



Title	Gilteritinib enhances graft-versus-leukemia effects against FLT3-ITD mutant leukemia after allogeneic hematopoietic stem cell transplantation
Author(s)	Zhang, Zixuan; Hasegawa, Yuta; Hashimoto, Daigo; Senjo, Hajime; Kikuchi, Ryo; Chen, Xuanzhong; Yoneda, Kazuki; Sekiguchi, Tomoko; Kawase, Tatsuya; Tsuzuki, Hirofumi; Ishio, Takashi; Ara, Takahide; Ohigashi, Hiroyuki; Nakagawa, Masao; Teshima, Takanori
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1 **Gilteritinib enhances graft-versus-leukemia effects against FLT3-ITD mutant**
2 **leukemia after allogeneic hematopoietic stem cell transplantation**

3 Zixuan Zhang^{1*}, Yuta Hasegawa^{1*}, Daigo Hashimoto¹, Hajime Senjo¹, Ryo Kikuchi¹,
4 Xuanzhong Chen¹, Kazuki Yoneda¹, Tomoko Sekiguchi¹, Tatsuya Kawase², Hirofumi
5 Tsuzuki², Takashi Ishio¹, Takahide Ara¹, Hiroyuki Ohigashi¹, Masao Nakagawa¹,
6 Takanori Teshima¹

7
8 ¹Department of Hematology, Hokkaido University Faculty of Medicine, Graduate
9 School of Medicine, Sapporo, 060-8638, Japan

10 ²Drug Discovery Research, Astellas Pharma Inc.,

11

12 * These authors contributed equally to this work

13

14 **Corresponding author:** Daigo Hashimoto, M.D., Ph.D.

15 N15 W7, Kita-ku, Sapporo 060-8638, Japan

16 Tel: +81-11-706-7214; Fax: +81-11-706-7823

17 E-mail: D5hash@pop.med.hokudai.ac.jp

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Abstract

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Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a potentially curative therapy for FLT3 internal tandem duplication mutant (FLT3-ITD⁺) acute myeloid leukemia, but relapse rate is high. A recent study showed that sorafenib, a first generation FLT3 and multikinase inhibitor, enhanced graft-versus-leukemia (GVL) effects against FLT3-ITD⁺ leukemia via interleukin-15 (IL-15) production. However, it remains to be clarified whether this effect could be mediated by selective FLT3 inhibition. We investigated whether gilteritinib, a selective FLT3 inhibitor, could enhance GVL effects against FLT3-ITD transfected Ba/F3 leukemia (Ba/F3-FLT3-ITD) in mice. Oral administration of gilteritinib from day +5 to +14 after allo-SCT reduced expression of the co-inhibitory receptors PD-1 and TIGIT on donor CD8⁺ T cells and enhanced IL-15 expression in Ba/F3-FLT3-ITD. Bioluminescent imaging using luciferase-transfected Ba/F3-FLT3-ITD demonstrated that gilteritinib significantly suppressed leukemia expansion after allo-SCT, whereas it did not impact the morbidity or mortality of graft-versus-host disease (GVHD), resulting in significant improvement of overall survival. In conclusion, short-term administration of gilteritinib after allo-SCT enhanced GVL effects against FLT3-ITD⁺ leukemia without exacerbating GVHD.

39 **Introduction**

40 FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD), which is
41 attributed to a head-to-tail duplication of 3-400 base pairs in the juxtamembrane-domain
42 coding sequence, is the most frequent (20-25%) mutation in adult acute myeloid
43 leukemia (AML)^{1,2}. The FLT3-ITD mutation is associated with a high leukemic burden
44 and a poor prognosis of AML³⁻⁵. Although allogeneic hematopoietic stem cell
45 transplantation (allo-SCT) potentially improves the outcomes of FLT3-ITD⁺ AML, the
46 relapse rates are significantly higher in FLT3-ITD⁺ AML than those in FLT3-ITD
47 negative AML after allo-SCT⁶⁻¹⁰.

48

49 FLT3 inhibitors are being developed to target constitutively activated
50 FLT3-ITD signaling, which induces the proliferation and survival of leukemia cells.
51 First-generation FLT3 inhibitors, such as sorafenib, are not specific to FLT3 and are
52 categorized as multikinase inhibitors, whereas the second-generation FLT3 inhibitors,
53 such as gilteritinib and quizartinib, are more specific to targeting mutated FLT3. In
54 murine models of allo-SCT, sorafenib has been shown to induce the interferon
55 regulatory factor 7 (IRF7)-dependent production of IL-15 in leukemia cells, which
56 promotes the expansion of donor cytotoxic T cells and potentiates graft-versus-leukemia
57 (GVL) effects against FLT3-ITD⁺ leukemia⁵. However, it remains to be clarified as to

58 whether second-generation FLT3 inhibitors can promote GVL effects after allo-SCT. In
59 the current study, we explored the impacts of the short-term administration of
60 gilteritinib on GVHD and GVL effects after mouse allo-SCT.

61

62 **Materials and methods**

63 **Mice:** Female C57BL/6 (H-2^b) and B6C3F1 (H-2^{b/k}) mice were purchased from CLEA
64 Japan (Shimizu, Japan) and Charles River Japan (Kanagawa, Japan), respectively. Mice
65 were 8-12 weeks of age at the time of transplantation and were maintained in a specific
66 pathogen-free environment. The recipient mice were randomly allocated to each
67 experimental group, thus ensuring that the mean body weight in each group
68 was similar. All the animal experiments were performed in a nonblinded fashion and
69 under the auspices of the Institutional Animal Care and Research Advisory Committee.

70 **Cell line:** FLT3-ITD transfected C3H-derived Ba/F3 leukemia cells (Ba/F3-FLT3-ITD),
71 FLT3-ITD-untransfected Ba/F3, and human myeloid leukemia cell line harboring
72 FLT3-ITD (MOLM-13 cells) were provided by Astellas Pharma Inc. (Tokyo, Japan)¹¹.
73 C57BL/6-derived myeloid leukemia cell line C1498 was purchased from ATCC. To
74 create a luciferase expressing Ba/F3-FLT3-ITD cell line (Ba/F3-FLT3-ITD-luc⁺), the
75 retroviral vector pMSCV-luc-IRES-YFP was kindly provided by Dr. Gerard Grosveld
76 (Department of Genetics, St. Jude Children's Research Hospital). Briefly,
77 Ba/F3-FLT3-ITD cells were infected with virus particles generated from HEK293T
78 cells that were transfected with pMSCV-luc-IRES-YFP plasmid and pCL-Eco (Addgene,
79 Cambridge, MA) in the RPMI 1640 medium supplemented with 1 µg/ml puromycin

80 (Sigma-Aldrich Japan, Tokyo, Japan) and 6 $\mu\text{g/ml}$ polybrene (Nakarai Tesque, Kyoto,
81 Japan)¹². pCL-Eco was obtained from Dr. Inder Verma (Addgene plasmid #12371)¹³.
82 After infection, a single YFP expressing clone was recovered by using the limiting
83 dilution method.

84 **SCT:** On day 0, B6C3F1 (H-2^{b/k}) recipients were lethally irradiated with 10.5 Gy total
85 body irradiation, and split into 2 doses with 4 hr intervals, followed by i.v. injection
86 with 5×10^6 T-cell depleted BM (TCD-BM) cells either alone or in combination with 1
87 $\times 10^6$ purified T cells from major histocompatibility complex haploidentical B6 (H-2^b)
88 on day 0. The purification of T cells and TCD-BM was performed by using Pan T Cell
89 Isolation Kit II, mouse (Miltenyi Biotec Japan, Tokyo, Japan) and
90 anti-CD90-MicroBeads (Miltenyi Biotec Japan), respectively, as well as and the
91 AutoMACS Pro Separator (Miltenyi Biotec Japan). To assess the GVL effects, recipient
92 mice were transferred with 5×10^4 or 5×10^5 Ba/F3-FLT3-ITD-luc⁺ cells on day 0.
93 Gilteritinib, which was provided by Astellas Pharma Inc., was dissolved in 0.5%
94 methylcellulose at a concentration of 1.25 mg/ml, and recipient mice were orally
95 administered with either gilteritinib at 10 mg/kg/day or vehicle from day +5 to day +14
96 after allo-SCT.

97 **Evaluation of GVHD and GVL:** Survival was monitored daily, and the severity of

98 GVHD was assessed by using the GVHD scoring system with five parameters¹⁴.
99 Bioluminescent imaging (BLI) was conducted weekly to evaluate the GVL effects. Mice
100 were anesthetized by using isoflurane and were subcutaneously injected with 500 μ g
101 d-luciferin (Promega, Madison, WI) at 5 min before *in vivo* imaging. Luciferase⁺ cells
102 were detected by using IVIS Imaging System ver. 4.3.1 (Perkin Elmer, Waltham, MA).
103 Light emission is presented as photons per second per square centimeter per ster-
104 radiant ($\text{ph/s/cm}^2/\text{sr}$). Leukemia death was defined by the existence of
105 hepatosplenomegaly with macroscopic tumor nodules, hinder leg paralysis¹⁵, or
106 leukemia expansion on BLI. Deaths without these signs were attributed to GVHD. To
107 evaluate allospecific cytotoxic T lymphocyte (CTL) responses, vehicle- or
108 gilteritinib-treated recipients of TCD-BM plus T cells were i.v. injected with 3×10^6
109 untransfected Ba/F3 (allogeneic) or C1498 (syngeneic) labeled with 5 μ M CellTraceTM
110 Violet (CTV, Thermo Fisher Scientific) on day +8 after allo-SCT. CTV positive cells in
111 the bone marrow were enumerated 2 hrs after the transfer.

112 **Culture:** Ba/F3-FLT3-ITD cells or MOLM-13 cells (5×10^5 cells/well) were cultured in
113 RPM I1640 medium containing 10% FCS in 6-well plates. Cells were stimulated with
114 either gilteritinib or quizartinib (Selleck Biotech, Tokyo, Japan) at concentrations of 1.0,
115 10, or 100 nM, or sorafenib (SYNKinase, Shanghai, China) at concentrations of 0.1, 1.0,

116 or 10 μ M for 24 hrs.

117 **Q-PCR:** The total RNA extracted from the leukemia cells by using either ISOGEN II
118 (Nippon Gene, Toyama, Japan) or RNeasy Kit (QIAGEN) was subjected to cDNA
119 synthesis by using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo
120 FSQ-301, Osaka, Japan) or SuperScript IV VILO Master Mix (Thermo Fisher
121 Scientific). Quantitative PCR (Q-PCR) was performed on the CFX96 Touch Real-Time
122 PCR Detection System (Bio-Rad, Redmond, WA) by using TaqMan Fast Advanced
123 Master Mix (Thermo Fisher Scientific, Waltham, MA) or on the QuantStudio 12K Flex
124 Real-Time PCR System (Thermo Fisher Scientific), with TaqMan Gene Expression
125 Master Mix (Thermo Fisher Scientific) and the following primer-probe sets. The mouse
126 18S rRNA primer probe set (Sigma Aldrich) was
127 5'-GCTCTTTCTCGATTCCGTGGG-3' for the forward primer,
128 5'-ATGCCAGAGTCTCGTTCGTTATC-3' for the reverse primer, and
129 FAM-CTCCACCAAC TAAGAACGGCCATGCACC-TAMRA for the probe, or
130 4319413E set (Thermo Fisher Scientific). The human GAPDH primer probe set was
131 4319413E set (Thermo Fisher Scientific). The IL-15 primer-probe sets were
132 Mm00434210_m1 (Thermo Fisher Scientific) for mouse cells and RT² qPCR Primer
133 Assay PPH00694B (QIAGEN) for human cells. The expression levels of 18S rRNA or

134 GAPDH were used as a standard.

135 **Flow cytometric analysis:** The monoclonal antibodies (mAbs) that were used were
136 PE-CF594-, PE-Cy7-, PerCP-Cy5.5-, APC-, BV510-, BV605-, or BV786-conjugated
137 anti-mouse CD4 (Cat#: 553051), CD8 (Cat#: 563152), CD44 (Cat#: 563114), CD62L
138 (Cat#: 560516), TCR β (Cat#: 560657), TIGIT (Cat#: 744215), and PD-1 (Cat#: 562523)
139 purchased from BD Pharmingen (San Diego, CA). The dead cells were excluded from
140 the analyses, based on the positivity of the Fixable viability stain 780 from BD
141 Pharmingen. Cells were analyzed by using a FACS Aria III (BD Bioscience, Tokyo,
142 Japan) and FlowJo software (BD Biosciences).

143 **Statistical analysis:** Dunnett's multiple comparison test was used to compare *in vitro*
144 IL-15 expression, and Mann-Whitney U tests were used to compare other data. The
145 Kaplan–Meier product limit method was used to obtain survival probability and the
146 log-rank test was applied to compare the survival curves. Cumulative incidences of
147 leukemia or GVHD death in each experimental group were compared by using the
148 Gray's test. Analyses were performed by using GraphPad Prism 8 software (GraphPad
149 Software, San Diego, CA) and EZR (Saitama Medical Center, Jichi Medical University,
150 Japan), the graphic user interface for R (The R Foundation for Statistical Computing,
151 Vienna, Austria)¹⁶. Results with $P < 0.05$ were statistically significant.

153 **Results**

154 *Gilteritinib promotes IL-15 production from FLT3-ITD⁺ leukemia*

155 First, we tested whether gilteritinib could stimulate FLT3-ITD-expressing
156 leukemia cells to produce IL-15. Ba/F3-FLT3-ITD cells and human myeloid leukemia
157 cells harboring FLT3-ITD (MOLM-13) were cultured in the presence or absence of
158 gilteritinib, quizartinib, or sorafenib for 24 hrs. All three reagents significantly
159 upregulated IL-15 expression both in Ba/F3-FLT3-ITD and MOLM/13 cells in a
160 partially dose-dependent manner (**Figure 1, A and B**). To confirm these findings *in vivo*,
161 lethally irradiated B6C3F1 mice were transplanted with 5×10^6 TCD-BM cells and $1 \times$
162 10^6 purified T cells from allogeneic B6 donors combined with 1×10^5 YFP-labeled
163 Ba/F3-FLT3-ITD cells. Gilteritinib at 10 mg/kg/day or vehicle was orally administered
164 from day +5 to day +8 after allo-SCT. The Q-PCR analysis showed a significant
165 upregulation of IL-15 in Ba/F3-ITD cells that were purified from the spleen and bone
166 marrow of the gilteritinib-treated mice on day +8 (**Figure 1, C and D**). The flow
167 cytometric analysis showed that gilteritinib significantly reduced the co-inhibitory
168 receptors, PD-1 and TIGIT on donor CD8⁺ T cells in the spleen, thus suggesting that
169 IL-15 production induced by gilteritinib inhibited T-cell exhaustion after allo-SCT
170 (**Figure 1, E to G**). To evaluate allospecific donor CTL responses, vehicle- or

171 gilteritinib-treated recipients of TCD-BM plus T cells were i.v. injected with
172 CTV-labeled untransfected Ba/F3 (allogeneic) or C57BL/6-derived C1498 (syngeneic)
173 cells on day +8 after allo-SCT, and CTV⁺ leukemia cells were enumerated in the bone
174 marrow 2 hrs later. We found that untransfected Ba/F3 cells were significantly reduced
175 in gilteritinib-treated mice compared to vehicle treated recipients, while the number of
176 donor-derived C1498 were comparable in vehicle- and gilteritinib-treated recipients,
177 indicating that gilteritinib enhanced allospecific donor CTL responses (**Figure 1, H and**
178 **I**).

179

180 *Gilteritinib enhances graft-versus-leukemia effects against FLT3-ITD-mutant*
181 *leukemia*

182 Next, we tested whether gilteritinib could enhance GVL effects against
183 Ba/F3-FLT3-ITD cells after allo-SCT. Lethally irradiated B6C3F1 mice were
184 transplanted with 5×10^6 TCD-BM cells either alone or in combination with 1×10^6
185 purified T cells from allogeneic B6 donors. Recipient mice were transferred with $5 \times$
186 10^4 Ba/F3-FLT3-ITD-luc⁺ cells on day 0, and gilteritinib was administered from day +5
187 to day +14 after allo-SCT. The BLI demonstrated that gilteritinib had only modest
188 anti-leukemic effects in the recipients of TCD-BM; all vehicle-treated and

189 gilteritinib-treated recipients of TCD-BM died with leukemia by day +15 and day +22,
190 respectively (**Figure 2, A and B**). Leukemia death was significantly suppressed in the
191 recipients of T cells compared to TCD-BM controls, thus indicating GVL effects.
192 However, all the mice succumbed to leukemia or GVHD by day +46 (**Figure 2, A and**
193 **F**). In GVHD mice, short-term administration of gilteritinib significantly suppressed the
194 expansion of Ba/F3-FLT3-ITD-luc⁺ cells and reduced leukemia death after T-cell
195 replete allo-SCT; 12 out of 15 vehicle-treated recipients and 8 out of 15
196 gilteritinib-treated recipients after T-cell replete allo-SCT succumbed to leukemia
197 (**Figure 2A**). Cumulative incidence of leukemia death in gilteritinib-treated recipients
198 was 60% at day +80 and significantly lower than that in vehicle-treated allogeneic
199 recipients (**Figure 2B**). Importantly, gilteritinib did not impact the morbidity or
200 mortality of GVHD, thus resulting in significantly prolonged overall survival in the
201 recipients of T cells (**Figure 2, C to F**). Taken together, short-term gilteritinib
202 administration enhanced GVL effects against FLT3-ITD⁺ leukemia without
203 exacerbating GVHD.

204

205 **Discussion**

206 Emerging evidence has suggested that FLT3 inhibitor is more effective in the
207 posttransplant setting than in the nontransplant setting¹⁷. The first-generation FLT3 and
208 multi-kinase inhibitor, sorafenib has been shown to induce more long-lasting remission
209 in patients after allo-SCT than in patients relapsing after chemotherapy¹⁸⁻²⁰. Recent
210 randomized trials have demonstrated that sorafenib maintenance after allo-SCT
211 significantly reduced the risks of relapse and death of FLT3-ITD positive AML^{21, 22}.
212 These findings suggest that FLT3 inhibitors can enhance GVL effects in addition to their
213 direct cytotoxic effects on FLT3-ITD-mutated AML cells. In preclinical models,
214 sorafenib activates IRF7 by downregulating activating transcription factor 4 (ATF4),
215 which enhances IL-15 production from leukemia cells⁵. IL-15 from leukemia cells has
216 been shown to expand CD8⁺CD107a⁺IFN- γ ⁺ donor cytotoxic T cells with increased
217 Bcl-2 and reduced PD-1 expression levels, which enhanced GVL effects against
218 FLT3-ITD-positive leukemia⁵. Recently, it has been reported that leukemia-derived
219 lactic acid impaired metabolic activity of T cells inducing dysfunctional T cells²³.
220 Because metabolic reprogramming is also associated with T cell exhaustion, it is
221 intriguing to evaluate if FLT3 inhibitors could modulate production of lactic acid in
222 leukemia cells in the future studies²⁴.

223

224 Despite the findings that support the GVL-potentiating effects of sorafenib, the
225 impacts of selective FLT3 inhibitors such as gilteritinib on GVL effects have not been
226 well studied. We herein demonstrated that the administration of gilteritinib after
227 allo-SCT enhances IL-15 production from mouse FLT3-ITD-positive leukemia,
228 potentiates CTL responses of donor T cells, and promotes GVL effects after allo-SCT.
229 Long-term suppression of leukemia growth was observed in some of the
230 gilteritinib-treated T-cell-replete recipients, thus suggesting that gilteritinib contributed
231 to an immune-mediated cure of FLT3-ITD-positive leukemia after allo-HCT. Although
232 we haven't tested if other selective FLT3 inhibitors such as quizartinib affect donor
233 T-cell profiles or GVL effects after allo-SCT, enhanced IL-15 expression in
234 quizartinib-treated leukemia cells suggests that FLT3 inhibitors in general can mitigate
235 exhaustion of donor T cells and enhance GVL effects after allo-SCT.

236

237 IL-15 production from leukemia cells significantly reduces T-cell expression of
238 PD-1, which is a critical molecule for tolerance induction after allo-SCT^{25, 26}, which
239 raises the concern that FLT3-ITD inhibition can exaggerate GVHD. A randomized trial
240 showed that maintenance therapy with sorafenib after allo-SCT can reduce the risk of

241 relapse without increasing acute or chronic GVHD²², whereas there was a trend towards
242 increased GVHD in another randomized trial²¹. It is worth noting that gilteritinib did not
243 impact the clinical GVHD scores, body weight changes, or mortality of GVHD in our
244 model, even though GVHD was more severe with shorter survival in our model,
245 compared to the previous sorafenib study, in which the delayed infusion of donor T cells
246 after allo-SCT mitigated GVHD, as has been previously shown^{5, 27, 28}. It has been
247 suggested that sorafenib-treated leukemia cells produce both IL-15 and IL-15Ra and can
248 trans-present the IL-15/IL-15Ra complex to donor T cells²⁹. Thus, FLT3-ITD inhibition
249 may activate donor T cells localizing around leukemia cells, without activating T cells
250 in the GVHD target organs. Furthermore, FLT3-ITD inhibitors likely activate immunity
251 only in the presence of leukemia cells, because they require IL-15 production from
252 leukemia cells. This nature of action of FLT3-ITD inhibitors makes FLT3-ITD
253 inhibition after allo-SCT safer and enables us to elicit just enough immunity to eradicate
254 leukemia cells.

255

256 It is important to assess whether gilteritinib reduced leukemia death by
257 enhancing GVL effects or solely by direct anti-leukemia effects of FLT3 inhibition. We
258 consider gilteritinib enhanced GVL effects for following reasons. First, BLI analyses

259 demonstrated that leukemia cells started to expand during gilteritinib-treatment in the
260 recipients transplanted with TCD-BM alone (Figure 2A, day12), indicating that
261 gilteritinib alone is not enough to reject Ba/F3-FLT3-ITD cells after T-cell deplete
262 allo-SCT. Second, *in vivo* CTL assay demonstrated that gilteritinib significantly
263 enhanced allospecific CTL responses against FLT3-ITD-untransfected Ba/F3. Therefore,
264 we concluded that gilteritinib reduced leukemia death at least partially by enhancing
265 GVL effects. We used short-term administration of gilteritinib to minimize the direct
266 anti-leukemia effect against Ba/F3-FLT3-ITD.

267

268 In summary, short-term gilteritinib administration after allo-SCT promotes
269 IL-15 production from FLT3-ITD-positive leukemia cells and enhances GVL effects
270 without affecting GVHD severity. Our data showing that gilteritinib enhanced GVL
271 effects support the use of gilteritinib as a maintenance. However, since gilteritinib
272 effects require residual AML cells, it is unclear if maintenance is effective in the
273 recipients with minimal amount of residual AML cells⁵. Thus, the safety and efficacy of
274 gilteritinib administration after clinical allo-SCT need to be studied in future and
275 ongoing clinical trials.

276

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284

285 **Author contributions**

286 D.H. and T.T. developed the conceptual framework of the study, designed the
287 experiments, analyzed the data and wrote the paper. Z.Z and Y.H. conducted
288 experiments, analyzed data, and wrote the paper. H.S., R.K., X.C, K.Y., T.S., T.K., H.T.,
289 T.I., T.A., and H.O. conducted experiments. M.N. supervised experiments.

290

291 **Conflict of Interest**

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430 **Figure Legends**

431 ***Figure 1. FLT3-ITD inhibitors promote IL-15 expression in Ba/F3-FLT3-ITD cells***

432 **(A,B)** Ba/F3-FLT3-ITD **(A)** or MOLM-13 **(B)** cells were cultured in the presence or

433 absence of gilteritinib or quizartinib at concentrations of 1.0, 10, or 100 nM or sorafenib

434 at concentrations of 0.1, 1.0, or 10 μ M for 24 hrs. RNA extracted from cells was

435 subjected to qPCR targeting IL-15. The expression levels of IL-15 were normalized to

436 18S rRNA **(A)** or GAPDH **(B)** expression levels. Data were collected in triplicate and

437 are shown as the means \pm SDs. **(C-G)** Lethally irradiated B6C3F1 mice were

438 transplanted with 5×10^6 TCD-BM cells and 1×10^6 purified T cells from allogeneic B6

439 donors combined with 1×10^5 Ba/F3-FLT3-ITD cells, followed by oral administrations

440 of 10 mg/kg/day gilteritinib or vehicle from day +5 to day +8 after allo-SCT. **(C,D)**

441 Relative expression levels of IL-15 in Ba/F3-FLT3-ITD cells purified from the spleen

442 **(C)** and BM **(D)** on day +8 after allo-SCT (n = 14/group). Data from three independent

443 experiments are combined and shown as the means \pm SEs. **(D-F)** Flow cytometric

444 analysis of spleens was performed on day +8 after allo-SCT. Histograms **(E)** and mean

445 fluorescence intensities of PD-1 **(F)** and TIGIT **(G)** on donor

446 TCR β^+ CD8 $^+$ CD62L $^-$ CD44 $^+$ cells. Data from two independent experiments are

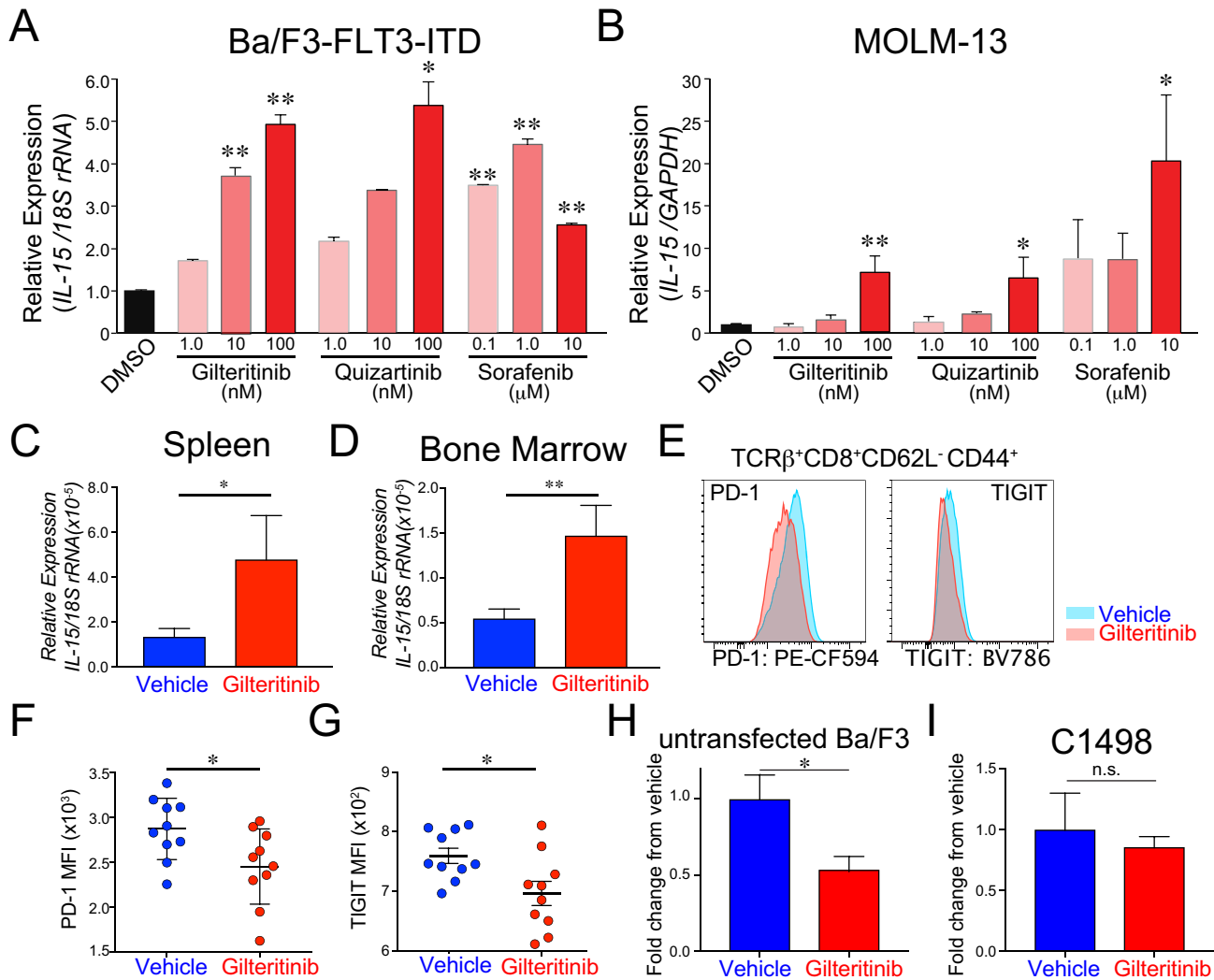
447 combined and shown as the means \pm SEs (n = 10/group). **(H,I)** Vehicle- or

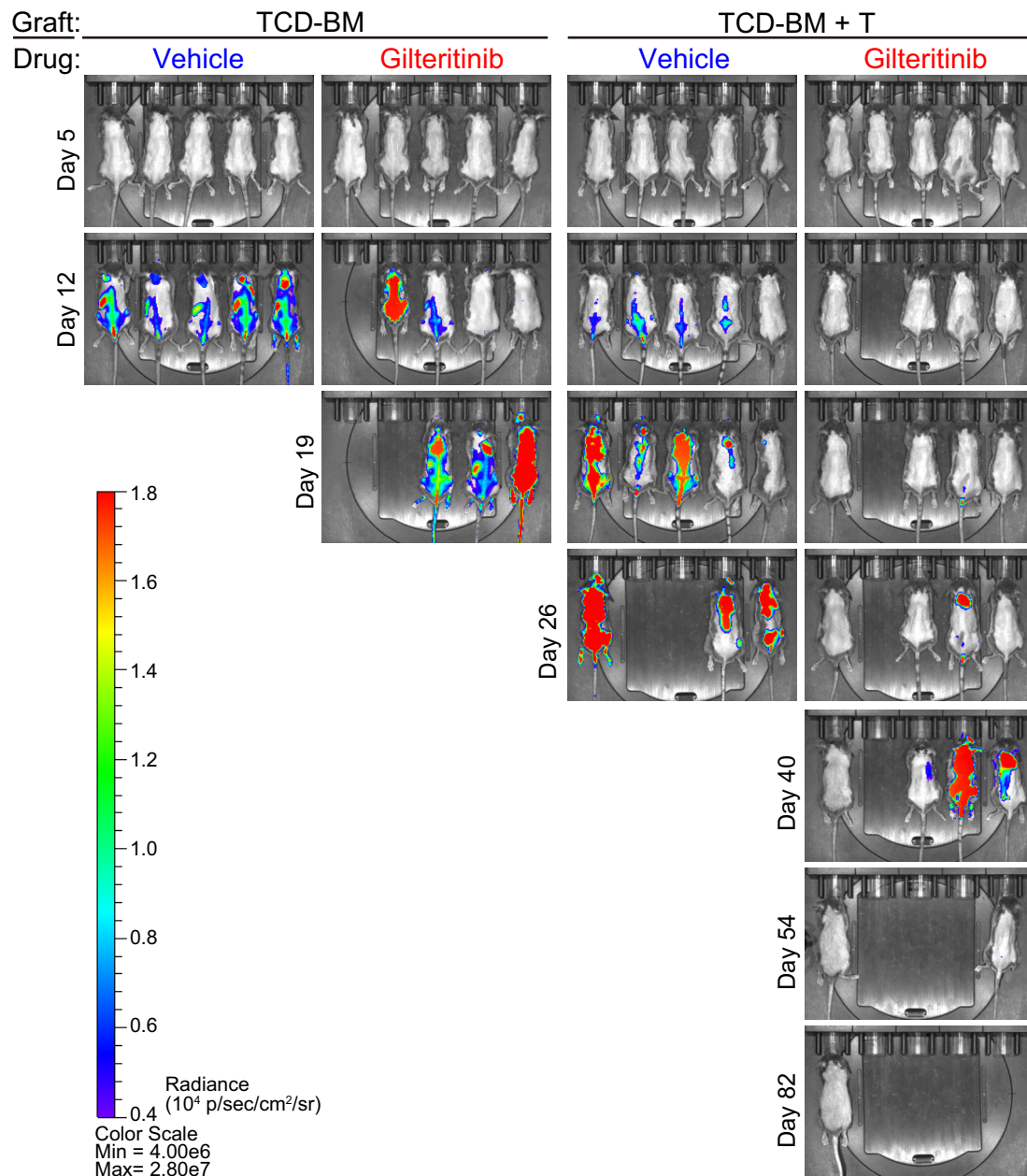
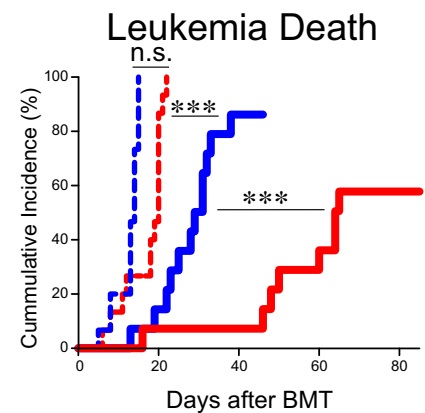
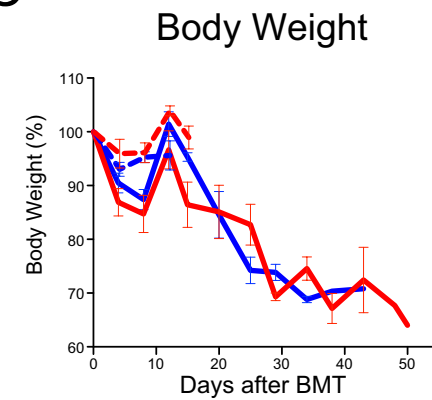
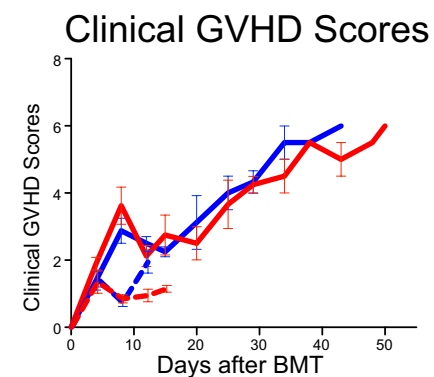
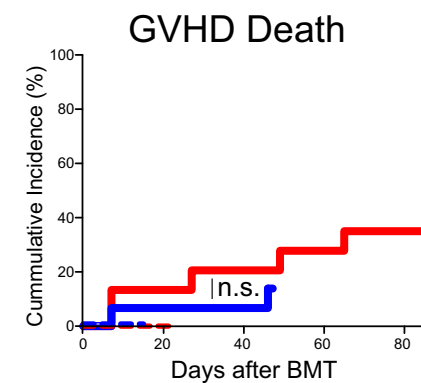
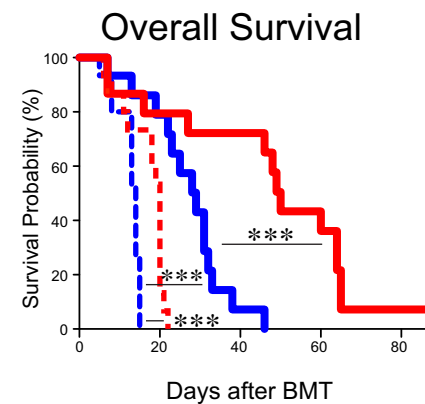
448 gilteritinib-treated recipients of TCD-BM plus T cells were i.v. injected with 3×10^6
449 CTV-labeled untransfected Ba/F3 (C, n=7/group) or C1498 (D, n=5/group) cells on day
450 +8 after allo-SCT. CTV-labeled cells in the bone marrow were enumerated 2 hrs after
451 the injection. Data from two independent experiments are combined. *, $P < 0.05$; **, P
452 < 0.01 .

453

454 ***Figure 2. Gilteritinib enhances GVL effects against Ba/F3-FLT3-ITD-luc leukemia***

455 Lethally irradiated B6C3F1 mice were transplanted with 5×10^6 TCD-BM cells either
456 alone or in combination with 1×10^6 T cells from allogeneic B6 donors and transferred
457 with 5×10^4 Ba/F3-FLT3-ITD-luc⁺ cells on day 0. Recipients were orally administered
458 with either gilteritinib or vehicle from day +5 to +14 after allo-SCT. Representative
459 images of BLI (A), cumulative incidences of leukemia death (B), body weight (C,
460 means \pm SDs), clinical GVHD scores (D, means \pm SDs), cumulative incidences of
461 GVHD death (E), and overall survival (F) in the recipients of TCD-BM treated with
462 either vehicle or gilteritinib, and in the recipients of TCD-BM plus T cells treated with
463 either vehicle or gilteritinib (n=15/group). (A) Images from one of three similar
464 experiments are shown. (B-F) Data from three independent experiments were
465 combined.



A**B****C****D****E****F**

- TCD-BM + Vehicle
- TCD-BM + Gilteritinib
- TCD-BM + T + Vehicle
- TCD-BM + T + Gilteritinib