Tracking the Evolution of Resistant Mutant Combinations

In 1997, a young boy died suddenly from severe flu symptoms after playing with chickens at a Hong Kong farm. Virologists were alarmed, as no human, not even poultry workers, had contracted this particular strain of influenza before. By the end of the year, 18 people in Hong Kong had been infected with the H5N1 virus, and six people had died. The Hong Kong government responded quickly, closing schools to avoid the risk of the infection spreading further, and ordering all live poultry to be killed.

Home from school, eight-year-old Nicholas Wu watched these events unfold. Over a period of three days, one and a half million birds—chickens, partridge, geese, and quail—were killed. Traditional Chinese meals took place without a poultry course. The familiar sights and sounds of his local market were absent. For an eight-year old, it was overwhelming “I was very surprised by the power of this virus to change my life, and the lives of the people around me,” Wu remembers.

But despite all the measures by government, and by the poultry industry, influenza returned to Hong Kong and other parts of Asia in 2003, and at periodic intervals since then. Dangerous new mutations and strains have emerged, including the H7N9 strain in 2013.

Twenty years later, at the Scripps Institute in California, Wu is still asking questions about the influenza virus.

In order to replicate, an influenza virus must attach to special receptors located on the membrane that envelops human upper respiratory cells or red blood cells. A protein on the surface of the influenza virus called hemagglutinin binds to these receptors, which are composed of sialic acid, a type of sugar. The virus fuses to the receptors on the cell membrane, enabling the virus to penetrate the cell and begin propagating. The antibodies produced by an influenza flu vaccination target the binding site to prevent the virus from entering the cell.

The reason influenza is able to evolve so quickly lies in its ability to generate numerous mutations—abrupt changes to the genetic information contained in the virus’ DNA. Most of these mutations quickly kill off the virus but about one percent are survivable, allowing the virus

to start propagating as a new strain.

Up until now, scientists have been in the position of “chasing” the flu—seeking out new strains and developing vaccines that build up immunity against those strains. During his PhD studies, at UCLA, Wu and his supervisor wanted to take a more proactive approach: “If we know ahead of time what kind of mutations can be accommodated at a particular site on the virus, we'll have a better idea of how to develop drugs or antibodies to target that site.” Wu says.

The experimental process was two-fold: Wu needed to create all possible mutation for each viral strain, then test every combination of mutations possible to see which ones were virus-tolerant.

Using a genetic engineering technique known as saturation mutagenesis, Wu introduced 30,000 mutant variants, each containing a single mutation, into the binding sites of H1N1 and H3N2 flu strains. More than ninety-five percent of the variants died—not an unexpected result given that most single mutations tend to disrupt the binding site, killing the virus.

Wu then combined the same mutations into aggregates of twos and threes and repeated the process. A surprising twenty percent of the mutant combinations not only didn’t kill the virus, they actually strengthened the viral strains, allowing them to keep replicating.

This discovery implied that certain mutations are able to bond together in predictable patterns to help the influenza virus thrive and propagate. Wu points out that the evolution of these resistant mutant combinations was not random—each new mutation developed in such a way as to cancel out the destructive effects of the previous one—a process known as epistasis.

“Epistasis refers to the combined effect of two individual mutations that can't be predicted ahead of time," Wu explains. "Individually, each of these mutations kills the virus, but together, they compensate for each other's harmful effects."

Using DNA sequencing technology, Wu and his colleagues identified each viable mutant combination and observed that many of them occurred in an area of the influenza binding site known as the 220-loop, a region already known for ease in facilitating the viral jump from birds to humans. What they didn’t realize was just how tolerant the 220-loop would be when exposed to such a wide variety of mutant combinations. Contrary to the single mutations that interfered

with the function of the binding site, certain mutant combinations did *not* disrupt the receptor binding function.

“These results suggest that perhaps we should avoid targeting regions such as the 220-loop when developing antibodies or drug therapies since these regions appear to be relatively tolerant of mutations,” Wu states.

“Predicting the evolution of mutations, that is, knowing in advance what potential mutations will come up, could help us develop effective antibodies and target sites,” Wu continues. “For each combination of mutations we are able to predict and generate, we are one step closer to forecasting how flu evolves—potentially leading to its elimination entirely.”