White paper

EUDRACAP® Select

EXAMINING A CASE FROM DEVELOPMENT TO CLINICAL TRIAL

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Summary

A growing body of scientific evidence suggests that the microbiome plays a critical role in various diseases and negatively impacts the overall survival of patients undergoing allogenic hematopoietic stem cell transplantation. Microbiome therapy can help maintain immune homeostasis and optimize gut function. However, microorganisms are typically difficult to formulate for oral delivery, due to their sensitivity to the acidic conditions present in stomach, as well as to the heat and moisture of standard enteric coating processes.

The objective of this study was to develop a suitable oral delivery system for a full ecosystem of fecal microbiota and to test its safety and tolerability in a Phase 1b clinical trial. Pre-locked hard HPMC capsules were coated with a proprietary combination of EUDRAGIT® polymers to achieve a targeted enteric behavior.

The capsules developed were tested under simulated physicochemical conditions of the human gastrointestinal tract before clinical trials and demonstrated the ability to deliver their contents to the distal small intestine and proximal colon. The Phase 1b clinical trial showed that the engraftment and richness of the microbiota improved considerably in patients with acute myeloid leukemia.

The empty, ready-to-fill, enteric capsule developed, EUDRACAP® Select, was effective and robust as a drug delivery system for the sensitive microbiome tested in this study, and also reduced development time, and scale-up and validation complexity.

Introduction

There are three key challenges in developing an oral drug. First, targeted drug delivery is often required to ensure precise drug targeting within a narrow absorption window. This approach reduces adverse effects, improves treatment efficacy, and increases patient compliance. Second, there is an increasing interest in the oral delivery of sensitive molecules such as nucleotides, peptides, and live biotherapeutics, which require protection from gastric acidity and the moisture and temperature of coating processes. Finally, there is a critical need to accelerate drug development time and reduce the complexity and risk in drug development programs, clinical trials, and scale-up.

This white paper focuses on the development of a customized functional coated capsule, EUDRACAP® Select, for the delivery of live biotherapeutics and demonstrates its effectiveness in the oral delivery of a sensitive proprietary microbiome ecosystem while simplifying the drug development process.

Gut microbiota – A new pillar in medicine

The human gut microbiota is a diverse and complex ecosystem that is unique to each individual. It consists of numerous types of bacteria that outnumber the genes in the human genome by 10 to 50 times. Growing scientific evidence strongly suggests that the microbiome plays a crucial role in various diseases, impacting everything from immune function and inflammation to metabolism and mental health. Understanding these implications could lead to groundbreaking advancements in disease prevention, diagnosis, and treatment. Maintaining a stable and resilient microbiota is therefore essential for overall health and immune system function. Certain diseases or medical treatments can disrupt the microbiota, leading to an imbalance known as dysbiosis. Restoring the complete gut microbiota ecosystem is then a promising therapeutic tool to improve clinical outcomes in patients.

Microbiome therapy for cancer patients

The richness and diversity of the gut microbiota has been shown to be associated with cancer outcomes. Cancer and its treatments can disrupt the gut microbiota, impair gut epithelial repair mechanisms, and compromise immune homeostasis and responsiveness. Microbiome therapy can prevent the decay of the gut ecosystem, preserve immune homeostasis, and optimize gut function. Therapy involves three key steps: prevention of dysbiosis, restoration and optimization, and maintenance. To achieve the desired clinical outcome, a robust and targeted delivery system is crucial.

There are four main methods of delivering live microorganisms: naso-duodenal delivery, transcolonoscopic delivery, enema-based delivery, and oral formulations. This white paper focuses on oral formulations due to their convenience and increased patient compliance. The study discussed here is a collaboration between Evonik and the clinical-stage biotechnology company MaaT Pharma, with Evonik providing functional ready-to-fill EUDRACAP® Select capsules and MaaT Pharma providing stable, pooled, full ecosystem microbiota.

Challenges of robust oral delivery of live microorganisms

Microorganisms are normally sensitive to acidic conditions and therefore require an acid-resistant delivery system. At the same time, they should not be exposed to the high moisture and temperatures of standard enteric coating processes, which could lead to a reduction in the number of viable microorganisms. The use of a customized, empty, ready-to-fill, modified-release coated capsule is then a viable alternative for this type of therapy.

Dissolution of modified-release products can be significantly influenced by physical stress events of biorelevant magnitude, such as those found in the human gastrointestinal (GI) tract. The *in vivo* disintegration and release of functional oral dosage forms are influenced by physiological variables, not only inter- but also intrasubject when exposed to different conditions. Three of the major challenges to robust oral delivery of live microorganisms are presented below.

Variability of pH and residence time: Each individual experiences a different GI transit time depending on their state, i.e. fasted or fed. Even under the same conditions, the literature[1] has reported significant differences in the variability of pH and residence time in different compartments of the GI tract. A robust formulation must be able to protect its contents from the conditions of the stomach even when exposed to higher pH and longer residence times.

Low gastrointestinal fluid volumes and buffering capacity: The low fluid volumes of the GI tract, coupled with the low buffering capacity of the ileum and jejunum, also play an important role, as the developed enteric functional capsule must withstand the conditions of the stomach, but be able to release its contents even under the mild buffering conditions of the more distal area of the GI tract.

High pressure and jet-like propulsions: The most critical stress zones in the GI tract are the pyloric and the ileocecal regions, where sphincters of high motor activity are present. During passage through these regions, dosage forms can be exposed to pressure waves of up to 350 mbar and jet-like propulsions of up to 50-70 cm/s, which adds additional challenges for product development.

Objective and solution – developing a suitable oral delivery system

The objective of this study was to develop a suitable oral delivery system for the full ecosystem fecal microbiota and test its safety and tolerability in acute myeloid leukemia patients exposed to intensive rounds of chemotherapy and antibiotics in a Phase 1b clinical trial sponsored by MaaT Pharma (identifier NCT04150393). EUDRACAP® Select functional capsules were used to address the above challenges.

These capsules enable targeted drug delivery with precise pH targeting, protecting sensitive actives from heat, moisture, and gastric acids. Evonik's EUDRACAP® capsules are easy to fill on standard filling machines and provide functionality through the use of EUDRAGIT® polymers, which have a long track record in the industry. Evonik also offers comprehensive formulation development and cGMP services.

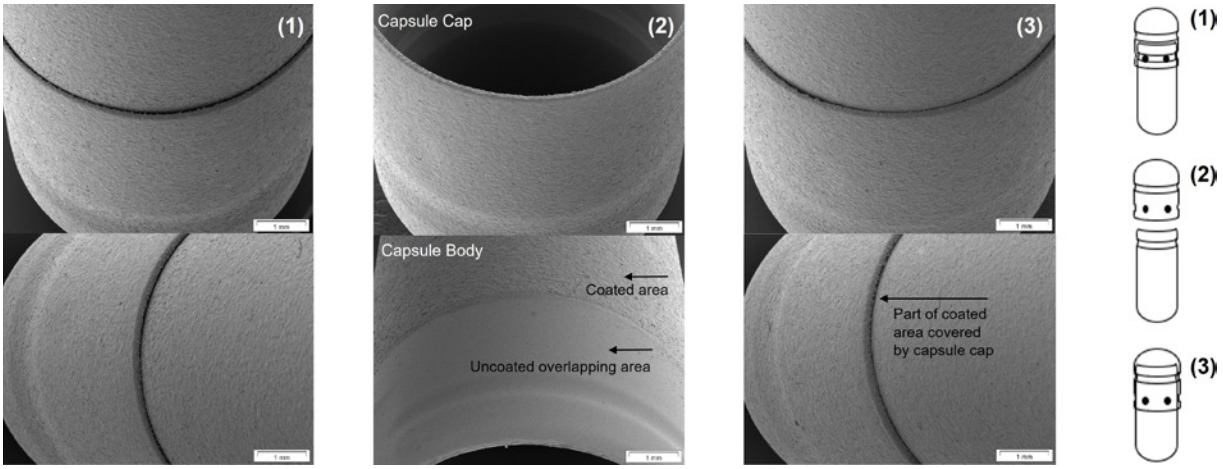
Development of customized EUDRACAP® Select capsules

Pre-locked, hard HPMC capsules were used for this study. Empty capsules were coated with a proprietary combination of EUDRAGIT® polymers to a specific weight gain based on their surface area. These ready-to-fill, enteric capsules were compatible with standard filling equipment, and required no banding or additional downstream processing.

Coating performance was evaluated via a three-stage dissolution test and acid resistance test. Caffeine:lactose blends were filled into enteric coated size 0 capsules. Dissolution tests were performed in USP Type II apparatus at 37 °C, with a basket speed of 75 rpm; filled capsules were exposed to 0.1 N HCl media for 2 hours followed by pH 6.8 potassium phosphate monobasic buffer for 1 hour and pH 7.2 buffer for 2 hours. The developed capsules were also subjected to a biorelevant dissolution test that is described later.

A blend of hydroxy naphthol blue dye/MCC was used as indicator for the acid resistance test. Upon exposure to acid, hydroxy naphthol blue immediately changes color to pink, providing a visual indication of enteric performance; the capsules were observed for visual changes during the disintegration test in 0.1 N HCl for up to 4 h.

For the phase 1b clinical study, the capsules developed were filled with a specific amount of the MaaT's proprietary standardized, high-richness, high-diverse microbiome ecosystem, containing a group of bacterial species, the Butycore®, known to produce anti-inflammatory short-chain-fatty acids. The study was performed according to the protocol described later (identifier of the study: NCT04150393).



Microscope: JEOL JSM IT300, Acc: 10 kV, El.Mag: 20 x, Detector: SED
Pictures are representative and taken from the EUDRACAP® enteric functional coated-capsules.

Figure 1: *SEM pictures of coated capsules in the pre-locked (1), opened (2) and locked (3) stages*

For illustrative purposes, Figure 1 shows capsules coated in the pre-locked (1), opened (2) and locked (3) stages, these capsules were produced by a process similar to that described here. Part of the capsule body is not coated as shown in Figure 1 (2). This part of the body is fully covered when the capsule is in the final locked stage, as observed in Figure 1 (3). In the final locked stage, the cap also covers part of the coated area of the body, this helps to form a hermetic seal between the cap and the body of the capsule.

Acid resistance test

This hermetic seal is designed to prevent any liquid from entering the capsule. To prove this functionality, an acid resistance test with dye was carried out on capsules manufactured with the same technology. Transparent capsules were used for better visibility, filled with a dye blend and then completely locked.

No banding or sealing was used. A red color appears when the dye comes into contact with a small amount of acid. Figure 2 shows that uncoated HPMC capsules change color very quickly when a few drops of 0.1 M HCl are applied. This indicates that moisture and acid are passing right through the capsule shell.

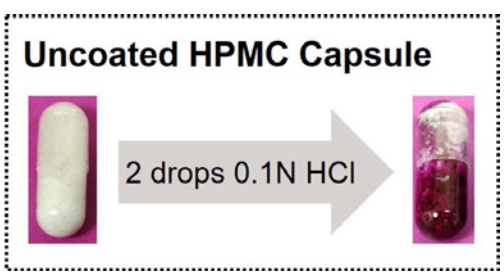


Figure 2: *Uncoated HPMC capsule before and after 2 drops of 0.1N HCl*

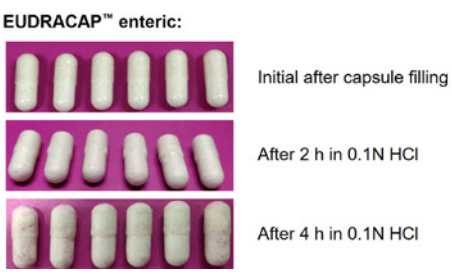


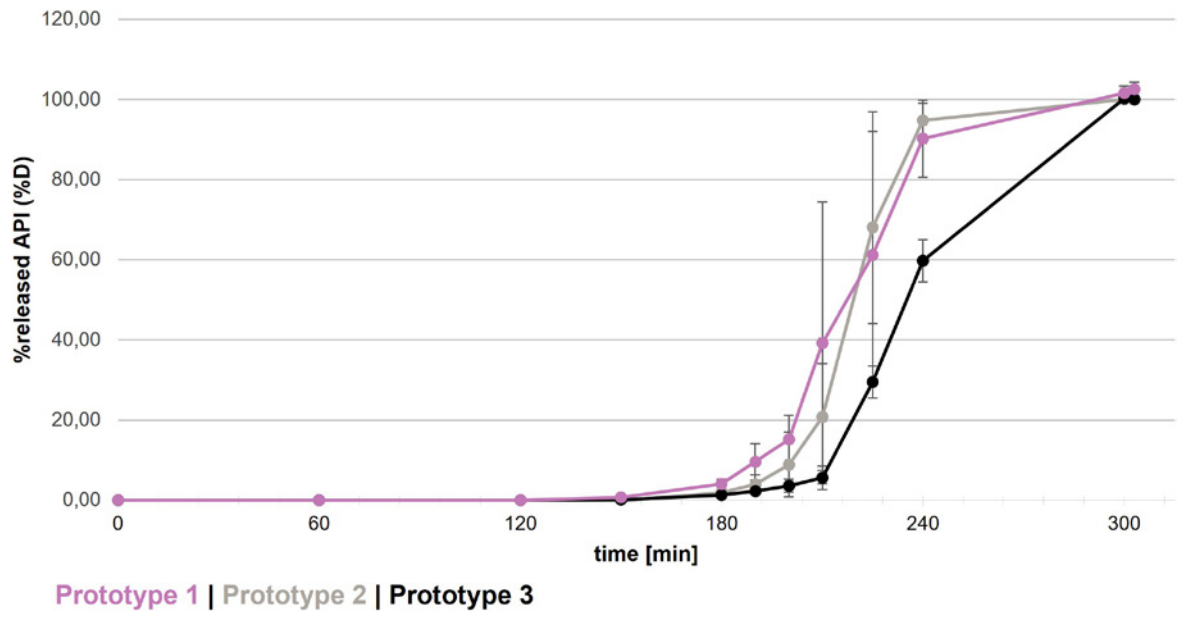
Figure 3: *EUDRACAP enteric capsules in acid resistance test*

Figure 3 shows functionalized coated capsules filled with the same dye after two and four hours in 0.1 M HCl. After four hours only a slight coloration was observed. When the capsules were opened, the capsule fillings were intact. There is a slight bulge between the cap and the body due to the hermetic seal that has been formed, which does not allow any moisture or acid to pass through. A perfect seal is needed to ensure that the capsule fillings are protected.

Prototypes matching the targeted profile

During the development phase, different formulations and weight gains were tested to target the lower end of the gut. The targeted release is expected to occur at the ileocecal junction. For this purpose, three prototypes were tested to find the optimal amount of coating and combination of polymers. Figure 4 shows that Prototype 1 is not robust enough and has a considerable level of standard deviation. On the other hand, Prototype 3, despite being more robust, delays the release tested *in vitro*. This behavior could indicate that the amount of polymer applied to the capsule is higher than necessary and could possibly delay the release *in vivo*. Therefore, Prototypes 1 and 3 were not considered for further steps. Prototype 2 showed full protection in HCl 0.1 M up to 120 min and also for a further 60 minutes in pH 6.8, after which its contents were rapidly released in pH 7.2. Therefore, Prototype 2 was selected as the most promising formulation and was then submitted for a stability study.

Dissolution profile, [%] average of 6



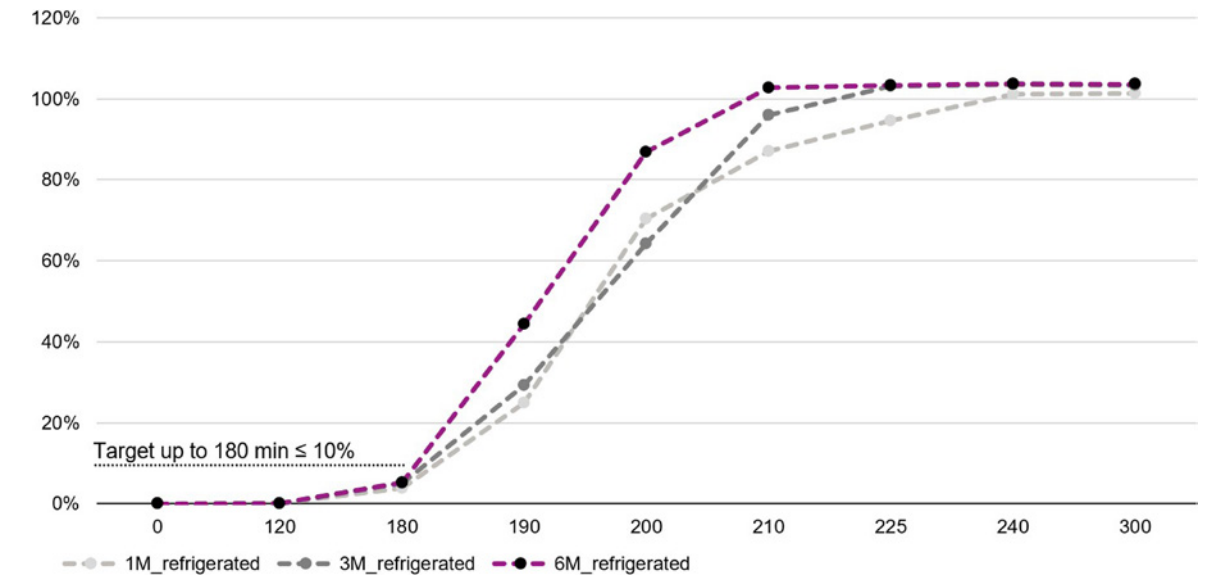
USP type 2, 75 rpm, Dissolution medium: 2h HCl 0.1 M;1h pH 6.8; 2h pH 7.2

Figure 4: *Prototypes matching the targeted profile*

Empty capsules were tested for stability

To test the stability of the capsules, a stability program was established with four different conditions: (a) long term; (b) intermediate; (c) accelerated; and (d) long term in refrigerated conditions. The latter was included in the stability study program to ensure that lower temperatures would not cause the capsule to become brittle at some point in time, which could be a risk factor for the final product formulation. The capsules included in the stability study were sampled from a representative scaled-up technical batch. The capsules were stable under all the different conditions tested, including accelerated and refrigerated. Considering the higher importance for this project, where the final pooled full ecosystem microbiota needs to be stored under refrigerated conditions, only the results of this condition are shown in Figure 5. The six-month stability study carried out so far has yielded positive results for all conditions tested, ensuring that a robust functionally coated capsule has been achieved. The long-term stability study is still ongoing.

Dissolution profile, [%] average of 6



USP type 2, 75 rpm, Dissolution medium: 2h HCl 0.1 M;1h pH 6.8; 2h pH 7.2

Figure 5: *Stability tests for empty capsules*

In vitro prediction of capsule behavior and robustness

The cost of clinical trials in drug development has been increasing in recent years and has a huge influence on the overall development costs. Therefore, it is advisable to stress the developed drug as much as possible in *in vitro* tests to reduce the risk of failure in later clinical studies. As discussed earlier, dissolution of modified release products can be significantly influenced by physical stress in the human GI tract, and this is not easy to predict by *in vitro* studies.

In order to simulate the physicochemical conditions of the GI tract, the EUDRACAP® Select capsules were subjected to a dissolution stress test performed by a specific device capable of simulating the levels of physiological mechanical stress that occur during the passage of a solid dosage form through the GI tract. In this test, the dosage form is subjected to sequences of agitation, including movement and pressure fluctuations, alternating with static phases, as observed *in vivo*. The device also allows simulation of intermittent contact of the dosage form with the dissolution medium. In addition, the intestinal pH profiles characteristic of fasting intake conditions were simulated with a biorelevant medium.

Figure 6 shows a schematic of the test setup. Movements and pressures are applied according to a defined protocol as detailed in Figure 7. Figure 7 also shows that there is no release of capsule contents for up to 2 hours. During simulated gastric emptying (1) the capsules yielded no deformation and signs of leakage. Low intensity mechanical stress simulated at 1 h (2) and 2 h (3) did not affect drug release. The mechanical agitation simulated at 3 h (4) triggered fast dissolution of part of the tested capsules. Ileocecal passage at 4 h (5) triggered fast drug release (deformation and perforation) from the capsules.

The results indicate that Prototype 2 capsules are capable of delivering live biotherapeutics to the distal small intestine and proximal colon, making them suitable for first-in-human trials.

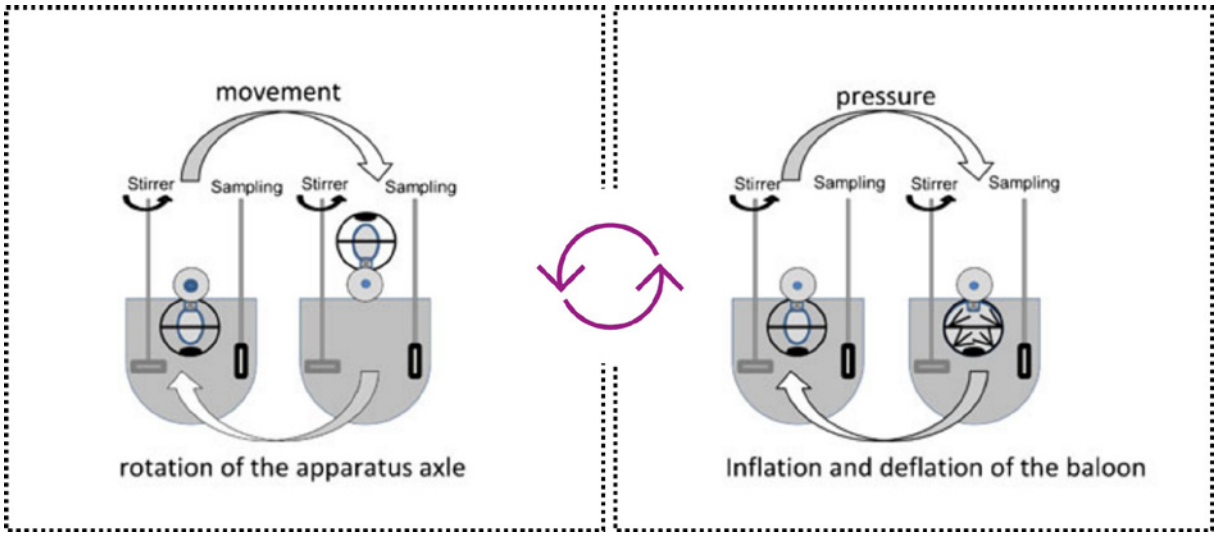


Figure 6: *Stress test apparatus to simulate high pressures and velocity of the GIT*

Dissolution profile obtained by the stress test, [%] average of 6

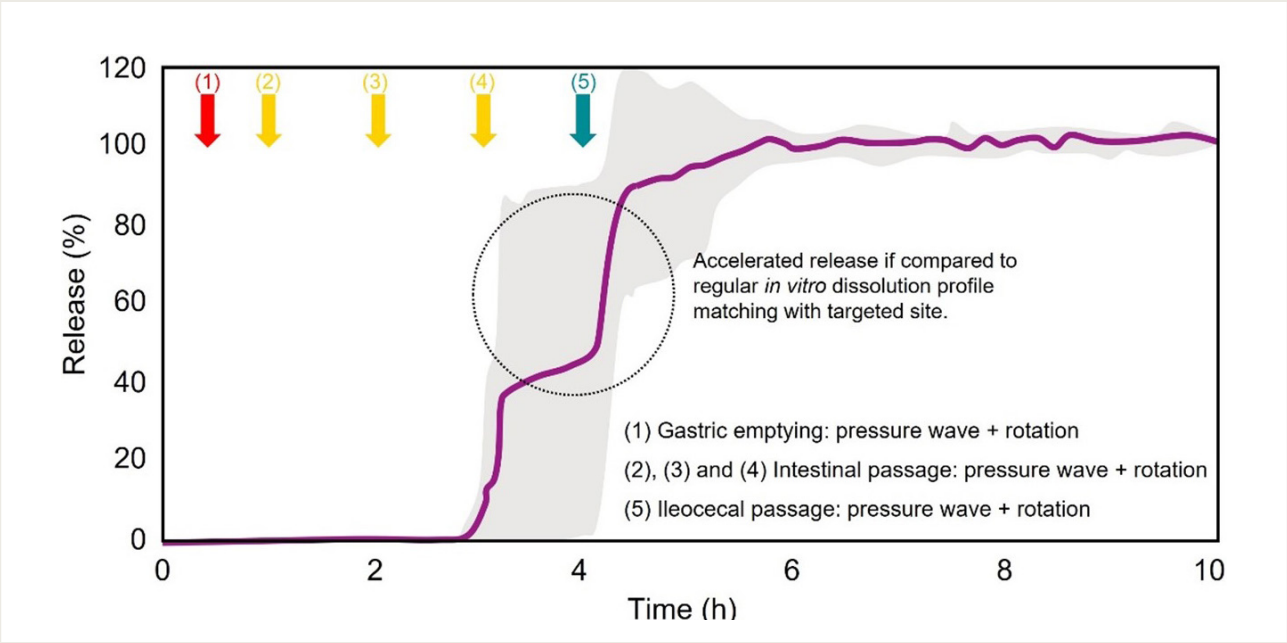


Figure 7: *Dissolution profile obtained by the stress test*

Encapsulated full-ecosystem microbiota was tested in a phase 1b clinical trial

Acute myeloid leukemia (AML) treatment combines intensive chemotherapy (IC) with broad-spectrum antibiotics (ATB) that induces a strong gut microbiota dysbiosis, promoting pathological conditions and increasing incidence of complications. Growing evidence[2] suggests that loss of diversity in the gut microbiota due to conditioning regimen, chemotherapy, antibiotics and reduced dietary intake promotes the development of Graft-versus-Host disease (GvHD) and impact negatively overall survival of patients receiving allogenic hematopoietic stem cell transplantation.

The developed EUDRACAP® Select capsule containing lyophilized pooled full ecosystem fecal microbiota drug candidate (MaaT033) was tested for tolerability, safety and efficacy in a Phase 1b clinical trial. The study was an open-label, single-arm study with 21 patients divided into 5 different cohorts. This study, sponsored by MaaT Pharma, took place at six investigational sites in France. The dose was scaled and administered according to Figure 8. The objectives of the study were to test tolerability, dose regimen (safety and activity, engraftment), patient compliance and to select the dose for Phase 2 study.

Cohort 5 was not performed because sufficient data were obtained from cohorts 1 to 4. Engraftment and richness in operational taxonomic units were examined on day 0 (baseline) of the treatment with MaaT033 and then on days 7 (±2) (V2), 19 (±5) (V3), and 44 (±10) (V4).

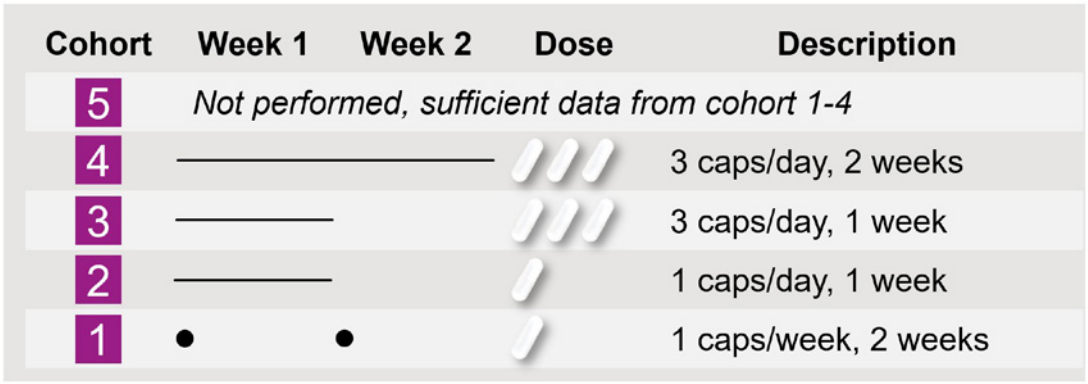


Figure 8: *Cohort, Dose and Description*

Strong engraftment and increased microbiota richness observed

The results of the Phase 1b clinical trial were first reported at the 64th edition of the American Society of Hematology[3]. Figure 9 shows that strong and sustained engraftment was observed in all 4 cohorts, even stronger for cohort 3 and cohort 4, where 3 capsules per day were administered. The engraftment level refers to the ratio of operational taxonomic units (OTU) that were not present in the patient at baseline, but were present in MaaT033 and were found in the patient after treatment, i.e. the treatment induced related engraftment. For this analysis, shared OTUs between MaaT033 and patients at baseline were excluded (values starting from zero). Persistent engraftment can be observed by relatively stable OTU levels at V4, after consolidation of chemotherapy and about 4 weeks after the treatment with MaaT033 was finished. It was also observed that MaaT033 bacterial engraftment is inversely correlated with patients' baseline microbiota richness (data not shown).

MaaT033 engraftment (mean, SD)*

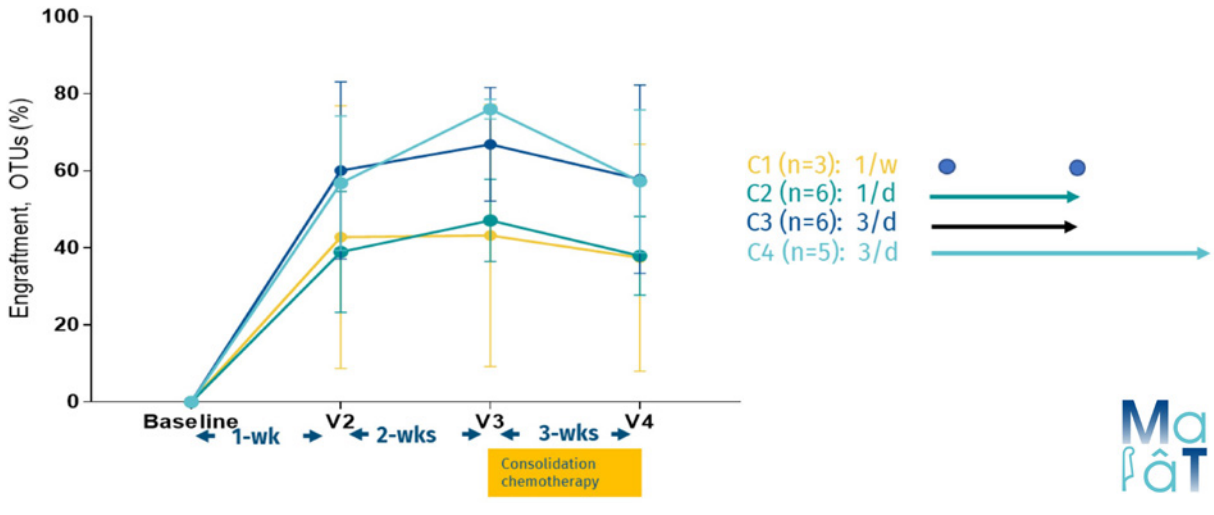


Figure 9: *Engraftment at different time points of the study – data MaaT Pharma- Phase 1b trial*

The richness of the microbiota was also evaluated in terms of variety of engrafted OTUs. Similar to the engraftment results, an increase in the number of OTUs was induced by MaaT033, which was also persistent, especially for cohort 2, cohort 3 and cohort 4, as shown in Figure 10.

Richness, OTU level (mean, SD)*

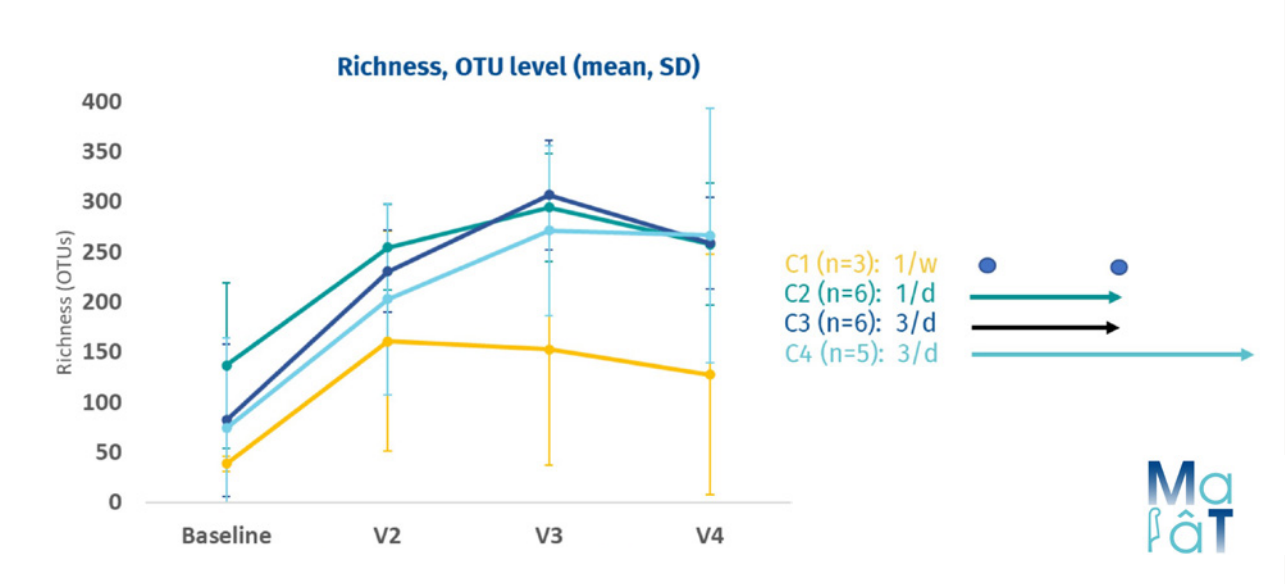


Figure 10: *Richness, OTU level – data MaaT Pharma- Phase 1b trial*

During the study, 4 serious adverse events were reported in 4 patients, but only 1 was considered as possibly related by the investigator. This event was an infectious diarrhea with enteropathogenic *E. coli* that started 3 days after MaaT033 treatment initiation. Genome sequencing was performed, and it was concluded that the E. coli that caused the reported event was not found in the MaaT033 or in the patient before the treatment started, so although the association between the event and MaaT033 is highly unlikely, it cannot be formally excluded. Other events were not reported as serious or potentially related to MaaT033.

The developed functional coated empty capsule EUDRACAP® Select was shown to be an excellent delivery system for safe and effective gut microbiota restoration in AML patients undergoing IC and ATB therapies.

Results and conclusion

In vitro testing has demonstrated the efficacy of the developed **EUDRACAP® Select** used in the **MaaT033** formulation, even under stressful conditions. Promising results were observed during the Phase 1b trial of the final formulation with MaaT Pharma's proprietary microbiome technology.

MaaT033 formulated with **EUDRACAP® Select** was shown to induce an increased microbiota richness at the OTUs level. It shows a strong and sustained bacterial engraftment higher when administered 3 times per day (1 or 2 weeks). Only one possible related serious adverse event was reported (infectious diarrhea 3 days after treatment initiation).

MaaT033 formulated with **EUDRACAP® Select** appears to be safe and effective in restoring gut microbiota restoration in AML patients receiving IC and ATB. 3 MaaT033 capsules per day for 1 week induce an increase in microbiota richness and an effective and persistent engraftment in AML patients. A Phase IIb trial is underway in 2023 to evaluate MaaT033 as an adjunctive in patients with hematological malignancies receiving allogeneic hematopoietic stem cell transplantation.

Evonik has provided a capsule that is effective and robust enough to deliver even sensitive molecules. The capsule is ideal for powders, pellets, granules and other dosage forms and is compatible with high-speed capsule filling machines. Using EUDRACAP® capsules saves developers time in process scale-up and validation, and has the benefit of using EUDRAGIT® polymers, which have been around for more than 65 years.

To find out more about how EUDRACAP® Select can be customized to meet your pre-clinical, clinical and commercial needs, please get in touch: healthcare@evonik.com

About MaaT

MaaT Pharma is a clinical-stage biotechnology company listed on Euronext Paris, is a leader in developing Microbiome Ecosystem Therapies (METTM) to enhance survival outcomes for cancer patients with two drug candidates (MaaT013 and MaaT033) currently being evaluating in hemato-oncology.

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