and protection elicited by the vaccine in the elderly is modest at best [6], though recent studies suggest that increasing the dose of antigen in subunit vaccines significantly improves efficacy [7]. Several factors have been proposed to explain hyporesponsiveness to influenza vaccination in the elderly, including host-related factors like genetics, immune status, health status, frailty and nutritional status [8]. The complex changes in the immune system occurring with age collectively termed immunosenescence, affects innate and adaptive immunity and may contribute to the decreased efficacy of vaccines in the elderly [9, 10]. Recent data also suggest that age-associated decline in antibody responses could reflect the effect of repeated annual influenza vaccination rather than age or frailty [11], however this hypothesis is being queried [12]. Systems biology approaches have been utilized in recent studies to acquire a global picture of vaccine-induced immunity in humans, which has enabled the identification of early innate signatures predicting vaccine immunogenicity and the elucidation of novel mechanisms of immune regulation [13]. Several reports have established age-dependent predictive signatures of influenza vaccine responses, however such studies typically considered the elderly as a homogeneous population, which is clearly not the case [14]. We address this concern in the current study and show data confirming that a healthy elderly population, selected for lack of comorbidity and not just chronology, is likely to respond to influenza vaccination.

Description of the study
The SENIEUR protocol clearly suggested that addressing chronological ageing alone was insufficient in immunogerontological studies [15] as the elderly of the SENIEUR category (healthy elderly as defined by the SENIEUR
Clinical seropositivity (100% in older adults and 45% in the young group, \( p < 0.0001 \)), the CD4/CD8 ratio \( (p < 0.01) \) and the level of C-Reactive Protein (CRP, \( p < 0.01 \)) (Table 1).

**Sustained antibody titers following influenza vaccination of healthy elderly**

We vaccinated the healthy elderly and the young healthy volunteers with Vaxigrip© (Sanofi-Pasteur) following 2014/2015 seasonal recommendations and subsequently collected fasting blood specimens on day 0 (baseline, D0), day 2 (D2), day 7 (D7) and day 28 (D28). We then determined the hemagglutination-inhibition (HAI) antibody titer at baseline and D28 post-vaccination as shown in Fig. 1a. Both groups responded to vaccination with a significant increase in HAI titers against all three strains tested at day 28 (Fig. 1a). Following the current international guidelines for seroconversion (>4-fold increase in HAI titers over baseline) and seroprotection (HAI titers \( \geq 40 \)) \([19, 20]\), fewer young subjects had seroconverted compare with the elderly, which was primarily due to higher baseline titers in the young subjects (GMT values: Flu B: \( 411 \pm 3 \) vs. \( 56 \pm 4 \); H1N1: \( 149 \pm 6 \) vs. \( 15 \pm 5 \); and H3N2: \( 156 \pm 4 \) vs. \( 38 \pm 5 \) in young vs. elderly subjects). A high number of young individuals were already seroprotected at baseline against each of the three strains contained in the vaccine compared to the elderly (young versus old: 100% vs 63.6% against the B strain \( p =

### Table 1 Study subject characteristics

| Demography | Young cohort (\( N = 29 \)) | Healthy elderly cohort (\( N = 22 \)) | \( p \) |
|------------|-----------------------------|--------------------------------------|------|
| Age        | 27.97 (23–33)               | 72.41 (65–84)                       | ****|
| Gender     | 18 females (63.3%)          | 10 females (45.5%)                  | ns   |
| BMI        | 22.8 (16.2–29.1)            | 23.6 (17.4–32.6)                    | ns   |
| Physical activity (% of time) | | | |
| Sedentary  | 60.3 (46.5–98.9)            | 60.2 (43.4–92.5)                    | ns   |
| Light activity | 35.1 (0.9–47.9)          | 35.2 (7.4–48.4)                     | ns   |
| Moderate activity | 4.5 (0.1–11.1)          | 3.8 (0.1–15.4)                      | ns   |
| Vigorous activity | 0.1 (0–1.8)               | 0.8 (0–12.1)                        | ns   |
| Clinical   |                            |                                      |      |
| CMV positivity | 13 (45%)                   | 22 (100%)                           | ****|
| CD4/CD8 ratio | 1.33 (0.62–2.42)          | 2.94 (0.52–14.71)                   | **   |
| CRP (mg/L) | 1.2 (0.2–4.1)              | 2.7 (0.3–9.3)                       | **   |
| Comorbidities | –                         | 0.36 (0–1)                          | ns   |
| MMSE/MoCA | –                          | 28 (22–30) / 25.59 (18–30)          | ns   |
| Pre/Post FEV1/FVC | –                     | 0.73 (0.5–0.91/0.71 (0.29–0.85) | ns   |
| Medications | –                          | 1.05 (0–4)                          | ns   |
| Hospitalizations | –                     | 0                                   | ns   |
| MNA        | –                          | 12.8 (11–14)                        |      |

BM* body mass index, CMV human cytomegalovirus, CRP C-reactive protein, MMSE mini mental state examination, MoCA Montreal cognitive assessment, FEV* forced expiratory volume, FVC forced vital capacity, pre/post inhalation of a bronchodilator, MNA mini nutritional assessment score. Physical activity was measured during 2 weeks using actigraphy watches (Phillips Respironics). Comorbidities are expressed as the number of diagnosed conditions, medications are defined as the number of prescribed drugs and hospitalizations is the number of time the individual have been hospitalized in the past year.
Fig. 1 (See legend on next page.)
In our study, we demonstrate that overall, healthy elderly are fully capable to mount a robust humoral response via expansion of plasmablast, HAI response and neutralizing antibodies, comparable to that of young subjects. Herein, these data extends our previous findings [22] with community-living elderly subjects from Singapore, and demonstrates that humoral immunity after influenza vaccination is preserved in healthy elderly. The studied healthy elderly group showed all signs of aging as previously described, including a higher prevalence of persistent infections (eg, CMV), high levels of pro-inflammatory molecules (eg, CRP) and features of immune aging (higher frequencies of CD28− and CD27− T cells, data not shown). The fact that plasmablast population dynamics is preserved in the healthy elderly from the SLAS cohort confirms earlier studies utilizing the SENIEUR protocol for immunogerontological studies. An important question to address in the near future will involve identifying which age-associated co-morbidity may interfere with optimal vaccine responses in the elderly. We recently ruled out type-2 diabetes as a condition that may interfere with influenza vaccine responses in a study of community-living elderly in Canada [23]. Older adults commonly take statins to reduce
cholesterol levels in order to manage cardiovascular risk, however recent published reports have raised concerns that statin use may impair vaccine-induced antibody responses, and reduce vaccine-induced protection, particularly for influenza A H3N2 [24]. Whether subjects with hypercholesterolemia or those on statin medication should be considered at-risk and benefit from improved vaccines is debatable, and in our study such subjects were excluded. Other conditions such as frailty have been proposed to alter influenza or pneumococcal vaccine responses, however this has also recently been challenged [25, 26]. Discrepancies in study outcome may be explained by different mean ages of the subjects included into the studies, the definition of frailty used, and living conditions (community vs. institution). A recent review highlighted the need to consider several approaches to stratify the older population in order to provide with the best vaccine strategy [27]. We are currently tackling these issues and other pertinent questions in an ongoing study aimed at understanding the associations between immunosenescence, hypo-responsiveness to vaccination and clinical phenotypes.

Abbreviations
CMV: Cytomegalovirus; CRP: C-reactive protein; HAI: Hemagglutination-inhibition; HAI: Hemagglutination-inhibition; SLAS: Singapore Longitudinal Ageing Study

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Availability of data and materials
Please contact author for data requests: anis_larbi@immunol.a-star.edu.sg

Authors’ contributions
AL, NTP, PT, and NBu conceived the study. MSZN and NTP recruited volunteers and performed clinical and frailty assessments. PT supervised the Vaxigrip clinical trial. XC and CTTY performed Luminex, immunophenotyping, microarray and serological assays. LV, NBu, CC, and SS analyzed microarray data and supervised the antibody titre assays performed at GCI and Sanofi Pasteur. VN, XC, MP, NBu, BA and AL performed data analysis, wrote and critically reviewed the manuscript. AL and NTP ensure the accuracy of the data and supervised the antibody titre assays performed at GCI and Sanofi Pasteur, Clinical Sciences, Marcy L’Etetle, France. Nestle Research Center Asia, 21 Biopolis Road, Singapore, Singapore. Department of Infectious Diseases, National University Health System, Singapore, Singapore.

Ethics approval and consent to participate
This study was approved by the National Healthcare Group’s Domain Specific Institutional Review Board and registered at clinicaltrials.gov under the registration number NCT03266237.

Consent for publication
“Not applicable”.

Competing interests
The authors Christophe Carre, Nicolas Burdin, Sanie Sesay and Lucian Visan were employed by Sanofi Pasteur, Marcy L’Etetle, France, and the author Nabil Bosco was employed by Nestlé Research Centre, Singapore. All other authors declare no competing interests.

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