Akelos continues to evaluate its proprietary series of HCN1 inverse agonists, including lead compound AKE-1018, for formal Development including IND Enablement. The latest data indicates that AKE-1018 and the chemical series possess favorable drug-like properties.

AKE-1018 was evaluated in a series of physiochemical, ADME and pharmacological assays. The data is summarized below:

*Solubility:*

AKE-1018 exhibits very good solubility in pharmaceutically-acceptable GRAS oils such as peanut oil (which was used in preclinical animal efficacy studies). AKE-1018 also displays very good solubility in aqueous vehicles containing appropriate solubilizing agents such as ethanol, polyethylene glycol (PEG) and a common cyclodextrin; all are pharmaceutically-acceptable diluents. As expected due to its chemical structure and high lipophilicity, AKE-1018 has low solubility at physiologic pH in the absence of added diluents.

| # | Approach | Medium | Solubility, Mean (n=3) ± SD | Solubility, Mean (n=3) ± SD |
|---|---|---|---|---|
| 1 | | Phosphate buffered saline (pH 7.4) | - | - |
| 2 | | Simulated gastric fluid | 17.61 ± 3.09 µg/mL | 34.12 ± 6.00 µMol |
| 3 | | Simulated intestinal fluid | - | - |
| 4 | | 80% EtOH in water | 51.93* ± 1.12 µg/mL | 100.62* ± 2.16 µMol |
| 5 | Kinetic | 20% hydroxypropyl-β-cyclodextrin in water | 48.25 ± 0.45 µg/mL | 93.49 ± 0.88 µMol |
| 6 | | 80% PEG 400 in water | 50.24* ± 0.64 µg/mL | 97.34* ± 1.24 µMol |
| 7 | | 20% PEG 400 / 20% Cremaphor EL / water | 51.35* ± 0.36 µg/mL | 99.50* ± 0.69 µMol |
| 8 | Thermodynamic | Sesame oil | 31.40* ± 0.81 mg/mL | 60.75* ± 1.57 mMol |
| 9 | | Peanut oil | 31.52* ± 1.72 mg/mL | 60.99* ± 3.32 mMol |

* - The upper solubility limit was reached. The actual solubility is **likely higher than reported**.

*Microsomal stability:*

AKE-1018 displays exceptional stability upon incubation with human liver microsomes. The calculated intrinsic clearance indicates a half-life of greater than 10 h, suggesting QD testing may be adequate in humans. This stability may be due, in part, to high protein binding.

| Compound I.D. | Client Compound I.D. | Test Concentration | Incubation (minutes) | % Compound Remaining (% remaining) | | | Half-Life (minutes) | | | Clint (µl/min/mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1st | 2nd | Mean | 1st | 2nd | Mean | |
| **Intrinsic clearance (liver microsomes- human)** | | | | | | | | | | |
| 100078078-1 | AKE-1018 | 1.0E-07 M | 0 | 100.00 | 100.00 | 100.00 | 732.6 | 627.9 | 680.2 | 10.2 |
| 100078078-1 | AKE-1018 | 1.0E-07 M | 15 | 98.53 | 111.94 | 105.24 | | | | |
| 100078078-1 | AKE-1018 | 1.0E-07 M | 30 | 102.74 | 100.67 | 101.71 | | | | |
| 100078078-1 | AKE-1018 | 1.0E-07 M | 45 | 103.49 | 103.47 | 103.48 | | | | |
| 100078078-1 | AKE-1018 | 1.0E-07 M | 60 | 90.89 | 95.75 | 93.32 | | | | |

Note: Unit of Clint is µL/min/mg for microsomes, S9 and UGT assays; µL/min/pmol for CYP assays; µL/min/Million cells for hepatocyte assays

In a second study aimed at evaluating microsomal stability across species, AKE-1018 similarly showed exceptional robustness ($T_{1/2}$ >> 1h).

*Compound stability in **mouse** liver microsomes*

| *Compound* | *Time, min* | *% remaining (n=2)* | *Negative control (% remaining)* |
|---|---|---|---|
| **AKE-1018** | 0 | 100.0 | 100.0 |
| | 15 | 98.1 | ND |
| | 30 | 96.4 | ND |
| | 45 | 96.7 | ND |
| | **60** | **95.2** | **99.8** |
| **Verapamil** | 0 | 100.0 | 100.0 |
| | 15 | 57.0 | ND |
| | 30 | 41.8 | ND |
| | 45 | 35.4 | ND |
| | **60** | **28.6** | **93.8** |

*Compound stability in **rat** liver microsomes*

| *Compound* | *Time, min* | *% remaining (n=2)* | *Negative control (% remaining)* |
|---|---|---|---|
| **AKE-1018** | 0 | 100.0 | 100.0 |
| | 15 | 96.4 | ND |
| | 30 | 98.0 | ND |
| | 45 | 99.1 | ND |
| | **60** | **104.6** | **95.6** |
| **Verapamil** | 0 | 100.0 | 100.0 |
| | 15 | 82.9 | ND |
| | 30 | 70.5 | ND |
| | 45 | 58.2 | ND |
| | **60** | **46.0** | **100.5** |

*Compound stability in **human** liver microsomes*

| *Compound* | *Time, min* | *% remaining (n=2)* | *Negative control (% remaining)* |
|---|---|---|---|
| **AKE-1018** | 0 | 100.0 | 100.0 |
| | 15 | 96.7 | ND |
| | 30 | 94.2 | ND |
| | 45 | 99.2 | ND |
| | **60** | **91.6** | **97.5** |
| **Verapamil** | 0 | 100.0 | 100.0 |
| | 15 | 88.8 | ND |
| | 30 | 79.6 | ND |
| | 45 | 68.4 | ND |
| | **60** | **59.0** | **97.6** |

*Protein Binding:*

AKE-1018 is very highly protein bound.

*PPB data obtained for tested compound and control samples in mouse plasma*

| *Test compound* | *Peak area (response) for plasma compartment* | *Peak area (response) for buffer compartment* | *Unbound, %* | *Bound, %* | *Protein binding (%)* | *PPB classification* |
|---|---|---|---|---|---|---|
| **AKE-1018** | 1.00 | ND | 0.00 | 100.00 | **100** | High |
| | 1.38 | ND | 0.00 | 100.00 | | |
| Propanolol (positive control) | 2.75 | 0.43 | 15.49 | 84.51 | 86 | High |
| | 2.80 | 0.38 | 13.46 | 86.54 | | |
| Fluconazol (negative control) | 1.15 | 0.89 | 77.54 | 22.46 | 20 | Low |
| | 1.19 | 0.97 | 81.58 | 18.42 | | |

*PPB data obtained for tested compound and control samples in rat plasma*

| *Test compound* | *Peak area (response) for plasma compartment* | *Peak area (response) for buffer compartment* | *Unbound, %* | *Bound, %* | *Protein binding (%)* | *PPB classification* |
|---|---|---|---|---|---|---|
| **AKE-1018** | 0.77 | ND | 0.00 | 100.00 | **100** | High |
| | 0.73 | ND | 0.00 | 100.00 | | |
| Propanolol (positive control) | 2.94 | 0.34 | 11.64 | 88.36 | 89 | High |
| | 2.90 | 0.30 | 10.47 | 89.53 | | |
| Fluconazol (negative control) | 1.00 | 0.81 | 80.79 | 19.21 | 22 | Low |
| | 1.07 | 0.81 | 75.80 | 24.20 | | |

*PPB data obtained for tested compound and control samples in human plasma*

| *Test compound* | *Peak area (response) for plasma compartment* | *Peak area (response) for buffer compartment* | *Unbound, %* | *Bound, %* | *Protein binding (%)* | *PPB classification* |
|---|---|---|---|---|---|---|
| **AKE-1018** | 0.68 | ND | 0.00 | 100.00 | **100** | High |
| | 0.67 | ND | 0.00 | 100.00 | | |
| Propanolol (positive control) | 2.65 | 0.45 | 16.96 | 83.04 | 84 | High |
| | 2.70 | 0.41 | 15.08 | 84.92 | | |
| Fluconazol (negative control) | 1.11 | 0.78 | 70.00 | 30.00 | 30 | Low |
| | 1.10 | 0.76 | 69.19 | 30.81 | | |

Despite high protein binding, AKE-1018 achieves good plasma exposures upon oral dosing and exhibits efficacy in a rodent model of neuropathic pain, indicating adequate free drug engages the pharmacological target.

*Permeability:*

AKE-1018 exhibits low but appreciate permeability in the standard Caco2 cell line assay. There is no efflux (A->B/B->A ratio) observed. This data is supportive of the appreciable plasma exposure levels observed in rats orally dosed with AKE-1018 and may reflect good oral bioavailability.

| Compound I.D. | Client Compound I.D. | Test Concentration | Permeability ( $10^{-6}$ cm/s) | | | Percent Recovery(%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1st | 2nd | Mean | 1st | 2nd | Mean |
| **A-B permeability (Caco-2, pH 6.5/7.4)** | | | | | | | | |
| 100078078-1 | AKE-1018 | 1.0E-05 M | 0.12 | 0.02 | 0.1 | 38 | 43 | 41 |
| **B-A permeability (Caco-2, pH 6.5/7.4)** | | | | | | | | |
| 100078078-1 | AKE-1018 | 1.0E-05 M | 0.04 | 0.01 | 0.0 | 35 | 37 | 36 |

*hERG Channel inhibition:*

AKE-1018 does not inhibit the cardiac ion channel hERG at all concentrations tested:

## CiPA hERG Assay Data Table

| Compound ID | Client Compound ID | Batch Number | Concentration (μM) | % inhibition | | |
|---|---|---|---|---|---|---|
| | | | | n1 | n2 | mean |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 0.1 | 4.19 | 7.45 | 5.82 |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 0.3 | 4.56 | 6.27 | 5.41 |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 1 | 3.08 | 0.27 | 1.67 |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 3 | 7.66 | 7.70 | 7.68 |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 10 | 7.19 | 3.30 | 5.24 |
| US034-0030073-1 | AKE-1018 | OSPT-3389 | 30 | 12.84 | 12.69 | 12.76 |
| Time-Matched Vehicle Control | 0.33% DMSO Addition 1 | | | 5.69 | 5.63 | 5.66 |

*CYP Inhibition:*

AKE-1018 did not appreciably inhibit any of the five major isoforms tested: CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4 (both the midazolam and testosterone binding sites), across the concentration range.

| CYP1A2 (Phenacetin) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (μM) |
| | (μM) | (Log) | | | | |
| α-Napthoflavone (PC) | 3.00 | 0.477 | 4.52E-03 | 13.9% | 86.1% | |
| | 0.00 | | 3.26E-02 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 3.17E-02 | 97.2% | 2.76% | > 100 |
| | 33.3 | 1.522 | 3.18E-02 | 97.5% | 2.45% | |
| | 11.1 | 1.045 | 3.20E-02 | 98.2% | 1.84% | |
| | 3.70 | 0.568 | 3.24E-02 | 99.4% | 0.61% | |
| | 1.23 | 0.090 | 3.23E-02 | 99.1% | 0.92% | |
| | 0.412 | -0.385 | 3.25E-02 | 99.7% | 0.31% | |
| | 0.137 | -0.863 | 3.24E-02 | 99.4% | 0.61% | |
| | 0.00 | | 3.26E-02 | 100% | 0.00% | |

| CYP2C9 (Tolbutamide) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (µM) |
| | (µM) | (Log) | | | | |
| Sulfaphenazole (PC) | 20.0 | 1.301 | 3.93E-04 | 9.63% | 90.4% | |
| | 0.00 | | 4.08E-03 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 1.65E-03 | 40.4% | 59.6% | 75.9 |
| | 33.3 | 1.522 | 3.10E-03 | 76.0% | 24.0% | |
| | 11.1 | 1.045 | 3.91E-03 | 95.8% | 4.17% | |
| | 3.70 | 0.568 | 4.05E-03 | 99.3% | 0.74% | |
| | 1.23 | 0.090 | 4.06E-03 | 99.5% | 0.49% | |
| | 0.412 | -0.385 | 4.07E-03 | 99.8% | 0.25% | |
| | 0.137 | -0.863 | 4.07E-03 | 99.8% | 0.25% | |
| | 0.00 | | 4.08E-03 | 100% | 0.00% | |

| CYP2C19 (S-Mephenytoin) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (µM) |
| | (µM) | (Log) | | | | |
| Nootkatone (PC) | 20.0 | 1.301 | 2.66E-04 | 18.5% | 81.5% | |
| | 0.00 | | 1.44E-03 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 1.16E-03 | 80.6% | 19.44% | > 100 |
| | 33.3 | 1.522 | 1.32E-03 | 91.7% | 8.33% | |
| | 11.1 | 1.045 | 1.38E-03 | 95.8% | 4.17% | |
| | 3.70 | 0.568 | 1.41E-03 | 97.9% | 2.08% | |
| | 1.23 | 0.090 | 1.43E-03 | 99.3% | 0.69% | |
| | 0.412 | -0.385 | 1.42E-03 | 98.6% | 1.39% | |
| | 0.137 | -0.863 | 1.43E-03 | 99.3% | 0.69% | |
| | 0.00 | | 1.44E-03 | 100% | 0.00% | |

| CYP2D6 (Dextromethorphan) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (µM) |
| | (µM) | (Log) | | | | |
| Quinidine (PC) | 3.00 | 0.477 | 1.29E-02 | 8.38% | 91.6% | |
| | 0.00 | | 1.54E-01 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 1.47E-01 | 95.5% | 4.55% | > 100 |
| | 33.3 | 1.522 | 1.48E-01 | 96.1% | 3.90% | |
| | 11.1 | 1.045 | 1.50E-01 | 97.4% | 2.60% | |
| | 3.70 | 0.568 | 1.51E-01 | 98.1% | 1.95% | |
| | 1.23 | 0.090 | 1.53E-01 | 99.4% | 0.65% | |
| | 0.412 | -0.385 | 1.52E-01 | 98.7% | 1.30% | |
| | 0.137 | -0.863 | 1.53E-01 | 99.4% | 0.65% | |
| | 0.00 | | 1.54E-01 | 100% | 0.00% | |

| CYP3A4 (Midazolam) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (µM) |
| | (µM) | (Log) | | | | |
| Ketoconazole (PC) | 5.00 | 0.699 | 1.59E-03 | 0.73% | 99.3% | |
| | 0.00 | | 2.17E-01 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 2.12E-01 | 97.7% | 2.30% | > 100 |
| | 33.3 | 1.522 | 2.13E-01 | 98.2% | 1.84% | |
| | 11.1 | 1.045 | 2.13E-01 | 98.2% | 1.84% | |
| | 3.70 | 0.568 | 2.14E-01 | 98.6% | 1.38% | |
| | 1.23 | 0.090 | 2.15E-01 | 99.1% | 0.92% | |
| | 0.412 | -0.385 | 2.14E-01 | 98.6% | 1.38% | |
| | 0.137 | -0.863 | 2.16E-01 | 99.5% | 0.46% | |
| | 0.00 | | 2.17E-01 | 100% | 0.00% | |

| CYP3A4 (Testosterone) | | | | | | |
|---|---|---|---|---|---|---|
| Compound ID | Conc | | Area Ratio | Relative Activity (% of NC) | % Inhibition | IC50 (µM) |
| | (µM) | (Log) | | | | |
| Ketoconazole (PC) | 1.00 | 0.000 | 2.66E-03 | 8.50% | 91.5% | |
| | 0.00 | | 3.13E-02 | 100% | 0.00% | |
| AKE-1018 | 100 | 2.000 | 1.43E-02 | 45.7% | 54.3% | 81.7 |
| | 33.3 | 1.522 | 2.19E-02 | 70.0% | 30.0% | |
| | 11.1 | 1.045 | 2.76E-02 | 88.2% | 11.8% | |
| | 3.70 | 0.568 | 3.09E-02 | 98.7% | 1.28% | |
| | 1.23 | 0.090 | 3.11E-02 | 99.4% | 0.64% | |
| | 0.412 | -0.385 | 3.12E-02 | 99.7% | 0.32% | |
| | 0.137 | -0.863 | 3.12E-02 | 99.7% | 0.32% | |
| | 0.00 | | 3.13E-02 | 100% | 0.00% | |

*Summary:*

The data (QC reports available) presented in this report are consistent with rodent pharmacokinetic and efficacy studies that demonstrated substantial plasma exposures and the reversal of mechanical allodynia and thermal hyperalgesia in a neuropathic pain model (rat Spared Nerve Injury).

AKE-1018 possesses favorable drug-like properties and should continue to be vetted as a possible Development Candidate for IND Enablement. In parallel, more potent analogs with lower protein binding should be pursued.

-SLD-