Meeting Report: AvATher Working Group & SAChA Project
15 May 2025, 17:00–19:00 (Virtual meeting via TEAMS)

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Meeting Objective: To present the SAChA project, which aims to develop an innovative therapy for the treatment of severe influenza.

A. Context

Currently available antivirals, such as the neuraminidase inhibitor oseltamivir, have limited effectiveness in severe influenza, especially when administered more than 48 hours after symptom onset. This limitation highlights the urgent need to develop new therapies capable of addressing two key challenges: slowing viral replication and controlling the excessive inflammatory response, which is the main driver of severity and mortality in severe influenza infections.

The SAChA project builds on the emerging field of immunometabolism, which explores the close connections between energy metabolism and immunity. This innovative approach views certain metabolites, such as succinate, not merely as simple energetic intermediates but as true bioactive compounds with antimicrobial and immunoregulatory properties.

In this context, **SAChA aims to identify and leverage cis-aconitate and its chemical analogues** as new compounds capable of both inhibiting viral replication and modulating inflammation, thereby paving the way for novel curative therapies against influenza and other viral immunopathologies.

B. Research Data

B.1: Previous results obtained with succinate

- **In vivo results: murine model**

The Metabolomic analysis of bronchoalveolar lavage fluid from mice infected with influenza A virus or the bacterium *Streptococcus pneumoniae* revealed distinct metabolic signatures specific to each pathogen. Principal component analysis (PCA) showed a clear separation between infected and control groups, as well as between the two types of infection. Among the discriminating metabolites, succinate, known for its immunoregulatory properties (PMID: 32333837), stood out with particularly high levels in the lungs of influenza-infected mice.

- **Clinical relevance: patient data**

In respiratory fluids collected from intubated patients with severe influenza, succinate levels were significantly higher than in ICU control patients presenting a comparable degree of pulmonary inflammation (for example, similar levels of IL-6 and IL-8). The control group consisted of brain-injured patients who had developed lung inflammation and purulent bronchorrhea due to micro-aspirations. This specific elevation of succinate in influenza patients suggests a regulatory mechanism unique to the infection and supports the hypothesis of a beneficial role for succinate in the host response to influenza infection.

- **In vitro results: epithelial cell model**

Experiments conducted on human bronchial epithelial cells confirmed that succinate, when administered alone at non-cytotoxic concentrations, does not induce any inflammatory response. In contrast, influenza virus infection triggers a strong IL-6 production, which is markedly reduced by succinate in a dose-dependent manner. This anti-inflammatory effect extends to several other pro-inflammatory cytokines.

Furthermore, electron microscopy revealed that human epithelial cells infected with influenza virus produced numerous budding virions, indicating active replication. In contrast, in succinate-treated cells, the viral cycle appeared to be interrupted, with very few budding particles observed, suggesting a marked inhibition of influenza virus replication.

- **In vivo validation: murine model**

In mice infected with influenza virus, intranasal administration of succinate significantly reduced lung inflammation and preserved pulmonary tissue architecture, indicating a protective effect on respiratory function. Clinically, succinate-treated mice maintained their body weight and achieved a 100% survival rate after a lethal challenge (LD50). In contrast, untreated mice experienced substantial weight loss and approximately 50% mortality, confirming the therapeutic benefit of succinate in this severe influenza model.

C. Mechanism of Action – Nuclear Retention and Succinylation of the NP Protein

Confocal microscopy of influenza-infected epithelial cells showed that, 20 hours post-infection, the viral nucleoprotein (NP), a key component of the viral ribonucleoprotein complex (vRNP), normally exits the nucleus to reach the cytoplasm, a crucial step for the assembly of newly formed virions. In contrast, in succinate-treated cells, this process is profoundly altered: NP remains confined within the nucleus, indicating a block in the viral cycle upstream of vRNP export.

Complementary mechanistic studies revealed that succinate induces a post-translational modification of NP through succinylation of lysine K87, a highly conserved residue in influenza A viruses and located within the genomic viral RNA-binding pocket. This modification alters the conformation and charge of lysine K87, thereby disrupting NP's ability to bind viral RNA. Notably, NP is the only influenza protein identified as a target of succinate-induced succinylation. The inability of NP to correctly interact with viral RNA prevents the association and assembly of functional vRNP complexes.

In the absence of functional vRNPs, export to the cytoplasm cannot occur, leading to a marked reduction in the production of infectious particles and consequently a decrease in associated inflammation.

B.2 : Results related to the SAChA program

D. Cis-aconitate: a dual-action metabolite, acting as a potent inhibitor of both viral replication and inflammation

Building on the discovery of succinate's antiviral effect, the team extended its analysis to other endogenous metabolites that might modulate influenza virus replication. Among numerous candidates, particularly those derived from the Krebs cycle, cis-aconitate proved to be especially potent, showing a marked inhibition of both viral replication and host inflammation.

Electron microscopy of human bronchial epithelial cells infected with influenza virus and treated with cis-aconitate showed an almost complete absence of viral budding. In parallel, Western blot analyses revealed a marked reduction in viral protein levels, and qPCR analyses confirmed decreased expression of viral RNAs. Mechanistic studies further demonstrated that cis-aconitate interferes with viral polymerase activity, leading to reduced expression of a reporter gene specifically controlled by this enzyme.

Treatment of human bronchial epithelial cells with cis-aconitate followed by stimulation with poly(I:C) (a TLR3 agonist) showed a clear, dose-dependent reduction in inflammation. This indicates that cis-aconitate has an intrinsic anti-inflammatory effect, independent of its antiviral activity, which distinguishes it from succinate. This effect was also observed when inflammation was induced by other stimuli, such as PMA or TNF-alpha, confirming the robust anti-inflammatory properties of cis-aconitate.

In mice expressing the transcription factor NF-κB, which is essential for regulating the inflammatory response, intranasal administration of LPS induced strong inflammation, evidenced by a marked increase in luminescence. Treatment with cis-aconitate led to a clear reduction in this signal, demonstrating a major anti-inflammatory effect in vivo.

Finally, in a murine influenza infection model, administration of cis-aconitate or oseltamivir 20 minutes after infection provided complete protection, both in terms of survival and prevention of weight loss. In contrast, when treatment was delayed by two days, oseltamivir lost all efficacy, whereas a single dose of cis-aconitate still conferred protection. Remarkably, cis-aconitate maintained its protective effect even when administered four days after infection, demonstrating an extended therapeutic window.

B.3: From discovery to therapeutic innovation: launching a start-up to accelerate development

Building on mechanistic data demonstrating the antiviral properties of succinate and cis-aconitate against influenza virus, the team launched an ambitious research and development program focused on these natural compounds and their chemical analogues, within the framework of the ANR SuccesS program [ANR-21-CE18-0061, in partnership with S. Messaoudi (CNRS & École Polytechnique X, Saclay)]. More than 60 optimized chemical analogues have been synthesized, several of which exhibit antiviral activity superior to that of cis-aconitate.
A patent describing the antiviral activity of cis-aconitate has been validated at the international level (WO 2024126742) and is currently under national evaluation. In parallel, two additional patent families have been filed to protect and expand the innovation portfolio. The SAChA project was also awarded the Grand Prize of the i-PhD 2024 competition.
With a focus on transfer and valorization, the team is now working to translate its discoveries into therapeutic innovation through the creation of a start-up. This initiative is supported by the Technology Transfer Office of the University of Tours, the regional transfer structure C-Valo (Centre-Val de Loire), as well as national funding mechanisms such as France 2030-CATRIEM. The objective is to position cis-aconitate as an innovative therapeutic solution to address the clinical and economic burden of influenza infections.

E. Discussions:

1. How do you explain the fact that no efficacy was observed on day 4 (post-infection)?

By day 4, the mice had already lost 20% of their body weight and were moribund. At this stage, observing any treatment effect is already remarkable. The efficacy of cis-aconitate observed on day 4 is likely linked to its dual antiviral and anti-inflammatory action. The hypothesis is that, through its combined effects on viral replication and inflammatory signaling, cis-aconitate remains effective even at an advanced stage of infection. It is worth noting that this late protective effect is not observed with succinate, which does not possess anti-inflammatory activity.

2. Does succinate treatment affect the nuclear localization of influenza nucleoprotein (NP), and what could be the implications for viral replication?

Confocal microscopy images revealed an unusual accumulation of NP in the nucleus of succinate-treated cells, suggesting interference with the intracellular trafficking of viral ribonucleoprotein complexes (vRNPs). Normally, during influenza infection, viral RNA is transcribed in the host cell nucleus, while NP, synthesized in the cytoplasm, is imported into the nucleus to associate with newly transcribed viral

RNA segments. The resulting vRNP complexes are then exported to the cytoplasm for assembly into new virions. The nuclear accumulation of NP under succinate treatment suggests inhibition of vRNP export, leading to interruption of the viral cycle and reduced production of newly formed virions.

3. **Is the efficacy of cis-aconitate limited to early-phase nasal administration, or does it extend to systemic routes and deep lung tissues?**

Cis-aconitate has demonstrated antiviral efficacy well beyond the nasal mucosa. Its activity has been confirmed in bronchial and alveolar cells, as well as in human lung tissue sections. In addition, animal studies are currently underway using systemic routes of administration (oral gavage, IV injection) to determine whether its antiviral and anti-inflammatory effects can be reproduced through routes other than local administration.

4. **With intrapulmonary instillation, similar to aerosol administration, do you think the observed effect is purely local on the pulmonary epithelium, or could it also exert systemic effects on distant sites, such as the brain, where influenza can cause neurotoxicity?**

No brain analysis was performed in the current murine model of influenza infection, making it impossible to directly assess systemic effects, particularly in the brain. However, preliminary pharmacokinetic data suggest that intranasal administration results in very low or even undetectable levels of cis-aconitate in the bloodstream, indicating that the therapeutic effect observed is primarily local, within the lungs. Interestingly, in a collaborative study with the VIRIMI team (CIRI, Lyon), cis-aconitate showed antiviral activity in brain tissue slices infected with a pathogenic neurotropic virus. This suggests that under certain conditions, or via alternative routes of administration, its effects could potentially extend beyond the lungs. Further studies will be needed to confirm this possibility and to evaluate its impact on the systemic manifestations of influenza infection.

5. **Has cis-aconitate demonstrated antiviral activity beyond influenza, and what are the dosing requirements and next steps in its preclinical development?**

Although both succinate and cis-aconitate were initially investigated, attention is now focused on cis-aconitate due to its superior antiviral efficacy. Robust activity has been confirmed against influenza virus as well as SARS-CoV-2. In addition, exploratory data suggest potential efficacy against other pathogens, though these results still require further validation.

With regard to dosing, preliminary animal studies indicate that cis-aconitate is effective at relatively low doses, around 5 mg/kg, based on single-dose administration. These levels are considered compatible with a viable drug development pathway. Additional work is planned to refine dosing strategies and optimize pharmacokinetic (PK) and pharmacodynamic (PD) parameters.

The compound's development is following a classical preclinical trajectory:

- Toxicology studies are underway,
- Efficacy tests are planned in animal models such as ferrets,
- Promising results obtained in human lung tissue models support continued development of the compound.

6. **What is the duration of the antiviral effect after a single dose? Based on your current PK/PD data, do you have indications on the duration of action of these metabolites and on the possible need for repeated administrations?**

Current pharmacokinetic data suggest a very short exposure time, indicating a rapid "hit-and-run" mechanism of action. Although a repeated-dose regimen could be considered, the observed response is sufficiently robust that, at this stage, multiple administrations do not appear necessary. If toxicity were to

emerge, dose reduction or split dosing could help maintain efficacy while reducing exposure. These aspects are still under evaluation.

7. **Since these metabolites are naturally produced by the body, do we have data on their toxicity at high doses, and are these aspects being further investigated? Why was cis-aconitate selected as the lead compound rather than S10 or S11, which showed better survival rates?**

   Although cis-aconitate is a metabolite physiologically produced by the body, an in-depth toxicological evaluation remains necessary at therapeutic doses. A toxicity study is currently underway. In murine models, the effective dose proved to be non-toxic, even with repeated administration over a 15-day period, and no organ damage was observed. Toxic effects appeared only at supra-therapeutic doses, which the ongoing study aims to characterize more precisely.

   Regarding the choice of the lead compound, cis-aconitate was selected over the synthetic analogues S10 and S11 due to the robustness of the available preclinical data and the most advanced mechanistic understanding to date. Although S10 and S11 showed promising survival benefits, focusing development efforts on a single, well-characterized candidate simplifies the strategy and provides greater clarity for investors. The other candidates remain part of the development pipeline as strategic alternatives to mitigate risk.
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8. **Are succinate and cis-aconitate specifically induced during influenza virus infection, and if so, what mechanisms underlie the production of these natural metabolites?**

   It has been clearly demonstrated that succinate increases during influenza virus infection, but cis-aconitate is more difficult to measure due to analytical sensitivity limits. Therefore, it has not yet been definitively shown that cis-aconitate is physiologically induced by influenza virus infection.

   More generally, this type of metabolic reprogramming is not specific to influenza. Over the past decade, many studies have shown that various stressors, such as infections, cancer, or inflammation, drive immune cells to alter their metabolic pathways. These changes can be beneficial for the host, but some pathogens may also exploit these metabolic resources to facilitate their replication.

   Interestingly, some metabolites that accumulate under stress conditions can act as powerful immunoregulatory agents. For example, cis-aconitate exhibits strong anti-inflammatory and antiviral properties. This supports the hypothesis that metabolites are part of an evolutionarily conserved defense arsenal and represent a promising source of new therapeutic candidates.

9. **Have you also observed increased succinate levels in hospitalized influenza cases that were not severely ill (i.e., not in intensive care)?**

   We do not have an answer to this question, as the analysis requires pulmonary fluid samples, and we do not have specimens from hospitalized patients who were not severely affected.

10. **Can the luciferase-based animal model provide kinetic data to better understand the timeline of cis-aconitate's antiviral effects, particularly in severe cases or when assessing systemic action?**

    Yes, kinetic analysis is possible and has likely already been performed. The luciferase experiment was conducted in collaboration with an INRAe team in Jouy-en-Josas (Ronan Le Goffic's group). The data currently available correspond to the 20-hour post-challenge time point, but image acquisitions at earlier and intermediate time points were also obtained. These could help clarify the timeline and dynamics of the therapeutic effects of cis-aconitate. Such information would be particularly valuable for determining whether the treatment acts very early in the course of infection, an essential aspect for targeting the most severe patients and for assessing potential systemic benefits.

Conclusion:

The AvATher experts consider the approach of the SAChA project, which aims to use metabolites as curative agents in influenza infections, to be promising. The project is well structured, already at an advanced stage, and currently shows no major weaknesses. The preliminary results are encouraging and suggest a potential therapeutic benefit.

Another advantage of this approach is that, unlike proteins which often exhibit species-specific differences, metabolites are generally less affected by such interspecies variations. Their mechanisms of action tend to be more conserved across species, making the transition from preclinical models to clinical studies in humans more predictable and reducing the risk of translational difficulties.

AvATher members' suggestions on the positioning of this approach

To ensure both clinical impact and development feasibility, it would be ideal to initially target treatment at hospitalized patients at high risk of severe influenza, particularly young immunocompromised individuals, including those with cardiopulmonary comorbidities, hematologic malignancies, or organ transplants.

This strategy presents several key advantages:

- **Medically**, it addresses an unmet need: current antivirals have limited effectiveness in severe forms, and these high-risk populations remain exposed to significant morbidity and mortality, even outside intensive care.
- **Strategically**, it enables the establishment of targeted proof of concept in a controlled medical environment, where diagnosis is confirmed and clinical outcomes can be reliably measured.
- **Operationally**, it avoids the logistical challenges of early outpatient management, where diagnosis is often delayed and care organization fragmented (e.g., in nursing homes or community medicine).

In contrast, an initial rollout in the general outpatient population or in nursing homes would face several obstacles: delayed diagnosis, irregular medical follow-up, and difficulty in accurately documenting therapeutic impact.

Once efficacy and safety are established in the hospital setting, evaluation could then be logically extended to post-exposure prophylaxis in vulnerable outpatient populations, particularly during influenza epidemics, where rapid intervention could prevent disease progression even in the absence of formal virological diagnosis.