

NFL BIOSCIENCES: SUCCESS OF THE STUDY WITH THE CEA

Disruptive new mechanism of action determined for smoking cessation: NFL-101 demonstrates restoration of normal activity in the brain area associated with craving during withdrawal

NFL BIOSCIENCES (Euronext Growth Paris – FR0014003XT0 – ALNFL), a biopharmaceutical company developing botanical drugs for the treatment of addictions, is announcing the demonstration during a molecular imaging study, conducted by the French Alternative Energies and Atomic Energy Commission (CEA), of the disruptive new mechanism of action concerning its drug candidate for smoking cessation:

- **The ability of NFL-101 to reduce craving is demonstrated by the restoration of normal brain activity in the region of the brain associated with this craving;**
- **The results suggests that there is communication between the immune system and the central nervous system, a different mode of action than with current smoking cessation drugs, which directly target nicotinic receptors.**

Launched in February 2023, and conducted by the CEA's Pharmacological Neuroimaging team, the study demonstrates the mechanism of action of NFL 101, a drug candidate for smoking cessation. The three stages of the study with the CEA have been validated and show the central effects of the treatment with NFL-101. These observations open up new possibilities for smoking cessation, as well as for the treatment of related co-addictions, tobacco/cannabis/alcohol.

For Nicolas Tournier, pharmacologist, radiopharmacist and head of the Pharmacological Neuroimaging team at the CEA / SHFJ: *“This collaboration has made it possible to achieve several major advances. Firstly, this project has enabled our team to develop and validate a robust model for exposure to tobacco in mice. Then, we have demonstrated for the first time that TEP molecular imaging at 18F-FDG, an indicator of cerebral metabolism, is a relevant biomarker for monitoring the neural substrates* of smoking cessation. We have been able to determine NFL-101's specific action on cerebral metabolism in the smoking cessation situation. These initial results are original and promising, opening up new possibilities for the treatment and therapeutic monitoring of smoking cessation in people”.*

Bruno Lafont, Chief Operating Officer and co-founder of NFL Biosciences: *“We are of course extremely satisfied with this study's results and would like to sincerely thank the CEA team for their outstanding work. This represents a major step forward for our project: NFL-101 induces a cerebral action on craving, and this action is specific. The determination of this mechanism further strengthens our development program's attractive positioning for our potential partners. We plan to continue our work with the CEA and we are considering extending it to study cessation with tobacco/cannabis co-addictions and also the presence of alcohol. The potential of the NFL-201 project, a cannabis extract dedicated to the treatment of cannabis addiction, has also been further strengthened through this discovery”.*

NFL-101 is a nicotine-free tobacco extract derived from a subcutaneous desensitization treatment that was initially developed by the Pasteur Institute against tobacco allergies in tobacco factory workers. NFL-101 has already been tested in two clinical trials: a Phase 1 study - CESTO - confirmed its safety and a Phase 2a study - PRECESTO - confirmed its ability to significantly reduce smoking satisfaction in smokers who do not want to quit. A Phase 2b clinical study - CESTO 2 - is underway at nine clinical centers in France with 318 smokers who wish to quit, with the results expected for July 2024. The development of a botanical drug often starts with an empirical observation of a plant's therapeutic properties, which can help increase the chances of success during subsequent studies to demonstrate its efficacy. Initially, a full understanding of the mechanism of action is not necessary. As the development progresses, more in-depth knowledge of this mechanism is built up. This is what led to the study with the CEA.

Method validated and results demonstrating the effect of NFL-101 on brain activity

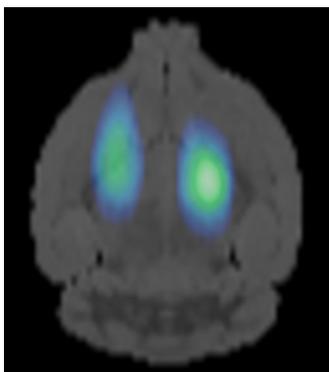
Study method validated

The study of the mechanism of action was conducted by the CEA Pharmacological Neuroimaging team at the Service Hospitalier Frédéric Joliot (SHFJ) research center in Orsay. It involved monitoring, with positron emission tomography (PET) imaging, the modifications in cerebral function associated with the development of tobacco addiction in mice in order to highlight the central effects of NFL-101 treatment in this context. The analysis was based on three stages:

1. **Model validation:** involves confirming that the model effectively makes it possible to study a potential effect on brain activity in smoking cessation conditions. For this, brain scans of mice exposed to tobacco then placed in cessation conditions are compared with brain scans of mice not exposed to tobacco. A significant difference in terms of activation in certain areas made it possible to validate the model.
2. **Efficacy analysis:** involves comparing the effect of the product NFL-101 and that of a placebo on mice presenting the condition to be treated. Brain scans of mice exposed to tobacco and placed in cessation conditions following the administration of NFL-101 are compared with those following the administration of the placebo. The difference observed reveals a significant effect.
3. **Specificity analysis:** involves comparing the effect obtained between the product and the placebo on mice not presenting the condition to be treated. Brain scans of mice not exposed to tobacco following the administration of NFL-101 are compared with those following the administration of the placebo. No difference was observed during this stage, which confirms that the product's effect is specific to the condition to be treated.

Results demonstrating the effect of NFL-101 on brain activity

1. Model validation (exposure to tobacco then cessation / non-exposure to tobacco)



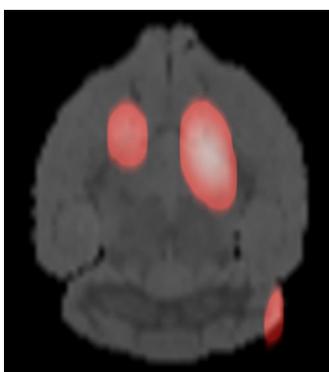
The significant differences ($p < 0.05$), in green/blue, are located in areas that correspond to the thalamus region. This region is under-activated in the mice exposed to tobacco and placed in cessation conditions.

These results make it possible to validate the study model.

As the thalamus region is known to be associated with craving^{**}, i.e. the irrepressible desire to smoke, the model is therefore also particularly adapted for studying cravings.

Image 1: representation of the significant differences (blue/green areas) between the scans of mice exposed to tobacco then placed in cessation conditions and the scans of mice not exposed to tobacco.

2. Efficacy analysis (during cessation: NFL-101 / placebo)



The significant differences ($p < 0.05$), in red, are located in areas that correspond to the thalamus region. The activation by NFL-101 is significantly stronger there than with the placebo.

The comparison between the two images shows that the over-activated areas (red) with NFL-101 correspond to the under-activated areas (green/blue) with the application of cessation conditions after exposure to tobacco. The effects of under-activation and over-activation are also on a comparable scale. This indicates that NFL-101 enables a lasting restoration of brain activity following exposure to tobacco and after being placed in cessation conditions.

Image 2: For the mice exposed to tobacco then placed in cessation conditions, representation of the significant differences (red) between the scans of mice that received NFL-101 and the scans of mice that received the placebo.

3. Specificity analysis (without exposure to tobacco: NFL-101 / placebo)

For the mice not exposed to tobacco, the administration of NFL-101 did not result in any changes in brain activity. The effect, which therefore only exists when there is prior exposure to tobacco, corrects a modification of brain activity triggered when placed in a smoking cessation situation.

These observations indicate that NFL-101 acts in a targeted and specific way.

Conclusion: NFL-101 demonstrates the restoration of normal activity in the area of the brain associated with smoking craving in a cessation situation

The results obtained support the existence of a disruptive new mechanism of action for NFL-101.

Following the pre-clinical studies and the CESTO clinical study, which demonstrated that NFL-101 triggered a specific immune response, this latest advance demonstrates the sustainable restoration of thalamus activity in a smoking cessation situation. This suggests that there is communication between the immune system and the central nervous system, a different mode of action than with the current smoking cessation drugs, which directly target nicotinic receptors.

Reducing craving on a lasting basis is recognized as the major challenge with tackling smoking through drugs. Craving limits the success of attempts to quit and then encourages relapses. This opens up possibilities for the fight against tobacco addiction in particular, as well as other addictions. Tobacco/cannabis/alcohol co-addictions with related reinforcements are frequent, and reducing craving on a lasting basis would make it possible to approach their treatment from a new angle.

The results, which may be the subject of a joint scientific publication in a leading international scientific journal, complement the CESTO and PRECESTO clinical studies with a view to increasing the attractiveness of NFL-101 to pharmaceutical companies that may be interested in licensing it.

* Neural substrates include all the biological components that are involved in the complex processes of the nervous system, including information transmission, neuronal activity regulation and the biological bases for cognitive functions and behaviors. The studying of neural substrates and cerebral metabolism is closely linked to that of brain activity.

** Examples of publications associating craving with the thalamus region:

Lor CS, Haugg A, Zhang M, Schneider L, Herdener M, Quednow BB, Golestani N, Scharnowski F. Thalamic volume and functional connectivity are associated with nicotine dependence severity and craving. *Addict Biol.* 2023 Jan;28(1):e13261. doi: 10.1111/adb.13261. PMID: 36577730; PMCID: PMC10078543.

Wang C, Zhang Y, Yan C, Sun M, Cheng J. The thalamo-cortical resting state functional connectivity and abstinence-induced craving in young smokers. *Brain Imaging Behav.* 2018 Oct;12(5):1450-1456. doi: 10.1007/s11682-017-9809-5. PMID: 29297152.

Hommer DW. Functional imaging of craving. *Alcohol Res Health.* 1999;23(3):187-96. PMID: 10890814; PMCID: PMC6760372.

About NFL Biosciences

NFL Biosciences is a biopharmaceutical company based in the Montpellier area which develops botanical drug candidates for the treatment of addictions. NFL Biosciences' ambition is to bring new, natural, safer and more effective therapeutic solutions to the entire world population, including low- and middle-income countries. Its most advanced product, called NFL-101, is a standardized, nicotine free tobacco leaf extract protected by two patent families. NFL Biosciences intends to offer smokers who want to quit a natural, safe, easy-to-administer and personalized alternative. NFL Biosciences is also developing NFL-301, a natural drug candidate for the reduction of alcohol consumption and has a drug development project for the treatment of cannabis use disorder.

The shares of NFL Biosciences are listed on Euronext Growth Paris (FR0014003XT0 – ALNFL). Find out more at www.nflbiosciences.com

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