Pegtibatinase
Mechanism of Action and Pharmacology

Summary

Background

- Pegtibatinase is an injectable, PEGylated, truncated human CBS under investigation as an ERT for the treatment of classical homocystinuria (HCU)[1,2]
- PK of pegtibatinase after single and multiple doses was evaluated in murine animal models of HCU, normal mice, normal rats, and NHPs[1,3]

Study Data

- In CBS KO mice, pegtibatinase decreased neonatal mortality by preventing severe liver disease and significantly reduced plasma and tissue levels of Hcy and methionine[4]
- Pegtibatinase substantially improved body composition, decreased facial alopecia and ciliary zonules, improved cognition, and rescued endothelial dysfunction in transgenic I278T mice[5,6]
- Pegtibatinase elimination $t_{1/2}$ was 48 hours in mice and 66.7±1.2 hours in monkeys. Peak plasma level increases were less than dose proportional[1,3]
- SC administration of pegtibatinase resulted in slow bloodstream absorption in all 3 species, with C_{max} and T_{max} ≥24 hours[1]
- In transgenic HO mice and NHPs, pegtibatinase demonstrated ~80% bioavailability and predictable PK.[1] Bioavailability was only 20% in male rats and 36% in female rats[1,3]

Background

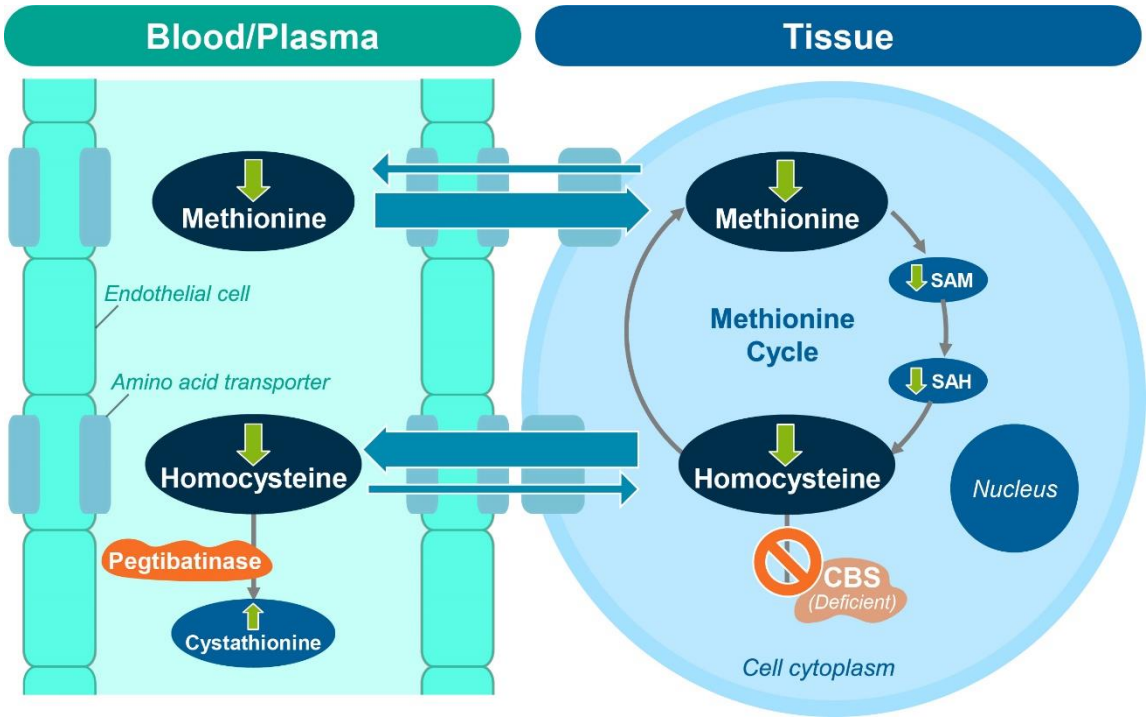
Pegtibatinase

Pegtibatinase is a genetically engineered CBS protein that metabolizes Hcy to cystathionine in the bloodstream. It is a recombinantly produced, modified catalytic core of hCBS that is chemically conjugated with PEG chains. PEGylation with a linear 20 kDa *N*-hydroxysuccinimide ester-activated methoxy-PEG resulted in modification of each CBS subunit by 5 PEG chains on average, improving enzyme half-life in circulation by ~10×.[7]

ERT Sink Hypothesis

The proposed concept for pegtibatinase mechanism of action is based on the metabolic sink hypothesis, wherein pegtibatinase, as an ERT, restores metabolic balance by creating an Hcy concentration gradient from tissues to plasma, thus reducing both plasma and tissue levels of Hcy and methionine (**Figure 1**).[2,8]

Figure 1. Metabolic Sink Hypothesis of Pegtibatinase Mechanism of Action



Preclinical Studies

Efficacy and PK of pegtibatinase have been studied in preclinical rodent models and in NHPs. Models vary in regard to the expression of the HCU disease state. The CBS KO model creates mice completely lacking in CBS, resulting in severe growth delay, neonatal lethality, bone loss, liver damage, low body weight and fat content. Transgenic I278T mice express human CBS I278T mutant cDNA and display facial alopecia, moderate liver steatosis, growth delay, bone loss, low weight and body fat, and decreased survival.[9,10] Transgenic HO mice express low levels of human CBS, ranging from 2% CBS WT activity in kidneys to 16% CBS WT activity in brain. This model reproduces the biochemical profile of human HCU without decreased neonatal survival or liver damage.[9]

Pharmacokinetic Studies

Pegtibatinase PK was evaluated after single and multiple doses in murine animal models of HCU, normal mice, normal rats, and NHPs.[1,3]

HO Model of HCU

Pegtibatinase PK was evaluated in male and female HO mice (n=3-4/group) after SC administration of 5, 10, or 15 mg/kg, and an IV dose of 10 mg/kg.[3,11] The PK/PD relationship and correlation of plasma CBS activity with levels of biomarkers (Hcy, Cth, Cys) were also assessed.[1]

In evaluations of repeated pegtibatinase dosing, HO mice (3M + 3F/group) received SC injections of 4, 8, 12, and 24 mg/kg/day over 10 days.[11]

Studies in WT Rodents

Single- and multiple-dose studies were performed in WT Sprague-Dawley rats. The first study included male and female rats but encountered technical difficulties and high inter-subject variability. Levels of sulfur amino acids (tHcy, Cth, and Cys) were measured to correlate with plasma CBS activity and determine the PK/PD relationship. A multiple-dose study examined differences in absorption in male vs female rats, in addition to safety and tolerability of PEG-CBS and to provide additional data to complement incomplete data sets from the previous 2 studies. SC injections of PBS vehicle or PEG-CBS were administered 1×/48 hours on study days 1, 3, 5, 7, 9, 11, 13, 15 and 17 for a total of 9 injections.[1]

Studies in NHPs

Pegtibatinase PK after IV and SC delivery was further assessed in a study of NHPs. A single-dose study utilized 3 groups of cynomolgus monkeys (n=2M and 2F/group). Group 1 received 2 mg/kg pegtibatinase by bolus IV injection and Group 2 and Group 3 received 2 and 6 mg/kg pegtibatinase, respectively, by SC injection. Measures included levels of sulfur amino acid metabolites (tHcy, Cth and Cys) as potential indicators of a PD effect.[1,11]

In a multi-dose study, 1, 3, and 10 mg/kg doses of PEG-CBS were alternately delivered via SC injection into right and left thoracic sites on study days 1, 4, 7, 10, 13, and 16.[1]

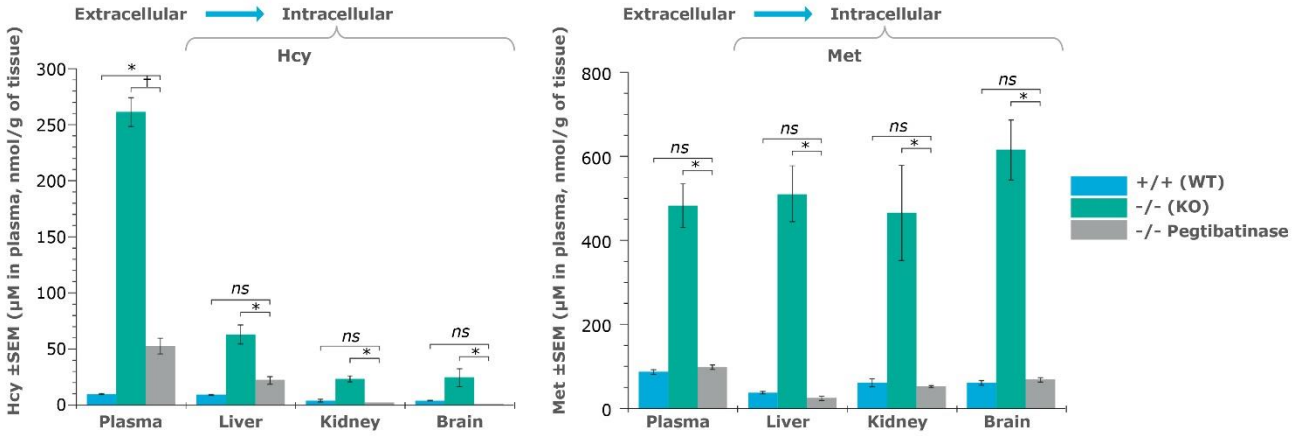
Study Data

Efficacy in Preclinical Studies

In preclinical mouse models, pegtibatinase demonstrated efficacy in all organ systems most affected by HCU: cardiovascular, ocular, skeletal, and CNS.[1,4-6]

In CBS KO mice, pegtibatinase prevented severe liver disease, completely rescued bone mineralization, improved body composition, and greatly prevented neonatal mortality. Treatment with pegtibatinase was associated with a significant (~80%) decrease in plasma and tissue concentrations of tHcy and reduction of methionine (**Figure 2**), and restoration of normal metabolic balance in the liver, kidneys, and brain. These results support the concept of pegtibatinase acting as a metabolic sink in treating HCU.[4,7]

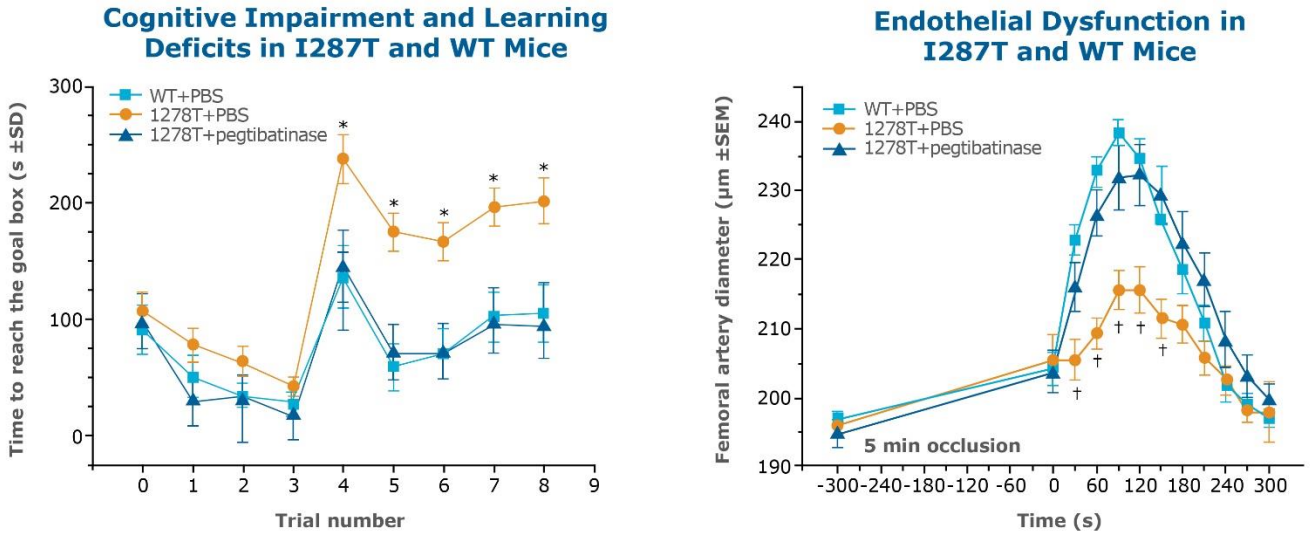
Figure 2. Reduced Hcy and Methionine Concentrations With Pegtibatinase Treatment



Plasma and tissue levels of sulfur metabolites in untreated or 20NHS PEG-CBS-injected KO mice compared with untreated WT mice. *P<0.05; †P<0.001.

In the transgenic I278T model, pegtibatinase was first assessed under normal dietary conditions including unrestricted methionine intake. Treatment with pegtibatinase completely rescued bone mineralization, improved body composition, prevented or reversed facial alopecia, rescued structure of ciliary zonules in the eye lens, alleviated endothelial dysfunction, and improved cognitive and learning deficits (**Figure 3**).[5-7]

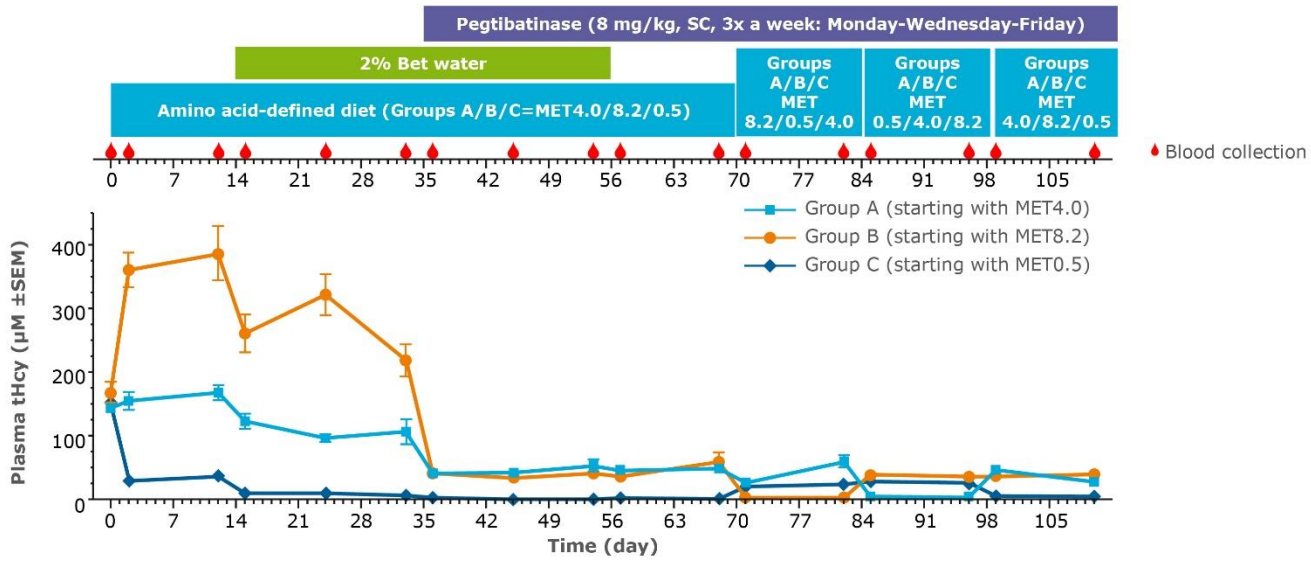
Figure 3. Improved Cognition and Decreased Endothelial Dysfunction With Pegtibatinase Treatment



*P<0.001; †P<0.01.

Pegtibatinase was then evaluated under simulated SOC conditions, including betaine therapy and restricted methionine intake. Treatment efficacy was similar under these conditions, including normalization of plasma tHcy. Following betaine withdrawal, pegtibatinase sustained metabolic balance; this effect was maintained during periods of dietary noncompliance simulated by varying high-, normal-, and low-methionine diets (**Figure 4**).[7,12]

Figure 4. Evaluation of Pegtibatinase Under Varied Dietary Conditions



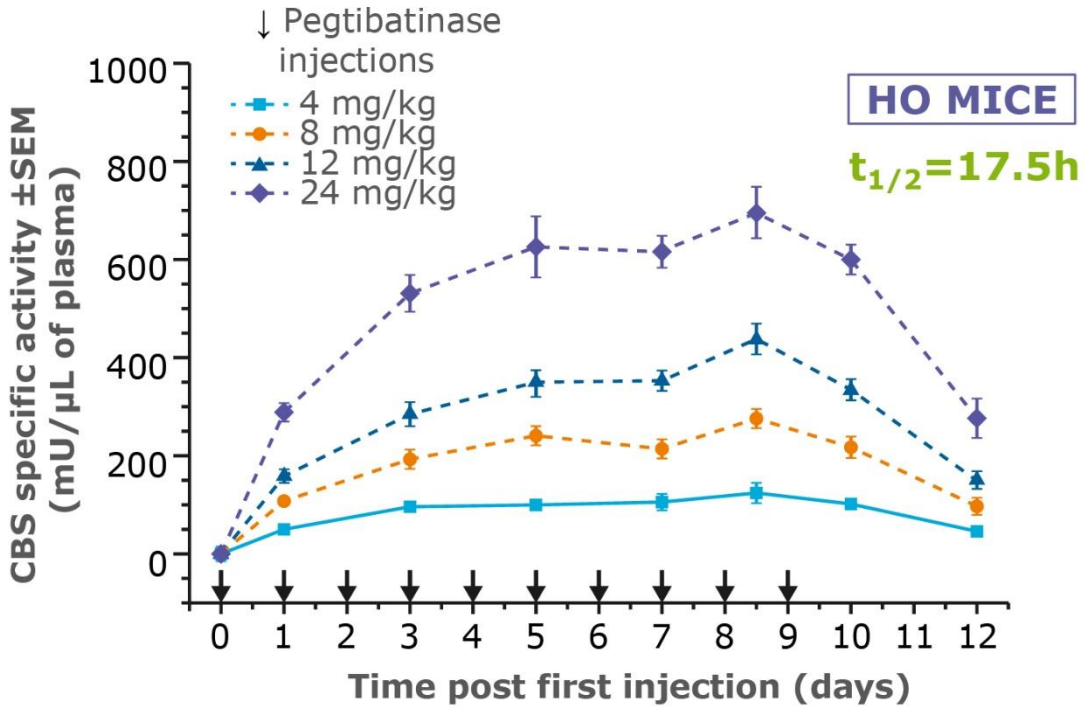
Long-term evaluation of OT-58. Three HO mouse cohorts (n=4M+4F each) were set on diets with reduced (MET0.5), normal (MET4.0), and increased (MET8.2) Met content later combined with Bet supplementation. OT-58 efficacy was evaluated on the background of these dietary regimes and later challenged by switching cohorts between the 3 diets.

Pegtibatinase Pharmacokinetics in Preclinical Studies

HO Model of HCU

In HO mice, accumulation of CBS activity in plasma was linear and dose-dependent following single or repeated SC administration (**Figure 5**).[1,7] SC administration of 5, 10, and 15 mg/kg pegtibatinase resulted in elimination $t_{1/2}$ of 137±95 hours, 17.5±1.27 hours, and 22.0±1.7 hours, respectively. Observed C_{max} was 51.3±3.7 hours for SC 5 mg/kg, 89.9±10.2 hours for SC 10 mg/kg, and 104.2±11.3 hours for 15 mg/kg. Bioavailability of the 5, 10, and 15 mg/kg doses was 82.6%, 64.6%, and 51.5%, respectively.[1]

Figure 5. Plasma CBS Activity Over Time in HO Mice After Repeated SC Injections of Pegtibatinase



Plasma CBS activities after repeated SC injections of 4 (solid line with squares), 8 (dashed line with circles), 12 (dotted line with triangles) and 24 mg/kg PEG-CBS (dash dotted line with diamonds). Arrows designate the dosing time points for a total of 10 injections administered once a day. The data points represent average values and the error bars show SEMs.

Studies in WT Rodents

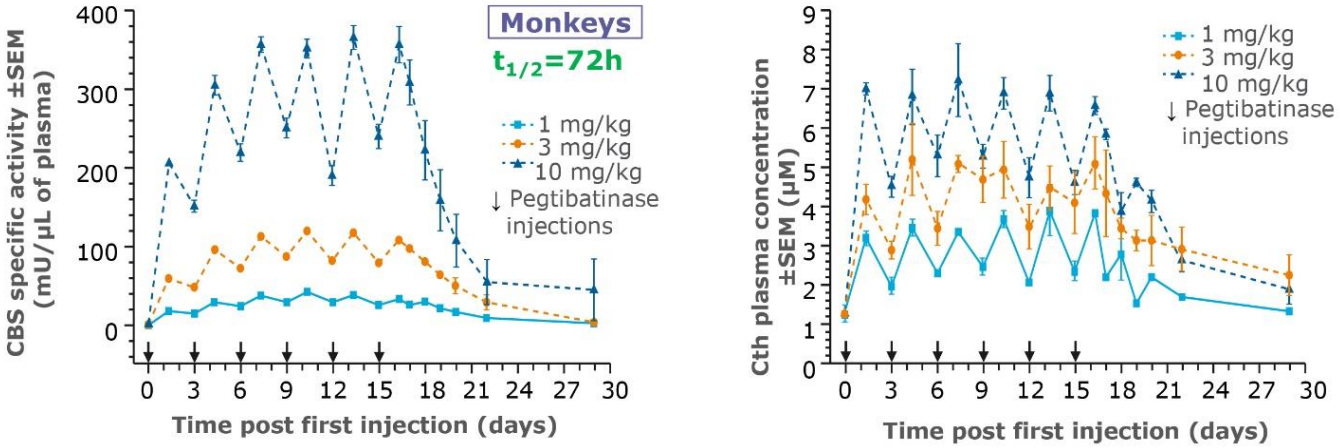
In the multiple-dose study of differences in absorption in male vs female WT Sprague-Dawley rats, bioavailability was 20% in male rats and 36% in female rats.[1,3]

Studies in NHPs

PK parameters were not different between male and female monkeys, allowing results to be pooled. After single 2 mg/kg dose IV administration, elimination $t_{1/2}$ was 66.7±1.2 hours.[1] V_{dss} was 73±4 mL (72,978 µL), indicating pegtibatinase distribution mainly to the plasma compartment.[3]

With repeated dosing, pegtibatinase demonstrated predictable PK, with ~80% bioavailability. Plasma levels of pegtibatinase were dose proportional across all doses studied (**Figure 6**).[1]

Figure 6. Plasma CBS Activity and Cth Over Time in Monkeys After Repeated SC Injections of Pegtibatinase



Plasma CBS activities and Cth levels in Cynomolgus monkeys (n=2M+2F in each group) after repeated SC injections of 1 (solid line with squares), 3 (dashed line with circles) and 10 mg/kg PEG-CBS (dotted line with triangles). Arrows designate dosing time points for a total of 6 doses administered every 3 days. Data points in all plots represent average values and error bars show SEMs.

Consistent with regulatory guidance for biologics, studies of pegtibatinase have not been conducted regarding tissue distribution, metabolism, or excretion.[3]

Abbreviations

Bet, betaine; CBS, cystathionine-β-synthase; cDNA, copy DNA; CNS, central nervous system; C_{max}, maximum drug concentration; Cth, cystathionine; Cys, cysteine; ERT, enzyme replacement therapy; F, female; hCBS, human CBS; HCU, homocystinuria; Hcy, homocysteine; HO, human only; IV, intravenous; KO, knockout; M, male; Met, methionine; NHP, non-human primate; ns, not significant; PBS, phosphate-buffered saline; PD, pharmacodynamics; PEG, polyethylene glycol; PEG-CBS, PEGylated human truncated cystathionine beta-synthase; PK, pharmacokinetics; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SC, subcutaneous; SEM, standard error mean; SOC, standard of care; $t_{1/2}$, half-life; tHcy, total homocysteine; T_{max}, time to maximum drug concentration; V_{dss}, volume of distribution at steady state; WT, wild type.

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