Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Basophil activation tests with cryopreserved mRNA-based COVID-19 vaccines

Dear Editor,

The management and mechanisms involved in allergies to mRNA anti-SARS Cov2 vaccines remain debated.[1] The major suspected allergen is polyethylene glycol (PEG), necessary for forming and stabilizing lipid nanoparticles forming the BNT162b2 vaccine from Pfizer and the mRNA-1273 vaccine from Moderna.[2]

Skin testing and *in vitro* basophil activation test (BAT) for the vaccines and their components have been included in the European Academy of Allergy and Clinical Immunology (EAACI) statement for the diagnosis, management, and prevention of severe allergic reactions to COVID-19 vaccines.[3] However, cellular tests, and more specifically BAT, are still not standardized for their implementation in clinical practice. Indeed, difficulties in assessing reliable cut-off values, as well as the limited availability of these vaccines for *in vitro* tests have impaired the standardization of BAT assays for mRNA COVID-19 vaccines.

To avoid vaccine waste, cryopreservation of the vaccine remnants for allergy workup and diagnostic tests could help to better standardize and explore the cause and nature of anaphylactic reactions. Thus, the World Health Organization (WHO) states that once the doses of mRNA COVID-19 vaccines are withdrawn or reconstituted, the time for usage is 6 h when held between 2 °C and 25 °C to ensure the safety and stability of the vaccine and adequate immunization.[4]

In this study, we first evaluate BAT in individuals with a history of hypersensitivity reaction (HSR) to mRNA COVID-19 vaccine excipients or to the first dose of mRNA COVID-19 vaccines where IDR tests to mRNA vaccines were performed. We tested the accuracy of leftover fresh or cryopreserved mRNA vaccines for BAT in those individuals. Finally, by measuring Spike protein expression, we tried to understand whether the BAT results with mRNA vaccines depended on its functional activity *in vitro*.

We performed BAT in 10 patients with positive IDR and 10 patients with negative IDR. BAT results with fresh vaccines confirmed that individuals with positive IDR had a significantly higher CD63 response and stimulation index than individuals with negative IDR (Fig. 1A, Supplementary Table 1). We then assessed the discrimination ability of BAT assays to identify positive IDR according to the expression of CD63 and stimulation index (SI) in basophils by performing ROC curves. The area under the curve (AUC) for CD63 expression was 0.8571 (IC95%, 0.5945–1.0000) and 0.7429 (IC95%, 0.4315–1.000) for mRNA-1273 and BNT162b2, respectively. Cut-off values were selected for mRNA-1273 vaccine at 4.2% (sensitivity 83.33%, specificity 85.71%) and for BNT162b2 vaccine at 3.99 (sensitivity 71.43%, specificity 100%) (Supplementary Fig. 1A, B). The AUC for SI was 0.88 (IC95%, 0.65–1.0000) and 0.74 (IC95%, 0.43–1.000) for mRNA-1273 and BNT162b2 respectively. Cut-off values were selected for mRNA-1273 vaccine at 1.99 (sensitivity 83.33%, specificity 85.71%) (Supplementary Fig. 1C), and for BNT162b2 vaccine at 2.83 (sensitivity of 71.43% and a specificity of 80%) (Supplementary Fig. 1D).

Since getting freshly prepared vaccines can be challenging for many centers, we next aimed to compare BAT results with fresh (<6 h after reconstitution into the syringe) or cryopreserved (from a different batch) mRNA vaccines. We found a strong correlation between freshly prepared BNT162b2 and mRNA-1273 vaccines for CD63 expression on basophils suggesting that shared epitopes between both vaccines trigger basophils degranulation and CD63 upregulation (Fig. 1B). Importantly, cryopreserving mRNA-1273 and BNT162b2 vaccines did not alter CD63 upregulation in basophils. Finally, a significant correlation was observed when comparing cryopreserved mRNA-1273 and BNT162b2 vaccines (Fig. 1B).

We next wanted to understand whether there is a relationship between the functional activity of the vaccine and the BAT results. To address this question, we assessed the Spike protein expression on Jurkat cells with increasing concentrations of fresh (<6 h) mRNA-1273 and BNT162b2 vaccines. After 24 h, the mRNA-1273 vaccine induced a dose-dependent spike expression in Jurkat (Fig. 2A). Yet and unexpectedly, the BNT162b2 vaccine resulted in only poor/no Spike protein expression, suggesting already that there is no correlation between the functional activity of the vaccine and the BAT results.

To further understand if cryopreservation could alter the *in vitro* functionality of the mRNA-1273 vaccine, we compared the spike expression in Jurkat cells exposed to fresh or cryopreserved (D0) mRNA-1273 vaccine. No differences were found (Fig. 2B). Instead, our results suggest that the *in vitro* functional activity of the mRNA-1273 vaccine decreases over time independently of its cryopreservation status (Fig. 2C). This outcome was validated by the Bland–Altmann plot contrasting with the mRNA-1273 vaccine capacity to induce consistent CD63 expression in basophils even 16 days after being retrieved and cryopreserved (Fig. 2D, Supplementary Fig. 2).

BAT is increasingly being used as an ex-vivo correlate to identify sensitization and document clinically relevant allergens. Positive BAT with mRNA vaccines have been reported by many centers.[5,6] Yet, we are the first group to consistently compare their results with IDR (1:100 dilution). Importantly, IDR with mRNA vaccines at a 1:100 dilution has been shown to be non-irritative.[7] Thus, the increased basophil reactivity in individuals with positive IDR

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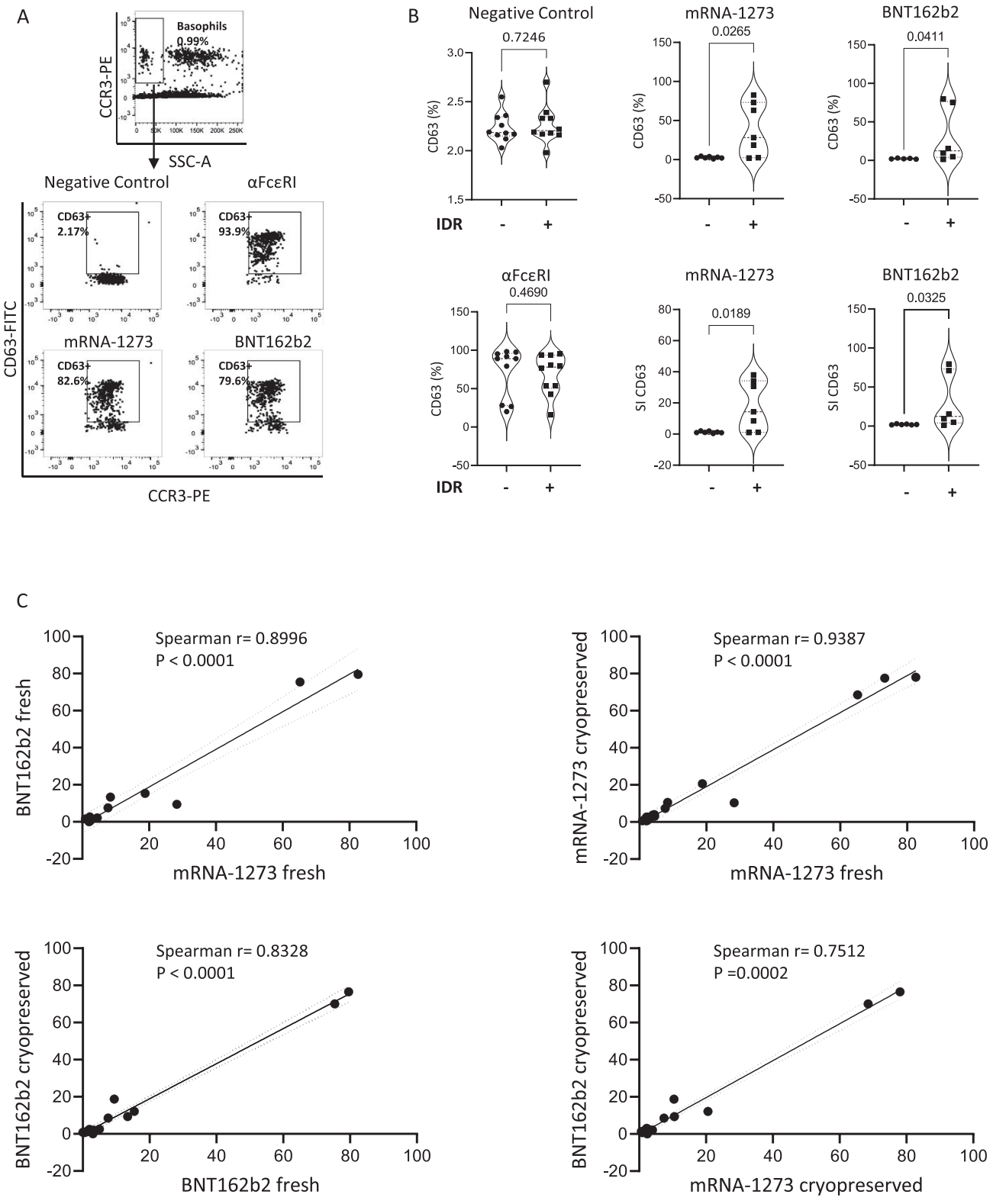

Fig. 1. Ex vivo BAT in response to mRNA1273 and BNT162b2 vaccines. **(A)** Gating strategy for identifying basophils and defining CD63 expression after stimulation with mRNA vaccines. (**B**) CD63 expression and SI for CD63 after stimulation with mRNA-1273 and BNT162b2 vaccines in patient with positive (mRNA-1273 N = 7; BNT162b2 N = 6) or negative (mRNA-1273 N = 7, BNT162b2 N = 6) IDR for mRNA vaccines. Abbreviation: intradermal testing (IDR). (**C**) Correlations for the percentage of CD63 expression comparing leftover fresh mRNA-1273 and BNT162b2 vaccines fresh and cryopreserved mRNA-1273 vaccines, fresh and cryopreserved BNT162b2 vaccines, cryopreserved mRNA-1273 and BNT162b2 vaccines.

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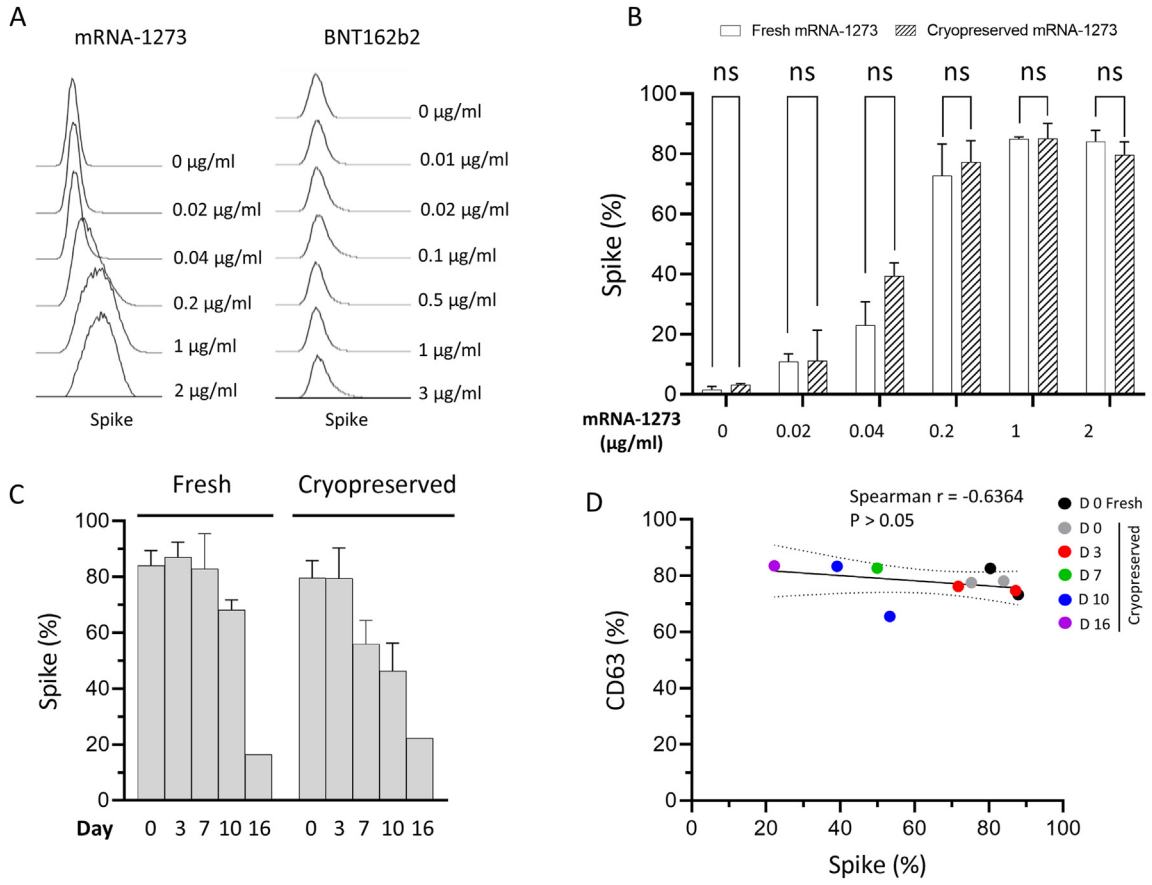

Fig. 2. In vitro functionality of fresh leftover vs cryopreserved mRNA COVID vaccines. **(A)** Spike protein expression on Jurkat cells after 24 h exposure with fresh mRNA-1273 or BNT162b2 vaccines at different concentrations. **(B)** Two independent experiments were performed to assess spike protein expression on Jurkat cells after the addition of different concentrations of fresh (white) and cryopreserved (dashed) mRNA-1273 vaccine at day 0. Median and range is shown. **(C)** Spike protein expression on Jurkat cells after the addition of 1% of fresh or cryopreserved mRNA-1273 vaccine at different timepoints was performed in two independent experiments. Median and range is shown. **(D)** Scatter plot showing correlation between the percentage of CD63 expression in basophils and Spike protein expression in Jurkat cells using the same batch of fresh or cryopreserved (at different time points) mRNA-1273 vaccines.

suggests that the mechanisms driving the positivity are similar and specific likely mediated by ethylene glycol motifs present in mRNA COVID-19 vaccines.[8] The recent demonstration of anti-PEG- IgE in patients with immediate allergic reaction to mRNA COVID-19 vaccines reinforce the notion that PEG is the main culprit.[9] Future studies should compare the sensitivity/specificity of anti-PEG- IgE and BAT.

BAT results were independent of the *in vitro* capacity of the vaccine to induce Spike expression possibly because PEG in suspension remains stable at −80 °C. In contrast to mRNA-1273 vaccines, the BNT162b2-vaccine induced no/low spike expression. These results may be explained by the storage condition of the mRNA-1273 vaccine, which comes already resuspended (as opposed to the BNT162b2 vaccine) to its final concentration and therefore may remain more stable even after being extracted from the vial.

Limitations include a lack of correlation between BAT positivity and clinical outcomes as patients with positive IDR were not challenged but eligible only to a five-step tolerance induction therapy.[10] Thus, we cannot conclude on the causative allergen of the immediate reactions. Yet, the BAT cross-positivity between the mRNA-1273 vaccine (containing tromethamine) and BNT162b2 vaccine (tromethamine-free) suggest that the tromethamine is not responsible for vaccine hypersensitivity. Finally, we have not compared the performance of PEG nor polysorbate (containing ethylene glycol motifs) with mRNA vaccines for BAT.

In conclusion, our results showed that BAT can be used as IDR surrogates to identify vaccine-sensitized individuals. Both mRNA vaccines are interchangeable and can be cryopreserved after the 6 h recommended for immunization purposes. For practical and economic reasons, we propose to use only one mRNA vaccine for BAT; the most convenient to obtain, and to cryopreserve it from unused doses which would be otherwise discarded.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2023.03.005.

Conflict of interest

The authors have no conflict of interest to declare.

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