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HOW AFFINITY TAGS ARE SPEEDING UP MALARIA VACCINE DEVELOPMENT

Source: Thermo Fisher Scientific

In 2020, there were 241 million malaria cases and 627,000 malaria deaths worldwide, according to the World Health Organization (WHO).[1] About 95% of the cases occurred in Africa, with most deaths in this region involving children under 5.

"Most of the deaths were caused by *Plasmodium falciparum,* one of the five malaria parasites that can cause disease in humans," explains Rebecca Ashfield, a senior project manager at the University of Oxford's Jenner Institute.

On October 6, 2021, the WHO approved a malaria vaccine for the first time: RTS,S/AS01. The vaccine is a viruslike particle, a construct that closely resembles a virus but contains no genetic material and therefore isn't infectious. It's known under the tradename Mosquirix but is better known as RTS,S. The WHO recommended the vaccine for widespread use in children living in sub-Saharan Africa and other regions with moderate to high *P. falciparum*

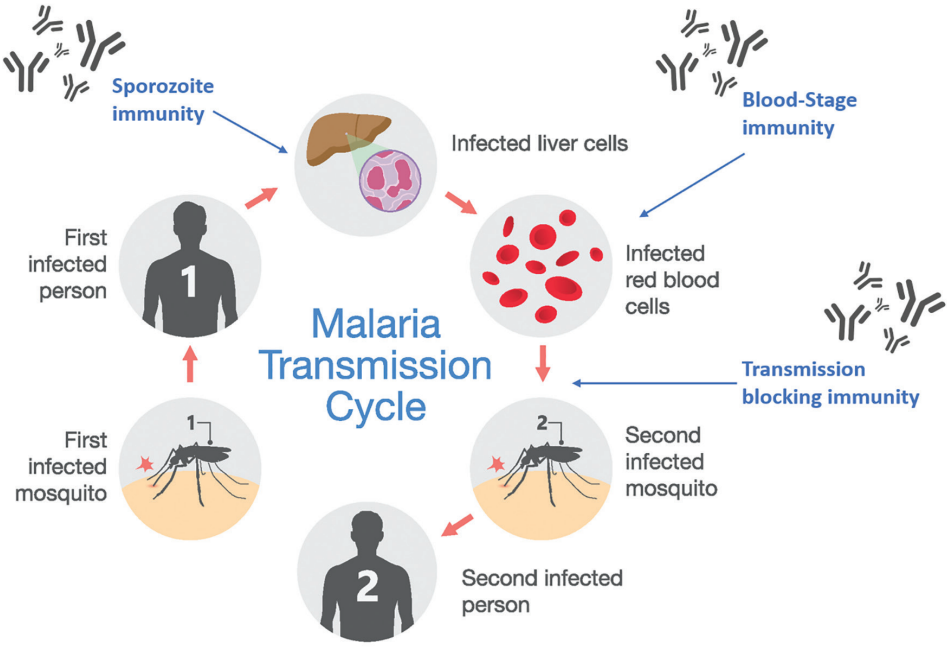
malaria transmission rates. This approval was the cumulation of over 30 years of research and development by GlaxoSmithKline, the nonprofit health organization Path, and other partners, including the Bill and Melinda Gates Foundation. “In field trials in Africa, RTS,S is approximately 30–50% effective,” Ashfield says. “It’s a very good start.”

But researchers have no intention at stopping there, and Ashfield and her colleagues have a pipeline of malaria vaccines in development that they hope will boast even higher efficacy. The manufacture of recent Oxford malaria vaccine candidates follow a blueprint that includes a novel purification approach for a biotherapeutic: chromatography using an affinity tag system. The affinity tag has enabled the team to overcome issues with purity, keep costs down, and rapidly advance its vaccine candidates into the clinic.

TESTING TIMES

Ten COVID-19 vaccines have been approved by the WHO since the coronavirus that causes it was discovered in humans just a few years ago, while scientists have spent decades working toward effective malaria vaccines. What’s holding things up in the malaria field?

For one, malaria research isn’t particularly well funded.[1] But even for those with the grants, a lack of a clear biological target on *P. falciparum* remains a huge challenge for malaria vaccine developers. “*Plasmodium falciparum* has about 7,000 genes, about 25% of the number of a human,” Ashfield says. “So unlike a virus—for example, SARS-CoV-2, where it was obvious that we had to target the



The vaccines being developed for malaria are each designed to disrupt one of three different stages of the *P. falciparum’s* complicated life cycle: how it infects liver cells, how it multiplies within red blood cells, and how it transmits to a new host.

Source: Thermo Fisher Scientific

spike protein on the surface of the virus—it is by no means obvious which of the parasite's 7,000 genes would make a good subunit vaccine."

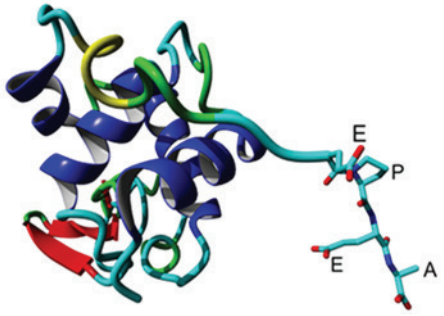
That lack of a biological target hinders researchers' ability to assess predictions of suitable vaccine designs in the lab. The Oxford team has concluded that clinical trials are the only appropriate way of evaluating hypotheses. "The overall strategy is to test as many different candidates as possible in the clinic, because at the moment, we don't know whether a vaccine is going to be effective until we test it in a clinical trial," Ashfield says.

The Oxford team has three vaccine *P. falciparum* candidates in clinical trials, each targeting a different stage of the parasite's life cycle. (The life cycle of this parasite is particularly complex and involves both the mosquito and human hosts.)

TAGS TO THE RESCUE

Figuring out how to manufacture vaccine candidates in accordance with good manufacturing practices (GMPs) without breaking the bank was a significant challenge for the Oxford team. For an academic group, vaccine manufacturing development can be prohibitively expensive, Ashfield says.

The Oxford scientists devised a standardized manufacturing blueprint while developing the manufacturing route to one of their vaccine candidates—the recombinant protein RH5.1.[2] The goal was to speed up manufacture and increase yields, thereby decreasing short-term costs. That blueprint has since been used, at least for early-stage manufacture, for all three of the vaccines in clinical trials and for a handful of others that are close to reaching the clinic.



The C-tag, a sequence of glutamic acid (E), proline (P), glutamic acid (E), and alanine (A), is the smallest affinity tag that can be fused at the C-terminus of recombinant proteins. Thermo Fisher produces a C-tag affinity resin suitable for GMP manufacture.

Source: Thermo Fisher Scientific

To make a vaccine, the antigen—the biomolecule that stimulates the immune response in the body—must first be cultivated in a bacteria, yeast, or cell culture in higher eukaryotes. Using a technology developed in collaboration with the Danish firm ExpreS2ion Biotechnologies, the Oxford team grows many of its recombinant proteins (including RH5.1) in *Drosophila* Schneider 2 cells. "This particular cell line is very good at refolding plasmodium proteins that are difficult to express in other systems," Ashfield says. The others are cultivated in the yeast *Pichia pastoris*.

The next step in vaccine manufacture is to fish the antigen out of the crude growth medium and purify it. The growth-medium soup contains a complex

mixture of biomolecules, and selectively pulling out just one of these can be highly challenging. Traditionally, chromatography columns separate mixtures by exploiting differences in their sizes or charges. Differences between biomolecules in these soups can be extremely small, however, so multiple different column types must be used in succession to meet GMP purity requirements. Developing the methodology and executing a purification protocol with multiple steps is time consuming, and each additional step reduces the yield of the desired antigen, according to Pim Hermans, head of ligand discovery at Thermo Fisher Scientific.

Affinity chromatography is an established tool for reducing the number of steps required when biomolecules are being purified. Rather than relying on size or charge differences, affinity columns separate biomolecules according to how well they bind—their affinity—to heavy metal ions or to other biomolecules immobilized on the chromatography resin. As the mixture passes through

INTRODUCING C-TAG

The C-tag is a short peptide sequence of four amino acids (E-P-E-A) that can be attached to the carboxy-terminus (the free carboxyl group, better known as the C-terminus) of any recombinant protein. The potential to use this tag in affinity chromatography was identified over a decade ago by the University of Cambridge chemist Christopher M. Dobson and his collaborators.[6]

The immobilized biomolecules on the chromatography resin that capture C-tags are based on a pared-down version of single-domain immunoglobulin G (IgG) fragments.

Using a recombinant fragment rather than the whole antibody has multiple advantages. Fragments of single-domain antibodies are more robust and stable than whole ones, Hermans says. “They can also be expressed in microbial systems, like the yeast system that we use, in high yields,” he adds.

Thermo Fisher started manufacturing its CaptureSelect C-tag affinity resin in 2012, at first for research purposes only. It wasn’t until the collaboration with Ashfield and the other malaria vaccine developers at Oxford that Hermans and his team seriously considered the possibility of their C-tag system being used for biotherapeutic manufacture. “We worked together to make this resin suitable for large-scale purification,” Hermans says.

The hope is that the success of the malaria vaccines’ GMP manufacture using a C-tag will open the door for other vaccine developers, targeting a wide range of diseases, to do the same and get their much-needed vaccines to populations at risk of infection faster.

the column, biomolecules with high affinity for the immobilized molecules bind to the resin. After everything else has passed through the column, the eluting solvent is changed to release the captured biomolecules. For GMP manufacturing, one or two polishing chromatography rounds to remove final traces of impurities will likely be all that's needed.

The most well-known affinity resin contains immobilized protein A, a bacterial surface protein with high affinity toward immunoglobulins. Protein A affinity resins are widely used in the manufacture of therapeutic monoclonal antibodies. Affinity chromatography's potential has been limited by an inherent problem: challenges associated with making affinity resins for other types of biologics. "For the majority of biomolecules, no affinity capture resin is available," Hermans says.

About 30 years ago, a workaround was devised: affinity tags. A short peptide sequence—the affinity tag—is attached to the target biomolecule using genetic engineering. The target can then be captured by an affinity tag–specific ligand that's attached to the resin. Once the target biomolecule has been released from the affinity column, the tag can be removed.

Affinity tags are now used extensively in the research lab. So far, however, safety concerns have hampered their use in biotherapeutic manufacture. Removing affinity tags during a biotherapeutic manufacturing process is laborious and can lead to product loss. In addition, if the tag is left on a biotherapeutic, it's possible that antibodies may be made in the body against the affinity tag rather than the therapeutic molecule.

While struggling to develop a traditional chromatography GMP process for RH5.1, the Oxford team looked at the possibility of utilizing an affinity tag. The scientists tested some commercial affinity tag systems in the research lab, and identified one—the C-tag—that met their purity requirements.

The C-tag is four amino acids long—glutamic acid (E), proline (P), glutamic acid (E), alanine (A)—and believed to be the smallest tag for which an affinity resin has been created. Thermo Fisher developed the affinity chromatography resin for the capture of C-tagged biomolecules under the brand name CaptureSelect™ C-tagXL Affinity Matrix.

The purity achieved using the C-tag system is very high, and the resin "is really exquisitely specific," Ashfield says. "It really does not bind to the other proteins." All that's needed to meet GMP purity requirements for the Oxford team's vaccines manufactured in *Drosophila* Schneider 2 cells is one polishing step using size-exclusion chromatography and a virus filtration step (to remove viruses in the growth medium). The yields are also pleasing, Ashfield says. For RH5.1, the C-tag chromatography step has an 85% yield.

Another reason the scientists selected the C-tag system was because they predicted that the four-amino-acid-long tag was too small to elicit unwanted

immune response in the body. They were therefore hopeful that the regulatory authorities would allow the tags to remain permanently on their vaccines, which eliminates the need for a cleaving step. "We consulted with the MHRA [Medicines and Healthcare products Regulatory Agency], the regulatory body in the UK, before we took RH5.1 into a clinical trial, and they indicated that they were very happy for the C-tag to be used throughout development," Ashfield says. "It's such a small sequence—and indeed it's smaller than an antibody epitope—so it's hard to see how you'd get a lot of immunogenicity building up against that tag."

Once it looked likely that leaving the C-tag on for clinical trials would be approved, Hermans's team at Thermo Fisher redesigned the CaptureSelect™ resin to boost its binding capacity and meet other GMP requirements.[2]

VACCINES IN THE FIELD

The approved GlaxoSmithKline RTS,S malaria vaccine intervenes immediately after a mosquito bites a human and releases sporozoite parasites into the bloodstream. The vaccine's goal is to prevent the parasite from reaching and infecting the liver cells, where it multiplies and reenters the bloodstream to infect red blood cells and cause disease symptoms.

The Oxford team has also developed a vaccine, R21, targeting that same stage in *P. falciparum*'s life cycle. This vaccine's development is spearheaded by Ashfield's colleague Adrian Hill. R21 is similar to RTS,S in many ways and has shown over 70% efficacy in Phase 2b field trials in Burkina Faso.[3] Further clinical trials are underway there, as well as in Kenya, Mali, and Tanzania.

TABLE 1: MALARIA VACCINE OVERVIEW

Target in parasite life cycle	Vaccine name	Lead developer	Vaccine type	Development progress
Sporozoite stage	RTS,S/AS01	GlaxoSmithKline	Viruslike particle	Approved by WHO in October 2021
Sporozoite stage	R21	Adrian Hill (Oxford)	Viruslike particle	Phase 3
Blood stage	RH5.1	Simon Draper (Oxford)	Soluble protein	Phase 1b
Blood stage	RH5.2	Simon Draper (Oxford)	Viruslike particle	Scheduled to start trials
Blood stage	CyRPA-Ripr	Simon Draper (Oxford)	Fusion protein	Scheduled to start trials shortly
Transmission stage	Pfs25-IMX313	Sumi Biswas (Oxford)	Nanoparticle	Phase 1b
Transmission stage	Pfs48/45	Sumi Biswas (Oxford)	Soluble protein	Scheduled to start trials shortly

While C-tag was initially used for the GMP manufacture of R21, it isn't currently employed for that purpose. "For R21, we moved away from C-tag purification because large-scale manufacture was transferred to the Serum Institute of India and they preferred a process that didn't involve affinity purification," Ashfield says. The current C-tag resins work better with small soluble proteins than larger virus-like-particles. "Thermo Fisher is developing different purification resins to cope with this issue," she adds.

The next stage of the *P. falciparum* life cycle that the Oxford vaccine makers are targeting is multiplication within red blood cells. The *P. falciparum* multiplies inside the cells until they burst and release the parasites, which go on to invade other red blood cells.

Simon Draper is leading the Oxford effort to develop vaccines that hinder parasite multiplication in red blood cells. The first-generation vaccine candidate of this type is RH5.1. The standardized capture-and-purification GMP process involving C-tag was designed during RH5.1's development.

A Phase 1/2a UK study of the vaccine in healthy adults demonstrated that it was safe and well tolerated and that antibodies against malaria remained in the body for over 2 years after treatment.[4] A Phase 1b clinical trial for RH5.1 began in early 2021 in Tanzania.

Two other candidates that target parasite multiplication in red blood cells, the viruslike particle RH5.2 and the fusion protein CyRPA-Ripr, are expected to start trials shortly.

The Oxford team is also trying to inhibit the transmission stage of the *P. falciparum* life cycle, when the mosquito collects parasite-laden blood from a person with malaria and transfers it to a new host. Stopping transmission means that though the individual who was originally vaccinated isn't protected from disease, "other individuals in the community will be protected when they're bitten by the mosquito," Ashfield says. "This is a way of building up herd immunity."

Sumi Biswas leads the Oxford transmission-blocking malaria vaccine project. A UK-based Phase 1a clinical trial of the first vaccine candidate of this type—the nanoparticle Pfs25-IMX313—recently finished.[5] A Phase 1b trial began in Tanzania in mid-2021.

The GMP manufacturing process using C-tag has also been completed for a second transmission-blocking vaccine candidate, the soluble protein Pfs48/45. The first clinical trial is scheduled for this year, according to Ashfield.

The Oxford team has other *P. falciparum* vaccine candidates in earlier stages of development. Ashfield predicts that eventually people will receive multiple malaria vaccines targeting different stages of the P. falciparum life cycle. The

team has secured funding for clinical trials of combinations of its vaccines. "We're very hopeful that that's going to be the way forward," Ashfield says. The vaccines initially will be made separately and mixed in the clinic but in time will be prepared in one formulation, similar to the MMR (measles, mumps, and rubella) vaccine.

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