GB5121 is an Oral, CNS-Penetrant, Potent and Selective BTK Inhibitor for the Treatment of CNS Malignancies

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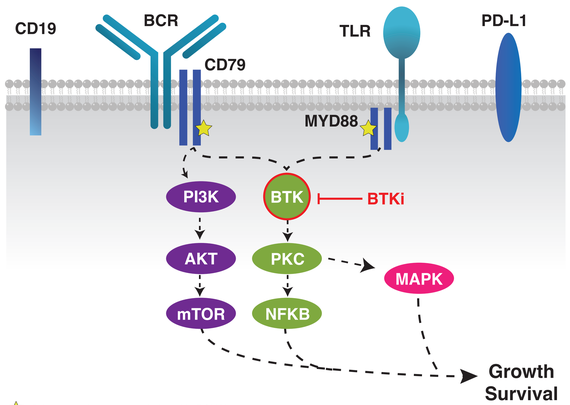
BACKGROUND

- Primary central nervous system lymphoma (PCNSL) is a rare and aggressive form of non-Hodgkin lymphoma that is restricted to the CNS
- Bruton's tyrosine kinase inhibitors (BTKi) are approved treatments for several B cell lymphomas, but are characterized by modest selectivity and/or low CNS exposure
- GB5121 has been designed to be a CNS-penetrant, potent, selective, irreversible, oral BTKi in development for B cell malignancies with CNS involvement
- These features differentiate GB5121 from other BTKi and support its use in clinical trials for CNS malignancies

Figure 1. BTK inhibition targets a key survival node in PCNSL



Adapted from Graham MS et al. 2018

OBJECTIVE

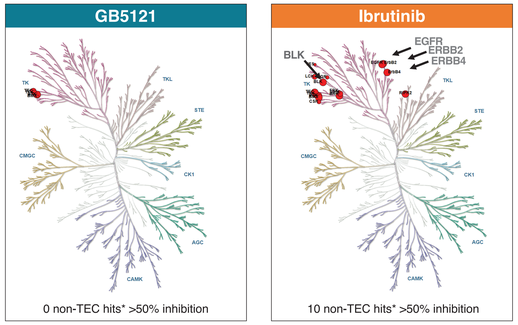
- Profile GB5121 for selectivity and CNS penetration in comparison to ibrutinib

METHODS

- Selectivity of GB5121 and ibrutinib was profiled in kinome scans against 349 kinases at 1 μM under conditions using 1 mM ATP concentration; EGFR activity was further evaluated in a cell-based assay
- Irreversible and covalent nature of GB5121 was evaluated using enzymatic assays and no warhead control compounds
- *In vitro* potency and inactivation kinetics of GB5121 were evaluated using biochemical and cell-based assays
- Comparative BTK target occupancy of GB5121 and ibrutinib was assessed in perfused mice with an intact blood brain barrier (BBB) using a probe-based ELISA
- Pharmacokinetic profiles of GB5121 were obtained in multiple species using intravenous and oral dosing

RESULTS

Figure 2. GB5121 is a highly selective BTK inhibitor



- GB5121 demonstrates >50% inhibition of only two kinases besides BTK (TEC and TXK)
- Compared to ibrutinib, GB5121 lacks activity against EGFR, ERBB2 (HER2) and ERBB4 (HER4)

Figure 3. GB5121 does not inhibit phosphorylation of EGFR in A431 cell line

Data are representative of 10 independent studies. Dose response curves were generated from duplicate values at each concentration of test article using a nonlinear, 4-parameter, variable slope curve-fitting function using GraphPad Prism software.

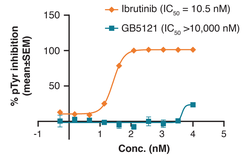


Table 1. BTK enzymatic mechanism of inhibition studies demonstrate properties of an irreversible covalent inhibitor

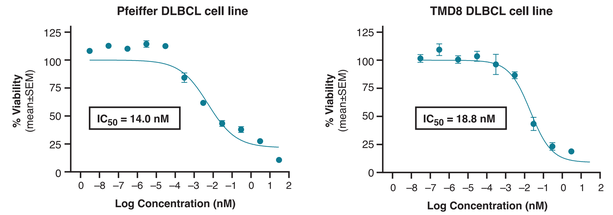
Assay		GB5121	GBD-07476 (GB5121 with unreactive warhead)	GBD-07547 (GB5121 with no warhead)
BTK Enzyme Assay Preincubation (nM IC_{50})	0 min	69	9010	3830
	30 min	14	8610	3780
	60 min	6	7210	4900
BTK enzyme recovery/Residence Time		>300 min	<5 min	<5 min
Irreversible Inhibition		yes	no	no

Table 2. Potency and inactivation kinetics in cells and whole blood

Assay	GB5121
hWB pBTK IC_{50}	63.5 nM
Ramos calcium flux IC_{50}	23.8 nM
hWB CD69 IC_{50}	181.3 nM
B cell proliferation IC_{50}	4.7 nM
hWB kinact/KI	4.7 10^{-4} nM^{-1} min^{-1}
mWB kinact/KI	7.2 10^{-4} nM^{-1} min^{-1}
mB kinact/KI	3.8 10^{-4} nM^{-1} min^{-1}
Pfeiffer DLBCL viability IC_{50}	14.0 nM
TMD8 DLBCL viability IC_{50}	23.5 nM

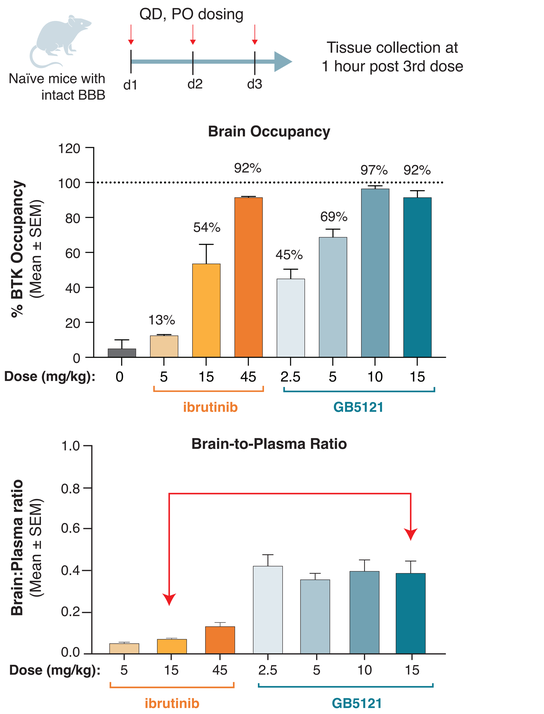
hWB = human whole blood, mWB = mouse whole blood, mB = mouse brain lysate

Figure 4. GB5121 shows potent activity *in vitro* in diffuse large B cell lymphoma (DLBCL) cell lines regardless of phenotype and mutational profile



Pfeiffer cell line: Germinal Center (GC) phenotype with wild type MyD88. **TMD8 cell line:** Activated B Cell (ABC) phenotype carrying MyD88 driver mutation. Dose response curves were generated from triplicate (Pfeiffer) or duplicate (TMD8) values at each concentration of test article using a nonlinear, 4-parameter, variable slope curve-fitting function using GraphPad Prism software

Figure 5. GB5121 shows superior CNS exposure with lower plasma exposures compared to ibrutinib in naïve mice with intact BBB

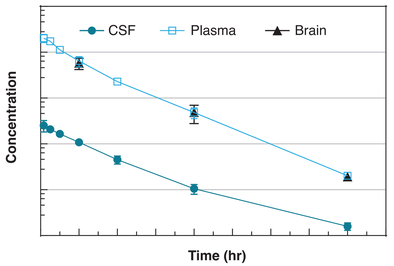


0 mg/kg = vehicle; 15 mg/kg = Mouse comparable dose of ibrutinib 560 mg QD clinical dose based on plasma AUC.
Data are representative of 2 independent experiments. Graphs were generated in GraphPad Prism software. Study included 3 mice per group.

Table 3. GB5121 brain-to-plasma ratios after single or multiple daily oral (PO) dosing in multiple species

Species Strain	N	PO Dose (mg/kg)	Day (Time)	Brain/Plasma Ratio (Mean ± SD)
Mouse C57BL/6	15	10	3 (1 hr)	0.187 ± 0.022
Rat Wistar Han	3	10	1 (1 hr)	0.285 ± 0.044
NHP Cyno	4	30	15 (4 hr)	1.60 ± 0.42

Figure 6. GB5121 demonstrates a 1:1 brain-to-plasma ratio in non-human primates (n = 3) after an IV dose



SUMMARY AND CONCLUSIONS

- **GB5121 is an oral irreversible BTK inhibitor that is currently being investigated in a phase 1b/2 trial in patients with relapsed/refractory primary/secondary CNS lymphoma and primary vitreoretinal lymphoma (NCT05242146)**
- **Preclinical studies demonstrated GB5121 to exhibit several characteristics differentiating it from approved BTK inhibitors and those currently under clinical investigation, including**
  - **High selectivity over other kinases**
  - **Rapid BTK inactivation kinetics across multiple tissues**
  - **Rapid equilibrium between plasma and brain concentrations**
  - **Superior target occupancy in the brain compared to ibrutinib**
- **Excellent CNS penetration and selectivity combined with activity against DLBCL cell lines support the use of GB5121 as a novel molecule to treat human BTK-driven malignancies including CNS lymphoma**

REFERENCES

1. Graham MS, DeAngelis LM. *Best Pract Res Clin Haematol.* 2018; 31: 262-269.

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