Sustained T cell immunity, protection and boosting using extended dosing intervals of BNT162b2 mRNA vaccine

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Competing Interests

AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI), but does not participate in policy decisions on COVID-19 vaccines. He is a member of the WHO's SAGE. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, or WHO. AJP is chief investigator on clinical trials of Oxford University's COVID-19 vaccine funded by NIHR. Oxford University has entered a joint COVID-19 vaccine development partnership with AstraZeneca.

Abstract

Extension of the interval between vaccine doses for the BNT162b2 mRNA vaccine was introduced in the UK to accelerate population coverage with a single dose. In a study of 503 healthcare workers, we show that after priming following the first vaccine there is a marked decline in SARS-CoV-2 neutralizing antibody (NAb) levels, but, in contrast, a sustained T cell response to spike protein. This divergent immune profile was accompanied by robust protection from infection over this period from the circulating alpha (B.1.1.7) variant. Importantly, following the second vaccine dose, NAb levels were higher after the extended dosing interval (6-14 weeks) compared to the conventional 3-4 week regimen, accompanied by a clear enrichment of CD4+ T cells expressing IL2. These data on dynamic cellular and humoral responses indicate that extension of the dosing interval

is an effective, immunogenic protocol and that antiviral T cell responses are a potential mechanism of protection.

Introduction

On December 31, 2020, the UK Chief Medical Officers announced changes to the dosing regimen for the second dose of both the Pfizer/BioNTech BNT162b2 and Oxford/AstraZeneca SARS-CoV-2 vaccines, with the interval between the first and second dose extended from 3-4 weeks, to up to 12 weeks. This UK policy was implemented in a bid to avert deaths, and prevent hospitalization due to severe COVID-19, and facilitated the rapid roll-out of one dose of SARS-CoV-2 vaccine providing a degree of cover as quickly as possible to prevent disease in a large proportion of higher-risk groups (Department of Health and Social Care UK, 2021).

The government's strategy to change the dosing regimen was based on estimates of efficacy following a single dose from clinical trials, mathematical modelling, and knowledge transfer from previous published clinical trials and studies of vaccines for other diseases. Whilst Pfizer's clinical trial data described a vaccine efficacy of 52% against symptomatic infection after a single dose (Polack et al., 2020), the UK's Joint Committee on Vaccination and Immunisation (JCVI) revised these figures to an estimated efficacy of 89% against symptomatic infection, having removed infection data from within the first 14 days following first-dose, on the basis that vaccine immune responses take approximately 2 weeks to become established (Joint Committee on Vaccination and Immunisation, 2020). However, the BNT162b2 vaccine is based on novel mRNA technology and currently little is known about the durability of mRNA-elicited vaccine responses after a single dose and about the impact of extension of the dosing interval.

The success of this strategy depends both on the real-world effectiveness of the single dose of vaccine, but also on how long single-dose immunity persists during the extended interval. In particular, the impact of viral variants may also have a substantial impact on the protective efficacy of the immune responses generated. Reports are emerging from Israel of reduced vaccine effectiveness from BNT162b2 against the SARS-CoV-2 delta (B.1.167.2) variant infection (Reuters, 2021). Relatively modest NAb against the early pandemic virus strain are reported after a single dose of BNT162b2 (Food and Drug Administration, 2020). We currently lack very clear correlates of protection against SARS-CoV-2, although recent attempts to compare vaccines have given some indication of the average binding and neutralizing antibody measures which accompany the efficacy of different vaccine approaches (Earle et al., 2021; Feng et al., 2021; Khoury et al., 2021). Most importantly, large scale clinical trial data, on which these estimates are based, do not include measures of T cells. Since T cells are an increasing focus of interest for providing an important component of protection (Rydyznski Moderbacher et al., 2020; Tan et al., 2021), human data to support a role for cellular immunity in providing protection over time are very valuable.

The UK SARS-CoV-2 Immunity & REinfection EvaluatioN (SIREN) Study is a large, multicentre prospective cohort study of health-care workers and support staff in National Health Service (NHS) hospitals in the UK (Hall et al., 2021a). Due to the rapid and extensive roll out of vaccines to NHS workers in December 2020, and the high burden of infection with the alpha variant, which emerged in Kent during the UK's second wave, SIREN has emerged as a leading study for investigating the real-world effectiveness of vaccines for SARS-CoV-2 (Hall et al., 2021b). The PITCH Study (Protective Immunity from T cells in Healthcare workers) is a multi-centre study nested within SIREN and aligned healthcare worker studies, with the goal of undertaking a deeper mechanistic study, including T cell responses, in immunity induced by natural infection and vaccination (Angyal et al., 2021). Samples from the PITCH study have helped define many features of the serologic response to SARS-CoV-2

variants following natural infection and different vaccine regimens (Dejnirattisai et al., 2021; Liu et al., 2021; Skelly et al., 2021; Supasa et al., 2021; Zhou et al., 2021), and confirmed robust antibody and T cell immune responses following a single dose of vaccine in previously infected donors (Angyal et al., 2021).

We aimed to describe the dynamics of T cell and Ab responses after the first dose of BNT162b2 mRNA vaccine over an extended dosing interval, and to compare the magnitude of Ab and T cell responses 4 weeks after dose 2 between short and long vaccination regimens. We coupled this comparison with the protective efficacy data from the parent SIREN study. We observed waning of NAb over the period between the 1st and 2nd doses during the extended dosing interval, whereas the T cell response was maintained through this period, which accompanied a high level of clinical protection in the face of the alpha variant. The extension of the interval also led to a higher level of NAb following second dose. This striking disjunct between NAb and T cells during the early part of the vaccination programme provides insight into the potential mechanisms of vaccine-induced protection.

Results

A single dose of BNT162b2 is associated with sustained protection against infection over an extended dosing interval
To demonstrate the impact of the extended dosing interval on vaccine effectiveness against infection, we include data from the entire SIREN cohort. This study undertook clinical follow-up of 25,066 healthcare workers (HCWs) between 7 December to 12 March 2021 with asymptomatic screening by PCR over a period of up to 95 days (13.6 weeks) from the first dose of BNT162b2. At this time, the alpha (B.1.1.7) variant was the dominant circulating virus in the UK, as confirmed by the Public Health England (PHE) sequencing programme. These data are derived from careful, prospective follow up of the cohort as described (Hall et al., 2021b). The time-resolved data show a gradual increase in the estimate of protection against all infection (asymptomatic and symptomatic) afforded by the vaccine following the single dose in individuals who were seronegative prior to vaccination (**Figure 1A**). A hazard ratio for infection of less than 50% is reached after about 14 days, with protection maintained at high levels until second dose, and then until the end of the follow up period (although with wide confidence intervals due to decreasing numbers). The hazard ratios are adjusted (including for age, ethnicity, comorbidities, and region), and the lower hazard ratio seen on days 0-3 is explained by deferral of vaccination in symptomatic individuals. Overall, these data show robust protection against infection following the first dose of BNT162b2, with vaccine effectiveness reaching 72% by 3 weeks after dose 1 and maintained after that point.

Participants - PITCH Study
503 participants received two doses of the BNT162b2 Pfizer/BioNTech vaccine between 09 December 2020 and 23 May 2021 in 5 UK National Health Service (NHS) Hospital centres. The median age was 43 years (IQR 33-52, range 21-71) with 75% (376) female, reflecting the demographics of the parent SIREN study (Hall et al., 2021b), and 11% (55/392 reported) from an ethnic minority group (**Table 1**). Individuals were defined as SARS-CoV-2 naïve (280, 56%) or previously infected (223, 44%) based on documented PCR and/or serology results from local NHS trusts, or the Mesoscale Discovery (MSD) assay spike (S) and nucleocapsid (N) antibody results if these data were not available locally. In those with previous infection, 53% (119/223) had a documented SARS-CoV-2 positive PCR test result a median of 8.6 (IQR 7.4-9.4) months prior to first vaccination.

The vaccine dosing interval was either the conventional "short" 2-5 week interval (n=75, median 24 days, IQR 21-26, range 14-34), or a "long" 6-14 week interval (n=428, median 70 days, IQR 63-77,

range 44-99). (**Figure 1B** and **Table 1**). An overview of assays performed is detailed in **Supplementary Figure 1**.

Neutralizing antibody levels against SARS-CoV-2 and viral variants drop markedly during the extended dosing interval

We next explored the immune responses that accompany this protection in the time between doses in the extended dosing schedule, using SARS-CoV-2 naïve individuals in the smaller immunology cohort (PITCH). NAb levels were tested using a live virus micro-neutralization assay as previously reported (Dejnirattisai et al., 2021; Liu et al., 2021; Supasa et al., 2021; Zhou et al., 2021). Measurable NAb titers against the ancestral early pandemic virus (Victoria) were observed in the majority of patients tested 4 weeks after the first dose (all infection-naïve), with a median 50% focus reduction neutralization titer ($FRNT_{50}$) of around 10^2 at the first time point, 4 weeks after the first dose (**Figure 2A**). For the variant viruses tested – beta (B.1.351), gamma (P.1) and delta (B.1.167.2), there was very limited detection of NAb against beta and delta after one dose, but only minimal reduction in titers against gamma compared with Victoria. Although the alpha variant was not assayed in this set of experiments, extensive previous comparisons have indicated a consistent drop in titer of around 3-fold in the same assay (Supasa et al., 2021). These titers were all markedly boosted following the second dose, an effect observed across all the variants tested.

We addressed a similar question for binding antibodies obtained using a multiplex ELISA approach (MSD) to measure anti-spike binding antibody. Here we split the cohort into those previously infected and a naïve group, since we know that prior infection can impact on the responses following a single dose. Focusing on the naïve group, we again observed detectable antibodies induced after a single dose of vaccine – but again a clear drop between 28 days and 70 days after the first dose (**Figure 2B**). There was marked boosting following the second dose. A parallel phenomenon was seen in the previously infected group, with the baseline levels of anti-spike antibody prior to the first dose already present at a level similar to that seen after a single dose in the naïve group, and boosted substantially by the first vaccine dose. Linear mixed-effects regression models were used to confirm these findings after adjusting for age and sex. Due to interactions between several post-vaccine timepoints and previous infection status, separate models were run for naïve and previously infected participants. A significant reduction in anti-spike binding antibodies was seen between 4 weeks and 10 weeks after the first dose in both the naïve (-0.38 AU/ml (Log_{10}), P<0.001) and previous infected HCWs (-0.18 AU/ml (Log_{10}), P=0.002, **Table 2**). As expected, the antibody level at 4 weeks following the second dose was significantly higher than at 4 weeks following the first dose in the naïve participants (+0.97 AU/ml (Log_{10}), P<0.001), but not in those who were previously infected (-0.02 AU/ml (Log_{10}), P=0.793).

Overall, a NAb response against the vaccine strain virus is induced by a single dose of vaccine across the cohort, as previously noted, but is not well maintained – in contrast to the clinical protection that was seen against the alpha variant. In addition, NAb against the delta variant were poorly induced after a single dose, and not maintained at all during the interval before the second dose, despite a degree of protection against delta variant now reported following a single dose. Neutralization correlates with spike and receptor binding domain (RBD) binding titers (**Supplementary Figures 2A to 2D**), but binding antibodies are better maintained, although a drop in these levels is also observed (2.6 fold drop for infection-naïve subjects, and a lesser drop of 1.6 fold for previously infected HCWs).

Induction and maintenance of anti-spike T cell responses over the extended doing interval

Given the decay of antibody titers between the doses, despite apparent clinical protection, we next investigated the T cell response over this extended dosing interval, using an interferon-gamma (IFNγ) ELISpot assay. This protocol has proven sensitive and specific, with minimal reactivity in pre-

pandemic samples to peptide pools representing the Victoria (B) spike protein (Angyal et al., 2021; Ogbe et al., 2021). In contrast to antibody titers, the spike-specific T cell response was clearly maintained during the 10 weeks following the primary vaccine, a phenomenon that was equally seen in the previously infected and naïve groups (**Figure 2C**). T cells in the naïve group were further boosted by the second dose, although no further statistically significant boosting effect was seen for the previously infected group. Linear mixed-effects regression models were performed to confirm these results after adjusting for age and sex. Again, an interaction between post-vaccine timepoints and previously infection status led to separate models for naïve and previously infected HCWs. No significant reduction in anti-spike T-cell response was seen between 4 weeks and 10 weeks after the first dose in either naïve (+0.28 SFU (Log$_{10}$), P=0.06) or previously infected HCWs (+0.08 SFU (Log$_{10}$), P=0.466, **Table 2**). A significant increase in anti-spike T-cell response between 4 weeks following the first dose and 4 weeks following the second dose was seen in both naïve (+0.85 SFU (Log$_{10}$), P<0.001) and previously infected HCWs (+0.31 SFU (Log$_{10}$), P<0.003).

Overall, these data reveal an important disjunct between circulating NAb and circulating IFNγ-secreting effector-memory T cell pools over this critical period post first dose, with the robust maintenance of T cells mirroring more closely the maintained protection seen in the larger cohort. We did not see a significant relationship between NAb and the T cell response to spike (**Supplementary Figures 2E-2H**).

Extension of the dosing interval leads to an increase in peak NAb but not T cells
We next compared a SARS-CoV-2 naïve cohort vaccinated using the longer dosing interval with those vaccinated using the conventional 3-4 week interval (short) as tested in the initial clinical trials leading to licensing of BNT162b2. We noted higher NAb titers (at 4 weeks post second dose of both regimens, all infection-naïve) in individuals vaccinated using the long interval regimen, with a 2-4 fold increase in titer, depending on the variant tested (**Figure 3A**), with a correlation across time (**Supplementary Figure 3A**). In each case, titers against the Victoria (B) virus were greater than against beta, gamma and delta variants tested, with the greatest reduction in titer noted against the beta variant, where the benefit of the longer dosing interval was also greatest. These data were confirmed using a secondary assay based on RBD binding inhibition to ACE2 (MSD) (**Figure 3B**). Again, a clear increase is seen with the extended dosing interval, visible across the variants tested (including here the alpha variant which was circulating widely during the period of this study), and this was most clearly evident in the infection-naïve cohort.

We also tested the impact of dosing interval on binding antibodies using spike, RBD and N as targets, splitting the cohort by previous exposure. A clear advantage of the longer interval was seen again, although only in the naïve cohort (**Figure 3C**). The previously infected cohort had equally high levels of binding to spike and RBD regardless of vaccine regimen (**Figure 3C**), and levels of N which appeared to drop over the longer dosing interval (**Figure 2B**). Generalised linear regression models were performed to confirm these findings after adjustment for age, sex and previous infection status. Due to an interaction between vaccine dosing interval and previous infection, separate models were run for naïve and previously infected individuals. The extended dosing interval was associated with a significantly higher anti-spike binding antibody level at 4 weeks after the second dose in naïve (+0.20 AU/ml (Log$_{10}$), P=0.001) but not in previously infected HCWs (+0.01 AU/ml (Log$_{10}$), P=0.886, Table 2). A modest effect of older age associating with lower antibody levels was seen in naïve HCWs (-0.01 AU/ml (Log$_{10}$), per year of age, P=0.020) but not those who were previously infected (0.00 AU/ml (Log$_{10}$), P=0.554). No impact of ethnicity was seen in a reduced dataset (n=143) where this information was available (**Supplementary Table 1**).

Comparing anti-spike responses with dosing interval grouped around 4, 6, 8, 10 and 12 week intervals, we saw a significant difference for infection-naïve HCWs between dose intervals of 4 versus 10 or 12 weeks, but no significant differences between other intervals such as 8 weeks versus 12 weeks, and no difference for previously infected HCWs (**Supplementary Figure 3B**). A number of individuals previously defined as naïve at baseline on the basis of serology and infection history (14/138, 10.1%) showed reactivity to N in this assay 4 weeks after the second dose, indicating exposure during the period of observation. We tested whether removing these individuals from the analyses for T cell responses impacted on the result obtained (**Supplementary Figure 4**), and found the higher binding antibody responses to spike and RBD remained significant.

In contrast to the antibody results, extension of the dosing interval did not lead to greater induction of T cell responses following the second dose (**Figure 3D**). Indeed, while for those previously infected, there was no effect detected, for those previously naïve we found a modestly lower T cell response weeks after the longer dosing interval compared to the shorter interval. Responses to control antigens (CEF - CMV, EBV, Influenza) were unaffected by prior exposure or regimen, while responses to SARS-CoV-2 membrane (M) and N proteins were, as expected, associated with prior exposure but stable over time. There was a low level correlation between binding antibodies and T cell response 4 weeks after the second dose for all HCWs studied, with no obvious impact of reported ethnicity (**Supplementary Figure 5**) although the study was underpowered for full evaluation of ethnicity. Generalised linear regression models were performed to confirm these findings after adjustment for age, sex and previous infection status. The anti-spike T-cell response at 4 weeks after the second dose was significantly higher in previously infected individuals (+0.38 SFU (Log_{10}), P<0.001) and modestly reduced with an extended dosing interval (-0.19 SFU (Log_{10}), P=0.041, Table 2). No impact of ethnicity was seen in a reduced dataset where this information was available (n = 277, **Supplementary Table 1**).

We also compared the effector and helper cell quality of these T cell responses 4 weeks after the second dose in more depth in 86 HCWs using intracellular cytokine staining (ICS). For this analysis, we selected only participants with positive ELISpot assays, which we defined as >40/cells per million PBMC, the mean of the DMSO negative values + 2 standard deviations, since in previous studies we have observed that the smaller frequency populations are hard to detect by flow cytometry, and also prone to inaccuracy in analysis of T cell functionality due to low cell numbers. We saw a marked skewing towards a CD4+ T cell response with the long dosing interval, but more balanced between CD4+ and CD8+ for the short interval (**Figure 4A**). These experiments revealed that, in those who made an IFNγ response on ELISpot, infection-naïve recipients of the long dosing interval generated a higher IL2 CD4+ response to spike compared with the short dosing interval, along with higher IFNγ and TNF CD4+ responses (**Figure 4B**), whilst the CD8+ response was reversed with lower IFNγ response for the long interval (**Figure 4C**). Comparing polyfunctional CD4+ T cells between the groups, there were significant, though modest differences in functionality between all the groups except those previously infected and vaccinated with a short interval, who had a higher proportion of polyfunctional CD4 + T cells (**Supplementary Figure 6**). This comparison for CD8+ T cells was limited by the small number of HCWs who showed CD8 responses sufficient for this analysis, but did not suggest any large differences between the groups.

Combined, the ELISpot and ICS data supports the shorter dosing interval in infection-naïve HCWs giving a modestly higher effector T cell response and the longer interval giving a deeper T cell memory response. The comparison of T cell functionality for HCWs with previous SARS-CoV-2 infection was more heterogeneous, as seen after a single dose (Angyal et al., 2021).

Taken together, these data again reveal another disjunct between the NAb and T cells. In relation to the impact of second dose boosting, there are clear differing effects following extension of the dosing interval.

Dynamics of immune responses following boosting recapitulate those following priming
Given the waning in NAb following a single dose of vaccine, we addressed whether the same effects could be seen following a second dose boost. Tracking NAb responses following the short interval regimen, we found a peak at 1 week post second dose, with a subsequent clear decline in circulating NAb titers against Victoria and variant viruses over the subsequent time points (4 weeks and 13 weeks post-second dose, all infection-naïve, **Figure 5A**). The decline against Victoria strain between weeks 1 and 4 is approximately 4-fold, which compares with around 3-fold between weeks 4 and 13, and thus around 12-fold over the overall 3-month period.

In contrast to the antibody data, once again we observed maintenance of T cell responses over the same period (**Figure 5B**). These responses showed a very modest, but statistically significant loss of activity against peptide pools representing the beta (1.18-fold lower, P = 0.0001) and gamma (1.1-fold lower, P = 0.0016) variants compared to wild-type sequence, but no loss of activity against delta variant (fold change = 1.00, P = 0.2058) **Figure 5C**).

We also looked at the results to compare the NAb response at set points in time between HCWs who received the vaccine second dose at 3-4 weeks and those on the longer dosing interval. Unsurprisingly, in January 2021, when alpha variant was circulating in the UK, those who had received a second vaccine dose at 3-4 weeks had levels of NAb against Victoria and variant viruses that were substantially higher than in those who were sampled 4 weeks after the first dose of the longer dosing regimen (**Figure 5D**). This is most evident against the variant viruses. However, looking at a later time point in April 2021, i.e. 13 weeks after the second dose in the short course regimen, but 4 weeks post-second dose in the longer regimen, the reverse is true: titers are significantly higher against Victoria and all the variants in those who completed vaccination more recently with the longer dosing interval (**Figure 5E**).

Overall these data recapitulate the data seen after a single dose. That is, there is a clear decline in circulating antibodies over a 3 month period, with strong maintenance of the T cell response. This is a period well studied in the clinical trials where robust protection following a second dose was observed (Polack et al., 2020).

Discussion

Extended dose intervals for boosting Covid-19 mRNA vaccines were introduced based on an interpretation of clinical trial data, but without a strong dataset to support the immunology or real-world clinical effectiveness. Here we provide an extensive serological and T cell assay dataset to explore the vaccine effectiveness seen in the parent SIREN study. The serologic response to one or two doses of BNT162b2 falls over time, and is higher after an extended dosing interval compared with the 3-4 week dosing interval that was tested in the licensing trials. By contrast, the T cell response is well maintained after one then two doses, and is of a marginally lower magnitude after the longer dosing interval when measured by the ELISpot assay of T cell effector function, yet shows a more developed memory cell phenotype compared with the 3-4 week dosing interval. This clear dichotomy of immunological assays after BNT162b2 vaccination provides a challenge to current dogma surrounding correlates or indeed mediators of immune protection.

The data provided from the larger clinical study in SIREN provide clear evidence of a protective impact of a single dose over an extended period. The SIREN study is based on prospective screening for infection rather than reactive testing based on symptoms alone, and therefore provides a robust measurement of protection. The number of unvaccinated individuals dropped over the period of study, thus leading to a wide confidence interval for the estimates of efficacy, but there is no evidence of a decline over the first 3 months and if anything, a trend to increasing protection. This feature is at odds with the profile of NAb seen, which decline sharply both after priming and interestingly also over time after the much higher levels achieved from boosting with second dose. The decline in NAb parallels a smaller but significant decline in binding antibody measured by ELISA, but contrasts with a robust T cell response over this period. The discordance of NAb with real-world estimates of vaccine effectiveness is important to bear in mind for policy makers around vaccine implementations.

Although this provides critical data of the dynamics of responses in the weeks after a single vaccine dose, the relative role of antibodies with different functionalities vs T cells in protection cannot be answered in a correlative study alone. Several studies have demonstrated functional antibody responses beyond neutralizing function against SARS-CoV-2 after natural infection and vaccination (Barrett et al., 2021; Bartsch et al., 2021; Tomic et al., 2021). Other interesting observations that support a role for T cells in protection come from analyses in the setting of the beta variant outbreak in South Africa (Moore et al., 2021). As we have shown, T cell responses are not substantially impacted overall by the emerging variants, in contrast to NAb, which show poor cross-reactivity in particular with the beta variant. The protective efficacy of vaccines such as Ad26 in South African trials has therefore been hypothesized to link to cellular immunity. Other data that have explored the relative role of antibody and T cells in protection come from more mechanistic animal studies, where depletion of CD8+ T cells in SARS-CoV-2 convalescent macaques partially abolished protective natural immunity against re-challenge when antibody levels were waning (McMahan et al., 2021).

The second major observation of this study is that boosting after a longer interval leads to a distinct impact on the anti-spike responses, with an increase in NAb but a reduction in T cell effector responses compared with conventional (short) interval dosing. Although the differences are quite small, they are reproducible with different assays and indeed similar data have recently been obtained in a study of an elderly UK cohort (Parry et al., 2021). The overall clinical impact of this is currently unclear – both the short and long dosing regimens result in induction of substantial T cell responses. The short dosing interval gives a slightly higher effector T cell response, with both CD4+ and CD8+ contributions, whilst the longer dosing interval results in a CD4+ dominated memory phenotype with marked IL2 production to spike. For antibody responses, given the rapid declines seen here post boosting, we expect that such a difference would be relatively short-lived, although it is possible that overall the levels of antibody induced would remain higher over a longer period. There is currently no agreed correlate of protection but it has been suggested that within a study of AZD122, an average level of 40,923 arbitrary units (AU)/ml at 4 weeks post second dose for anti-spike IgG using the MSD assay used in this study, equivalent to 264 binding antibody units (BAU)/ml using the WHO international standard, is associated with 80% protection from symptomatic infection for recipients receiving that vaccine regimen (Feng et al., 2021).

The marked CD4+ helper T cell phenotype induced by the extended dosing interval gives mechanistic insight into the increased antibody boost as IL2 provides important help for B cells to develop into plasmablasts (Hipp et al., 2017; Le Gallou et al., 2012). Ongoing work will further explore this mechanism and observe the impact of dosing interval on longer term durability of antibody and T cell responses, but benefits of longer intervals between vaccine doses have been observed for other vaccines in mouse and human studies, where early boosting results in higher numbers of terminally differentiated and effector T cells, whilst later boosting promotes efficient T cell expansion and

enhanced long term memory cell persistence (Capone et al., 2020; Steffensen et al., 2013). Therefore, a longer interval between vaccine doses may allow spike-specific T cells to fully differentiate into memory T cells that respond optimally to spike re-exposure.

Regardless of the regimen, there remains a large amount of inter-individual variability in the vaccine responsiveness for both humoral and cellular responses. Overall, we see a correlation between the two measures when responses across all time points are considered, but there was no relationship between NAb and T cell responses (ELISpot) when the time point 4 weeks after second dose was considered alone. Interestingly, the impact of the dosing interval and the effect of boosting on individuals with prior infection was much less evident than in naïve individuals. We also examined the influence of age, sex and ethnicity in our cohort. Although these observations are limited by the numbers studied and balance of the cohort, we did not observe any substantial effect in simple comparative or multivariate analyses, except for a modest effect of older age associating with lower antibody levels in naïve HCWs. It remains likely however that genetic effects such as HLA type do play a role as this has been shown in other vaccine settings (Mentzer et al., 2015), and the impact of exposure to other beta coronaviruses remains to be further explored.

In conclusion, the immunogenicity of longer dose regimens appears robust, and indeed for antibody measurements, improved over the conventional 3-4 week regimen. We provide evidence that T cells are induced and sustained during the longer period between doses in the 6-14 week regimen, but there is an impact of dosing interval on the relative proportion of T cell subsets. T cell populations may contribute to important protection against SARS-CoV-2 including the alpha variant during this extended dosing interval, and indeed beyond the second dose. The ultimate impact of distinct memory pools in long-term protection following either regimen requires longer term follow up of such highly-exposed and biologically informative cohorts.

Limitations of the Study
This study provides detailed information at relative scale about the immune response to the BNT162b2 vaccine in a healthy, working age population to help understanding of the vaccine effectiveness against the alpha variant seen in the SIREN study and in the UK as a whole. The limitations of this study include firstly the predominance of females and white ethnicity, reflective of the HCWs we were able to recruit, although neither female sex nor ethnicity were revealed as significant variables in our modelling analysis. Complementary studies in other populations including ethnic minorities, older adults, and those with immune compromise will further inform knowledge of vaccine response and susceptibility to SARS-CoV-2. Secondly, laboratory measurements such as low neutralizing antibody levels are subject to threshold effects and may not reflect true functional immunity on re-exposure to spike protein. Thirdly, ongoing follow-up to evaluate the impact of dosing interval on the durability of the immune response is needed. Fourthly, the high heterogeneity of antibody and T cell responses that we observed means that our findings, of higher levels of antibodies and T cell memory function after an extended dosing interval, are of most relevance at a population level rather than at an individual level. Finally, though we know the vaccine efficacy in this cohort, we did not measure this directly in the same individuals in whom we made T cell and antibody measurements, meaning that we cannot directly measure novel correlates of protection in this study.

The importance of immune memory and the multiple ways in which the immune system post vaccination can prevent severe disease, including functional properties of binding antibodies and cellular responses must be borne in mind to avoid excessive focus on NAb as a singular proxy for vaccine-induced immunity. Our study demonstrates that two doses of BNT162b2 are highly immunogenic for antibodies and T cells across the range of dosing intervals studied. When current and anticipated levels of circulating virus are low, the extended dosing interval appears optimal for

immunogenicity, but this needs to be weighed against the more immediate benefits of two doses over one. Policy decisions around vaccine dosing interval and the use of third booster doses will depend on several factors including current prevalence of SARS-CoV-2, which variants of concern are emerging, population susceptibility and vaccine supply.

METHODS

The SIREN Study
The SIREN study is a separate ongoing study with the methodology published previously (Hall et al., 2021b). SIREN is a large prospective cohort study of healthcare workers and allied staff aged 18 years and above working in UK National Health Service (publicly funded) hospitals. The vaccine effectiveness analysis included in Figure 1A of this paper presents a repeat analysis after an extended follow-up period (up to 12 March 2021) to that previously published (Angyal et al., 2021); data up to 5 February 2021. In brief, effectiveness of the BNT162b2 vaccine against PCR-confirmed infection (asymptomatic and symptomatic) was estimated in SIREN participants followed up from 7 December 2020 to 12 March 2012, by comparing time to infection in vaccinated and unvaccinated participants. Participants underwent fortnightly asymptomatic SARS-CoV-2 PCR testing and monthly antibody testing, and all tests (including symptomatic testing) outside SIREN were captured. Baseline risk factors were collected at enrolment, symptom status was collected every 2 weeks, and vaccination status was collected through linkage to the National Immunisations Management System and questionnaires. Historic SARS-CoV-2 PCR and antibody testing data was used to determine each participants prior SARS-CoV-2 infection status (positive or negative cohort) at the beginning of the analysis period (7 December 2020). Vaccine effectiveness of the BNT162b2 vaccine was calculated using a piecewise exponential hazard mixed-effects model (shared frailty-type model) using a Poisson distribution, which adjusted for the variable incidence during the follow-up period and important confounders. The study is registered with ISRCTN, number ISRCTN11041050, and is ongoing.

Study Design
In this prospective, observational, cohort study, HCWs were recruited into the PITCH Consortium Study at NHS hospitals in five centres in England (University Hospitals Birmingham NHS Foundation Trust, Liverpool University Hospitals NHS Foundation Trust, Newcastle upon Tyne Hospitals NHS Foundation Trust, Oxford University Hospitals NHS Foundation Trust, and Sheffield Teaching Hospitals NHS Foundation Trust). Individuals consenting to participate were recruited by word of mouth, hospital e-mail communications and from hospital-based staff screening programmes for SARS-CoV-2, including HCWs enrolled in the national SIREN study at three sites (Liverpool, Newcastle and Sheffield). Eligible participants were adults aged 18 or over currently working as an HCW, including allied support and laboratory staff, and were sampled for the current study between 4 December 2020 and 27 May 2021.

PITCH is a sub-study of the SIREN study which was approved by the Berkshire Research Ethics Committee, Health Research 250 Authority (IRAS ID 284460, REC reference 20/SC/0230), with PITCH recognised as a sub-study on 2 December 2020. SIREN is registered with ISRCTN (Trial ID:252 ISRCTN11041050). Some participants were recruited under aligned study protocols. In Birmingham participants were recruited under the Determining the immune response to SARS-CoV-2 infection in convalescent health care workers (COCO) study (IRAS ID: 282525). In Liverpool some participants were recruited under the "Human immune responses to acute virus infections" Study (16/NW/0170), approved by North West - Liverpool Central Research Ethics Committee on 8 March 2016, and amended on 14th September 2020 and 4th May 2021. In Oxford, participants were recruited under the GI Biobank Study 16/YH/0247, approved by the research ethics committee (REC) at Yorkshire & The Humber - Sheffield Research Ethics Committee on 29 July 2016, which has been amended for this purpose on 8 June 2020. In Sheffield, participants were recruited under the Observational Biobanking study STHObs (18/YH/0441), which was amended for this study on 10 September 2020. The study was conducted in compliance with all relevant ethical regulations for work with human participants, and according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. Written informed consent was obtained for all patients enrolled in the study.

Participants
HCWs were recruited to the PITCH study from across the five centres, both with and without a history of SARS-CoV-2 infection. Individuals were defined as SARS-CoV-2 naïve or previously infected based on documented PCR and/or serology results from local NHS trusts, or the MSD assay S and N antibody results if these data were not available locally. All participants received the BNT162b2 Pfizer/BioNTech vaccine. The vaccine dosing interval was either a "short" 3-5 week interval (median 24 days, IQR 21-26, range 14-34), or a "long" 6-14 week interval (median 70 days, IQR 63-77, range 44-99). The immune response data from baseline and 4 weeks after the first dose has been previously reported, alongside data for 21 HCWs 4 weeks after the second vaccine in a short dosing regimen (Angyal et al., 2021).

Sample collection
Participants on the "long" dosing interval received phlebotomy for assessment of immune responses prior to first dose of vaccine (median 23 days, IQR 7/33), 4 weeks after the first dose (median 28 days, IQR 25/31), 8 weeks after the first dose (median 66 days, IQR 61/75) and 4 weeks after the second dose (median 29 days, IQR 27/33). Participants on the "short" dosing interval received phlebotomy for assessment of immune responses 1 week after the second dose (median 7 days, IQR 7/8,) 4 weeks after the second dose (median 28 days, IQR 27/32) and 13 weeks after the second dose (median 94 days, IQR 89/100). An overview of assays performed is detailed in **Supplementary Figure 1**. Clinical information including BNT162b2 immunisation dates, date of any prior SARS-CoV-2 infection defined by a positive PCR test and/or detection of antibodies to spike or nucleocapsid protein, presence or absence of symptoms, time between symptom onset and sampling, age, gender and ethnicity of participant was recorded.

Peripheral blood mononuclear cells (PBMCs) were separated from heparinised whole blood using density gradient centrifugation with Lymphoprep (StemCell Technology, 07861) in Leucosep tubes (GreinerBio, 227290) and cryopreserved in liquid nitrogen for later use. Extracted plasma was stored at -80°C until further analysis.

Neutralizing antibody titration
Viral Stocks
SARS-CoV-2/human/AUS/VIC01/2020 (Caly et al., 2020), SARS-CoV-2/B.1.1.7 and SARS-CoV-2/B.1.351 were provided by Public Health England, P.1 from a throat swab from Brazil were grown in Vero (ATCC CCL-81) cells. Cells were infected with the SARS-CoV-2 virus using an MOI of 0.0001. Virus containing supernatant was harvested at 80% CPE and spun at 3000 rpm at 4 °C before storage at -80 °C. Viral titers were determined by a focus-forming assay on Vero cells. Victoria passage 5, B.1.1.7 passage 2 and B.1.351 passage 4 stocks P.1 passage 1 stocks were sequenced to verify that they contained the expected spike protein sequence and no changes to the furin cleavage sites. The B.1.617.2 virus was kindly provided Wendy Barclay and Thushan De Silva and contained the following mutations compared to the Wuhan sequence: T19R, G142D, Δ156-157/R158G, A222V, L452R, T478K, D614G, P681R, D950N.

Focus Reduction Neutralization Assay (FRNT)
The neutralization potential of antibodies (Ab) was measured using a Focus Reduction Neutralization Test (FRNT), where the reduction in the number of the infected foci is compared to a negative control well without antibody. Briefly, serially diluted Ab or plasma was mixed with SARS-CoV-2 strain Victoria or P.1 and incubated for 1 hr at 37 °C. The mixtures were then transferred to 96-well, cell culture-treated, flat-bottom microplates containing confluent Vero cell monolayers in duplicate and incubated for a further 2 hrs followed by the addition of 1.5% semi-solid carboxymethyl cellulose (CMC) overlay medium to each well to limit virus diffusion. A focus forming assay was then performed by staining Vero cells with human anti-nucleocapsid monoclonal Ab (mAb206) followed by peroxidase-conjugated

goat anti-human IgG (A0170; Sigma). Finally, the foci (infected cells) approximately 100 per well in the absence of antibodies, were visualized by adding TrueBlue Peroxidase Substrate. Virus-infected cell foci were counted on the classic AID ELISpot reader using AID ELISpot software. The percentage of focus reduction was calculated and IC_{50} was determined using the probit program from the SPSS package.

Mesoscale Discovery (MSD) binding assays

IgG responses to SARS-CoV-2, SARS-CoV-1, MERS-CoV and seasonal coronaviruses were measured using a multiplexed MSD immunoassay: The V-PLEX COVID-19 Coronavirus Panel 3 (IgG) Kit (cat. no. K15399U) from Meso Scale Diagnostics, Rockville, MD USA. A MULTI-SPOT® 96-well, 10 spot plate was coated with three SARS CoV-2 antigens (S, RBD, N), SARS-CoV-1 and MERS-CoV spike trimers, as well as spike proteins from seasonal human coronaviruses, HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63, and bovine serum albumin. Antigens were spotted at 200–400 µg/mL (MSD® Coronavirus Plate 3). Multiplex MSD assays were performed as per the instructions of the manufacturer. To measure IgG antibodies, 96-well plates were blocked with MSD Blocker A for 30 minutes. Following washing with washing buffer, samples diluted 1:1,000-10,000 in diluent buffer, or MSD standard or undiluted internal MSD controls, were added to the wells. After 2-hour incubation and a washing step, detection antibody (MSD SULFO-TAG™ Anti-Human IgG Antibody, 1/200) was added. Following washing, MSD GOLD™ Read Buffer B was added and plates were read using a MESO® SECTOR S 600 Reader. The standard curve was established by fitting the signals from the standard using a 4-parameter logistic model. Concentrations of samples were determined from the electrochemiluminescence signals by back-fitting to the standard curve and multiplied by the dilution factor. Concentrations are expressed in Arbitrary Units/ml (AU/ml). Cut-offs were determined for each SARS-CoV-2 antigen (S, RBD and N) based on the concentrations measured in 103 pre-pandemic sera + 3 Standard Deviation. Cut-off for S: 1160 AU/ml; cut-off for RBD: 1169 AU/ml; cut-off for N: 3874 AU/ml.

A multiplexed MSD immunoassay (MSD, Rockville, MD) was also used to measure the ability of human sera to inhibit ACE2 binding to SARS-CoV-2 spike (B, B.1, B.1.1.7, B.1.351 or P.1). A MULTI-SPOT® 96-well, 10 spot plate was coated with five SARS-CoV-2 spike antigens (B, B.1, B.1.1.7, B.1.351 or P.1). Multiplex MSD Assays were performed as per manufacturer's instructions. To measure ACE2 inhibition, 96-well plates were blocked with MSD Blocker for 30 minutes. Plates were then washed in MSD washing buffer, and samples were diluted 1:10 – 1:100 in diluent buffer. Importantly, an ACE2 calibration curve which consists of a monoclonal antibody with equivalent activity against spike variants was used to interpolate results as arbitrary units (units/ml), with 1 unit being equivalent to 1ug/ml neutralising activity of the standard. Furthermore, internal controls and the WHO SARS-CoV-2 Immunoglobulin international standard (20/136) were added to each plate. After 1-hour incubation recombinant human ACE2-SULFO-TAG™ was added to all wells. After a further 1-hour plates were washed and MSD GOLD™ Read Buffer B was added, plates were then immediately read using a MESO® SECTOR S 600 Reader.

ELISpot Assays

The PITCH ELISpot Standard Operating Procedure has been published previously (Angyal et al., 2021). Interferon-gamma (IFNγ) ELISpot assays were set up from cryopreserved PBMCs using the Human IFNγ ELISpot Basic kit (Mabtech 3420-2A). A single protocol was agreed across the centres as previously published (Angyal et al., 2021), and we found no significant difference in magnitude of ELISpot response to spike 4 weeks after the second vaccine across the five centres (**Supplementary Figure 6**).

PBMCs were thawed and rested for 3-6 hours in R10 at 37°C prior to stimulation with peptides. MultiScreen-IP filter plates (Millipore, MAIPS4510) were coated with the capture antibody (clone 1-D1K) at 10µg/ml diluted in sterile phosphate buffered saline (PBS; Gibco, 10010031) and sterile carbonate bicarbonate (Sigma, C3041) at 50µl/well overnight at 4°C prior to application. The following

day, coated plates were washed four times with sterile PBS, then blocked with RPMI media supplemented with 10% fetal bovine serum and 10mM penicillin/streptomycin (R10) for two hours at 37°C. Overlapping peptide pools (18-mers with 10 amino acid overlap, Mimotopes) representing the spike (S), Membrane (M) or nucleocapsid (N) SARS-CoV-2 proteins were added to 250,000 PBMCs/well at a final concentration of 2μg/ml for 16–18 h. For selected individuals, pools representing the S1 and S2 subunits of variant of concern were also included (B.1.35/beta and P.1/gamma). Pools consisting of CMV, EBV and influenza peptides (2μg/ml, Proimmune, Oxford, UK) and phytohemagglutinin (PHA, Sigma L8754) were used as positive controls, along with negative control wells. Wells were then washed with PBS-0.05% Tween and incubated with the biotinylated detection antibody (clone 7-B6-1) diluted in PBS-0.05% Tween at 1μg/ml for 2 hours at room temperature (RT). After another washing step, streptavidin-ALP was added diluted in PBS at 1μg/ml for 45-90 minutes at RT. Wells were then washed with PBS and detection was carried out using the AP Conjugate kit (1706432, BioRad) for 15 minutes at RT. Plates were scanned and analysed with the AID Classic ELISpot reader (software version 8.0, Autoimmune Diagnostika GmbH, Germany). Antigen-specific responses were quantified by subtracting the mean spots of the control wells from the test wells and the results were expressed as spot-forming units (SFU)/10^6 PBMCs.

Intracellular cytokine staining

T cell responses in selected IFNγ ELISpot positive samples were characterised further using intracellular cytokine staining (ICS) after stimulation with overlapping spike peptide pools. In brief, 1-1.5 x 10^6 cells were plated in R10 and co-stimulatory antibodies (with anti-CD28, BD 555725 and anti-CD49d, BD 555501) in a 96 well U-bottom plate and peptide pools were added at 2μg/ml final concentration for each peptide. DMSO was used as the negative control at the equivalent concentration to the peptides. As a positive control, cells were simultaneously stimulated with ionomycin (Sigma, I0634-1MG) at 500ng/ml and PMA (Sigma, P1585-10MG) at 50ng/ml final concentrations. Degranulation of T cells (a functional marker of cytotoxicity (Betts et al., 2003) was measured by the addition of an anti-CD107a specific antibody (BD, 561348) at 1 in 20 dilution during the culture. The cells were then incubated at 37°C, 5% CO_2 for 1 hour before adding Brefeldin A (10 μg/ml). Samples were incubated at 37°C, 5% CO_2 for a further 5 hours before proceeding with staining for flow cytometry.

First, stimulated cells were stained with live/dead stain 1:500 at RT in the dark for 20 minutes then washed in DPBS followed by spinning the samples at 300g for 5 minutes. Cells were then fixed in 2% formaldehyde for 20 minutes, then frozen at -80°C in DPBS/1% BSA/10% DMSO prior to staining and analysis, which was done in batches. cells were thawed, centrifuged at 400g for 5 minutes to remove the freezing mix before permeabilization in 1x Perm/Wash buffer (BD, 554273) for 20-25 minutes at RT. Staining was performed in the dark at RT for 30 minutes in 1x Perm/Wash buffer with the antibodies listed in Supplementary Table S1, then the cells were washed and resuspended in DPBS. The samples were run on a FacsCanto II cytometer and the data were analysed using FlowJo software version 10 (Treestar). Polyfunctional T cell analysis was conducted using SPICE v6.1, with pre-processing in R version 4.0.2 using the tidyverse packages, and PESTLE version 2.0 (Roederer *et al*. 2011). A threshold of > 0.001 was applied in SPICE, and the data are presented as the relative fraction of cytokine positive cells, using the median as the base. The permutation test was used to compare pies, running 10,000 simulations.

Statistical analysis

Continuous variables are displayed with median and interquartile range (IQR). Paired comparisons were performed using the Wilcoxon matched pairs signed rank test. Unpaired comparisons across two groups were performed using the Mann-Whitney test. Two-tailed P values are displayed. Statistical analyses were done using R version 3.5.1 and GraphPad Prism 9.0.1.

Expression of multiple effector functions on a per-cell basis ("polyfunctional T cells") was assessed using Simplified Presentation of Incredibly Complex Evaluations (SPICE) software (Roederer et al., 2011). In order to reliably include specimens with enough events for the analysis, positive responses were defined as >0.02% IFNγ/TNF double positive cells, at least double the DMSO value, and with >50 events in any double positive gate. Data were background subtracted before importing into SPICE, and a threshold of > 0.001 was applied. The difference in T cell functionality between naïve and previously infected participants was assessed using the SPICE permutation test, running 10,000 simulations.

Statistical Regression Models

Multivariate regression models were created to estimate the associations between variables in the study cohort and antibody and T cell immune response. Variables included age, sex, ethnicity, previous infection, time point and vaccine dosing interval. Interactions and co-linearity between variables were explored and variables analysed in separate models where necessary. Generalized linear models were created to estimate associations between the variables sex (discrete), age (continuous), Ethnicity (discrete), previous infection (discrete), and vaccine interval regimen (discrete) on spike ELISpot response (spike B SFU/10^6; log transformed) or spike IgG response (SARS-Cov-2 S AU/ml; log transformed). Linear mixed-effect models were created to estimate associations between variables sex (discrete), age (continuous), sample timepoint (discrete), previous infection (discrete) and Ethnicity (discrete). on spike ELISpot response (spike B SFU/10^6; log transformed) or spike IgG response (SARS-Cov-2 S AU/ml; log transformed) in data from the Long dosing interval. Interactions were found between previous infection and vaccine dosing interval in model 1 and 2 for Spike IgG responses, so separate models were run for naïve and previously infected individuals. GLM and LMER models were performed in R /R studio. Summary tables were reported. To check assumptions were met, residuals vs fitted and Normal Q-Q diagnostic plots were created.

Model 1 <- glm (immune response ~ age + sex + previous infection + vaccine dosing interval + previous infection : vaccine dosing interval, data = data)

Model 2 <- glm (immune response ~ age + sex + previous infection + vaccine dosing interval + ethnicity + previous infection : vaccine dosing interval, data = data)

Model 3 <- lmer (immune response ~ age + sex + previous infection + sample timepoint + previous infection : timepoint, data = data)

References

Angyal, A., Longet, S., Moore, S., Payne, R., Harding, A., Tipton, T., Rongkard, P., Ali, M., Hering, L., Meardon, N*., et al.* (2021). T-Cell and Antibody Responses to First BNT162b2 Vaccine Dose in Previously SARS-CoV-2-Infected and Infection-Naive UK Healthcare Workers: A Multicentre, Prospective, Observational Cohort Study (SSRN).

Barrett, J.R., Belij-Rammerstorfer, S., Dold, C., Ewer, K.J., Folegatti, P.M., Gilbride, C., Halkerston, R., Hill, J., Jenkin, D., Stockdale, L*., et al.* (2021). Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. Nat Med *27*, 279-288.

Bartsch, Y.C., Wang, C., Zohar, T., Fischinger, S., Atyeo, C., Burke, J.S., Kang, J., Edlow, A.G., Fasano, A., Baden, L.R*., et al.* (2021). Humoral signatures of protective and pathological SARS-CoV-2 infection in children. Nat Med *27*, 454-462.

Betts, M.R., Brenchley, J.M., Price, D.A., De Rosa, S.C., Douek, D.C., Roederer, M., and Koup, R.A. (2003). Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J Immunol Methods *281*, 65-78.

Caly, L., Druce, J., Roberts, J., Bond, K., Tran, T., Kostecki, R., Yoga, Y., Naughton, W., Taiaroa, G., Seemann, T*., et al.* (2020). Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. The Medical journal of Australia *212*, 459-462.

Capone, S., Brown, A., Hartnell, F., Sorbo, M.D., Traboni, C., Vassilev, V., Colloca, S., Nicosia, A., Cortese, R., Folgori, A*., et al.* (2020). Optimising T cell (re)boosting strategies for adenoviral and modified vaccinia Ankara vaccine regimens in humans. NPJ vaccines *5*, 94-94.

Dejnirattisai, W., Zhou, D., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R*., et al.* (2021). Antibody evasion by the P.1 strain of SARS-CoV-2. Cell *184*, 2939-2954.e2939.

Department of Health and Social Care UK (2021). Optimising the COVID-19 vaccination programme for maximum short-term impact.

Earle, K.A., Ambrosino, D.M., Fiore-Gartland, A., Goldblatt, D., Gilbert, P.B., Siber, G.R., Dull, P., and Plotkin, S.A. (2021). Evidence for antibody as a protective correlate for COVID-19 vaccines. medRxiv, 2021.2003.2017.20200246.

Feng, S., Phillips, D.J., White, T., Sayal, H., Aley, P.K., Bibi, S., Dold, C., Fuskova, M., Gilbert, S.C., Hirsch, I*., et al.* (2021). Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. medRxiv, 2021.2006.2021.21258528.

Food and Drug Administration (2020). Vaccines and Related Biological Products Advisory Committee Meeting.

Hall, V.J., Foulkes, S., Charlett, A., Atti, A., Monk, E.J.M., Simmons, R., Wellington, E., Cole, M.J., Saei, A., Oguti, B.*, et al.* (2021a). SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet (London, England) *397*, 1459-1469.

Hall, V.J., Foulkes, S., Saei, A., Andrews, N., Oguti, B., Charlett, A., Wellington, E., Stowe, J., Gillson, N., Atti, A.*, et al.* (2021b). COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet (London, England) *397*, 1725-1735.

Hipp, N., Symington, H., Pastoret, C., Caron, G., Monvoisin, C., Tarte, K., Fest, T., and Delaloy, C. (2017). IL-2 imprints human naive B cell fate towards plasma cell through ERK/ELK1-mediated BACH2 repression. Nature Communications *8*, 1443.

Joint Committee on Vaccination and Immunisation (2020). Optimising the COVID-19 vaccination programme for maximum shortterm impact.

Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. (2021). What level of neutralising antibody protects from COVID-19? medRxiv, 2021.2003.2009.21252641.

Le Gallou, S., Caron, G., Delaloy, C., Rossille, D., Tarte, K., and Fest, T. (2012). IL-2 Requirement for Human Plasma Cell Generation: Coupling Differentiation and Proliferation by Enhancing MAPK–ERK Signaling. The Journal of Immunology *189*, 161.

Liu, C., Ginn, H.M., Dejnirattisai, W., Supasa, P., Wang, B., Tuekprakhon, A., Nutalai, R., Zhou, D., Mentzer, A.J., Zhao, Y.*, et al.* (2021). Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell.

McMahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandrashekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A.*, et al.* (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature *590*, 630-634.

Mentzer, A.J., O'Connor, D., Pollard, A.J., and Hill, A.V.S. (2015). Searching for the human genetic factors standing in the way of universally effective vaccines. Philosophical transactions of the Royal Society of London Series B, Biological sciences *370*, 20140341.

Moore, P.L., Moyo-Gwete, T., Hermanus, T., Kgagudi, P., Ayres, F., Makhado, Z., Sadoff, J., Le Gars, M., van Roey, G., Crowther, C.*, et al.* (2021). Neutralizing antibodies elicited by the Ad26.COV2.S COVID-19 vaccine show reduced activity against 501Y.V2 (B.1.351), despite protection against severe disease by this variant. bioRxiv, 2021.2006.2009.447722.

Ogbe, A., Kronsteiner, B., Skelly, D.T., Pace, M., Brown, A., Adland, E., Adair, K., Akhter, H.D., Ali, M., Ali, S.-E.*, et al.* (2021). T cell assays differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses. Nature Communications *12*, 2055.

Parry, H., Bruton, R., Stephens, C., Brown, K., Amirthalingam, G., Hallis, B., Otter, A., Zuo, J., and Moss, P. (2021). Extended interval BNT162b2 vaccination enhances peak antibody generation in older people. medRxiv, 2021.2005.2015.21257017.

Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Pérez Marc, G., Moreira, E.D., Zerbini, C.*, et al.* (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med *383*, 2603-2615.

Reuters (2021) Israel sees drop in Pfizer vaccine protection against infections. 5th July 2021 https://www.reuters.com/world/middle-east/israel-sees-drop-pfizer-vaccine-protection-against-infections-still-strong-2021-07-05/

Roederer, M., Nozzi, J.L., and Nason, M.C. (2011). SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry Part A : the journal of the International Society for Analytical Cytology *79*, 167-174.

Rydyznski Moderbacher, C., Ramirez, S.I., Dan, J.M., Grifoni, A., Hastie, K.M., Weiskopf, D., Belanger, S., Abbott, R.K., Kim, C., Choi, J.*, et al.* (2020). Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell *183*, 996-1012.e1019.

Skelly, D.T., Harding, A.C., Gilbert-Jaramillo, J., Knight, M.L., Longet, S., Brown, A., Adele, S., Adland, E., Brown, H., Medawar Laboratory Team*, et al.* (2021). Two doses of SARS-CoV-2 vaccination induce more robust immune responses to emerging SARS-CoV-2 variants of concern than does natural infection. Research Square.

Steffensen, M.A., Holst, P.J., Steengaard, S.S., Jensen, B.A.H., Bartholdy, C., Stryhn, A., Christensen, J.P., and Thomsen, A.R. (2013). Qualitative and quantitative analysis of adenovirus type 5 vector-induced memory CD8 T cells: not as bad as their reputation. J Virol *87*, 6283-6295.

Supasa, P., Zhou, D., Dejnirattisai, W., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Nutalai, R., Tuekprakhon, A.*, et al.* (2021). Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell *184*, 2201-2211.e2207.

Tan, A.T., Linster, M., Tan, C.W., Le Bert, N., Chia, W.N., Kunasegaran, K., Zhuang, Y., Tham, C.Y.L., Chia, A., Smith, G.J.D.*, et al.* (2021). Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Reports *34*.

Tomic, A., Skelly, D.T., Ogbe, A., O. Connor, D., Pace, M., Adland, E., Alexander, F., Ali, M., Allott, K., Ansari, M.A.*, et al.* (2021). Divergent trajectories of antiviral memory after SARS-Cov-2 infection. Research Square.

Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R.*, et al.* (2021). Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell *184*, 2348-2361.e2346.

Table 1
Characteristics of healthcare workers included in the study and dosing intervals
IQR = Interquartile range. N = number.

Table 2
Generalized linear models (GLM). Table shows three GLM models of T cell (naïve and previously infected individuals), antibody (naïve individuals) and antibody (previously infected individual) responses at 4 weeks after second dose. Variables include age, sex, previous infection and vaccine dose interval. Variable references are Sex; F (Female) versus M (Male), Previously infected; Yes versus No, Vaccine dose interval; Short versus Long. Linear mixed-effect models (LMER). Table shows four LMER models of T cell or antibody responses in naïve or previously infected individuals,
across vaccine timepoints in Long vaccine dose regimen. Variables include age, sex and timepoint. Variable references are Sex; F (Female) versus M (Male), Timepoint; Dose-1 plus 4 Weeks versus Pre-vaccine/Dose-1 plus 10 weeks/Dose-2 plus 4 weeks.

Figure 1
A) Adjusted hazard ratios for PCR confirmed case by interval after first and second doses of vaccination, source: SIREN study. Healthcare workers underwent regular asymptomatic PCR screening, n=25,066 (negative cohort = 16,423, positive cohort= 8,643), with follow-up to 95 days post the first dose of BNT162b2 vaccine. The hazard ratios are adjusted (including for age, ethnicity, comorbidities, and region), with full methodology described (Hall et al., 2021b).
B) Schematic to show dosing strategy of Short and Long vaccine interval and phlebotomy timepoints.

Figure 2.
Long dosing interval with BNT162b2 (Pfizer-BioNTech) vaccine elicit distinct neutralizing antibody titer profiles against SARS-CoV-2 variants of concern B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) and maintained T-cell responses.
2A) Neutralizing antibodies against the Victoria isolate, B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) taken from participants 4 (n=20) and 10 weeks (n=20) after the first vaccine dose and 4 weeks (n=20) after the second vaccine dose in the long interval cohort. The X axis signifies weeks since dose. Timepoints were compared with the Kruskal-Wallis nonparametric test and Dunn's multiple comparisons tests. P values are illustrated above linking lines and fold changes in brackets. Geometric mean neutralizing titers are immediately above each column and marked by a horizontal line on each column. Focus Reduction Neutralization Assay (FRNT). FRNT50, is the reciprocal dilution of the concentration of serum required to produce a 50% reduction in infectious focus forming units of virus in Vero cells (ATCC CCL-81)
2B) SARS-CoV-2 spike(S)-, receptor-binding domain (RBD)- and nucleocapsid(N)-specific IgG time course in naive (grey circles, n=29) and pre-infected individuals vaccinated (red circles, n=29) with a long interval between the doses. IgG responses were measured in paired sera before vaccination (pre), 4 weeks after the first dose (1-dose +4 wks), 8-12 weeks after 1st dose (1-dose + 10 wks), and 4 weeks after the second dose (2-dose + 4wks) using multiplexed MSD immunoassays. Data are shown in Arbitrary Units/ml (AU/ml). Bars represent the median with interquartile range. Paired comparisons between the groups were performed using a Wilcoxon test. Fold change values each refer to the P value comparisons directly below. Horizontal dotted lines represent the cut-offs of each assay based on pre-pandemic sera. Data from 51 of the pre-vaccine, and 51 of the 1-dose +4 wks responses were previously published (Angyal et al., 2021).

2C) Comparison of IFN-y ELISpot responses to Spike (Victoria) from cryo-preserved PBMCs in 26 naïve individuals and 26 previously-infected individuals matched for pre-vaccine (pre), 4 weeks after 1st dose (1-dose +4 wks), 8-12 weeks after 1st dose (1-dose +10 wks) and 4 weeks after 2nd dose (2-dose +4 wks). Paired comparisons were performed using the Wilcoxon matched pairs signed rank test. Fold change values each refer to the P value comparisons directly below. Grey symbols represent naïve individuals, red symbols represent previously infected individuals. Data from 51 of the pre-vaccine, and 51 of the 1-dose +4 wks responses were previously published (Angyal et al., 2021).

Figure 3
Comparison of IgG responses and T-cell responses 4 weeks after second dose of vaccine
3A) Comparison of neutralizing antibodies against the Victoria isolate, B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) 4 weeks after the second dose in short (n=20) and long (n=20) interval participants. Median of 3.3 weeks (range 2.4-4.3) between doses in the short interval cohort (n=25) and 8.4 weeks (range 6.4-10) in the long interval cohort (n=20). Timepoints were compared with two-tailed Mann-Whitney tests. P values are illustrated above linking lines and fold changes in brackets. Geometric mean neutralizing titers are shown.

3B) Impact of a short and a long vaccine dosing interval on the ability of sera to inhibit ACE2 binding to SARS-CoV-2 spike (Victoria, B.1.1.7 (alpha), B.1.351 (beta) or P.1 (gamma)) 28 days after the second dose. ACE2 inhibition was analysed using a multiplexed MSD assay. Data are shown in units/ml. Bars represent the median with 95% confidence intervals. Unpaired comparisons between the groups were performed using a Mann-Whitney test. Naïve, short: n= 23; Naïve, long: n=94; Pre inf, short: n=14; Pre inf, long: n=119.

3C) Effect of a short or a long vaccine dosing interval on SARS-CoV-2 S-, RBD- and N-specific IgG responses in naïve (grey symbols) and pre individuals (red symbols). IgG responses were measured in serum 4 weeks after the second dose using multiplexed MSD immunoassays and are shown in Arbitrary Units/ml (AU/ml). Bars represent the median with interquartile range. Unpaired comparisons between the groups were performed using a Mann-Whitney test. Fold change values each refer to the P value comparisons directly below. Naïve, short: n= 42; Naïve, long: n=151. Pre inf, short: n=18; Pre inf, long: n=169. Horizontal dotted lines represent the cut-offs of each assay based on pre-pandemic sera.

3D) IFN-y ELISpot responses from cryo-preserved peripheral blood mononuclear cells (PBMCs) 4 weeks after the second dose in naïve short (n=37), naïve long (n=188), previously infected short (n=20) and previously infected long (n=124) individuals. Values are expressed as spot forming units per million PBMCs (SFU/106). Displayed are responses to peptide pools representing S1 and S2 subunits of Spike (Victoria), peptide pools representing membrane (M) and N proteins (MN) and Cytomegalovirus, Epstein-Barr virus, influenza and tetanus antigens (CEFT). Unpaired comparisons across two groups were performed using the Mann Whitney test. Fold change values each refer to the P value comparisons directly below.

Figure 4. Analysis of Spike specific T cell responses by flow cytometry
86 healthcare workers with positive ELISpot responses and sufficient sample remaining were analysed by intracellular cytokine staining and flow cytometry. (A) The ratio of the response in CD4+ or CD8+ cells, calculated as % CD4+ [cytokine+] / (% CD4+ [cytokine+] + % CD8+ [cytokine+]). (B) Staining for individual cytokines in CD4+ T cells, (C) Staining for individual cytokines and CD107a (a marker of cytotoxicity) in CD8+ T cells.

Figure 5.
Short dosing interval with BNT162b2 (Pfizer-BioNTech) vaccine elicit distinct neutralizing antibody titer profiles against SARS-CoV-2 variants of concern B.1.351 (beta), P.1(gamma) and B.1.617.2(delta) that declines over time, whilst T-cell responses are maintained.

5A) Neutralizing antibody titers, measured by FRNT, against the early pandemic Victoria isolate, B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta), taken from participants in weeks 1 (n=25), 4 (n=19) and 13 (n=20) after receipt of the second vaccine in the short interval dose cohort (median dose interval 3.3 weeks (range 2.4-4.3). The X axis signifies weeks since dose. Timepoints were compared with the Kruskal-Wallis non-parametric test and Dunn's multiple comparisons tests. P values are illustrated above linking lines and fold changes in brackets. Geometric mean neutralizing titers shown.
5B) Comparison of IFN-y ELISpot responses to spike B, from cryo-preserved PBMCs in 37 naïve individuals who received the short interval dose, at 1, 4 and 13 weeks after the second dose of vaccine. Unpaired comparisons across two groups were performed using the Mann Whitney test. Fold change values each refer to the P value comparisons directly below. Grey symbols represent naïve individuals.
5C) Comparison of IFN-y ELISpot responses from cryo-preserved PBMCs in 40 naïve individuals and 42 previously-infected individuals matched for responses to Spike B, spike B.1.35/beta and spike P.1/gamma. Individuals received the Long interval dosing regimen and samples were taken 4 weeks after the second dose. Paired comparisons were performed using the Wilcoxon matched pairs signed rank test. Fold change values each refer to the P value comparisons directly below. Grey symbols represent naïve individuals, Red symbols represent previously infected individuals.
5D-E) Comparison of neutralizing antibodies against the Victoria isolate, B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) in short and long dose interval cohorts 4 weeks after second vaccine dose. Median of 3.3 weeks (range 2.4-4.3) between doses in the short interval cohort (n=25) and
8.4 weeks (range 6.4-10) in the long interval cohort (n=20). Timepoints were compared with two-tailed Mann-Whitney tests. P values are illustrated above linking lines and fold changes in brackets. FRNT50 represents the reciprocal serum dilution at which there was a 50% reduction in infected foci, compared with negative control. Geometric mean neutralizing titers are shown. Neutralization titers from dose2 +1 week (Victoria, B.1.351, P.1 & B.1.617.2), and dose-1 + 4weeks and 10
weeks (Victoria & B.1.617.2) have been previously reported in (Dejnirattisai et al., 2021; Liu et al., 2021; Supasa et al., 2021; Zhou et al., 2021).