Overcoming bioanalytical challenges during the preclinical and clinical development of risdiplam (Evrysdi®) for the treatment of spinal muscular atrophy in children and adults

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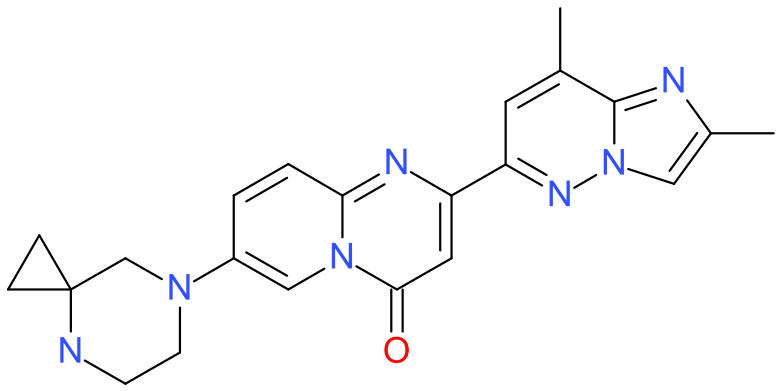
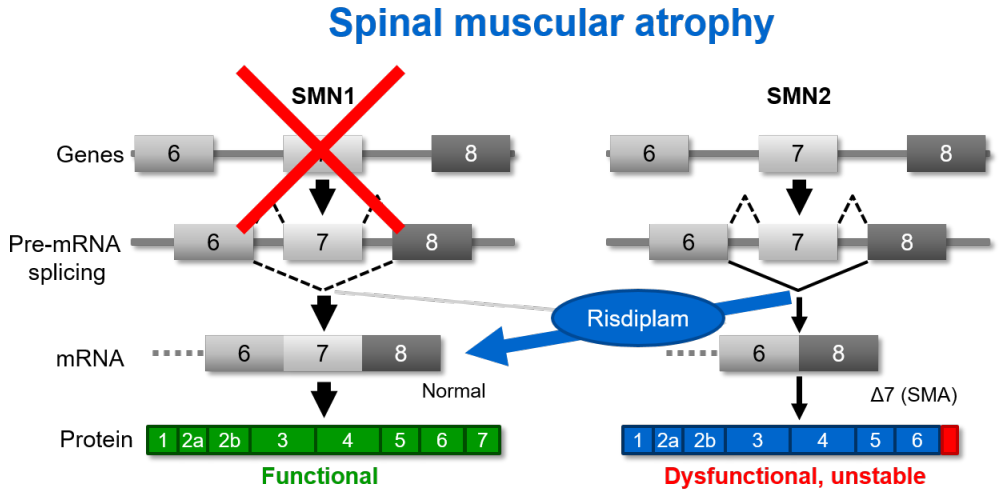


Risdiplam treats Spinal Muscular Atrophy (SMA)

- **SMA**: rare disease but leading genetic cause of death in babies and toddlers when remain untreated
- SMN1 gene deleted which causes low levels of Survival of Motor Neuron (SMN) protein

➢ Profound weakness and muscle atrophy with proximal and chest muscles most severely affected, respiratory failure is major cause of death (normal cognition & intellect)

Type	Age of onset	Symptoms	Lifespan
I	< 6 months	- Never sit - Respiratory failure	< 2 years
II	< 18 months	- Never walk - Wheelchair-bound	> 2 years
III	> 18 months	- Can stand unsupported - Muscle weakness	Normal



Risdiplam is a small molecule, orally administered Survival of Motor Neuron 2 (SMN2) pre-mRNA splicing modifier.

➢ Instead of unstable dysfunctional protein, the expression of functional SMN protein is increased in the SMN2 gene

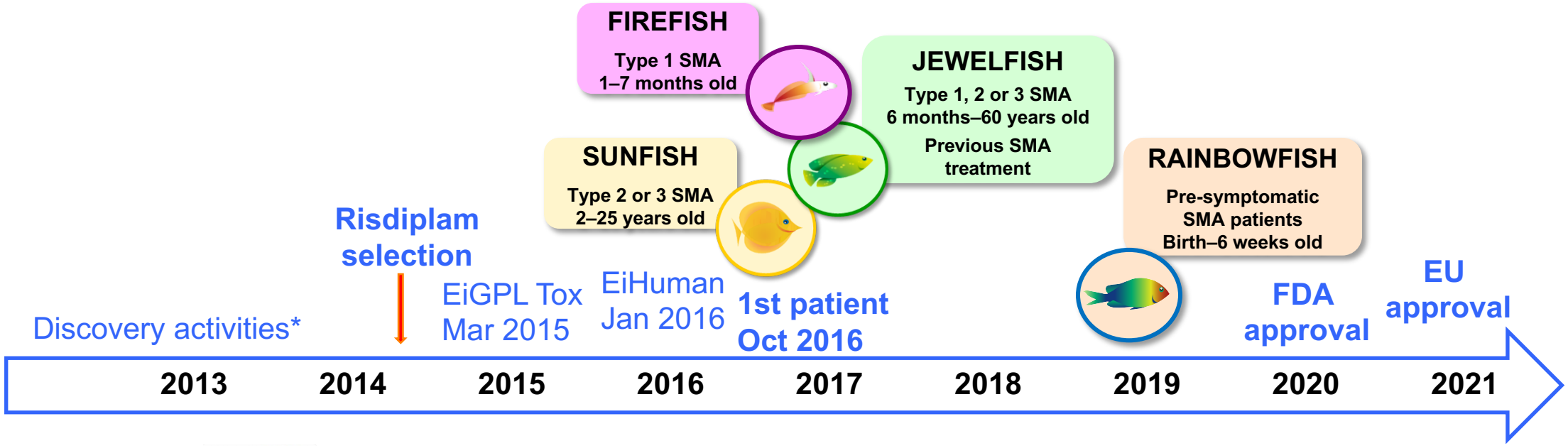
SMN protein levels in SMA patient's blood confirm desired 2-fold increase in SMN protein

Significant improvement in patients' motor function

K. Heinig, BA challenges during risdiplam drug development, 14th EBF Open Symposium, Barcelona, Nov 2021

Risdiplam development to the market

Highly unmet medical need, large competitive landscape → **accelerated development**



*Collaboration: PTC THERAPEUTICS, SMA FOUNDATION

Overcoming bioanalytical challenges during LC-MS/MS method development and validation
Contribution of bioanalysis to patient-centric approaches for safe and efficacious dose determination

Overcoming BA challenges related to molecule properties

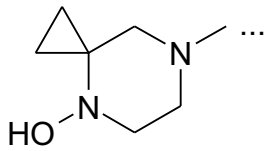
Photodegradation

- **Unstable** in clear vials in solutions and plasma (under natural and artificial light)
- **Stable** in whole blood even at daylight
- **Stable** in plasma in colored vial, in the dark & short time (~2h) at normal lab light

➢Implement light protection

- Use **amber vials**
- Work up samples under **sodium vapor light**

Degradation of major human metabolite M1



Unstable in solutions and biological matrices due to chemical and enzymatic hydrolysis with ring opening

Ascorbic acid **(anti-oxidant)**	**!** Tubes not commercially available, ascorbic acid solution not stable, fresh preparation & exact pipetting at clinics not feasible

- **Stable w/o ascorbic acid** in human blood & plasma few hours **at 4°C** and in plasma **1 year at -80°C**

= Addition of stabilizer not needed in clinics

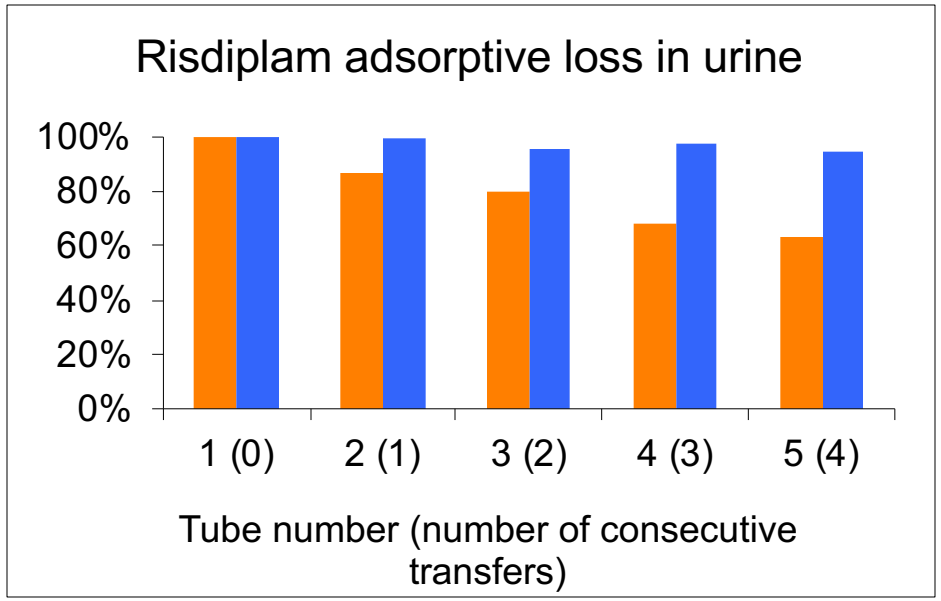
➢ Maintain cold chain storage

➢ Add **ascorbic acid (10 mM)** to study samples at BA lab before extraction
[Calibrators/QCs spiked in plasma containing ascorbic acid]

Overcoming BA challenges related to molecule properties

Nonspecific binding

- Significant **compound loss** after multiple transfers of urine
- In general, use LoBind plasticware
- Addition of 0.3% **Tween 80** to urine PK tube

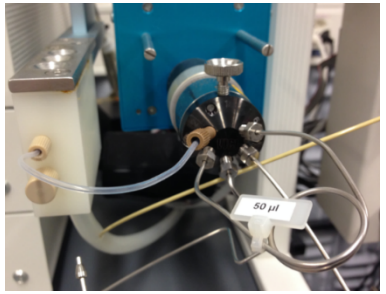


Carryover

Minimize:

- Remove column carryover

  Optimized gradient, prolonged wash at increased flow rate, add isopropanol/acetone in phase B
- Basic pH aqueous and strong organic autosampler wash, valve toggling
- Regular injector maintenance



Sporadic carryover ~30% of LLOQ

Mitigate:

- Do not randomize samples
- Insert blanks after high concentrations
- Monitor carryover in each run and re-analyze any impacted sample

- **Strategy accepted by FDA and EMA**

Tissue analysis for drug disposition and safety

- Elucidate **risdiplam distribution** into key tissues affected by SMA, including muscle, blood and brain of rodents and monkeys
  [Poirier A et al., Pharmacol Res Perspect. 2018]

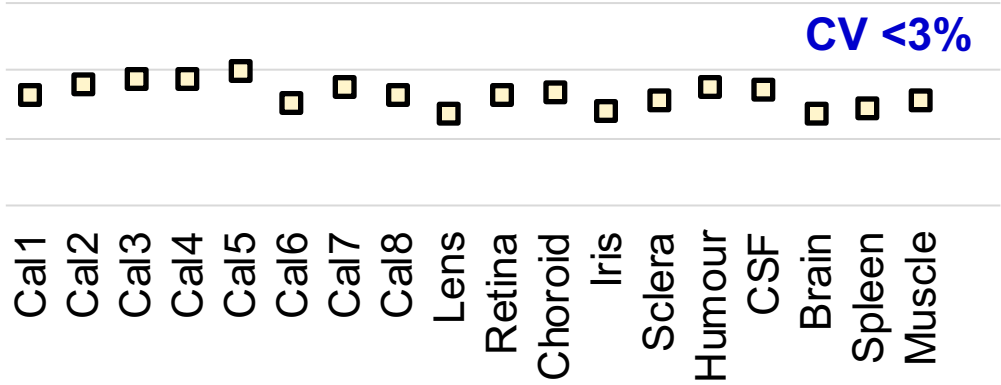
- Determination of **ocular tissue levels** triggered by retinal findings in monkeys after high doses
  (Ophthalmologic monitoring of patients in all studies did not reveal any safety issue [Sergott RC et al., Ann Clin Transl Neurol. 2021])

Recovery from tissue unknown, stability cannot be proven → **Fit-for-purpose assays**

Rare matrices – restrictions in blank material availability: ethical issues (3Rs) and costs

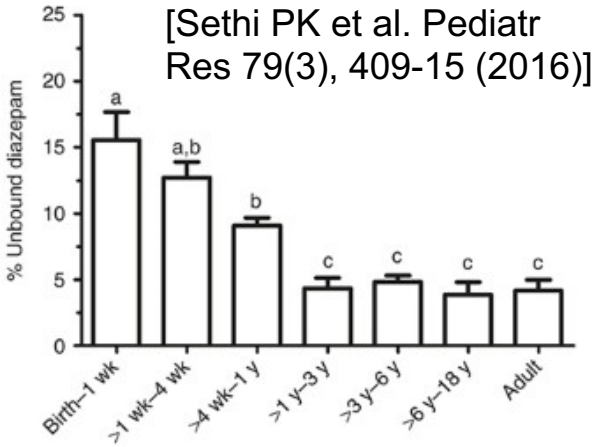
- ➢ Calibration standards in plasma, quality control samples prepared in tissue homogenate
- ➢ Surrogate matrix approach
- ➢ Small amount of matrix (particularly eye):
- Dilute ≥10-fold with plasma and measure with calibration standards and QCs prepared in plasma
- The use of **stable isotope labeled IS** increases ruggedness of the assay.
- **Monitor IS** anomalies/trends and repeat affected samples after further dilution.

IS response across matrices



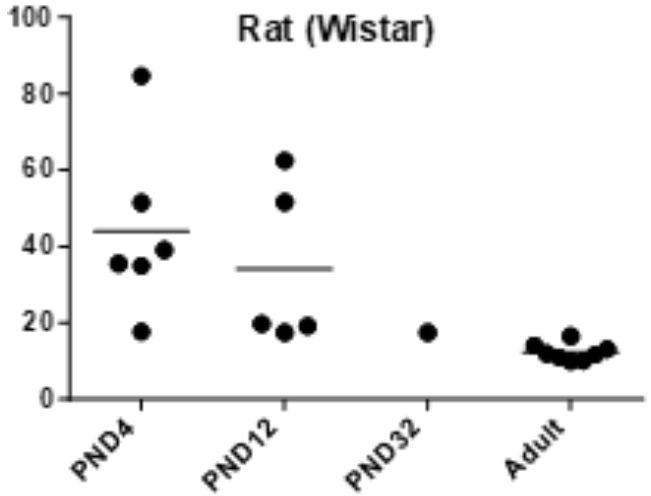
Addressing age-related differences in plasma protein binding (PPB)

- Only free drug (i.e. not bound to proteins) can interact with the target
- **PPB can be affected by physiological differences in children and adults**
- Reduced plasma protein content (e.g. albumin), higher displacer levels (e.g. fatty acids) in infants may lead to increased free drug



[Sethi PK et al. Pediatr Res 79(3), 409-15 (2016)]

Target population of risdiplam: very young to adult patients
→ free plasma fraction at different ages is crucial for efficacy and safety



- *In-vitro* testing of PPB in plasma from animals of different age ranges
- Free fraction **in rats: a strong age dependency** and high inter-individual variability; higher free fraction correlated with toxicity findings in Post-Natal Day 4 rodent pups (older animals tolerated the drug well)
- Minor effect of age on free drug observed in non-rodent
- Free fraction in human pediatric patients unknown

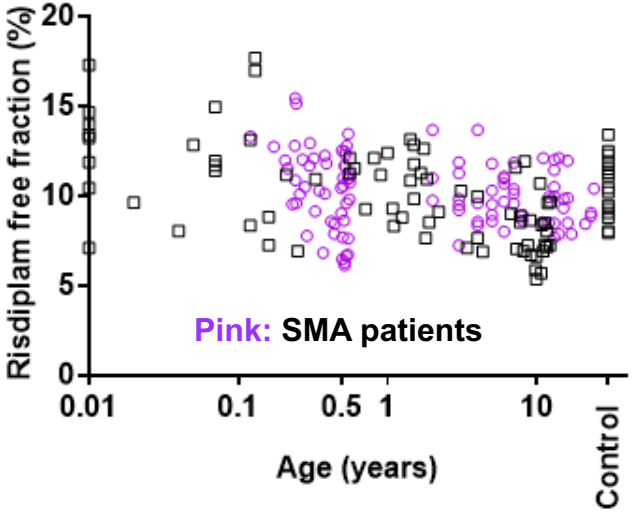
Determining PPB in pediatric patients

➢ **to ensure safety and appropriate dose and support health authority approval of studies**

a) Measure PPB in pre-dose samples collected at screening for all **pediatric patients in risdiplam studies**

b) Collaboration with Basel University Hospital to obtain PPB information from **non-SMA pediatric patients**

- **PPB**: Equilibrium dialysis of drug-spiked pediatric patient plasma. Low volume: 3-fold diluted with buffer. Adult plasma as a control reference and diazepam used to verify correctness of dialysis procedure.
- **Bioanalysis**: Matrix-matched samples (*buffer added to plasma samples from donor compartments and plasma added to buffer samples from acceptor compartments, plasma/buffer 1:5, v:v*). Partial validation. Stability tests in plasma/buffer: benchtop, short-term frozen, extract.



No age-dependency of risdiplam plasma protein binding.

➢**There should be no need to adjust by free fraction across age groups in human for efficacious and safe exposure levels.**

➢PPB monitoring continued in newborn babies (rapid turnaround shipment & analysis for starting dose confirmation)

RAINBOWFISH

Pre-symptomatic SMA patients Birth–6 weeks old

Patient Centricity – measuring PK for dose determination

Target exposure based on:

NOAEL for animal study toxicities (mean AUC 2000 ng.h/mL) to ensure safety margin

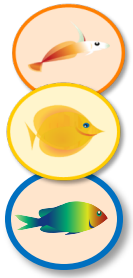
At least 2-fold increase in SMN protein predicted to lead to clinical efficacy

Wide age range of patients from newborns to 60 year old required sophisticated dosing regimen
Children are no small adults - different PKPD and less predictable dose-response relationship

➢ After confirming starting dose by PPB, frequent PK monitoring with rapid TAT for ultimate dose finding

Assay quality

Scientifically sound, **robust** and regulatory compliant assays. **«Do it right first time»!**



Run pass rate 99% risdiplam & 94% M1

Run pass rate 100% risdiplam & 97% M1

Run pass rate 97% risdiplam & M1

All ISR: >91% of samples within criteria

Operational aspects

- Difficult recruitment prediction requires high flexibility
- Frequent analysis of small batches blocks resources and generates additional costs
- Sample logistics (e.g. Shipment directly from site or via Central lab)
- Data flow and reconciliation

Substantial efforts in sample logistics, data provision and evaluation to support patient-centric treatment

Patient Centricity - Microsampling

Babies and children: limited volume of blood available for PK monitoring (*blood loss should not exceed 3% of total blood volume over four weeks or 1% of total blood volume at any single time*)

- Minimize volume and frequency of blood collection by **micro-volumes** and **sparse sampling**

Collect blood, harvest and analyze **plasma = same matrix across all species and studies**, no bridging for safety margins, dose extrapolation, population PK etc.

< 2 years: 0.25 mL MiniCollect, venipuncture or finger/heel prick

Site of blood sampling could make a difference to drug exposure: physiological aspects, drug properties, individual sampling issues (e.g., contamination with interstitial fluid)

- Drug levels from finger or heel prick sampling compared with venous collection **in a healthy volunteer trial.**

  Good agreement, slightly higher exposures of risdiplam in capillary plasma [S. Sturm et al., BJCP, 2018 85(1): p. 181-193]

- **Use either technique in patient studies depending on age, disease state and the preference of patients/caregivers**

Limited options for repeat analyses (analytical failures) or ISR

- Robust assays with high pass rate
- Conduct ISR only in a subset of studies and/or samples

Patient Centricity - Home Nursing

- Specialized clinics required patients to travel long distances and even across borders.
- ➢ PK sampling at home by local nurses was set up to alleviate travel burden for patients.
- ➢ Ramped up in light of COVID-19 pandemic with travel restrictions, quarantine rules and concerns about infections.

Home nursing provides quality care to patients conveniently and cost effective.

Sample integrity

- **Trainings and procedural documents** provided by sponsor and global care vendor
- Home visit lab kits provided by central lab (same materials as used for on-site visits)
- Home nurse carries centrifuge and wet ice for processing plasma at patient's home
- Plasma sample (venous or finger/heel prick) is placed on ice in the fridge
- Courier is ordered to bring dry ice and ship samples frozen to central lab
- Flag samples not picked up within 6 hours (stability: M1 without stabilizer only 6 hours on ice)
- **Documentation** of accurate drug administration date & time, blood collection date & time

Complex sample and data flow need close collaboration between sponsor, clinical site, central lab, ambulant care global provider, local nurses, bioanalytical lab

China strategy for risdiplam bioanalysis

Global studies with samples collected in China. To export or not to export, that is the question …

Challenges of analysis in China

- Second bioanalytical lab to be set up and managed
- 2 Assays (PPB & PK) transferred, validated and cross-validated
- Import of materials & cross-validation samples
- Risk of cross-validation failure
- Risk of data comparability issue
- Additional time and budget needed

Advantages

❖ **No sample export necessary.**

! Unpredictable patient recruitment, demands for rapid PPB and PK measurement

➢ **not feasible to export samples**
(lengthy process requiring several consecutive permits, regulated by Human Genetic Resources Administration of China)

➢**Local analysis in China.** Successful transfer, validations & sample analysis.

➢**Cross-validation:**- spiked QCs (patient samples not available in sufficient volumes due to low blood volumes collected), 21 individual samples, blank and 6 concentration levels
- all QCs within ±15% of nominal concentration for risdiplam and M1 at both labs
- bias of mean accuracies -2.7 to 8.5% for risdiplam, -1.8 to 4.7% for M1

Conclusions

“it takes a village to raise a child” … and a big city to develop a molecule

- The development of a pediatric drug poses many scientific, regulatory and operational challenges for bioanalytics, requiring innovative and flexible approaches.
- Risdiplam is an excellent example where an efficient cross-functional collaboration was established and all individual experts came together as a team “doing now what patients need next”.
- Partnership with regulators, pediatricians and patients as well as external vendors are of upmost importance.

Many thanks to hundreds of Roche colleagues, external partners, patients and their families, physicians and clinical staff world-wide!