**Phase 1 safety, tolerability, pharmacokinetics and pharmacodynamic results of KCL-286, a novel RARb agonist for treatment of spinal cord nerve injury, in male healthy participants**

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**Supplementary data**

**Methods**

**Analysis of RARβ2 expression in human WBCs**

Ten ml blood sample from each patient according to the standard operating procedure in tubes containing anticoagulant EDTA andRNAlater. Samples were centrifuged at ~1,500-2,000 ×g for 10-15 min at room temperature with no brake applied. Aliquots of plasma were then stored at -80°C until used. RNA was extracted (Qiagen 74104) and reverse transcription (Qiagen 205311) was carried out according to manufactures instructions. Primers used were human GAPDH and RARβ2. Samples were set up using a SYBR Green I Master mix (Roche 04707516001) and were carried out on a Roche lightcycler. Each reaction was run in triplicate and relative expression values obtained using a GAPDH standard curve. Primers used were, human gapdh forward gttcgtcatgggtgtgaacc and rev gcatggactgtggtcatgagt, product size 142 bp; human RARb2, forward tctacactgcgagtccgtct, and reverse tcaattgattgagcagtgtgc, product size, 105 bp. Products were identified by melting curve analysis

**An example using Bayesian statistics to predict dose for MAD cohort 5**

The fourth MAD cohort has been dosed and is based on 75 participants. The number of participants to be randomised to active dose in the next cohort is 6. Data is observed on day 1 for doses of 1, 2, 4, 6, 8, 12, 24, 48, 72 and 100mg, and on day 7 for doses of 6, 12, 24 and 72mg. Analysis is carried out using R version 4.0.3 (2020-10-10) and OpenBugs V3.2.3. Specifically, if P(At least one subject in the next cohort of patients has 𝐶𝑚𝑎𝑥 on either day> 3700|𝐷𝑎𝑡𝑎) < 𝛿1 and P(At least one subject in the next cohort of patients has 𝐴𝑈𝐶(0 − 24) on either day> 25100|𝐷𝑎𝑡𝑎) < 𝛿1, doses will be escalated to the next higher dose level. 𝛿1 = 0.05. The next higher dose-level is defined to be no more than 3 times the previous dose +5%. From this analysis (supplementary table 1), the highest dose that is suggested for the MAD cohort 5 that satisfies constraints for days 1 and 7 for both$ AUC\_{0-24}$ and $C\_{max}$ is 105 mg.

**Supplementary Table 1**: The predictive mean (95% CI) for $AUC\_{0-24}$ and Cmax values for a new participant, for doses given to previous cohorts and also for potential next doses. It also shows the probability that any participant in the next cohort of 6 exceeds the maximum allowable exposure.

| **Dose** | **AUC0-24** | **Cmax** |
| --- | --- | --- |
|  | **Day 1** | **Day 7** | **Probability exceed threshold** | **Day 1** | **Day 7** | **Probability exceed threshold** |
| 1 | 382(201,718) | 254(129,498) | 0.000 | 41(20,81) | 31(15,66) | 0.000 |
| 2 | 635(338,1189) | 388(203,743) | 0.000 | 70(36,139) | 51(25,104) | 0.000 |
| 4 | 1056(567,1959) | 593(314,1118) | 0.000 | 121(62,235) | 82(41,163) | 0.000 |
| 6 | 1424(762,2644) | 760(405,1428) | 0.000 | 166(85,324) | 108(55,216) | 0.000 |
| 8 | 1759(943,3255) | 908(483,1699) | 0.000 | 208(106,403) | 133(67,262) | 0.000 |
| 12 | 2367(1269,4385) | 1164(627,2154) | 0.000 | 285(146,553) | 175(90,341) | 0.000 |
| 16 | 2924(1572,5426) | 1389(745,2576) | 0.000 | 357(183,694) | 214(109,417) | 0.000 |
| 24 | 3941(2118,7318) | 1779(951,3314) | 0.000 | 490(251,955) | 283(144,553) | 0.000 |
| 48 | 6548(3495,12198) | 2722(1454,5085) | 0.000 | 841(430,1643) | 457(231,901) | 0.000 |
| 72 | 8824(4727,16515) | 3491(1846,6621) | 0.004 | 1155(591,2271) | 605(302,1216) | 0.003 |
| 100 | 11225(5961,21036) | 4266(2242,8127) | 0.037 | 1492(761,2940) | 759(376,1531) | 0.029 |
| 105 | 11644(6192,21873) | 4392(2292,8400) | 0.048 | 1551(784,3066) | 784(385,1592) | 0.037 |
| 110 | 12054(6393,22697) | 4522(2371,8673) | 0.063 | 1610(814,3184) | 810(400,1643) | 0.049 |
| 115 | 12440(6621,23389) | 4649(2433,8893) | 0.082 | 1664(843,3288) | 836(413,1708) | 0.064 |
| 120 | 12842(6816,24101) | 4770(2485,9154) | 0.101 | 1722(873,3402) | 861(423,1758) | 0.078 |
| 140 | 14393(7631,26903) | 5243(2733,10138) | 0.212 | 1944(982,3847) | 957(468,1970) | 0.172 |

**Supplementary Table 2**: Summary of Pharmacokinetic Parameters at Day 1 (PK Set) – SAD

|  |  |  |
| --- | --- | --- |
| Parameter  | Unit  | Treatment dose |
| Plasma |  | S1, 1 mgN=3 | S5, 2 mgN=4 | S3, 4 mgN=6 |
| AUC 0‑inf, D1 | h.ng/mL | 389.2 (114.99) | 492.6 (181.02) | 1125.6 (259.07) |
| AUC0‑t D1 | h.ng/mL | 374.3 (115.24) | 465.3 (219.61) | 1117.6 (257.26) |
| Cl/F | L/h | 2.713 (0.7379) | 4.728 (2.4709) | 3.694 (0.7414) |
| Cmax D1 | ng/ml | 35 (5.84) | 42.8 (13.03) | 110.9 (17.99) |
| T1/2 D1 | h | 7.506 (2.2935) | 5.469 (0.8528) | 5.802 (0.7651) |
| Lambda z D1 | 1/h | 0.0975 (0.02556) | 0.1293 (0.02173) | 0.1214 (0.0177) |
| Tlag D1 | h | 0.58 (0.382) | 0.31 (0.239) | 0.34 (0.263) |
| Tmax D1 | h | 3.333 (1.1547) | 4.013 (0.025) | 4.342 (1.9749) |
| Vz/F obs | L | 27.839 (1.823) | 35.196 (11.7668) | 30.652 (6.0795) |
| AUC0‑24h D1 | h.ng/mL | 334.1 (77.91) | 453.8 (158.31) | 1037.5 (225.66) |
| CL/F (norm) | L/h kg | 0.035 (0.0119 | 0.062 (0.043) | 0.046 (0.0086) |
| Vz/F (norm) | L/kg | 0.354 (0.0349) | 0.455 (0.2263) | 0.381 (0.0668) |
|  |  |  |  |  |

**Supplementary Table 3:** Evaluation of Dose-proportionality for PK Parameters of KCL-286

|  |  |  |
| --- | --- | --- |
| **PK parameter (units)** | **Cohort** | **Exponent of the power model** |
|  | **Estimate** | **90% CI** |
| AUC(0-inf) (ng\*h/mL) | SAD | 0.7506 | (0.6862, 0.8150) |
| Cmax (ng/mL) | SAD | 0.8069 | (0.7431, 0.8707) |
| AUC(0-inf) (ng\*h/mL) | MAD day1 | 0.7097 | (0.6061,0.8133) |
| Cmax (ng/mL) | MAD day 1 | 0.7708 | (0.6523,0.8893) |
| AUC(0-inf) (ng\*h/mL) | MAD day 7 | 0.6249 | (0.5485,0.7013) |
| Cmax (ng/mL) | MAD day 7 | 0.7298 | (0.6465,0.8132) |

A power model was fitted with log (PK parameter) as response variable and log(dose) as predictor. AUC0-inf, area under the plasma concentration-time curve from zero hours to infinity; CI, confidence interval; Cmax, maximum plasma concentration; PK, pharmacokinetic.

**Supplementary Table 4:** Evaluation of Dose-proportionality Food Effect for PK Parameters of KCL-286

|  |  |  |
| --- | --- | --- |
| PK parameter | **GM mean ratio****Fed/fasted** | **90% CI** |
| AUC(0-inf) (ng\*h/mL) | 0.874 | (0.769,0.992) |
| AUC(0-t)[ng\*h/mL] | 0.871 | (0.768,0.987) |
| Cmax (ng/mL) | 0.716 | (0.601,0.854) |

A mixed effect model was fitted with log (PK parameter) as response variable, the food status as fixed effect and the subject as random effect. AUC0-inf, area under the plasma concentration-time curve from zero hours to infinity; AUC(0-t), area under the plasma concentration-time curve from zero hours to last measurable concentration; CI, confidence interval; Cmax, maximum plasma concentration; PK, pharmacokinetic. The exponent for AUC(0-inf) is close to 1, which can be interpreted as a dose proportional relationship.

**Supplementary Table 5:** Treatment-Emergent Adverse Event (Safety Set) SAD

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Category  | S1 , 1 mg  N=3  n (%) E  | S2 - 2 mg  N = 4 n (%) E  | S3 - 4 mg  N = 6 n (%)E  |  S4 - 8 mg  N = 6n (%) E  | S5 - 12 mg  N = 6  n (%) E  | S6 - 24 mg  N = 6 n (%) E  | S7 - 48 mg  N = 5  n (%) E  | S8 - 100 mg  N = 6  n (%) E  |  Placebo  N =14  n (%) E  | Total  N = 56  n (%) E  |
| Any TEAE  |  2 (66.7) 4  |  2(50.0) 2  |  3(50.0) 9  |  4 (66.7) 6  | 5 (83.3) 8  |  3 (50.0)7  |  4 (80.0) 6  |  2(33.3) 2  | 6(42.9) 12  |  31(55.4) 56  |
| Any Serious TEAE  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Any TEAE Leading to Withdrawal  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| Any TEAE Leading to Death  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| TEAE by Relationship to IMP Related  |  0  |   1 (25.0) 1  |   1 (16.7) 2  |    1 (16.7) 1  |  1 (16.7) 1  |   1 (16.7) 1  |  3 (60.0) 3  |  0  |  3 (21.4) 3  | 11 (19.6) 12  |
| Not Related  |  2 (66.7) 4  |  1 (25.0) 1  |  3 (50.0) 7  |  4 (66.7) 5  | 4 (66.7) 7  |  3 (50.0) 6  | 2 (40.0) 3  | 2 (33.3) 2  | 5 (35.7) 9  | 26 (46.4) 44  |
| TEAE by Severity Mild  |   2(66.7) 4  |   2 (50.0) 2  |   3 (50.0) 9  |    4 (66.7) 6  |   5 (83.3) 8  |  3 (50.0) 7  |  4 (80.0) 5  |  2 (33.3) 2  |  6 (42.9) 12  | 31(55.4) 55  |
| Moderate  | 0  |  0  |  0  | 0  | 0  | 0  | 1 (20.0) 1  | 0  | 0  | 1 (1.8) 1  |
|  Severe 0 0 0 0 0 0 0 0 0 0  |

Abbreviation(s): AE - Adverse Event, IMP - Investigational medicinal product, TEAE - Treatment Emergent AE,

N - number of subjects at risk, n - number of subjects having an AE, E - number of events

Note(s): % - n/N\*100

**Supplementary Table 6:** Treatment-Emergent Adverse Event (Safety Set) MAD

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Category  | M1 - 6 mg N =6n(%)E  | M2 - 12 mg N=6 n(%)E  | M3 - 24 mg N =6 n(%)E  | M4 - 72 mg N =6 n(%)E  | M5 - 100 mg N =6 n(%)E  | Placebo N = 10 n (%) E  | Total N = 40 n (%) E  |
| Any TEAE  | 3 (50.0) 3  | 1 (16.7) 1  | 4 (66.7) 5  | 4 (66.7) 8  | 3 (50.0) 3  | 5 (50.0) 7  | 20 (50.0) 27  |
| Any Serious TEAE  |  0  |  0  |  0  |  0  |  0  |  0  |  0  |
| Any TEAE Leading to Withdrawal  |  0  |  0  |  0  |  0  |  0  |  0  |  0  |
| Any TEAE Leading to Death  |  0  |  0  |  0  |  0  |  0  |  0  |  0  |
| TEAE by Relationship to IMP Related  |   0  |   0  |    0  |  1 (16.7) 1  |  1 (16.7) 1  |  0  |   2 (5.0) 2  |
| Not Related  | 3 (50.0) 3  | 1 (16.7) 1  |  4 (66.7) 5  | 3 (50.0) 7  | 2 (33.3) 2  |  5 (50.0) 7  | 18 (45.0) 25  |
| TEAE by Severity Mild  |  3 (50.0) 3  |  1 (16.7) 1  |   4 (66.7) 5  |  4 (66.7) 8  |  3 (50.0) 3  |  5 (50.0) 7  |  20 (50.0) 27  |
| Moderate  |  0  |  0  |  0  |  0  |  0  |  0  | 0  |
| Severe  |  0  |  0  |  0   |  0  |  0  | 0  | 0  |

Abbreviation(s): AE - Adverse Event, IMP - Investigational medicinal product, TEAE - Treatment Emergent AE,

N - number of subjects at risk, n - number of subjects having an AE, E - number of events

Note(s): % - n/N\*100

|  |  |  |
| --- | --- | --- |
|  Period 1  |  Period 2  |  Overall  |
| Category  | 6 mg Fasted N = 4 n (%) E  |  6 mg Fed  N = 4  n (%) E  | 6 mg Fasted N = 3 n (%) E  | 6 mg Fed N = 3 n (%) E  | 6 mg Fasted N = 7 n (%) E  | 6mgFed N = 7n (%)E  |
| Any TEAE  | 4 (100.0) 6  | 1 (25.0) 1  | 2 (66.7) 2  | 1 (33.3) 1  | 6 (85.7) 8  | 2 (28.6) 2  |
| Any Serious TEAE  |  0  |  0  |  0  |  0  |  0  |  0  |
| Any TEAE Leading to Withdrawal  | 1 (25.0) 1  |  0  |  0  |  0  | 1 (14.3) 1  |  0  |
| Any TEAE Leading to Death  |  0  |  0   |  0  |  0  |  0  |  0  |
| TEAE by Relationship to IMP Related  |   0  |   0  |   0  |   0  |   0  |  0  |
| Not Related  | 4 (100.0) 6  | 1 (25.0)1   | 2 (66.7) 2  | 1 (33.3) 1  | 6 (85.7) 8  |  2 (28.6) 2  |
| TEAE by Severity Mild  |  4 (100.0) 6  |  1 (25.0) 1  |  2 (66.7) 2  |  1 (33.3) 1  |  6 (85.7) 8  | 2 (28.6) 2  |
| Moderate  |  0  |  0  |  0  |  0  |  0  |  0  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  Severe  |  0  |  0   |  0  |  0  |  0  |  0  |

**Supplementary Table 7:** Treatment-Emergent Adverse Event (Safety Set) FI

Abbreviation(s): AE - Adverse Event, IMP - Investigational medicinal product, TEAE - Treatment Emergent AE,

N - number of subjects at risk, n - number of subjects having an AE, E - number of events

Note(s): % - n/N\*100