Preclinical Characterization of a Novel, Wild-Type-Sparing, JAK2 V617F Mutant Selective Inhibitor

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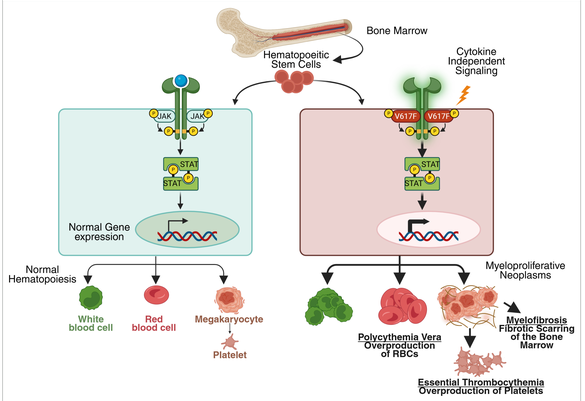
#3752

Introduction

- JAK2 V617F is the most prevalent molecular abnormality in BCR-ABL-negative myeloproliferative neoplasms (MPNs), occurring in approximately 95% of patients with polycythemia vera and 50% of patients with essential thrombocythemia or primary myelofibrosis[1]
- This acquired point mutation in the JAK2 JH2 pseudokinase domain results in the constitutive activation of JAK2 kinase signaling leading to cytokine-independent growth of hematopoietic cells (Figure 1)[1,2,3,4]
- Clinically approved JAK2 inhibitors, such as ruxolitinib, are JAK2 JH1 kinase domain binders that effectively alleviate symptoms and improve outcomes in patients with MPNs. However, these inhibitors rarely lead to significant reductions in variant allele frequency or molecular remission due to dose-limiting hematologic toxicities.[5,6]
- A JAK2 V617F mutant selective inhibitor that spares wild-type JAK2 has the potential to target and eliminate mutant clones and induce molecular remission while avoiding hematological tolerability issues

Figure 1 | JAK2 V617F Leads to Aberrant JAK/STAT Signaling and Drives MPN Disease

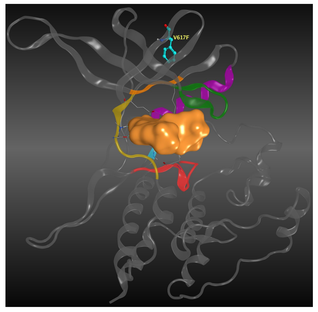


Results

Two Lead Series Were Identified with >10-Fold Selectivity for JAK2 V617F, from which CGT1145 emerged with >100-Fold Selectivity

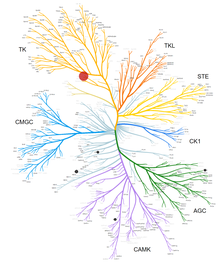
Results (cont'd)

X-Ray Crystallography Shows CGT1145 Bound in the JAK V617F JH2 Pseudokinase Domain



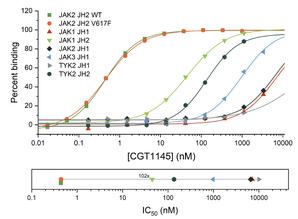
- Crystal structure of JAK2 JH2 pseudokinase domain co-crystallized with CGT1145 (orange surface) at 1.62 Å resolution
- Confirms binding in the JH2 ATP pocket near the V617F mutation
- Over 40 co-crystal structures enabled structure-based drug design of selective and potent compounds
- CGT1145 binds at the hinge region between the C- & N-lobes of JAK2 JH2 forming two hydrogen bonding interactions with backbone NH and C=O of Val629. It also forms hydrogen bonds with Lys581 and backbone C=O of Lys677. Additionally, CGT1145 forms several hydrophobic interactions with sidechains from both the C- & N-lobes of JAK2-JH2 and hydrogen bonds with water molecules.

CGT1145 is Highly Selective Across the Kinome



- CGT1145 was screened at a concentration of 100 nM, >200x IC_{50} for binding to the JAK2 V617F JH2 domain, against a panel of 377 kinases using 10 uM ATP assay conditions
- CGT1145 was highly selective inhibiting only four kinases greater than 50% (black circles on the kinome tree image)
- 100% target occupancy for the JAK2 V617F JH2 domain represented by red dot on the kinome tree image

CGT1145 is Selective for the JAK2 JH2 Domain



- CGT1145 binds to the JAK2 JH2 domain with sub-nM potency and is over 100x selective to JAK1 JH2, JAK1/2/3 JH1, and TYK2 enzymes

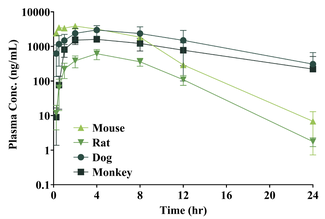
CGT1145 is a Potent JAK2 V617F Inhibitor with Best-In-Class Selectivity for JAK2 WT and JAK1/3 Isoforms

Cogent ID	CGT1145	Ruxolitinib	Momelotinib	Pacritinib	Fedratinib
JAK2 V617F Cell IC_{50} nM	76 nM	61 nM	499 nM	1.3 uM	622 nM
JAK2 WT Cell IC_{50} nM (Sel. vs V617F)	8.6 uM (170x)	34 nM (0.6x)	337 nM (0.7x)	1.3 uM (1x)	255 nM (0.4x)
JAK1/3 Cell IC_{50} nM (Sel vs V617F)	10 uM (>100x)	17 nM (0.3x)	273 nM (0.5x)	2.6 uM (2x)	256 nM (0.4x)

pSTAT5 was measured via HTRF in HEL92.1.7 cells (JAK2 V617F), GM-CSF stimulated TF-1 cells (JAK2 WT), and IL-2 stimulated Hut78 cells (JAK1/3).

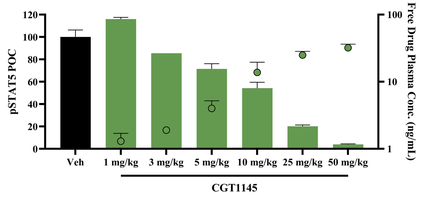
- CGT1145 is a potent inhibitor of JAK2 V617F, mechanistic cellular IC_{50} = 76 nM
- CGT1145 is 170-fold selective for JAK2 V617F over JAK2 WT in cellular assays
- Improved JAK isoform selectivity compared with approved JAK inhibitors would potentially reduce off-target immunosuppression

CGT1145 has High Oral Bioavailability and Low Clearance Across Preclinical Species



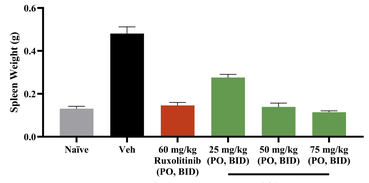
- A single dose of CGT1145 was administered orally at 10mg/kg to male CD-1 mice, SD rats, beagle dogs, and cynomolgus monkeys (n=3 animals per group)
- CGT1145 exhibited low clearance across preclinical species tested, extraction ratio (ER) = 4% to 21%
- High oral bioavailability (>80%) was achieved in cynomolgus monkey and beagle dog
- Based on modeling, CGT1145 is predicted to have low human clearance and high oral bioavailability

CGT1145 Dose-Dependently Inhibits pSTAT5 in a JAK2 V617F PK/PD Model



- Athymic nude mice bearing subcutaneous JAK2 V617F HEL 92.1.7 tumors were administered a single oral dose of CGT1145. At 4 hours post-dose, drug plasma concentrations and tumor phospho-STAT5 levels were measured.
- CGT1145 showed a dose dependent reduction in tumor phospho-STAT5 levels measured by AlphaLISA.
- CGT1145 administered at 50 mg/kg resulted in 96% inhibition of tumor pSTAT5.

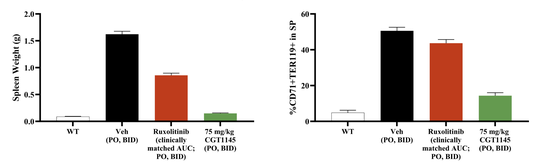
CGT1145 is Efficacious at 50 mg/kg BID in a JAK2 V617F Splenomegaly Model



- In a mouse model of splenomegaly which mimics human MPN, athymic nude mice were implanted intravenously with Ba/F3-EPOR-JAK2V617F cells. After 24 hours, CGT1145 was administered orally twice daily for 10 days. At the end of treatment, spleens were collected and weighed.
- CGT1145 treatment led to a dose dependent inhibition of splenomegaly.
- CGT1145 administered at 50 mg/kg BID restored spleen weight to that of naïve animals and was equivalent to the activity of 60 mg/kg BID ruxolitinib.

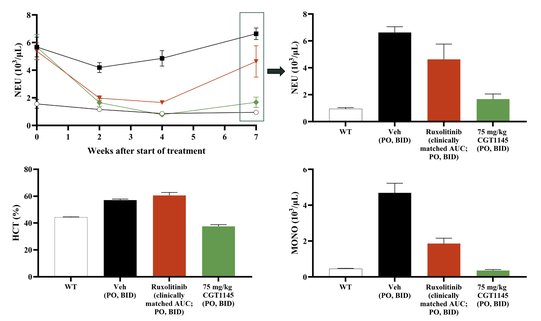
CGT1145 Decreases Erythroid Precursors in Spleen and Normalizes Hematological Parameters in a Transgenic Mouse Model of JAK2 V617F Driven MPN

Spleen Outcomes



- Transgenic mice heterozygous for JAK2 V617F and exhibiting a classic MPN-like phenotype were administered CGT1145 orally twice daily for 7 weeks vs. ruxolitinib dosed at clinically matched AUC/exposure. Blood was collected for CBC analysis and spleens were measured for weight and CD71+TER119+ erythroid precursor accumulation.
- Treatment with CGT1145 led to normalization of spleen weight and decreased CD71+TER119+ erythroid precursor accumulation in the spleen supporting the potential of CGT1145 as a disease modifying therapy through restoration of bone marrow function and attenuation of extramedullary hematopoiesis.

Hematological Parameters



- After 7-weeks of treatment, CGT1145 restored neutrophil counts to levels comparable to those of WT age-matched littermates, while ruxolitinib led to a brief normalization followed by re-elevation at clinically matched AUC/exposure.
- Treatment with CGT1145 also led to restoration of normal HCT and MONO levels compared with ruxolitinib at clinically matched AUC/exposure. WBC was normalized in this study with CGT1145 treatment. These data collectively suggest an enhanced ability of CGT1145 to mitigate thrombosis risk, fibrotic risk and inflammation.

Conclusion: CGT1145 Best-In-Class JAK2 V617F Mutant Selective Inhibitor

CGT1145 is a potent inhibitor of JAK2 V617F mutations and is >100x selective over JAK2 WT and the JAK1/3 isoforms. This inhibitor has high oral bioavailability and low clearance in mouse, rat, dog and cyno pharmacokinetic studies. CGT1145 provides dose-dependent inhibition of pSTAT5 in a JAK2 V617F PK/PD model with complete inhibition seen at 50 mg/kg. In a JAK2 V617F splenomegaly model, CGT1145 administered at 50 mg/kg BID restored spleen weight to that of naïve animals and was roughly equivalent to the activity of 60 mg/kg BID ruxolitinib. CGT1145 normalizes blood cell counts and spleen weights in a transgenic mouse model of JAK2 V617F driven MPN. CGT1145, a best-in-class JAK2 V617F mutant selective inhibitor that has the potential to eradicate JAK2 V617F myeloproliferative neoplasm propagating cells and induce molecular remission with improved hematological tolerability. Cogent plans to submit an IND application in 2026.

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References: [1]Baxter. 2005. Lancet. [2]James. 2005. Nature. [3]Levine. 2005. Cancer Cell. [4]Kralovics. 2005. N. Engl. J. Med. [5]Harrison. 2016. Leukemia. [6]Deininger. 2015. Blood.

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