

# Measuring potentiator activity using organoids

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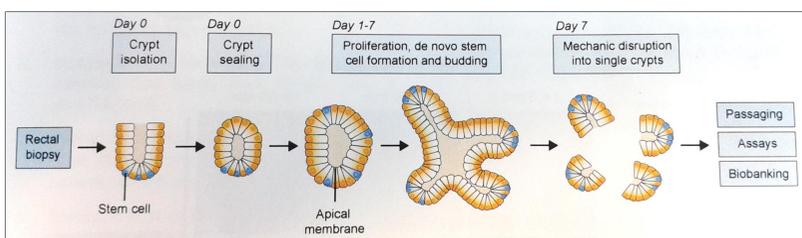
## Introduction

We already described the characterization of GLPG1837, derived from a previously disclosed potentiator series<sup>1</sup>. Here, the further characterization of the potentiator using patient derived organoids is presented.

The organoid technology as developed and described by Dekkers *et al.* (2013)<sup>2,3</sup> was used to characterize GLPG1837. For this, rectal biopsies were taken from several CF patients harbouring the G551D/F508del or S1251N/F508del CFTR mutations, since these are the patient populations that were selected for our Saphira 1 and 2 studies. The patient derived organoids were grown as described and used for forskolin induced swelling (FIS) assays. Sensitivity to forskolin was determined and for each mutation an optimal forskolin concentration was selected to determine potentiator potency.

## Organoid culturing

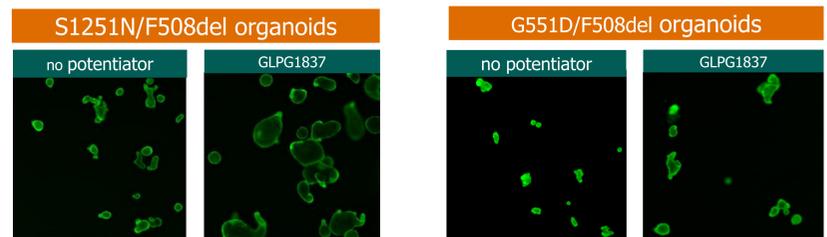
Crypts isolated from rectal biopsies of CF patients can self-organize into organoids. Organoids were cultured *in vitro* and the newly generated material was used for measuring forskolin-induced swelling (FIS) or for storage in liquid nitrogen.



Schematic overview of organoid generation from rectal biopsies and culturing (Dekkers, 2013).

## Organoid swelling

Stimulation of S1251N/F508del organoids with forskolin resulted in appreciable swelling, while this was not the case for G551D/F508del organoids. Incubation with the GLPG1837 potentiator resulted in increased organoid swelling showing a higher CFTR activity.

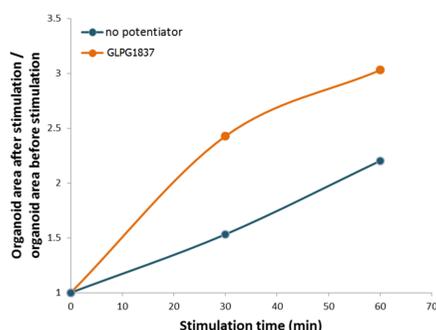
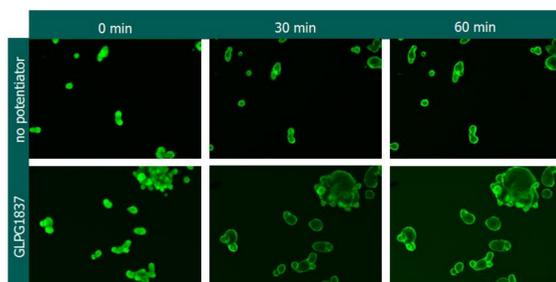


S1251N/F508del and G551D/F508del organoids were seeded, labeled with CalceinAM and treated with forskolin +/- GLPG1837. Organoid area was measured before and 1 h after forskolin + compound treatment.

## Swelling kinetics

The time-dependent organoid swelling was evaluated by measuring organoid area just before and at different timepoints after stimulation with forskolin w/wo potentiator.

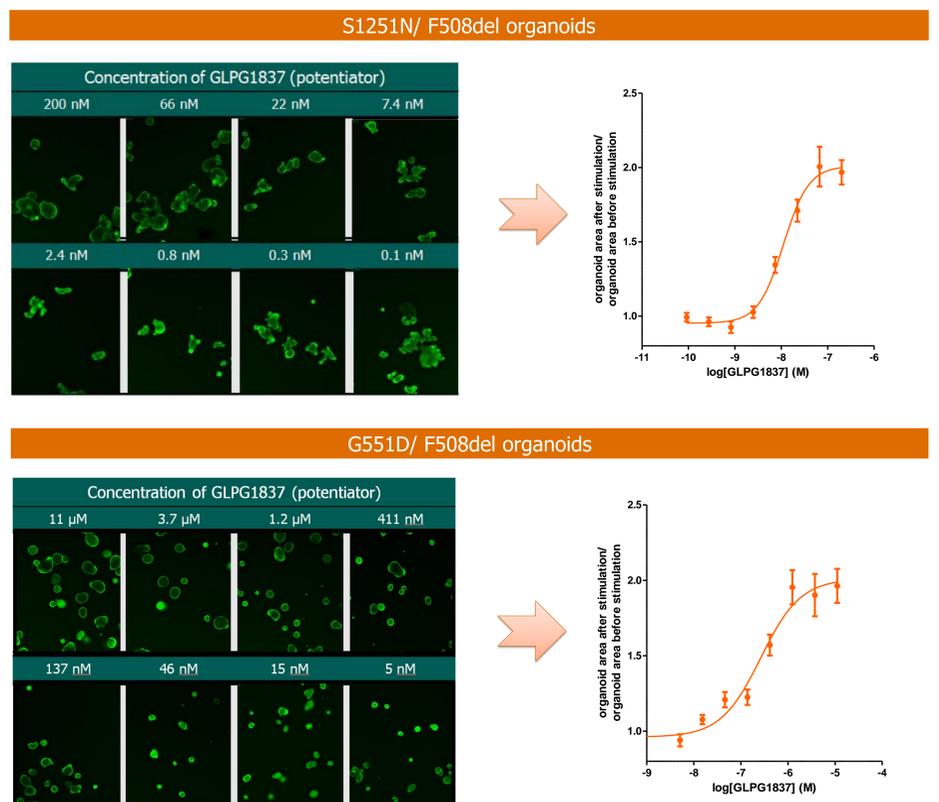
Potency of the potentiator was calculated from swelling observed after 1 hour treatment



S1251N/F508del organoids were seeded, labeled with CalceinAM and treated with forskolin +/- GLPG1837.

## GLPG1837 potency determination

The ability of GLPG1837 to restore CFTR function in S1251N/F508del and G551D/F508del organoids was evaluated by testing a concentration range in the FIS assay.



S1251N/F508del and G551D/F508del organoids were seeded, labeled with CalceinAM and treated with 1 µM forskolin + a concentration range of GLPG1837. Organoid area was measured before and 1 h after forskolin + compound treatment. Data were analyzed by calculating the ratio of the organoid area after/before forskolin + compound stimulation; this using custom made image analysis software.

## Summary

We present the development of assays using patient derived organoids and the data obtained in these assays for GLPG1837. GLPG1837 has a potency of 7.8 nM on S1251N/F508del organoids and of 262 nM on G551D/F508del organoids.

## References

- Conrath K, *et al.* (2013) Novel potentiators for treating Cystic Fibrosis NACFC
- Dekkers F, *et al.* (2013) Novel opportunities for CFTR-targeting drug development using organoids. Rare Diseases 1: e27112
- Dekkers F, *et al.* (2013) A functional assay using primary cystic fibrosis intestinal organoids. Nature Medicine 19(7): 939-945

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