Efficacy of vecabrutinib treatment in a murine model of sclerodermatous graft-versus-host disease

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Abstract

BACKGROUND. Chronic graft-versus-host disease (cGVHD) can manifest as a complication in patients following allogeneic hematopoietic stem cell transplant resulting in morbidity and mortality. Effective treatment strategies for cGVHD are currently lacking. Ibrutinib, an irreversible BTK inhibitor with activity against several other Tec-family kinases, has been clinically developed for cGVHD treatment due to regulation of pathogenetic B cells and T-cell subsets. Vecabrutinib is a more selective, reversible inhibitor of BTK with a distinct kinase domain interaction, which could result in differentiated safety and activity profiles compared to ibrutinib. Vecabrutinib has the capacity to overcome the C481S mutation that mediates resistance to ibrutinib. Vecabrutinib also also demonstrates activity against ITK, which is expressed in T cells. **In this study, we investigated the activity and immune modulation of vecabrutinib treatment in a murine model of sclerodermatous cGVHD.** Ibrutinib was utilized for comparison.

	TEC Family Kinases					Other Kinases				
	BTK	ITK	TEC†	TXK	BMX	LCK†	cSRC/SRC	BLK	NEK11	EGFR
Vecabrutinib IC_{50} nM[1]	3	14	14	474	224	8	84	23	90	>6000
Ibrutinib IC_{50} nM[2]	1.5	4.9	7	2	0.8	6.3	19	0.1	NA	5.3

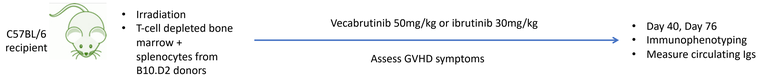
Table 1. In vitro kinase assays showing selectivity of vecabrutinib[1] and ibrutinib[2] against TEC-family and other kinases

RESULTS. Clinical symptoms, including appearance, activity, skin irritation, redness, alopecia and diarrhea were significantly reduced in vecabrutinib- and ibrutinib-treated groups. Furthermore, there was a trend toward a more favorable clinical score overall for the vecabrutinib-treated group, however no statistical difference was observed compared to ibrutinib possibly due to small sample size. Weight loss was slightly elevated during vecabrutinib and ibrutinib treatment compared to vehicle (trend), however mice recovered body weight post-treatment and continued to maintain benefit. On day 40 (during treatment) and day 76 (post-treatment) groups of mice were euthanized for immunophenotyping analysis utilizing a 22-color flow cytometry panel. During treatment, both vecabrutinib and ibrutinib-treated mice retained total B cell numbers but exhibited reduced B-cell activation, proliferation, and number of B220+ CD138+ plasma cells. In addition, B cells secreted less IL-10, and IL-4/5. Expression of co-stimulatory molecules CD80 and CD86 on B cells were unchanged. Total CD3+ T cells, activated and proliferating CD4+ and CD8+ T cells, and cytotoxic granzyme-B+ CD8+ T cells were reduced in treated mice. Interestingly, CD4+ CD25+ FoxP3+ Treg number, expression of PD-1 on Tregs and CD4+ CXCR5+ PD-1+ T follicular helper cells were also reduced in both treatment groups. In addition, there were globally reduced numbers of cytokine-secreting CD4+ cells but no differences in Th1/Th2 or Th17/Treg ratios were observed. Post-treatment, proliferation and cytokine secretion of B cells and T cells were still lowered but less impaired than during treatment. Tregs and PD-1 expression were still reduced post-treatment. Finally, circulating levels of IgA appeared lower during and post treatment in vecabrutinib-treated mice compared to vehicle, while IgG1, IgG2b were reduced in both treated groups post-treatment. No changes in IgM levels were observed.

[1]Jebaraj et al. Blood 2021. DOI 10.1182/blood.2021011516. [2]Byrd et al. New England Journal of Medicine 2016. DOI 10.1056/NEJMoa1509981. NA: not available.

Methods

CHRONIC GVHD MURINE MODEL. A murine model of sclerodermatous cGVHD (sc-GVHD) was initiated by adoptive transfer of $5x10^6$ T-cell depleted bone marrow plus $5x10^6$ whole splenocytes from B10.D2 donors into BALB/c recipients following sub-lethal irradiation. Total bone marrow and irradiation only groups were included as controls. Mice with established cGVHD were treated with vecabrutinib once daily at 50mg/kg by oral gavage or ibrutinib at 30mg/kg in drinking water 5 days per week for approximately 3 weeks beginning on day 27 post-adoptive transfer and ending on day 45 (n=8-10 mice per group). Vecabrutinib was gifted by Sunesis Pharmaceuticals and ibrutinib was commercially obtained.



IMMUNOPHENOTYPING & IMMUNOGLOBULINS. Spleens were collected from euthanized mice at two timepoints (day 40 during treatment and on day 76 post-treatment). Flow cytometry was performed to detect B cell and T cell subsets. Splenocytes were stimulated with PMA/ionomycin for 5hrs, stained with antibodies against cell surface markers, fixed/permed and stained with antibodies against intracellular markers. Samples were acquired on a BD FACS Symphony cytometer. Cell count was calculated using Accucheck counting beads (Thermofisher). Levels of circulating immunoglobulins in sera were measured by multiplex cytokine assay at both timepoints (Milliplex assay, Millipore Sigma).

Funding

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Conflict-of-interest

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Figure 1. sc-GVHD severity is alleviated by treatment with BTK inhibitors vecabrutinib and ibrutinib

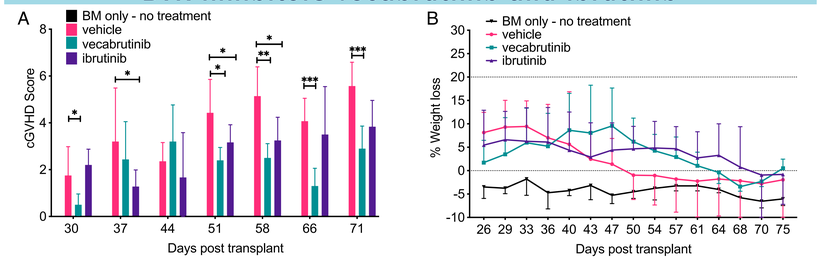

Figure 1. sc-GVHD severity is alleviated by treatment with BTK inhibitors vecabrutinib and ibrutinib. (A) Mice were assessed for symptoms including appearance, activity, skin irritation/redness/alopecia, diarrhea, conjunctivitis. Each mouse was assigned a score between 0-2 for each criteria with 0-absent/mild, 1-moderate, 2-severe. Scores were totaled for each mouse. Both treatments alleviated symptoms, and mice receiving vecabrutinib appeared to benefit longer post-treatment than mice receiving ibrutinib. Graph shows mean score per group + SD. (B) Body weight was assessed throughout the study. Weight loss was calculated as a percentage of pre-irradiation (day -1) weight. Graph displays mean % weight loss over time per group +/- SD. *p<0.05 **p<0.01 ***p<0.001 in a student's t-test.

Figure 2. Pathogenic B-cell characteristics are reduced by BTK inhibition in mice with sc-GVHD

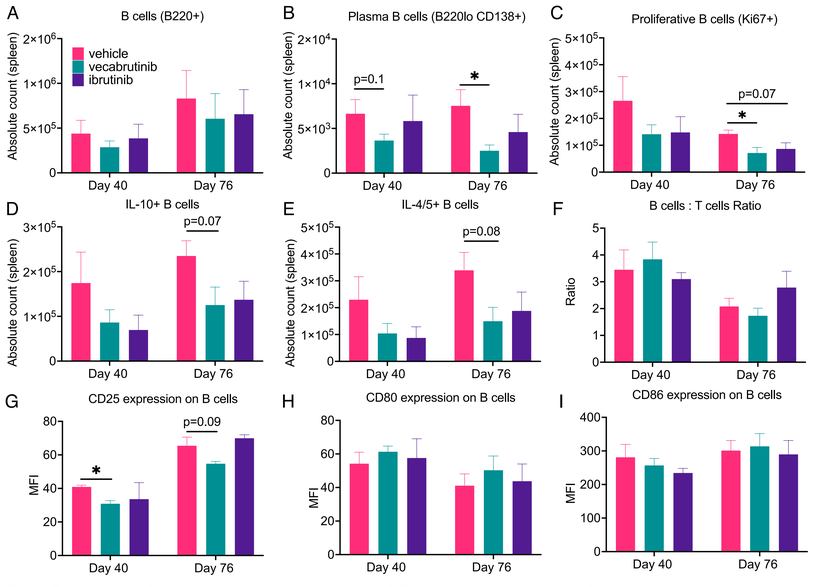

Figure 2. Pathogenic B-cell characteristics are reduced by BTK inhibition in mice with sc-GVHD. Mice from each group were euthanized at day 40 during treatment (n=3) or day 76 post-treatment (n=4-6). Flow cytometry analysis was performed on splenocytes that had been stimulated with PMA/ionomycin for 5h ex vivo. (A) Total B cell numbers (CD3- B220+) were not changed. (B-C) Plasma B cells (B220lo CD138+) and proliferating B cells (Ki67+) were lowered by treatment. (D-E) Numbers of IL-10 and IL-4/5 cytokine-secreting B cells were reduced by treatment. (F) B cell (B220+) to T cell (CD3+) ratio was not significantly altered in treated mice compared to vehicle. (G-I) Expression of activation marker CD25 on surface of B cells was reduced by vecabrutinib, however co-stimulatory molecules CD80 and CD86 were not altered. Graphs show mean + SEM. *p<0.05 in a student's t-test.

Figure 3. Treatment with BTK inhibitors alters T-cell phenotype and function in mice with sc-GVHD

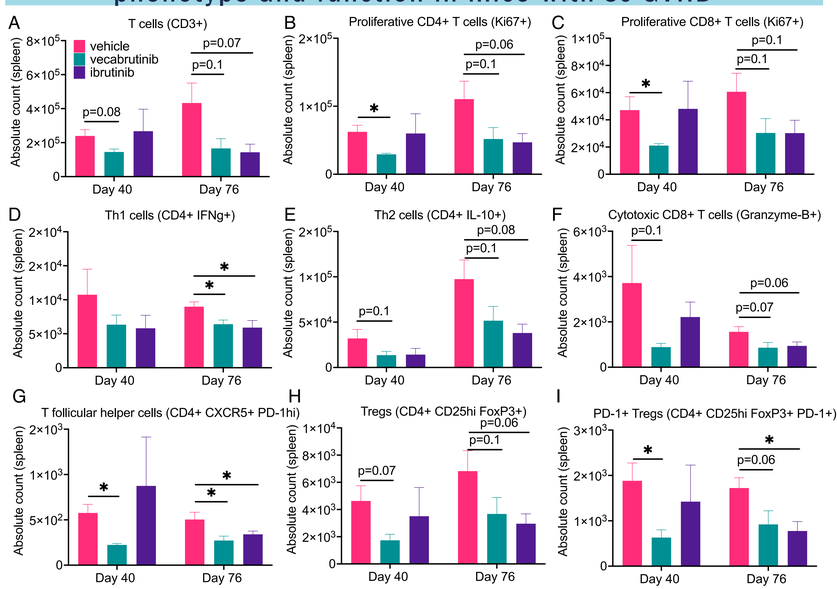

Figure 3. Treatment with BTK inhibitors alters T-cell phenotype and function in mice with sc-GVHD. Mice from each group were euthanized at day 40 during treatment (n=3) or day 76 post-treatment (n=4-6). Flow cytometry analysis was performed on splenocytes that had been stimulated with PMA/ionomycin for 5h ex vivo. (A) Total T cell (CD3+) numbers were lowered by treatments. (B-C) Proliferation of CD4+ and CD8+ T cells was also lowered. (D-E) Both Th1 and Th2 cytokine-secreting CD4+ T cells were reduced and this was more prominent at the later time point. (F) Granzyme-B+ CD8+ T cells were also reduced. (G) T follicular helper cells, that interact with germinal center B cells, were significantly reduced at both timepoints. (H-I) Tregs and PD-1+ Tregs were reduced by treatments. Graphs show mean + SEM. *p<0.05 in student's t-test.

Figure 4. BTK inhibition reduced circulating immunoglobulin levels in mice with sc-GVHD

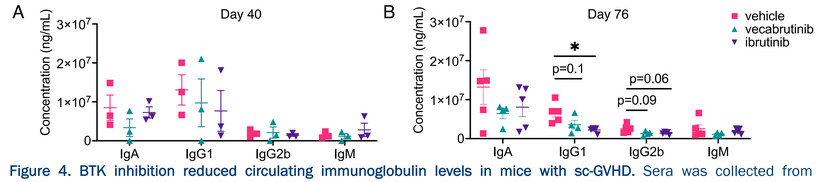

Figure 4. BTK inhibition reduced circulating immunoglobulin levels in mice with sc-GVHD. Sera was collected from euthanized mice at each time point and concentrations of circulating Igs were detected by multiplex cytokine assay (n=3-5). (A) IgA levels were lower in vecabrutinib-treated mice at day 40 (B) IgG1 and IgG2b levels were lower in both treatment groups at day 76. No differences observed in IgM levels. Graphs show mean +/- SEM and *p<0.05 in a student's t-test.

Conclusions

Vecabrutinib treatment reduced symptomatic severity, beneficially regulated B-cell and T-cell immune subsets and reduced circulating immunoglobulins in a preclinical murine model of sclerodermatous cGVHD. Vecabrutinib-treated mice displayed alleviated symptoms for a longer time period post-treatment than ibrutinib-treated mice. Further studies are ongoing to assess differences between vecabrutinib and ibrutinib.