Scientific Opinion of the AvATher Working Group on EV25: An Antibody-Recruiting Anti-Influenza Therapeutic Candidate

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A. Context

Influenza remains a major public health threat, responsible for substantial annual morbidity, mortality, and health-system burden. Beyond seasonal epidemics, the continued risk of novel influenza strain emergence, including zoonotic spillover and avian influenza outbreaks, supports ongoing preparedness efforts and the evaluation of antiviral candidates that could complement vaccination strategies and existing therapeutics.

Current antiviral options (including neuraminidase inhibitors and other approved agents) are useful but may face limitations in real-world effectiveness, timing of administration, and the emergence of resistant strains. Accordingly, the AvATher working group of the ANRS-MIE continues to identify and assess innovative antiviral approaches with the potential to expand the therapeutic window, improve clinical outcomes, and strengthen pandemic readiness. In this context, the working group invited Prof. Philip S. Low to present EV25, an investigational anti-influenza candidate built on a targeted antibody-recruiting strategy.

B. EV25: Summary of Mechanism of Action and Key Characteristics[1]

EV25 is an investigational antiviral designed to target influenza-infected cells through a dual-mechanism strategy combining neuraminidase binding with recruitment of endogenous natural antibodies against haptens. The molecule is a multifunctional conjugate comprising three principal components: (i) a neuraminidase-targeting ligand (NTL), and (ii) two distinct hapten moieties, all connected via long linker.

Following influenza infection, viral neuraminidase expressed on the surface of infected host cells provides the molecular anchor for a neuraminidase inhibitor targeting moiety, enabling selective binding of EV25 to infected cells and virions. The attached hapten groups are intended to recruit natural anti-hapten antibodies present in human serum, leading to antibody sensitization of virions and infected cells, which, in turn, is expected to promote immune recognition and clearance through multiple mechanisms, including antibody-dependent cell-mediated cytotoxicity, antibody-dependent cell-mediated

[1]Shahriar I, Kamra M, Kanduluru AK, Campbell CL, Nguyen TH, Srinivasarao M, Low PS. Targeted recruitment of immune effector cells for rapid eradication of influenza virus infections. Proc Natl Acad Sci U S A. 2024 Oct 8;121(41):e2408469121. doi: 10.1073/pnas.2408469121. Epub 2024 Sep 30. Erratum in: Proc Natl Acad Sci U S A. 2024 Dec 3;121(49):e2422717121. doi: 10.1073/pnas.2422717121.

phagocytosis, and complement activation. In parallel, the neuraminidase inhibitor component retains neuraminidase inhibitory activity, providing an additional direct antiviral effect.

C. Preclinical Efficacy and Safety Data on EV25

In vitro targeting

In vitro experiments demonstrate that the neuraminidase inhibitor-based targeting moiety of EV25 can selectively bind neuraminidase expressed on the surface of influenza-infected cells. To assess this, the neuraminidase inhibitor was conjugated to a rhodamine fluorophore and incubated with influenza-infected MDCK cells. Minimal binding was observed at early time points (approximately 5 hours post-infection), consistent with limited surface expression of neuraminidase. In contrast, marked cell-surface fluorescence was detected at later time points (approximately 15 hours post-infection), indicating efficient binding of neuraminidase inhibitor conjugate once neuraminidase became accessible at the infected cell surface.

These findings support the proposed targeting strategy of EV25, whereby viral neuraminidase expressed on infected host cells may serve as a molecular anchor for the conjugate. Although the experimental probe incorporated a rhodamine label rather than the dual-hapten configuration of EV25, the results can be considered as proof of principle for the molecule's ability to selectively target influenza-infected cells. Additional direct evaluations of the binding of EV25 to viral neuraminidase have shown the binding affinity for the unmodified construct to be ~7nM.

In vivo biodistribution and target specificity

Radiolabelled biodistribution studies were conducted to further assess the specificity of the neuraminidase inhibitor-based targeting approach *in vivo*. In this experiment, the neuraminidase inhibitor was conjugated to a technetium-99m radiotracer and administered to influenza-infected mice, enabling quantitative assessment of tissue distribution. A high level of accumulation of the radiolabelled conjugate was observed in the infected lungs, consistent with binding to neuraminidase expressed in infected tissue.

Target specificity was further examined using competitive inhibition strategy. Co-administration of a 100-fold excess of unlabelled neuraminidase inhibitor markedly reduced lung uptake of the radiolabelled compound, supporting the interpretation that tissue localization was predominantly mediated through neuraminidase recognition. In addition, minimal uptake was detected in uninfected mice, indicating preferential localization to infected tissue rather than nonspecific pulmonary distribution.

Overall, these findings provide *in vivo* proof of principle that the neuraminidase inhibitor -based construct can selectively target influenza-infected lungs. However, it should be

noted that the experiments were performed using a radiolabelled neuraminidase inhibitor probe rather than the full EV25 molecule, and therefore primarily support the neuraminidase-targeting side of the proposed mechanism. The potential influence of the hapten components on overall biodistribution cannot be assessed from these data. While a Quantitative Whole-Body Autoradiography has not yet been performed to evaluate distribution in the absence of a tracer, unconjugated EV25 accumulate in lungs tissues in the presence of infections.

Neuraminidase inhibition activity across influenza strains

The ability of the EV25 to retain neuraminidase inhibitory activity was evaluated using enzymatic assays. These studies were conducted across multiple influenza strains, including two H1N1 strains, one H3N2 strain, and one influenza B strain. The inhibitory potency of the conjugated molecule was reported to be broadly comparable to that of unmodified neuraminidase inhibitor, suggesting that incorporation of the hapten moieties does not substantially impair neuraminidase binding or enzymatic inhibition.

Neuraminidase inhibitory activity was also maintained against selected drug-resistant variants, including oseltamivir-resistant and baloxavir-resistant strains, with inhibition profiles described as favorable in these models. Overall, these findings suggest that EV25 preserves the direct antiviral function of the neuraminidase inhibitor at a nanomolar level while incorporating additional antibody-recruitment mechanisms.

Baseline anti-hapten antibody prevalence

The presence of naturally occurring anti-hapten antibodies in human serum was evaluated using 1137 blood samples. Antibody titers against both haptens were measured to assess whether the EV25 mechanism could rely on pre-existing humoral immunity. Detectable antibodies of both haptens were reported in the majority of individuals tested, , which is considered supportive of the proposed immune recruitment strategy. No individual was found to be lacking in anti-hapten antibodies.

Subgroup analyses showed broadly similar titers between males and females and across racial groups (Caucasian, African descent, and Asian). Detectable antibodies were also reported in healthy infants (approximately 2–12 months of age) and in individuals with selected comorbidities, including those receiving prednisone, organ transplant recipients, and patients with leukaemia, renal disease, heart failure, COPD, asthma, diabetes, or HIV infection.

Overall, these findings suggest that baseline anti-hapten antibodies are widely present in the populations tested and may support the intended antibody-recruitment mechanism.

***In vivo* efficacy and risk of resistance**

In murine influenza models (a setting that neutralizes the interindividual variability in humans), EV25 provided substantial protection following 10x LD50 lethal viral challenge. Treatment initiated up to 96 hours post-infection with single-dose intranasal administration resulted in marked reductions in lung viral titers (generally ≥3–4 log decreases) and improved survival compared with untreated controls. Activity was observed across multiple strains, including H1N1, H3N2, influenza B, and selected oseltamivir-, baloxavir- and zanamivir-resistant variants. In several experiments, single-dose intranasal administration achieved outcomes comparable or superior to multi-day dosing with standard antivirals.EV25 has been shown to have *in vitro* efficacy against both older and more recently isolated strains of H5N1 and H7N9. Microneutralization tests between H5N1 And H1N1 show similar efficacy. In early proof of concept testing with a H5N1 model, where drug is given 24 hours post infection early viral control and survival benefit were observed, although some late viral recurrences suggest that repeat dosing may be required for later intervention. This, in conjunction with its ability to block 90% of transmission in a guinea pig transmission model, suggest that EV25 could be a tool in combating pandemics in the future.

Serial passage in vivo studies did not show emergence of resistance under the tested conditions. Sequencing of strains isolated after treatment in 5 different mouse studies also reveal no drug induced mutations. Which corresponds with the data from serial passaging in vitro which did not generate any mutants after 10 passages.

D. Clinical development program and early clinical findings[2]

The clinical development program for EV25 includes a Phase I safety study followed by a randomized, double-blind human influenza challenge study. In the Phase I trial, single doses of 30mg, 100mg, and 300mg were evaluated in healthy participants (8 participants in each group). No meaningful differences were observed between placebo and treatment groups across safety parameters, and no clear safety signals were identified, suggesting the compound was generally well tolerated in this early evaluation.

In the Phase II human challenge study conducted in Belgium, participants (mean age approximately 43 years; 53% male, 47% female) were experimentally infected with influenza (A/Belgium/4217/2015 H3N2), developing clinical symptoms 24 hours later. They were treated approximately 24 hours post- inoculation with either placebo, EV25 30mg, or EV25 300mg administered intranasally. Treatment was associated with a dose-dependent reduction in infectious viral titers, assessed by area-under-the-curve parameter, with a significant effect observed at the higher dose. Improvements in clinical outcomes, (decreases in symptom burden measured by Flu-PRO and other symptom scores), associated with reductions in interferon-γ levels, were also reported. No treatment-emergent resistance mutations were detected in viral sequencing analyses.

[2]https://euclinicaltrials.eu/search-for-clinical-trials/?lang=en&EUCT=2024-514832-26-00

A clinical study was initiated to evaluate a dry intranasal formulation of EV25. The dry powder formulation was developed to increase bioavailability and simplify delivery. However, the dry powder formulation utilized for EV25 and the placebo control was poorly tolerated in both groups following initial dosing of 4 participants. The dry powder also did not show the anticipated increased bioavailability. To reduce the risk of future tolerability issues and ensure ready bioavailability of EV25, planned studies will utilize the liquid subcutaneous formulation. This formulation is being tested in a Phase 1 safety, tolerability and PK study in the EU as well as a Phase 1/2 safety and natural infection efficacy study in Bangladesh in Q2 2026.

Overall, these early clinical data were presented as supportive of antiviral activity and symptomatic benefit.

E. Discussion

Q1: It appears that the activity of EV25 depends in part on zanamivir binding. Could you clarify whether the compound remains active against zanamivir-resistant influenza strains?

EV25 does not rely solely on neuraminidase inhibition for antiviral activity but primarily on binding to neuraminidase to enable antibody recruitment. Although some reduction in binding potency may occur with zanamivir-resistant strains, the molecule was reported to retain sufficient binding to recruit endogenous antibodies. Recent studies, including *in vivo* work conducted with collaborators, show maintained antiviral activity against zanamivir-resistant viruses even when EV25 showed reduced neuraminidase inhibition against those strains *in vitro*.

Q2: Given that immunocompromised patients are an important target population for respiratory antivirals and may have lower levels of anti-hapten antibodies, is there a strategy to assess efficacy in immunocompromised patients or to mitigate potentially reduced antibody levels (e.g., through IVIG supplementation)

Evaluation of anti-hapten antibody levels in 50 humans sera with immunosuppression found that anti-hapten levels were maintained at normal levels for many of the most common causes of immunosuppression (leukemia, HIV, organ transplantation, immune suppressants drug usage, kidney failure). This suggests that in many cases anti-hapten antibodies may be present in sufficient quantities to provide sufficient support to immunosuppressed patients. However, the possibility of additional dosing or adjunctive strategies such as IVIG supplementation could form part of broader development considerations. Maintaining a single-dose regimen is a development objective, particularly to support global accessibility and cost considerations. In non-clinical studies, EV25 demonstrated significant efficacy in an immunocompromised mouse model of influenza, achieving 100% survival and the lowest lung viral loads with a modified dosing regimen, outperforming both vehicle control and baloxavir. While

EV25's mechanism relies on host immunity, these data indicate it can still provide meaningful protection in immunocompromised settings, although rebound infection was observed with single-dose treatment.

Further clinical evaluation in immunocompromised patients has not yet been fully defined, although a planned larger clinical study (e.g., the proposed study in Bangladesh) may contribute additional data relevant to this question.

Q3: Given that EV25 relies on anti-hapten antibodies, and acknowledging that some individuals may lack one of these antibodies, how might this variability affect treatment response? In addition, is there an identified minimal antibody threshold required for efficacy in humans?

EV25 activity does not require the simultaneous presence of both anti-hapten antibodies; binding to either antibody population is considered sufficient to support the mechanism of action. The dual-hapten design was introduced to increase population coverage in case one antibody type is absent. Preclinical analyses across a range of antibody titers (including approximately the 20th to 80th percentiles observed in humans) showed no meaningful difference in efficacy within this titer range. The combined levels of the anti-hapten antibody levels was assessed in >1000 samples of human plasma and were found to be at very high levels. Levels were also not impacted by age, sex, ethnicity, or influenza infection status. The levels remain high in high-risk patients that are immunocompromised or at risk of severe influenza infections. Individuals with very low antibody levels could theoretically exhibit reduced response, although based on the data generated to date, such cases would be considered incredibly uncommon. In situations of insufficient endogenous antibodies, administration of intravenous immunoglobulin (IVIG) could represent a potential mitigation strategy. As stated above, no individual lacked both antibodies in the >1000+ sera tested.

Q4: Given that the haptens targeted by EV25 are immunogenic and that asthmatic patients may have elevated antibody titers, is there a potential safety concern in these populations? Have any allergic manifestations been observed following intranasal administration, and how has safety in allergic patients been addressed?

EV25 has not yet been evaluated in asthmatic patients; however, such populations are planned for inclusion in upcoming clinical studies. The design of EV25 is intended to limit activation to sites where the viral target is present, with the aim of minimizing off-target immune activation. Non-clinical safety studies did not to show evidence of systemic immune activation or cytokine release syndrome, and no evidence was seen in the Phase 1/2a challenge study. However, clinical confirmation of safety in patients with asthma or other underlying respiratory conditions remains pending.

Q5: Given the apparent efficacy of EV25 when administered relatively late after infection in preclinical models, how do you plan to evaluate this feature clinically? Are there data from the human challenge model or plans in Phase II/III studies to assess efficacy when treatment is initiated several days after infection?

The human challenge study evaluated EV25 administered approximately 24 hours after viral inoculation that showed in participants with confirmed influenza infection, a single dose of 300 mg EV25 was able to significantly decrease viral load AUC (98% mean reduction) compared to placebo, and was also able to significantly decrease clinical symptoms as measured by FluPro compared to placebo (Total symptom score, Peak AUC, Lower Respiratory and body/Systemic Symptoms). To further address the potential to address later stage treatment, the upcoming Phase 1/2 trial in Bangladesh is designed to include patients treated at multiple time points following symptom onset (e.g., 24, 48, and 72 hours). The planned study, expected to enrol 620 patients, is intended to provide a broader assessment of treatment timing effects and capture potential variability in response.

Q6: In the context of avian influenza, are you considering combination therapy with other antivirals or repeated dosing of EV25? Additionally, at what time point after infection is EV25 intended to be administered in the H5N1 model?

Regarding treatment strategy, repeat dosing of EV25 is being considered where necessary, particularly in models such as H5N1 where viral control may require sustained exposure. Evaluation of potential combination therapy with Oseltamivir and Baloxavir has been performed. Moderate synergism was detected, opening the potential for EV25 to be dosed alongside some current standard of care treatments.

In the H5N1 mouse model, treatment was initiated approximately 24 hours after infection to remain within a therapeutic (rather than prophylactic) framework. Viral neuroinvasion observed in this model may represent a limitation, as EV25 is not expected to cross the blood-brain barrier, which could necessitate multiple dosing in certain settings. Efforts are ongoing to balance efficacy with the objective of maintaining a simple and cost-effective dosing regimen.

Q7: Is there a risk that EV25 could induce a long-term increase in anti-hapten antibodies in humans, and if so, are there potential clinical consequences?

Based on the proposed mechanism of action, a sustained increase in anti-hapten antibodies is considered unlikely. The molecule is not designed to form covalent adducts or to generate peptide antigens that would be presented via MHC pathways, and it is expected to be rapidly cleared if not bound to its target. Anti-hapten antibodies are present ubiquitously in the human population and are at such high levels due to

constant exposure from the normal environment. EV25 is not anticipated to stimulate more exposure than what one would encounter on a daily basis. These antibodies are IgGs and present at high levels so increased amounts is also not anticipated to have adverse effects.

Data from our non-clinical studies shows no meaningful changes in anti-hapten levels present in rats and dogs after 14 days of repeat dosing was found in the blood when measured 14 days post study. Additionally, no aberrations to any inflammatory markers or immune cell populations in response to the repeat dosing were detected.

Data from our Phase 1/2a study supports rapid clearance of the EV25 with a half life of 2.5-3 hrs, and treatment does not show a long-term increase in anti-hapten antibodies when measured out to 4 weeks post treatment. This cannot be definitively answered however until antibody titers are measured out 6-8 weeks post treatment.

F. AvATher Group Recommendations and Opinions on EV25

Key Strengths

- The AvATher group considered EV25 to be an innovative and scientifically promising approach, with particularly strong *in vitro* activity and encouraging early clinical data. Experts acknowledged the originality of the antibody-recruitment strategy and noted the reported reductions in viral load and symptom burden in early studies. The absence of major safety signals to date and the lack of detected resistance in the presented datasets were viewed as positive.

Main Uncertainties and Risks

- Several important reservations were raised. Experts noted that the intranasal administration evaluated in early studies appeared generally well tolerated and was associated with encouraging reductions in viral load and symptom burden. However, to reduce the risk of future tolerability issues and to increase the bioavailability of EV25, future planned studies will utilize a liquid subcutaneous formulation.
- These include the need to re-characterize pharmacokinetics following the potential shift from intranasal to parenteral administration discussed for future clinical studies. The group is also concerned by the loss of the practical advantages associated with intranasal delivery.

Additional comments by ERADIVIR after the meeting following these remarks :

Eradivir has conducted 11 nonclinical studies to evaluate and characterize the impacts the shifting from an internasal to subcutaneous formulation. This included the evaluation and assessment of subcutaneous EV25 in 4 different species. It was found that EV25 has very controlled and ideal pharmacokinetic properties when administered subcutaneously and the improvements in bioavailability will allow for much lower dose administration in humans. EV25s efficacy is not negatively impacted by this route of administration and is found to remain proportional to the serum concentration levels. These pharmacokinetic findings will be confirmed in a human bridging study in Belgium in May 2026 prior to our study in Bangladesh this summer where subcutaneous dosing will be used for to treat 620 naturally infected patients. While intranasal administration might have a compliance advantage, human bioavailability intranasally was ~15% and required higher than expected dose administration to achieve the required pharmacokinetic parameters. The dose volume and solubility limits of the drug make delivering that high of a dose via intranasal route commercially and practically unrealistic. With this drug being a single dose drug a single dose subcutaneous dose with >90% bioavailability allows for this drug to keeps its cost of goods in a margin that allows worldwide adoption. Sterile subcutaneous injectable drugs have become quite prevalent in the market and have been widely adopted, with administration causing minimal injection site pain. Eradivir is additionally exploring additional routes of administration such as subcutaneous patches to further reduce some of the convenience issues lost with moving to a single SC injection

- Safety concerns remain, particularly regarding possible IgE-mediated responses, use in asthmatic or allergic populations, and more generally increased anti-hapten immunogenicity due to the formation of neuraminidase-EV25 complexes (the neuraminidase providing helper T cell epitopes that are indeed absent in EV25).

Additional comments by ERADIVIR after the meeting following these remarks :

Eradivir will monitor for the formation of any IgE- related responses in its upcoming trials for EV25. The Bangladeshi and European populations being evaluated will include both asthmatics and those prone to allergies. EV25 thus far has shown no overactivation of the immune system in vitro, in vivo and in humans. In vitro human PBMC from multiple donors treated with EV25 and in the presence and absence of human antibodies has not elicited any activation in absence of an infection. In vivo in the presence of infection we have found both the local and system cytokines are decreased in nonclinical models, In the absence of infection no immune toxicity has been observed in any of the toxicity studies. In humans we saw a marked reduction in systemic proinflammatory cytokines with no evidence of changes in eosinophil populations post treatment during an infection in humans.

- Experts also highlighted limitations in the resistance package, notably the absence of comparison with zanamivir and the lack of robust data on whether resistance mutations may preserve binding.

> ***Additional comments by ERADIVIR after the meeting following these remarks :***
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> *Zanamivir resistant strains were also tested with strains proving to have significant resistance against NAI only activity of EV25. Later data suggests that EV25 is still effective against zanamivir resistant strains in animal models. These data suggest that mutations that impact the ability of the neuraminidase inhibitor to block the catalytic activity are not sufficient to block the immunological recruitment as the molecule just needs to stick to recruit the immune system. This allows EV25 the ability to address a much broader spectrum of strains than those that can solely be addressed by neuraminidase inhibition alone.*

- More broadly, the group noted a typical attenuation of effect from *in vitro* to animal and to human data and emphasized that current clinical results are based on small studies, with the highest dose appearing to drive most of the observed benefit.

> ***Additional comments by ERADIVIR after the meeting following these remarks :***
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> *The efficacy window being skewed more toward the high dose was due to the bioavailability of EV25 intranasal dosing in humans was lower than was observed in animals models so the desired pharmacokinetics parameters were not achieved at the lower dose. This is one of the main driving factors for our switch to subcutaneous dosing where it is anticipated we can get the same exposure as the 300mg dose intranasally with a 50mg dose subcutaneously.*

Recommendations

- First of all, it would be interesting to evaluate the *in vivo* distribution of radiolabelled EV25 to exclude hapten-mediated capture of the compound independently on the infection. It would be also interesting to have (1) a clearer view of the contribution of either hapten specificity at the individual level (data as diagrams and not cumulated histograms), (2) in a larger population of individuals, and (3) a more detailed analysis at the level of antibody classes and subclasses. This will be necessary to better analyse the potential interindividual variability and, maybe, to determine companion biomarkers that would allow to better identify the patients that could benefit from the product alone and/or would require IVIG administration.

> ***Additional information provided by ERADIVIR following the recommendation:***

The vast majority of antibodies are IgGs, and >1000 people were screened so far without having found someone low in both. The reason 2 haptens are present was to allow redundancies for inter-person variability. though the variation on the combined titer across the 1000+ people is quite minimal. With most people sitting at a combined pool of 500ug/ml. We anticipate that the vast majority of all patients will have quite a bit excess relative to what is needed to function and that only extreme case of low antibody levels would supplementation with IVIG would be needed. We have developed GCP validated assays to monitor both anti-DNP and anti-rhamnose levels in human serum. we will use these to continue to monitor this in our clinical trials to see if we run across any nonresponders that can be attributed to hapten level issues.

- The group considered that additional data, including full human PK characterization with the intended route of administration, expanded safety evaluation in the general population (anti-hapten IgE, increased anti-hapten immunogenicity) and in at-risk populations, strengthened resistance and comparator analyses, and confirmation of clinical efficacy in larger, well-powered studies conducted in naturally infected patients, would strengthen the development of EV25.

  Additional information provided by ERADIVIR following the recommendation:

  Several of these points are or will be assessed in ongoing studies

Overall assessment

Overall Assessment of EV25

Pre-exposure Prophylaxis	***Post-Exposure Prophylaxis***	***Out-patient Treatment***	***In-patient Treatment***
Insufficient	***Interesting***	***Interesting***	***Insufficient***

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