Fast evolution of SARS-CoV-2 BA.2·86 to JN.1 under heavy immune pressure

The SARS-CoV-2 saltation variant BA.2.86, which was quickly designated as a variant under monitoring after its emergence, has garnered global attention. Although BA.2.86 did not show substantial humoral immune escape and growth advantage compared with current dominant variants, such as EG.5.1 and HK.3, it showed remarkably high ACE2 binding affinity.[1–5] This increased binding affinity, coupled with its distinct antigenicity, could enable BA.2.86 to accumulate immune-evasive mutations during low-level populational transmission, akin to the previous evolution from BA.2.75 to CH.1.1 and XBB.[6–9] With just one additional receptor binding domain mutation (L455S) compared to its predecessor BA.2.86, the JN.1 variant rapidly became predominant in France (figure A; appendix 1 p 12), surpassing both BA.2.86 and the so-called FLip (L455F+F456L) strains. A thorough investigation into the immune evasion capability of JN.1, particularly given its few additional mutations, is imperative.

We first study the humoral immune evasion of JN.1 using pseudovirus-based neutralisation assays with plasma from individuals recovering after XBB infection. These individuals, having received three doses of inactivated vaccines, subsequently contracted XBB (XBB subvariants with S486P substitution) breakthrough infections. Our study included two cohorts, one with 27 participants who had post-vaccination XBB breakthrough infections and another with patients reinfected with XBB after BA.5 or BF.7 breakthrough infections (appendix 2). JN.1 displayed significantly enhanced immune escape compared with BA.2.86 (figure B). This finding was evidenced by a 2·1-fold decrease in 50% neutralisation titers (NT50) among individuals who were reinfected with XBB post-BA.5 or BF.7 infection and a 1·1-fold decrease in NT50 in individuals recovering from XBB breakthrough infections (figure B; appendix 1 p 13). Additionally, JN.1's plasma evasion surpassed that of competitive variants HV.1 (EG.5+L452R) and JD.1.1 (FLip+A475V). HV.1 and JD.1.1 also showed significantly lower plasma neutralisation titers compared with their parental strains after acquiring L452R and A475V mutations, respectively, explaining their growth advantages.

As L455 is located on the binding interface between human ACE2 and receptor binding domain (appendix 1 p 14),[10] L455S could change the binding affinity between ACE2 and the receptor binding domain of JN.1. By using surface plasmon resonance, we found a notable reduction in ACE2 binding affinity for JN.1 receptor binding domain, indicating that its enhanced immune escape capabilities come at the expense of reduced ACE2 binding (figure C). A475V mutation carried by JD.1.1 (XBB.1.5+FLip+A475V) also resulted in decreased binding affinity, enhancing immune evasion compared to HK.3 (XBB.1.5+FLip). However, the L452R mutation of HV.1 did not affect binding affinity.

Considering that the L455 is predominantly located at the epitope of receptor binding domain Class 1 antibodies, as indicated by earlier research, our study further examined the evasion capabilities of JN.1 in response to eight XBB.1·5-neutralising class 1 monoclonal antibodies.[7]

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See **Online** for appendix 2

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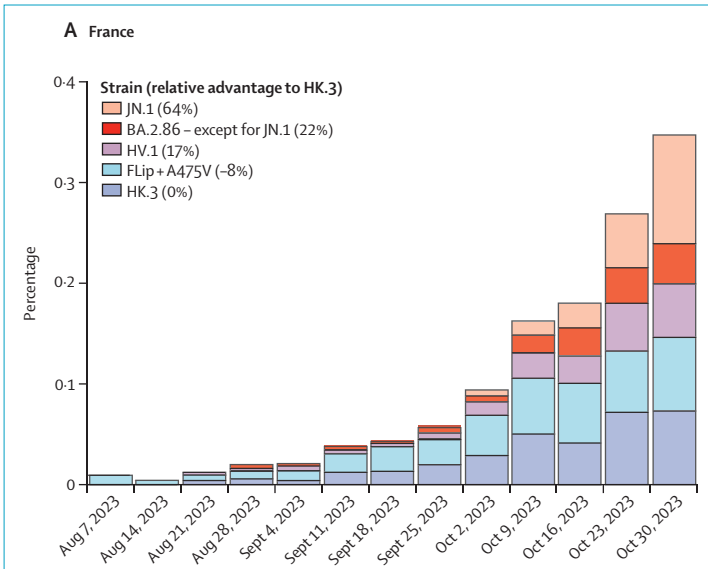

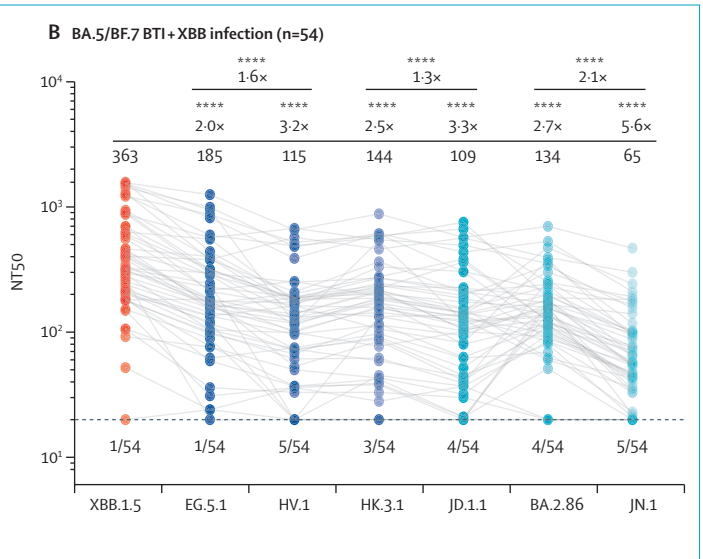

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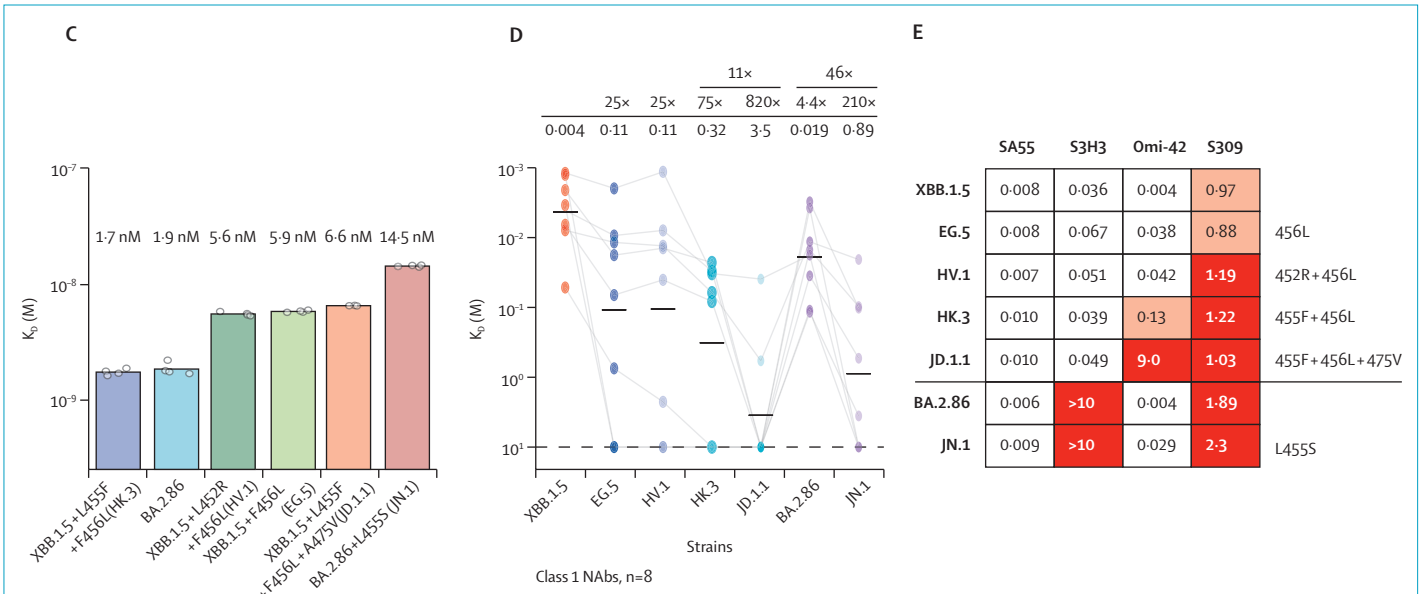

Figure: **JN.1 shows profound immune evasion and decreased ACE2 binding affinity**
(A) Sequence percentages of prevalent variants in France since August, 2023, including JN.1, BA.2·86 (the original BA.2.86 and its subvariants, except JN.1), HV.1, FLip+A475V, and HK.3. The growth advantages relative to HK.3 in past two months of these strains are denoted in the legend within parentheses. Data are collected from covSPECTRUM. (B) The 50% neutralising titer (NT50) of convalescent plasma against SARS-CoV-2 variants measured in individuals who received three CoronaVac doses and had breakthrough infection with BA.5 or BF.7 followed by XBB reinfection (n=54). Labels for geometric mean titers (GMT) are located above each group, with the fold changes and statistical significances indicated above the GMT labels. Below the dashed line are labels specifying the numbers of negative samples which are related to the limit of detection (NT50=20). Two-tailed Wilcoxon signed-rank tests of paired samples were used. *p<0·05, **p<0·01, ***p<0·001, and ****p<0·0001. (C) The human ACE2 (angiotensin-converting enzyme 2) binding affinities of HK.3 (XBB.1.5+L455F+F456L), BA.2.86, HV.1 (XBB.1.5+L452R+F456L), EG.5 (XBB.1.5+F456L), JD.1.1 (XBB.1.5+L455F+F456L+A475V), and JN.1 (BA.2.86+L455S) receptor binding domain determined by surface plasmon resonance sensorgrams. K^D values (nM) are displayed above the bars, and all replicates are represented as points. (D) Class 1 Nabs resistance against pseudovirus of XBB.1.5, EG.5, HV.1, HK.3, JD.1.1, BA.2.86, and JN.1 strains indicated by the IC50 values (n=8). The values and related fold changes when compared to D614G or other strains are labelled. The IC50 (μg per mL) of approved or candidate monoclonal neutralising antibody drugs targeting spike are assessed against XBB.1.5, EG.5, HV.1, HK.3, JD.1.1, BA.2.86, and JN.1 pseudovirus. IC50=50% inhibitory concentration; K^D=equilibrium dissociation constant; NAbs=neutralising antibodies; nM=nanomolar

Pseudovirus neutralisation assays showed that the addition of the L455S mutation enhanced JN.1's ability to evade class 1 antibodies (figure D). This mutation effectively compensated for BA.2.86's susceptibility to this antibody group (figure D). Similarly, the FLip+A475V variant (JD.1.1) also showed increased resistance to class 1 antibodies compared with the FLip variant (HK.3), offering insights into the trend of convergent A475V mutations among FLip variants. In terms of therapeutic antibodies, SA55 retained its neutralising efficacy against all examined variants including JN.1 (figure E). Together, these findings suggest that L455S greatly enhanced JN.1's resistance to humoral immunity through compensation of BA.2.86's weakness to class 1 antibodies.

In summary, JN.1, by inheriting BA.2.86's antigenic diversity and acquisition of L455S, rapidly achieved extensive resistance across receptor binding domain class 1, 2, and 3 antibodies,[1] and showed higher immune evasion compared with BA.2.86 and other resistant strains like HV.1 and JD.1·1, at the expense of reduced human ACE2 binding. This evolutionary pattern, similar to the previous transition from BA.2.75 to CH.1.1 and XBB,[2,3,9] highlights the importance of closely monitoring strains with high human ACE2 binding affinity and distinct antigenicity, like BA.2.86 and BA.2.75, despite their unremarkable immune evasion capabilities. Such strains could survive and transmit at low levels since their antigenic difference would allow them to target distinct populations compared with dominant strains and have the potential to quickly accumulate highly immune-evasive mutations at the cost of human ACE2 binding capabilities.

YC is the inventor of the provisional patent applications for BD series antibodies, which includes BD55–5514 (SA55). YC is the founder of Singlomics Biopharmaceuticals. All other authors declare no competing interests.

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