



Of Mice and (Wo)Men

CASSS Meeting

Simone Tomaschek
Roche Diagnostics GmbH
Penzberg, Germany

April 2025 | public use

Table of contents

Problem

Solution

Challenges on the way

Health Authority Approvals

“ Trouble with mice is
you always kill 'em. ”

- John Steinbeck, Of Mice and Men

Problem

Problem...

Bioassay testing at an external company

- In vivo
 - Parenteral application in mice
 - Blood sampling and testing
- Very expensive
- 5.000 mice per year



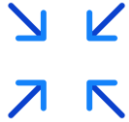
Solution

Solution...

- In vitro
 - Cell based reportergene assay
 - Binding of molecule to the receptor and activation of the signaling cascade



Perspectives



Internal testing

in house vs. external testing



Fast to patients

1 day vs. 3 weeks



Cost reduction

400.000€ per year



Animal welfare

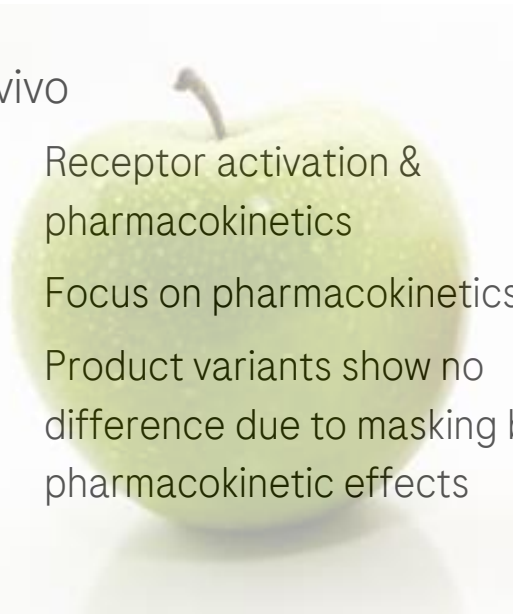
Zero vs. 5000 mice / year

Challenges on the way

Challenges on the way...

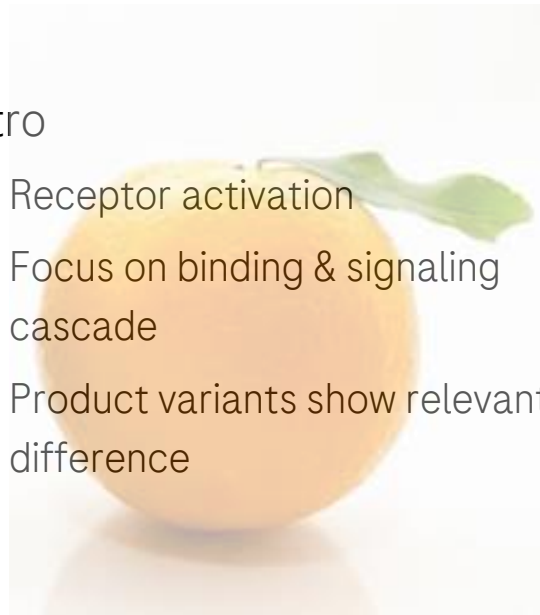
■ In vivo

- Receptor activation & pharmacokinetics
- Focus on pharmacokinetics
- Product variants show no difference due to masking by pharmacokinetic effects



■ In vitro

- Receptor activation
- Focus on binding & signaling cascade
- Product variants show relevant difference



- ➔ **Direct method bridging is NOT possible**
- ➔ **Taking all CQAs (Critical Quality Attributes) into account**

Challenges on the way...

Sensitivity of in vitro assay



Is the in vitro assay sensitive to new CQAs with impact on biological activity?



Method validation

Stress samples show sensitivity of assay to deamidation and oxidation

Characterization studies

Sensitivity of the assay toward non-glycosylated molecule

CQA methods

Control over non-glycosylated molecule and starting material size variants

Challenges on the way...

Release & stability acceptance criteria



How do we set the upper and lower release and stability acceptance criteria?



Testing of current and historic release samples

- Samples covering the whole manufacturing range
- Acceptance criterion = 2.5 SD of the method based on a method precision of 10% RSD
- Range typical of other cell-based potency assays
- Well-known impact of biochemical product variants on in vitro potency

Challenges on the way...

Release & stability acceptance criteria



During storage time



Related substances
Receptor affinity
In vitro potency



Pharmacokinetics



In vivo potency

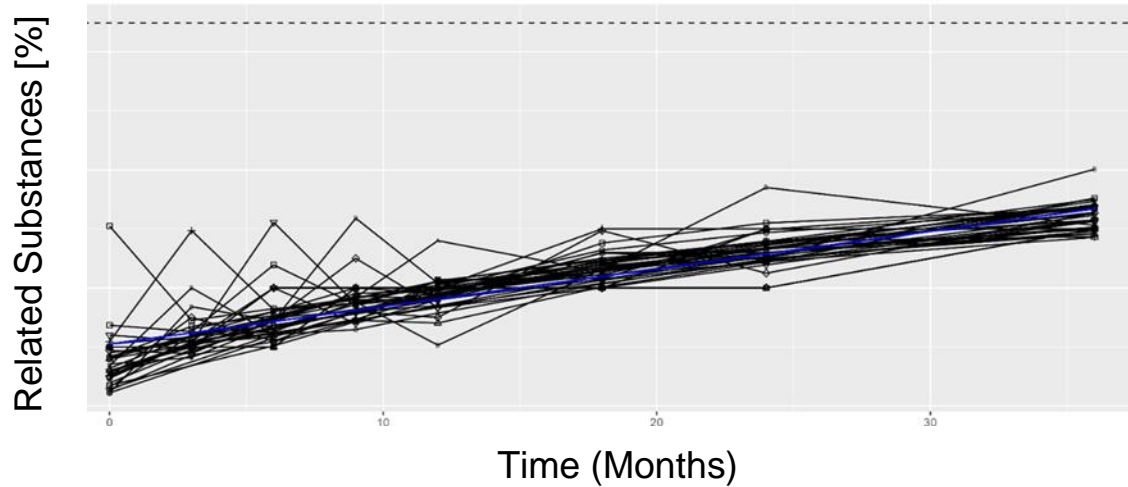


Challenges on the way...

Release & stability acceptance criteria



Related substances



Challenges on the way...

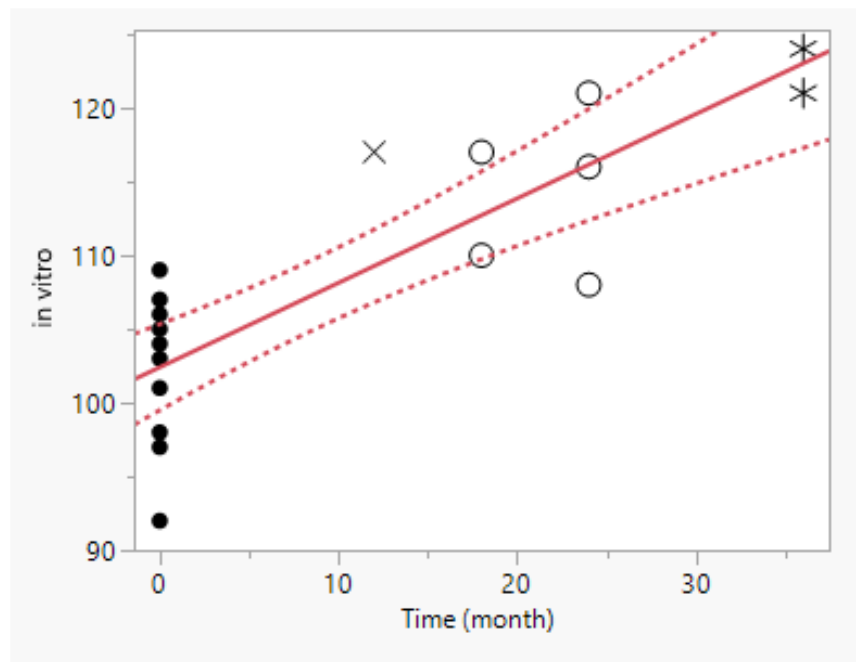
Release & stability acceptance criteria



In vitro potency

- Average increase of 0.573% relative potency per month
- Increase of ~ 21% relative potency over the shelf life of 36 months

The increase was added to the upper release acceptance criterion leading to the shelf life acceptance criterion



Challenges on the way...

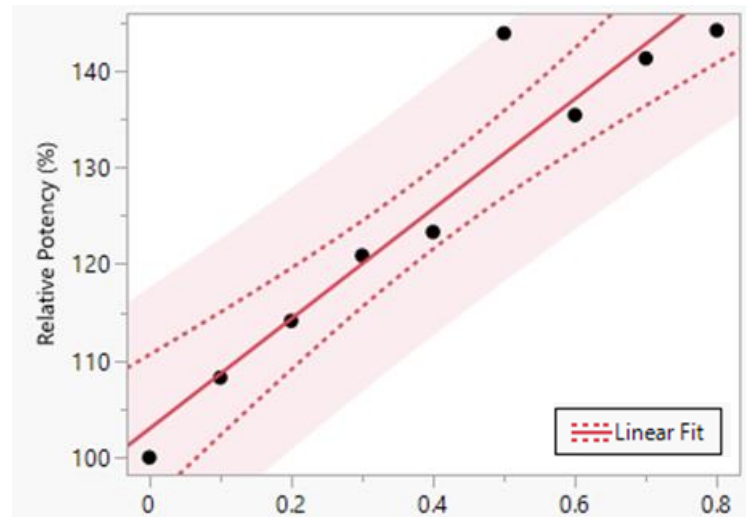
Release & stability acceptance criteria



In vitro potency

Spiking with non-modified molecule

- Note: The spiked non-modified molecule represents a worst case for related substances
- Linear correlation between spiked non-modified molecule and in vitro potency results
- Reflection of anticipated impact of these expected degradation products on in vitro potency

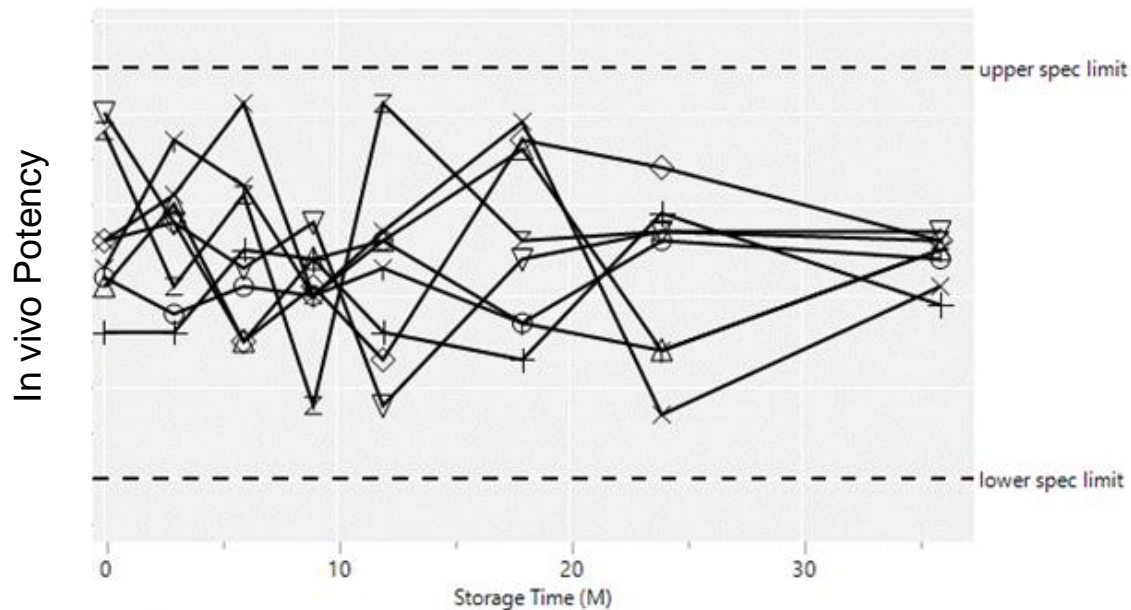


Challenges on the way...

Release & stability acceptance criteria



In vivo potency



Challenges on the way...

Release & stability acceptance criteria

	In vitro Potency by reporter gene assay	In vivo Potency by mouse assay
Sample A (more acidic)	76 % relative potency	comparable
Sample B (more basic)	127 % relative potency	comparable

Challenges on the way...

Risk of potential shift in sample potency due to future RS



Is there a risk regarding future Reference Standards without in vivo bioassay data?



Roche's reference standard management program

1. Qualification testing
2. Monitoring
3. cGMP processes

Health Authority Approval

Health authority approvals

Already approved by



FDA

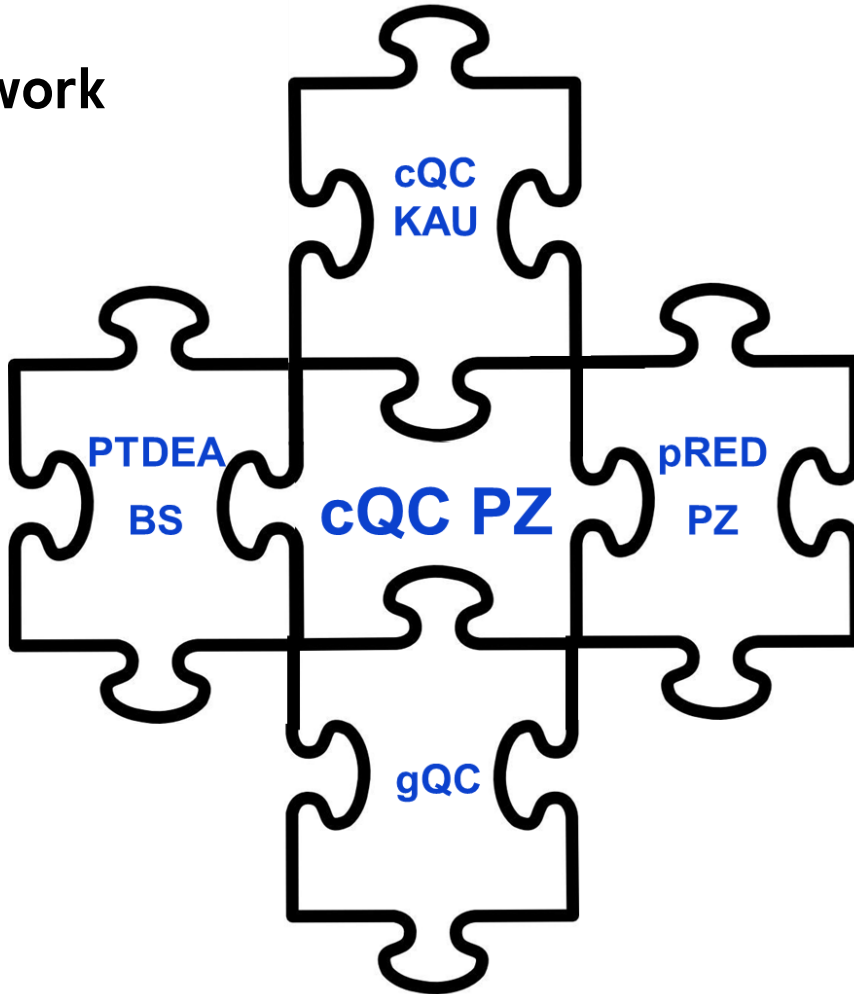


EMA



SwissMedic

Network



Simone Tomaschek
Klaus Leonhard
Alexandre Briguët
Stefan Elmlinger

Alexander Büttner
Jan Pollmann
Sebastian Krötz
Kathrin Ostermaier
Vera Kinscher
Bastian Hejda
Tanja Kössinger
Theresa Wakolbinger
Franziska Rolew
Joëlle Bisch
Madeleine Jehnich
Nadine Henneberge
Ulrike Faber
Nico Bartel
Adelheid Rohde



Doing now what patients need next