**Strategy for inclusion of modelling part in the TGFβ paper**

1. **Research question**

Everolimus induces SMAD2 phosphorylation.

In addition, we see an increase in Akt and ERK activity upon everolimus treatment.

Is the effect of everolimus on SMAD2 phosphorylation Akt, ERK or Akt+ERK dependent?

1. **Starting models**

We have 2 sets of 3 models

* Set 1: ERK can inhibit SMAD2
  + Model 1.A. Akt activates SMAD2
  + Model 1.B. ERK activates SMAD2
  + Model 1.C. Akt and ERK activate SMAD2
* Set 2: ERK cannot inhibit SMAD2
  + Model 2.A. Akt activates SMAD2
  + Model 2.B. ERK activates SMAD2
  + Model 2.C. Akt and ERK activate SMAD2

1. **First parameterization round**

We have 4 time course experiments:

* MK2206 and everolimus time course
* MK2206 time course
* AZD6244 and everolimus time course
* AZD6244 time course

**Expected results :**

For both Set 1 and Set 2, the model in which Akt activates SMAD2 (models 1.A and 2.A respectively), fit best the experimental data.

1. **Model prediction and experimental validation**

Models 1.A and 2.A are used to simulate a time course with MK2206, AZD6244 and everolimus.

Note: Experimental data still needs to be generated

**Expected results:**

Predictions of model 1.A at the level of SMAD2 phosphorylation are more similar to the experimental data than predictions of model 2.A

1. **Second parameterization round of Set 1 models (model 1.A, 1.B and 1.C)**

We have 5 time course experiments:

* MK2206 and everolimus time course
* MK2206 time course
* AZD6244 and everolimus time course
* AZD6244 time course
* Validation experiment: MK2206, AZD6244 and everolimus time course

**Expected result:**

After including the validation experiment for parameterization, model 1.A fits the experimental data better than models 1.B and 1.C