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The multi-kinase inhibitor Debio 0617B reduces maintenance and self-renewal of primary human AML CD34$^+$ stem/progenitor cells.

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Abbreviation list: AML, acute myelogenous leukemia; BM, bone marrow; CR, complete remission; FLT3, FMS-related tyrosine kinase 3; *FLT3-ITD*, FLT3-internal tandem duplication; RTKs, growth factor receptor tyrosine kinases; HSCs, hematopoietic stem cells; JAKs, Janus kinases; LSCs, leukemia stem cells; lin$^-$, lineage-negative; PK/PD, pharmacokinetics/pharmacodynamics; STAT; signal transducer and activator of transcription; TMA, tissue microarray; TFs, transcription factors; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor.

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Abstract

Acute myelogenous leukemia (AML) is initiated and maintained by leukemia stem cells (LSCs). LSCs are therapy-resistant, cause relapse and represent a major obstacle for the cure of AML. Resistance to therapy is often mediated by aberrant tyrosine kinase (TK) activation. These TKs primarily activate downstream signaling via STAT3/STAT5. In this study, we analyzed the potential to therapeutically target aberrant TK signaling and to eliminate LSCs via the multi-TK inhibitor Debio 0617B. Debio 0617B has a unique profile targeting key kinases upstream of STAT3/STAT5 signaling such as JAK, SRC, ABL and class III/V receptor TKs. We demonstrate that expression of phospho-STAT3 (pSTAT3) in AML blasts is an independent prognostic factor for overall survival. Furthermore, phospho-STAT5 (pSTAT5) signaling is increased in primary $CD34^+$ AML stem/progenitors. STAT3/STAT5 activation depends on tyrosine phosphorylation, mediated by several upstream TKs. Inhibition of single upstream TKs did not eliminate LSCs. In contrast, the multi-TK inhibitor Debio 0617B reduced maintenance and self-renewal of primary human AML $CD34^+$ stem/progenitor cells *in vitro* and in xenotransplantation experiments resulting in long-term elimination of human LSCs and leukemia. Therefore, inhibition of multiple TKs upstream of STAT3/5 may result in sustained therapeutic efficacy of targeted therapy in AML and prevent relapses.

Introduction

Acute myelogenous leukemia (AML) originates from oncogene-transformed hematopoietic stem/progenitor cells known as leukemia stem cells (LSCs)(1). LSCs self-renew by symmetric cell division or divide asymmetrically into a stem cell and into differentiated cells (2). Phenotypically human LSCs in AML are a very heterogeneous population (3). Therefore, LSCs are primarily characterized by their function and capacity to induce leukemia in xenotransplantation experiments (1,4,5). From a clinical perspective, LSCs are of fundamental interest in the therapy of AML as they are resistant to existing therapeutic interventions resulting in high rates of relapse (6,7). As a consequence, LSC numbers or LSC gene signatures in blasts are negative predictors for survival (8,9).

AML pathogenesis is based on serial acquisition and accumulation of various mutations in transcription factors (TFs) as well as growth factor receptor tyrosine kinases (RTKs) such as FMS-related tyrosine kinase 3 (FLT3) (10,11). Signal transducer and activator of transcription (STAT) proteins are TFs involved in cytokine signaling. STATs are activated through tyrosine phosphorylation, typically through cytokine receptor-associated Janus kinases (JAKs), cytoplasmic tyrosine kinases (TKs; SRC and ABL families of kinases) or subsets of RTKs (12). Cytoplasmic SRC, JAK and ABL TKs act upstream of STAT family of TFs (12,13). The STAT3/STAT5 pathway is crucial for oncogenesis in several cancer entities including leukemia (14-17). An activating mutation in JAK2 (*JAK2V617F*) was identified in patients with myeloproliferative neoplasms (18). Constitutive phosphorylation of JAK2, STAT3 and/or STAT5 has been reported in AML blasts and is associated with a shorter time to relapse and poor outcome (19-22). Treatment of primary human AML cells with JAK2 and FLT3 inhibitors has resulted in reduced proliferation (23). Recently, it has been demonstrated that JAK/STAT signaling is increased in $CD34^+$ AML LSCs compared to normal cord blood or peripheral blood stem cells, suggesting that JAK/STAT signaling supports AML LSC growth and survival (24). Furthermore, pSTAT5 signaling is increased and essential for maintenance of leukemic stem/progenitor cells (25).

Therefore, targeting key TKs upstream of pSTAT3/pSTAT5 may be a promising strategy to treat AML. A phase 2 study with the JAK2 inhibitor Ruxolitinib in relapsed/refractory AML patients showed partial clinical efficacy with complete remission (CR) in 3/38 patients (26). However, in all patients leukemia relapsed after a short time period, indicating that inhibiting a single upstream TK may not be sufficient to eliminate LSCs. We therefore analyzed the potential of combined inhibition of several key TKs upstream of STAT3/STAT5 to treat AML. The multi-TK inhibitor (TKI), Debio 0617B, has a unique

profile targeting key kinases upstream of STAT3/STAT5, including JAK, SRC, ABL and a subset of RTKs, class III (FLT3, c-KIT, CSF1R, PDGFRα and PDGFRβ) and V (VEGFR1-3) RTKs (27), which are also known to play a role in STAT3 activation (15). So far, Debio 0617B has documented efficacy in STAT3-driven solid tumors *in vitro* and *in vivo* (27).

In this study, we demonstrate that pSTAT5 signaling is increased in primary AML $CD34^+$ stem/progenitor cells compared to bulk leukemia blasts and that nuclear pSTAT3 is an independent negative prognostic factor in AML. The multi-TKI Debio 0617B reduced maintenance and self-renewal of primary human $CD34^+$ AML stem/progenitor cells *in vitro* and in xenotransplantation experiments resulting in elimination of human LSCs, without affecting non-malignant hematopoietic stem/progenitor cells.

Materials and Methods

Cell lines. NB4, HT-93, and MV-4-11 cells were kindly provided by Prof. Jürg Schwaller, Department of Biomedicine, University Hospital Basel, Switzerland and Prof. Mario Tschan, Institute of Pathology, University of Bern, Switzerland, who obtained the cell lines from ATCC and DSMZ in 2015 and 2016. Consequently, no additional authentication was performed by the authors. All cell lines were tested mycoplasma-free. Cell lines were grown in FCS containing medium recommended by ATCC (https://www.lgcstandards-atcc.org/?geo_country=ch) with GlutaMAX^{TM} supplemented 100 U/mL penicillin, and 100 µg/mL of streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Media were routinely changed every 3 days.

Patients and healthy controls. Peripheral blood samples and BM aspirates were obtained from untreated AML patients at diagnosis at the University Hospital of Bern, Switzerland during 2014-2016. The complete list of patients' characteristics is summarized in Supplementary Table S1. Informed written consent was collected from all patients involved in the study. Study data were collected and managed using REDCap electronic data capture tools hosted at the Department of Clinical Research (28). BM aspirates from negative-staging biopsies from lymphoma patients ("healthy individuals") were used as controls. Analysis of samples was approved by the local ethical committee of the Canton of Bern, Switzerland.

Animals. Female NOD/LtSz-scid IL2Rγnull (NSG) mice and female NOD-SCID mice (NOD.CB17-Prkdcscid/J) were purchased from Charles River (Sulzfeld, Germany). Female nude rats (Crl: NIH-Foxn1 rnu) were purchased from Charles River (L'Abresles, France). 6-8 weeks old mice and rats were housed under specific pathogen-free conditions in individually ventilated cages with food and water ad libitum and were regularly monitored for pathogens. Mice were assigned randomly to the different treatment groups. Experiments were conducted and analyzed in a non-blinded fashion. Experiments were performed one to two times. Details on replicates are indicated in the figure legends. Power calculation was not performed to predetermine sample size.

Human xenograft experiments were approved by the local experimental animal committee of the Canton of Bern and performed according to Swiss laws for animal protection.

Experimental studies using the disseminated MOLM-13-Luc model were approved by the Ethics Committee for Animal Experimentation of Baden-Wuerttemberg. The experimental protocol was registered by the Regierungspräsidium Freiburg (G-11/11).

Experimental studies using MV-4-11 rat model were approved by the commission nationale de l'expérimentation animale de France.

AML patients and construction of tissue microarray (TMA)

Case selection and construction of the TMA used in the present study was described before (29). Briefly, bone marrow hematoxylin and eosin (H&E) slides were reviewed to confirm the diagnosis and to identify the most representative and blast-rich areas. 0.6mm punches of these areas were then removed out from the formalin-fixed, paraffin-embedded (FFPE) tissue block using a fully automated arraying device (TMA Grandmaster, 3D Histech), and a TMA block containing 222 cases was assembled.

Reagents for treatment. The compounds Debio 0617B (27), Sunitinib, Quizartinib (AC220), Dasatinib, Saracatinib and Ruxolitinib as well as the vehicles EAD-Lip1 and Kleptose HPB solution were provided by Debiopharm.

Kinome scan. KINOMEscan™ was performed as described in (30). Compound activity is described as % Ctrl. % Ctrl was calculated as

$$\left(\frac{\text{test compound signal} - \text{positive control signal}}{\text{negative control signal} - \text{positive control signal}}\right) \times 100$$

Determination of pSTAT3/pSTAT5 levels by flow-cytometry. AML cell lines were fixed in pre-warmed cytofix buffer (BD Biosciences) for 10 minutes at 37°C. After washing, cells were fixed in chilled Perm Buffer III (BD Biosciences) for 30 minutes at 4°C, followed by washing and incubation in FACS buffer supplemented with appropriately diluted BD Phosphoflow antibodies or isotype controls for pSTAT3 and pSTAT5.

For primary human blood an BM samples of AML patients and healthy donors, samples were stained in FACS buffer supplemented with appropriately diluted for CD45, CD34, CD38, CD90, lineage

markers (CD2, CD24, CD16, CD19, CD56, CD235, CD3e) prior to intracellular pSTAT3/pSTAT5 staining.

Liquid cultures. $1x10^5$ FACS-purified $CD34^+$ stem/progenitor cells from the peripheral blood and BM of AML patients and healthy donors (Supplementary Table S1) were cultured in StemSpan SFEM medium (Stem Cell Technologies) supplemented with human cytokines (StemSpan CC100; Stem Cell Technologies) in the presence or absence of different concentration of Debio 0617B (0.001, 0.01, 0.1, 1, 10 μM) or vehicle in 96-well plates at 37°C and 5% CO_2. Numbers of viable cells were determined by trypan blue staining after 7 days of culture.

Colony assays. Colony assays of FACS-purified $CD34^+$ stem/progenitor cells from the peripheral blood or BM of newly diagnosed AML patients or BM of "healthy donors" (Supplementary Table S1) were performed as previously described with slight modifications (31,32). Briefly, $1x10^3$ $CD34^+$ cells were cultured overnight in 96-well plates at 37°C and 5% CO_2 in StemSpan SFEM medium in the presence or absence of GI_{50} concentration of Debio 0617B (Supplementary Table S1) followed by plating into MethoCult H4435 Enriched medium (methylcellulose, STEMCELL Technologies). Colonies and cells were enumerated after 14 days (≥30 cells/colony).

For re-exposure experiments to Debio 0617B (Fig. 4H), total cells from primary AML colony assays were collected from the methylcellulose and $1x10^4$ of these cells were re-exposed to GI_{50} concentrations of Debio 0617B or vehicle in StemSpan SFEM medium overnight followed by re-plating in methylcellulose. Colonies and cells were enumerated after 14 days.

For serial re-plating experiments (Supplementary Fig. S7), 10^4 cells were collected from preceding colony assays and were re-plated in methylcellulose without further addition of compound or vehicle. Colonies were enumerated 14 days later.

MOLM-13-luc disseminated AML model. All experiments were performed using 5-6 weeks old female NSG mice. On day 22, the study was terminated, all animals sacrificed and a necropsy performed. The study consisted of 5 experimental groups (each with 10 NSG mice). On days -3 and -2, all mice were pretreated with 100 mg/kg cyclophosphamide (i.p.). On day 0, $1x10^6$ MOLM-13-Luc cells in 100 µL 0.9% NaCl were injected i.v.. Leukemia growth was measured by using *in vivo* bioluminescence imaging on days 7, 15 and 20. From day 8 on, animals were treated once daily p.o.

with 10, 15 and 20 mg/kg/day of Debio 0617B, respectively or Sunitinib (40 mg/kg), on a "5 days on 2 days off treatment regimen". The vehicle controls were Blanose CMC, Kleptose HPB and Polysorbate 80. Selected organs (lumbar spine, brain, lymph nodes and peritoneal carcinomatosis samples from fatty tissues) were collected and analyzed for bioluminescence.

Murine patient-derived xenograft AML model and secondary transplantations. All experiments were performed in 6-8 week old female NSG mice. NSG mice were sublethally irradiated (2.75 Gy) on the day before injection. $1x10^6$ FACS-purified $CD34^+$ AML cells from the peripheral blood or BM of newly diagnosed AML patients (patient P145 and 149, Supplementary Table S1) were injected intravenously into the tail vein. Starting two weeks after transplantation, mice were randomized and subjected to treatment with Debio 0617B (10 mg/kg), Ruxolitinib (20 mg/kg), Sunitinib (40 mg/kg), Saracatinib (25mg/kg), Quizartinib (AC220, 10 mg/kg) on a "5 days on 2 days off treatment regimen" until the end of the study unless specified differently. Mice were monitored daily for signs of morbidity (significant weight loss, failure to groom, abnormal gait and posture) and were euthanized when terminally ill. In addition, disease state in the BM was verified by flow-cytometry in all sacrificed mice. All mice that succumbed to the disease harbored an overt leukemia.
Secondary transplantations were performed by injecting $1x10^7$ whole BM cells from primary xenografted animals i.v. into sublethally irradiated (2.75 Gy) female NSG mice.

Statistical analysis. Statistical analysis was performed using GraphPad Prism® software v5.0 (GraphPad) and SPSS v24 (IBM). The details of the test carried out are indicated in the figure legends. Data are represented as mean ± s.e.m and distributed approximately normally. Data were analyzed using one-way ANOVA and Tukey's or Dunnett's multiple comparison test or student's t-test (two-tailed). Cut-off was determined using X-tile software a bioinformatics program for outcome-based biomarker cut-off optimization (33). Survival time differences were plotted using Kaplan-Meier curves and analyzed using the log-rank test. After verification of the proportional hazards assumption, multiple Cox regression analysis was carried out using the continuous pSTAT3 and pSTAT5 values. Effect size was determined using hazard ratios (HR) and 95% confidence intervals (CI), with a baseline hazard of 1.0 and a greater risk of death with HR>1.0. All p-values were two-sided and considered significant when $P < 0.05$.

Details on materials and methods used for antibody stainings, IHC, siRNA knockdown and organ-specific bioluminescence are listed in the Supplementary Methods and Materials.

Results

STAT5 signaling is increased in leukemia-inducing CD34$^+$ AML cells.

Activation of STAT3/STAT5 signaling has been reported in AML blasts and constitutive activity of STAT3 has been associated with short disease-free survival (19-21,34). To analyze the phosphorylation status and sub-cellular localization of STAT3 and STAT5, we performed tissue microarrays (TMA) on bone marrow (BM) biopsies from untreated AML patients (29). pSTAT3- and pSTAT5-positive nuclei were detected in all biopsies analyzed but the expression level was very heterogeneous **(Supplementary Fig. S1A,B)**. The frequency of pSTAT3 showed a significant positive correlation with the frequency of pSTAT5 **(Supplementary Fig. S1C)**. Importantly, a high frequency of pSTAT3-positive nuclei correlated with poor overall survival of AML patients **(Fig. 1a)**. A similar, though not statistically significant trend was observed for pSTAT5-positive nuclei **(Fig. 1B).** Patient's age and frequency of pSTAT3/pSTAT5-positive nuclei did not significantly differ between patients in the three cytogenetic/molecular risk groups (35) **(Supplementary Table S1 and Supplementary Fig. S1D-J)**. Multivariate analysis for pSTAT3- and pSTAT5-positive nuclei adjusted for risk group and age confirmed pSTAT3 as an independent prognostic marker **(Fig. 1C)**.

Next, we investigated whether blasts of newly diagnosed AML patients express pSTAT3/pSTAT5 by flow-cytometry. In accordance with previous findings (19,20), AML blasts ($CD45^{dim}SSC^{lo}$ cells (36)) expressed pSTAT3 and pSTAT5 **(Fig. 1D-G and Supplementary Table S1).** In contrast, $CD45^{dim}SSC^{lo}$ cells from BM aspirates from negative-staging biopsies from lymphoma patients (from here on termed healthy donors) which were used as controls did not express pSTAT3/pSTAT5 **(Fig. 1H,I).**

In the majority of AML subtypes, the disease-initiating LSCs express CD34 (1,3,5). Furthermore, LSCs are phenotypically discriminated from non-leukemic hematopoietic stem cells (HSCs) by the lack of CD90 expression (lineage-negative (lin^-) $CD90^-CD34^+$ cells) (37). Interestingly, LSCs expressed significantly more pSTAT5 compared to lin^-CD34^- leukemic progenitors **(Fig. 1G)**. In contrast, pSTAT3 expression was not significantly different between AML cell subsets **(Fig. 1E and Supplementary Table S1).** Importantly, only marginal pSTAT3 and pSTAT5 levels (ΔMFI: 7.7 ± 5.7 and 10.2 ± 5.8, respectively) could be detected in $lin^-CD34^+CD90^+$ HSCs from BM aspirates of healthy donors compared to $CD34^+$ stem/progenitor cells from AML patients **(Fig. 1J).** These results indicate that blasts and $CD34^+$ AML stem/progenitor cells of newly diagnosed AML patients express pSTAT3 and pSTAT5 and that pSTAT5 signaling is increased in AML LSCs.

Debio 0617B induces apoptosis in human AML cell lines.

To assess the therapeutic potential of multiple TK inhibition upstream of STAT3/STAT5 signaling, we made use of the novel SRC/JAK/ABL/Class III/V RTKs TKI Debio 0617B (N-(4-chloro-3-(2-((4-(methylcarbamoyl)phenyl)amino)-7,8-dihydropyrido[4,3-d]pyrimidin-6(5H)yl)phenyl)-4(trifluoromethyl)picolinamide (27); **Supplementary Fig. S2A**). Debio 0617B was rationally designed and synthesized on the capacity to bind the ATP binding pocket of c-SRC, JAK1, JAK2, and ABL (27). In order to address the degree of activity and specificity in inhibiting these kinases, we performed a cell-based kinome scan analyzing 467 different kinases. The results of the kinome scan indicated that Debio 0617B targets JAK, SRC, ABL kinases with a high degree of efficacy **(Supplementary Table S2)**. In addition, Debio 0617B targets the mutated and oncogenic form of FLT3 (FLT3-internal tandem duplication (ITD) and FLT3-D835Y), BCR-ABL, c-KIT, CSF1R, PDGFRα/β, which play a role in AML and class V RTKs (VEGFR1, 2, 3) RTKs, which play a role in the leukemic cells-stroma interaction. **(Supplementary Table S2, S3, Fig. 2A).**

Debio 0617B was compared to selective TKIs against c-SRC/ABL (Saracatinib (38), Dasatinib (39)), JAK1/2 (Ruxolitinib (40)), and RTK (Sunitinib (41), Quizartinib (AC220) (42)) **(Fig. 2A)**. First, we analyzed the expression of pSTAT3/pSTAT5 in a panel of AML cell lines by flow-cytometry and Western blot. All AML cell lines tested expressed either pSTAT3 or pSTAT5 or both **(Fig. 2B, Supplementary Fig. S2B, S2C Supplementary Table S4)**. The effect of Debio 0617B on cell growth of five different AML cell lines was tested in titrated concentrations *in vitro*. Debio 0617B was highly potent with a mean GI_{50} of 0.2474 μM **(Supplementary Table S4).** We assessed the reduction of pSTAT3/pSTAT5 as a surrogate biomarker for the activity of Debio 0617B and compared the induction of cell death by Debio 0617B to other TKIs specifically targeting only one or few of the STAT3/5 upstream TKs. The pSTAT3/pSTAT5-positive HT-93 cells were cultured in the presence or absence of Debio 0617B or comparable concentrations of TKIs **(Fig. 2B, Supplementary Table S4)**. Debio0617B treatment strongly reduced pSTAT3/pSTAT5 signaling **(Fig. 2C,D)**. Importantly, total STAT3 and STAT5 levels were not affected by the Debio 0617B treatment which is in line with previous findings obtained in solid tumor models (27). In addition, Debio 0617B increased apoptosis **(Fig. 2E)** and reduced cell numbers **(Fig. 2F)**. Importantly, Debio 617B was more potent than any other TKI, partially targeting the key kinases upstream of STAT3/STAT5 in each of these assays **(Fig. 2C-F)**. Similarly, Debio 0617B induced cell death in FLT3-ITD-expressing MOLM-13 AML cells (43) **(Supplementary**

Fig. S2). Therefore, we conclude that Debio 0617B effectively inhibits and eliminates AML cells in vitro.

To explore whether the effect of Debio 0617B on cell viability is mainly driven by blockade of STAT3/5 signaling, we silenced *STAT3* or *STAT5a* genes by siRNA in MOLM-13 cells resulting in reduced STAT3 and STAT5 levels of 80-90% **(Supplementary Fig. S3A)**. Silencing of active STAT3 and STAT5a in MOLM-13 AML cells reduced cell viability. Importantly, siRNA-treated cells were protected from Debio 0617B-induced cell death, indicating that inhibition of STAT3/5 signaling is a main pathway for the activity of Debio 0617B **(Supplementary Fig. S3B)**.

Debio 0617B reduces leukemia progression and prolongs survival in the MOLM-13 AML mouse model.

First we evaluated pharmacokinetics/pharmacodynamics (PK/PD) for Debio 0617B in the subcutaneous MV-4-11 (*FLT3-ITD*-positive) AML rat model that allows for harvesting sufficient tumor tissue and blood for analysis (44). Debio 0617B demonstrated a strong dose- and concentration-dependent inhibition of phospho-FLT3 (pFLT3; a direct target of Debio 0617B) in plasma and pSTAT5 (downstream target of Debio 0617B) in the tumor after oral administration **(Supplementary Fig. S4)**. To study the efficacy of targeting multiple key TKs upstream of STAT3/5 by Debio 0617B in an *in vivo* mouse model, we made use of a luciferase-expressing *FLT3-ITD*/STAT5-positive MOLM-13 AML cell line (MOLM-13-luc, **Supplementary Table S4**). Seven days after injection of MOLM-13-luc cells intravenously, NSG mice were randomized and subjected to treatment with different concentrations of Debio 0617B (10, 15 and 20 mg/kg) or Sunitinib (40 mg/kg) for two weeks **(Fig. 3A)**(41). Of note, Sunitinib was chosen as comparator as it demonstrated the best potency in inhibiting FLT3-ITD in an activity-based kinase profiling study **(Supplementary Table S5)**. Sunitinib and Debio 0617B treatment significantly reduced leukemia progression compared to vehicle treatment as indicated by reduced levels of whole body bioluminescence 21 days after tumor injection **(Fig. 3B,C)**. Analysis of organ-specific luminescence in peritoneum, spines, brain and lymph nodes revealed similar results **(Fig. 3D and Supplementary Fig. S5A-C).** Importantly, the multi-TKI Debio 0617B reduced leukemia progression more efficiently than the TKI Sunitinib, which has a different kinase inhibition profile compared to Debio 0617B **(Fig. 3B,C)**. Because leukemia growth did not significantly differ between mice treated with different concentrations of Debio 0617B **(Fig. 3B,C)**, we selected a concentration of 10 mg/kg for all subsequent experiments. Importantly, no drug-related toxicities as assessed by

clinical monitoring and body control have been observed in naïve mice treated with 10 mg/kg Debio 0617B over treatment periods up to 60 days **(Supplementary Fig. S6)**.

In a second independent experiment, Debio 0671B (10 mg/kg) was additionally compared to Ruxolitinib (20 mg/kg; as JAK inhibitor comparator (40)) and Saracatinib (25 mg/kg, as SCR/ABL inhibitor comparator (38))(**Fig. 3E-G**)**.** After 14 days of treatment only Debio 0617B and Sunitinib reduced leukemia progression **(Fig. 3F,G).** To determine whether Debio 0617B- and Sunitinib-treated mice eliminated the disease after two cycles of treatment, four animals from the Debio 0617B-treated group and all animals from the Sunitinib-treated group were kept alive after treatment discontinuation in order to monitor leukemia relapse/progression. Leukemia progressed in all animals upon drug discontinuation, indicating that the leukemia was not completely eliminated after 14 days of treatment. However, leukemia progression was significantly slower in animals treated with Debio 0617B **(Fig. 3G)**. Similar results were obtained in a MV-4-11 disseminated AML mouse model. These data indicate that Debio 0617B treatment efficiently inhibits and targets leukemia cells *in vivo*.

Debio 0617B reduces growth and survival of primary CD34$^+$ AML cells *in vitro*.

To investigate the therapeutic potential of Debio 0617B in primary human AML, Debio 0617B was tested in liquid cultures of isolated primary human CD34$^+$ AML stem/progenitor cells. In this context, Debio 0617B demonstrated to be highly potent with a mean GI_{50} of 0.147 µM **(Fig. 4A, Supplementary Table S1)**. Importantly, treatment of CD34$^+$ and CD34$^-$ BM cells from healthy donors (H)" revealed a mean GI_{50} of 0.5 µM for Debio 0617B **(Fig. 4A)**. In line with the results obtained with AML cell lines **(Fig. 2 and Supplementary Table S4)**, Debio 0617B treatment significantly decreased pSTAT3/pSTAT5 phosphorylation compared to vehicle treatment **(Fig. 4B,C)**. Importantly, Debio 0617B increased apoptosis **(Fig. 4D)** and reduced cell growth of human CD34$^+$ AML cells **(Fig. 4E)**.

As a possibility to functionally assess the effect of Debio 0617B on stem/progenitor cells, we analyzed colony formation capacity of CD34$^+$ AML cells in methylcellulose in the presence and absence of Debio 0617B. FACS-purified CD34$^+$ AML cells were incubated overnight in the presence of vehicle (V) and GI_{50} concentrations for Debio 0617B (G_{IC50}) as determined in liquid culture experiments **(Fig. 4A, Supplementary Table S1)** followed by plating in methylcellulose. Debio 0617B treatment significantly reduced colony numbers and cell numbers per colony of primary human CD34$^+$ AML stem/progenitor cells compared to vehicle treatment, suggesting efficient elimination of LSCs **(Fig. 4G)**. To further

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assess the reduction in LSCs after Debio 0617B treatment, we performed serial re-plating experiments *in vitro*. Colony-forming capacity of Debio 0617B-treated AML $CD34^+$ stem/progenitors was significantly reduced in all re-platings compared to vehicle-treated cells indicating that treatment with Debio 0617B, a multi-TK inhibitor, reduced AML $CD34^+$ stem/progenitor numbers and function *in vitro* **(Supplementary Fig. S7A)**. Importantly, colony-forming capacity of $lin^-CD34^+CD90^+$ stem/progenitor from healthy controls was only marginally affected in serial re-plating experiments **(Supplementary Fig. S7B)**.

LSCs in AML have been previously shown to be resistant against standard treatment of care (6). To address the question whether the remaining colony-forming cells were still sensitive to the applied treatment, surviving cells from primary colony assays were washed out of the methylcellulose and re-exposed to GI_{50} concentrations of Debio 0617B or vehicle overnight followed by re-plating in methylcellulose. After 14 days, re-exposure to Debio 0617B further decreased colony formation **(Fig. 4H)**. These experiments indicate that Debio 0617B eliminates $CD34^+$ AML progenitor cells *in vitro*.

Debio 0617B eradicates human LSCs in AML patient-derived xenografts.

The effect of different TKIs on AML progression *in vivo* was further evaluated in an AML patient-derived xenograft model. In human AML, LSCs are present in, the $CD34^+CD38^-$ and the $CD34^+CD38^+$ fraction (1,5). Therefore, FACS-purified $lin^-CD34^+CD90^-$ AML cells from patients 145 ($FLT3\text{-}ITD^{mut}$) and 149 (JAK2V617F) were transplanted into NSG mice. Two weeks later, mice were subjected to treatment with vehicle, Debio 0617B (10 mg/kg) and the narrow-spectrum TKIs Ruxolitinib (JAK inhibitor), Quizartinib (AC220, FLT3 inhibitor) and/or Sunitinib (large spectrum TKI) **(Fig. 5A)**. All narrow-spectrum TKI-treated mice which succumbed to the disease harbored an overt leukemia. Debio 0617B-treated mice survived significantly longer than narrow-spectrum TKI-treated AML mice with 9/10 mice surviving long-term **(Fig. 5B,C)**. These data indicate that LSCs were eradicated or successfully controlled in Debio 0617B-treated mice. Importantly, no-drug-related changes in body weight as parameter of toxicity have been observed **(Supplementary Fig. S8).**

To analyze whether LSCs had been eliminated in surviving Debio 0617B-treated mice, we analyzed peripheral blood, spleen or BM for the presence of residual human $CD45^+$ cells. No residual human $CD45^+$ cells were detected in any organ analyzed. In addition, BM cells from surviving animals did not form human $CD45^+$ colonies in methylcellulose.

In vivo only a few remaining LSCs may transmit the disease to secondary recipients. To further prove that the findings from FACS analysis and colony assays *in vitro* actually account for absence of LSCs *in vivo* and to functionally investigate whether LSCs persisted, we transferred 10^7 BM cells from surviving mice into sublethally irradiated NSG recipients. None of the secondary recipients developed an AML and all animals survived long-term without any sign of leukemia **(Fig. 5D,E)**. These results indicate that treatment with Debio 0617B eliminated the disease-initiating LSCs in a majority of animals.

Debio 0617B inhibits growth and self-renewal of human LSCs *in vivo*.

Finally, we analyzed the effect of Debio 0617B treatment on disease-initiating $CD34^+$ AML LSCs *in vivo*. $CD34^+$ AML cells from patient 145 (*FLT3-ITD*) were transplanted into NSG mice. Two weeks later, Debio 0617B, Ruxolitinib, Quizartinib (AC220) or Sunitinib were administered by gavage for two weeks. Debio 0617B treatment significantly reduced spleen size and cellularity **(Supplementary Fig. S9)** and reduced the percentage and absolute numbers of human AML cells in murine BM compared to controls **(Fig. 6A,B)**. Similar to the *in vitro* experiments **(Fig. 2)**, Debio 0167B significantly reduced pSTAT3 **(Fig. 6C)** and pSTAT5 **(Fig. 6D)** expression in $CD34^+$ AML cells *in vivo*. Treatment with Quizartinib (AC220) and Sunitinib efficiently reduced pSTAT5 but had little effect on pSTAT3 expression in primary AML cells *in vivo*. Ruxolitinib neither reduced pSTAT3 nor pSTAT5 *in vivo* in this setting. In addition, Debio 0617B induced the highest rate of apoptosis in $CD34^+$ AML cells *in vivo* **(Fig. 6E)**. Importantly, Debio 0617B treatment reduced $CD34^+$ AML LSCs **(Fig. 6F)** resulting in fewer colonies in methylcellulose when compared to controls **(Fig. 6G)**.

In AML only LSCs are able to propagate the disease *in vivo* (1,4,5). To further prove that the findings from FACS analysis and colony assays in vitro actually account for reduced LSC numbers in vivo, we secondarily transplanted BM cells from AML-bearing NSG mice of all treatment groups into sublethally irradiated NSG mice. All animals transplanted with BM from Debio 0617B-treated mice survived long-term, whereas mice from all single TK-treated groups died. This indicates that Debio 0617B indeed targets leukemia-initiating cells **(Fig. 6H)**.

Discussion

AML is a very heterogeneous myeloid neoplasm resulting from sequential acquisition of multiple molecular abnormalities. These include various mutations in TFs as well as growth factor RTKs. As a common downstream signaling event, activated TK lead to increased signaling via STAT3/STAT5 and increased cell proliferation, survival and disease progression. Therefore, constitutive STAT3 signaling correlates with decreased disease-free survival (21). In this study, we confirmed and extended these earlier results in a large TMA analysis by showing that increased expression of pSTAT3, but not pSTAT5, is an independent negative prognostic marker for overall survival in AML patients. These results suggest that targeting STAT signaling is a promising strategy to treat AML. Unfortunately, the TFs STAT3 and STAT5 are difficult to inhibit directly. As multiple TKs play a critical role in STAT3/STAT5 phosphorylation and activation (45), there is substantial interest to target TKs upstream of STAT3/STAT5.

FLT3-ITD is the most common molecular aberration in AML, activates primarily pSTAT5 signaling and confers poor prognosis (24,46). A variety of TKIs targeting FLT3 activity have been evaluated in clinical phase I/II studies in relapsed/refractory AML patients (47). However, clinical responses to narrow-spectrum TKIs are moderate and not long-lasting. Midostaurin as single agent induced a CR in 2/20 relapsed/refractory AML patients (48). In a phase II study, Midostaurin in combination with standard chemotherapy followed by post-remission maintenance therapy with Midostaurin resulted in high rates of CR (33/40). This concept was prospectively tested in a randomized phase III study (NCT00651261). Treatment of *FLT3-ITD*-positive AML patients with Midostaurin in combination with standard induction and consolidation chemotherapy resulted in a 23% improvement in OS (49). Although, Sunitinib has a documented effect against *FLT3-ITD*-positive AML cells lines *in vitro* and *in vivo* (50,51) its efficacy *in vivo* was very variable ranging from limited and transient effects to CR in 59% elderly patients with mutant FLT3 in a clinical phase II trial (52). In contrast, Sorafenib in combination with standard chemotherapy failed to improve event-free and overall survival in a randomized clinical phase II trials in 197 elderly AML patients (53). Similarly, Lestaurinib demonstrated limited clinical efficacy and non-durable responses in elderly AML patients and in *FLT3*-mutated patients with advanced AML (54-56).

The most potent FLT3 inhibitor is Quizartinib (AC220). Quizartinib demonstrated strong anti-leukemia efficacy in preclinical MV-4-11 xenograft models as indicated by rapid and complete tumor progression and long-term survival of the mice. In contrast, low-dose Sunitinib treatment showed poor anti-

leukemic activity in the same model (42). In a first clinical phase I trial in refractory/relapsed AML patients, Quizartinib demonstrated promising results with response rates (CR and PR) in 10/76 and 13/76 patients, respectively (57). In a subsequent phase II study, Quizartinib conferred response rates of 54% and 31% in *FLT3-ITD* and *FLT3-WT* AML patients, respectively, although rates of CR were low (58).

In contrast, targeting TKs other than FLT3 upstream of STAT3/STAT5 are less well studied. Different JAK2 inhibitors reduce viability of AML cell lines *in vitro* (59,60). However, clinical studies using the JAK2 inhibitor Ruxolitinib resulted in only modest anti-leukemic efficacy in relapsed/refractory AML patients (26).

In the present studies *in vitro* and in xenotransplantation experiments show that TKIs specific for a single/narrow-spectrum TK upstream of STAT3/STAT5 are not sufficient to eliminate leukemia cells long-term. Similar studies in solid tumor models have demonstrated compensatory activation of STAT3 by JAK family kinases following inhibition of SRC, indicating the necessity of a dual JAK/SRC blockade when targeting STAT3 (61). Importantly, we could show that combined targeting of SRC, JAK, ABL and Class III/V RTK families of kinases via the multi-TKI Debio 0617B more effectively reduced cell growth and survival of AML cell lines and primary AML cells *in vitro* and *in vivo*. Our findings are in line with recent studies indicating that combining individual FLT3 and JAK TKIs improve anti-leukemic activity. Furthermore, they confirm the finding of improved activity of the JAK2/FLT3 inhibitor Pacritinib (62,63).

Therapy-resistant LSCs are the major obstacle in the treatment of AML (6,7). We demonstrate here that primary AML stem cells express pSTAT3 and pSTAT5. Interestingly pSTAT5 but not pSTAT3 was expressed in $lin^-CD34^+CD90^-$ LSCs at higher levels than in more differentiated AML cells. Preclinical studies using TKIs targeting single TKs upstream of STAT3/STAT5 indicated that LSCs are affected by the treatment. Selective targeting of JAK signaling in AML by AZD1480, AZ960 or Ruxolitinib induced apoptosis and reduced colony formation and *in vivo* engraftment of $CD34^+$ stem/progenitor cells (24). Similarly, the SRC/ABL inhibitor Dasatinib has been shown to increase apoptosis and reduce growth of $CD34^+$ AML cells in colony and long-term culture initiating cell assays *ex vivo* (64,65). In contrast, targeting FLT3-ITD by treatment with Sunitinib only moderately affects colony forming capacity of $CD34^+/CD38^-$ AML stem/progenitor cells in methylcellulose (60). Quizartinib treatment has been shown to reduce disease burden and result in long-term survival in a PDX model with cells from a single AML M5 patient (*FLT3-ITD, IDH2-R140Q DNMT3A-R882H* and *NPM1*

mutations) (66). We therefore compared the effect of targeting single/narrow spectrum TKs versus targeting multiple TKs by Debio 0617 in LSCs in a *FLT3-ITD*-positive xenotransplantation model. Targeting JAK signaling by Ruxolitinib did not significantly reduce LSC numbers *in vivo*. Inhibition of FLT3-ITD by Sunitinib and Quizartinib reduced disease burden but failed to completely eliminate LSCs (Fig. 6) resulting in a marginal prolongation of survival (Fig. 5). Only the combined inhibition of TKs by Debio 0617B completely eliminated LSCs as tested in a very sensitive re-transplantation assay in irradiated NSG mice. This indicates that Debio 0617B as single agent is highly active and eliminates LSCs by combined inhibition of multiple key TKs whereas inhibiting a single pathway is sufficiently compensated by other up-stream TKs. This is in contrast to our findings with Debio 0617B in STAT3-driven solid tumors models, where potent and sustained tumor inhibition required combination with an EGFR inhibitor (27).

Importantly, as compared to other TKIs used in the clinical management of myeloid malignancies, our data suggest that continuous exposure to Debio 0617B is not required to control/eradicate LSCs.

However, inhibition of TKs upstream of STAT3/STAT5 also activates other compensatory signaling pathway. For example FLT3 TKI-resistant primary samples and cell lines trigger a constant activation PI3K/AKT/JAK signaling (67) and BM stromal cells induce compensatory ERK signaling in response to FLT3-ITD inhibition in AML cells (68). A recent study suggests that a combination of FLT3 with JAK inhibitors may overcome these resistance mechanisms (62). In addition, incomplete elimination of LSCs will lead to the acquisition of secondary resistance mutations (47), e.g. different studies reported the emergence of secondary FLT3 TK domain point mutations in response to FLT3 inhibitor therapy (69,70). Remarkably, in our study, even after exposure of AML mice to Debio 0617B for more up to 130 days, no resistant clones to Debio 0617B could be detected. Consequently, studying resistance mechanisms to Debio 0617B could not be assessed in this study. Overall, this data indicates that Debio 0617B rapidly and efficiently eliminates LSCs in the long-term before secondary resistance mechanisms develop. Importantly, Debio 0617B did not reduce the viability of normal hematopoietic stem- or progenitor cells nor led to toxicity in treated animals supporting the potential further development and validation of this approach for potential use and induction of long term responses in AML patients in the clinics. Consequently, inhibition of multiple TKs by Debio 0617B improves the therapeutic efficacy of targeted therapy in AML.

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Figure legends

Figure 1. pSTAT3 is an independent prognostic marker in AML and pSTAT5 is increased in CD34$^+$ AML cells. (A,B) Kaplan-Meier survival curves of the entire AML patient cohort for **(A)** pSTAT3 expression (n=136) and **(B)** pSTAT5 expression (n=134). AML cohorts are divided into 2 groups at the pSTAT3 and pSTAT5 cut-off of 17.0 % and 17.6% positive nuclei, respectively. **(C)** Multivariate analysis for pSTAT3 and pSTAT5 expression adjusted for risk group and age. **(D)** Histogram of pSTAT3 expression on AML blasts (CD45^{dim}SSC^{lo}) and **(E)** ΔMFIs of pSTAT3 on AML blasts and AML stem/progenitor subsets from blood and BM of newly diagnosed AML patients measured by flow-cytometry. **(F)** Histogram of pSTAT5 expression on AML blasts and **(G)** ΔMFIs of pSTAT5 on AML blasts and AML stem/progenitor subsets. For **(D and F)** one representative FACS plot of 18-20 is shown. Staining is indicated in red, isotype in blue. ΔMFI: MFI [staining] – MFI [isotype]. **(H)** pSTAT3 and **(I)** pSTAT5 expression on CD45^{dim}SSC^{lo} cells of healthy donors. One representative FACS plot of 4 (H5-H8) is shown. **(J)** ΔMFIs of pSTAT3 and pSTAT5 on lin$^-$CD90$^+$CD34$^+$ BM cells from healthy donors measured by flow-cytometry. Statistics: **(A,B)** log-rank test, **(C)** multiple Cox regression. **(E,G)** Student's t-test. * P <0.05. Statistically not significant differences with P >0.05 are not indicated. CI, confidence interval; HR, hazard ratio. See also Supplementary Fig. S1 and Supplementary Table S1.

Figure 2. Debio 0617 B induces apoptosis of AML cell lines by inhibition pSTAT3/5 signaling. **(A)** Schematic representation of TKIs acting upstream of pSTAT3/pSTAT5. **(B)** Expression of pSTAT3 (red line, left panel) and pSTAT5 (red line, right panel) vs. the respective isotype controls (blue lines) in the AML cell line HT-93 as analyzed by flow-cytometry. **(C-F)** 10^5 HT-93 cells were cultured in triplicates with Quizartinib (AC220, 1.43 μM), Sunitinib (1.87 μM), Ruxolitinib (2.47 μM), Dasatinib (2.04 μM) and Debio 0617B (1.61 μM) or vehicle. **(C)** ΔMFI of pSTAT3 and **(D)** of pSTAT5 and **(E)** Annexin V^+ cells were determined by flow-cytometry after 72h. ΔMFI: MFI [staining] – MFI [isotype]. **(F)** Live cell numbers. One representative experiments of two is shown. Data are shown as mean±s.e.m. Statistics: **(C-F)**: one-way ANOVA. * *P* <0.05, ** *P* <0.01, *** *P* <0.001. Statistically not significant differences with *P* >0.05 are not indicated. See also Supplementary Tables S2 and S3 and supplementary Fig. S2.

Figure 3: Debio 0617B reduces leukemia progression and prolongs survival in MOLM-13-luc AML model. **(A)** Experimental setup. NSG mice were pre-conditioned with two intraperitoneal injections of 100 mg/kg cyclophosphamide 2 and 3 days prior to the intravenous administration of 10^5 luciferase-transduced MOLM-13 cells (MOLM-13-luc). **(A-D)** At day 8 after tumor injection, animals were randomized and subjected to treatment with vehicle, 40 mg/kg Sunitinib and 10, 15 or 20 mg/kg Debio 0617B by gavage for two weeks in a "5 days on 2 days off regimen" schedule (n=10 mice/group). One day after the last treatment (day 20 after tumor inoculation) **(B,C)** whole body bioluminescence and **(D)** *ex vivo* organ bioluminescence in the peritoneum were analyzed as measure for leukemia load. **(E)** Experimental setup as described in **(A)**. **(E-G)** Animals were randomized and subjected to treatment with vehicle (n=7), 40 mg/kg Sunitinib (n=4), 20 mg/kg Ruxolitinib (n=6), 25 mg/kg Saracatinib (n=6) and 10 mg/kg Debio 0617B (n=7) by gavage for two cycles (10 injections) 11 days after tumor inoculation. **(F,G)** Whole body bioluminescence was analyzed one day after the last treatment. Data are shown as mean±s.e.m. Statistics: **(C,D and G)** one-way ANOVA. * *P* <0.05, ** *P* <0.01, *** *P* <0.001. Statistically not significant differences with *P* >0.05 are not indicated. See also Supplementary Fig. S4.

Figure 4: Debio 0617B reduces survival and colony formation capacity of primary $CD34^+$ AML cells *in vitro*. **(A)** GI_{50} values of Debio 0617B determined in liquid cultures. $1x10^5$ FACS-purified

$CD34^+$ AML cells from the blood and BM of different newly diagnosed AML patients (P144-152), one relapsed AML Patient (P143) and $CD34^+$ BM cells from healthy donors were cultured in liquid cultures in duplicates in the presence of different concentration of Debio 0617B (0.001, 0.01, 0.1, 1, 10 μM) or vehicle. Live cell numbers were determined after 7 days of culture using trypan blue exclusion. **(B-E)** 10^5 FACS-purified $CD34^+$ AML cells from patients P144, P145 and P150 were cultured in the presence in of 1 μM Debio 0617B or vehicle in triplicates. **(B)** ΔMFI of pSTAT3, **(C)** ΔMFI of pSTAT5 and **(D)** Annexin V^+ cells were determined by flow-cytometry after 72h. ΔMFI: MFI [staining] – MFI [isotype]. **(E)** Live cell numbers. **(F-G)** $1x10^3$ FACS-purified $CD34^+$ AML cells from patients P1-P10 were cultured in duplicates in the presence of the individual GI_{50} concentrations of Debio 0617B for each patient as determined in liquid culture experiments **(see Supplementary Table S1)** or vehicle overnight followed by plating in methylcellulose. **(F)** Colonies per well and **(G)** cells per colony after two weeks. Cells from primary colonies of patients P144, P145, P146 and P150 were re-exposed to GI_{50} concentrations of Debio 0617B or vehicle overnight in duplicates followed by re-plating in methylcellulose. **(H)** Change in colony formation compared to 1^{st} plating (%). Data are shown as mean±s.e.m. Statistics: **(A-H)** Student's t-test. ** *P* <0.01, *** *P* <0.001. Statistically not significant differences with *P* >0.05 are not indicated. See also Supplementary Table S1.

Figure 5: Debio 0617B eradicates primary CD34$^+$ AML LSCs *in vivo*. (A) Experimental setup. **(B,C)** Kaplan-Meier survival curve of sublethally irradiated (2.75 Gy) NSG mice xenotransplanted intravenously with 1x10^6 FACS-purified lin$^-$CD34$^+$ AML from blood of patient **(B)** P149 and **(C)** P145. After engraftment of AML cells (2 weeks after transplantation), mice were treated with **(B)** 10 mg/kg Debio 0617B, 20 mg/kg Ruxolitinib, 10 mg/kg Quizartinib (AC220) or vehicle by gavage in a "5 days on 2 days off regimen" until the end of the study (n=5 mice/group). **(C)** Mice were treated as described in **(B)** and with 40 mg/kg Sunitinib. **(D,E)** Survival of sublethally irradiated (2.75 Gy) secondary NSG recipients that received 1x10^7 whole-BM (WBM) cells from Debio 0617B-treated primary AML mice (n=5) that were alive **(D)** 150 or **(E)** 300 days after primary transplantation. Numbers of mice that succumbed to AML of total transplanted mice are indicated. Data are shown as mean±s.e.m. Statistics: log-rank test. *** P <0.001. Statistically not significant differences with P >0.05 are not indicated.

Figure 6. Debio 0617B inhibits growth and self-renewal of human LSCs *in vivo*. (a-h) $1x10^6$ FACS-purified lin^-CD34^+ AML from the blood of patient P145 were injected into sublethally irradiated (2.75 Gy) NSG mice. Upon engraftment of cells (2 weeks after transplantation), mice were treated with 10 mg/kg Debio 0617B, 20 mg/kg Ruxolitinib, 10 mg/kg Quizartinib (AC220), 40 mg/kg Sunitinib or vehicle by gavage in a "5 days on 2 days off regimen" for a total of 10 injections (n=5 mice/group). **(A)** Frequency, **(B)** total number of human $CD45^+$ AML cells, **(C)** MFI of pSTAT3 and **(D)** pSTAT5 of $lin^-CD34^+CD90^-$ cells, **(E)** Annexin V^+ positive and **(F)** total $lin^-CD34^+CD90^-$ cells were determined by flow cytometry 24 days after transplantation. **(G)** Human colony forming capacity per mouse. **(H)** Survival of sublethally irradiated (2.75 Gy) secondary NSG recipients that received $1x10^7$ WBM cells from primary AML mice (n=5 mice/group). Data are shown as mean±s.e.m. Statistics: **(A-G)** one-way ANOVA, **(H)** log-rank test. * P <0.05, ** P <0.01, *** P <0.001. Statistically not significant differences with P >0.05 are not indicated.

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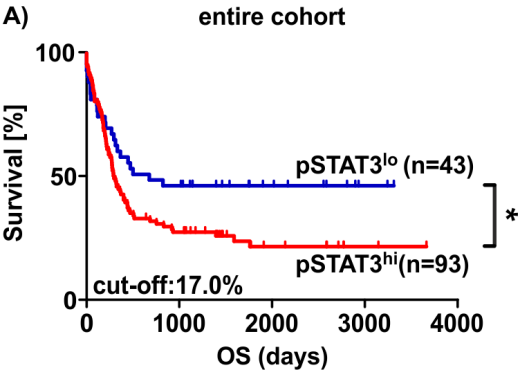
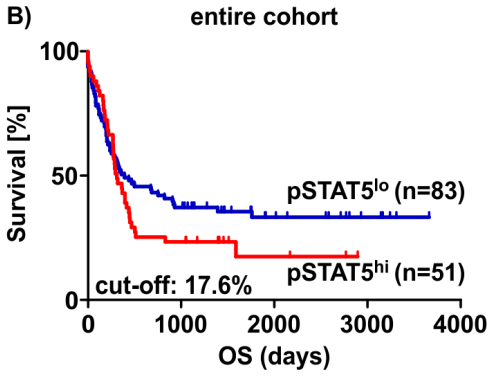
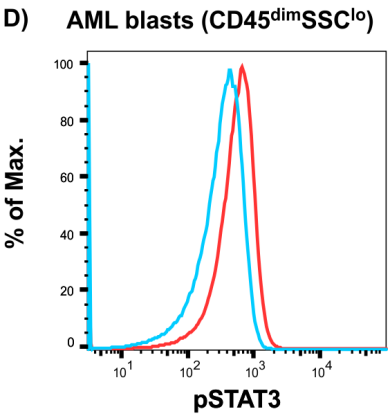
Figure 1

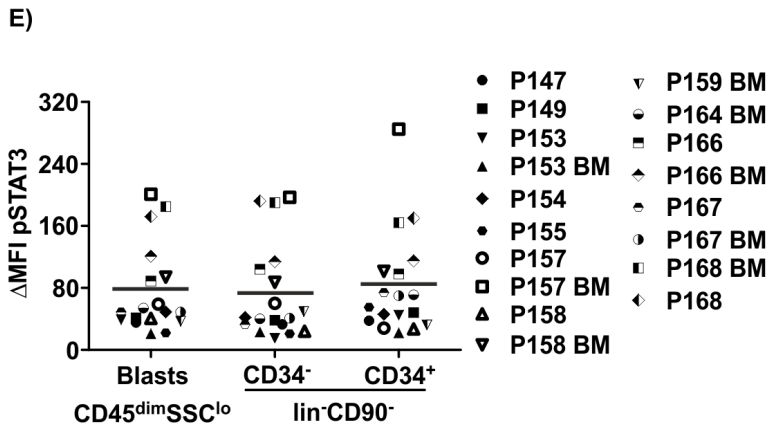


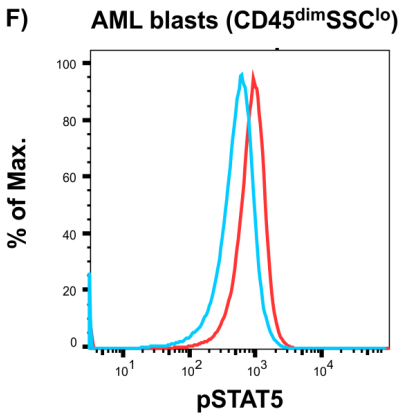


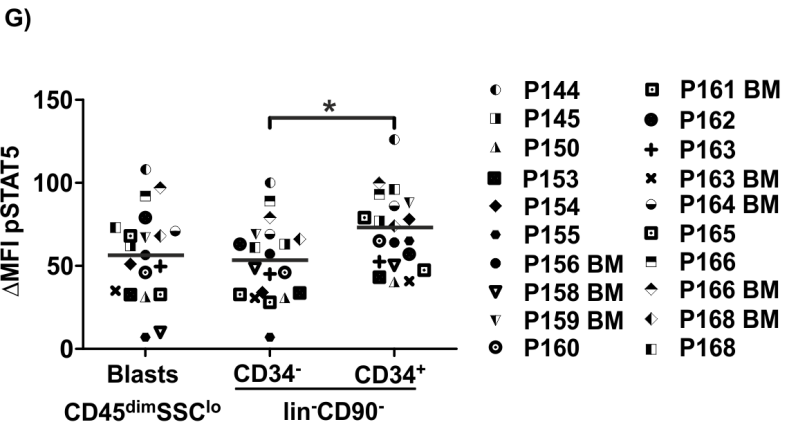
C)

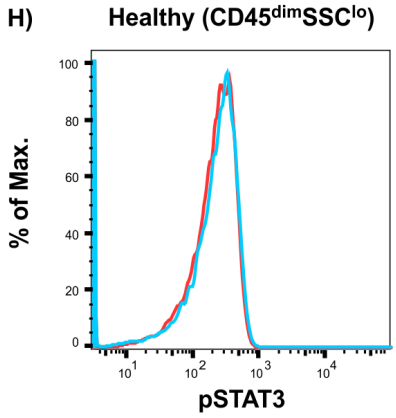
Parameter	HR (95%) CI	p-value
pSTAT3	1.016 (1.002 - 1.030)	.022
pSTAT5	0.996 (0.978 - 1.015)	.703
age	1.020 (1.001-1.038)	.036
risk	1.372 (1.022 - 1.842)	.035

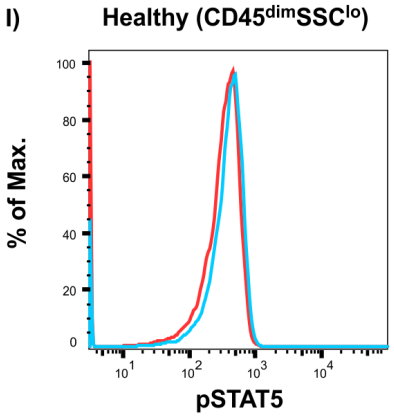












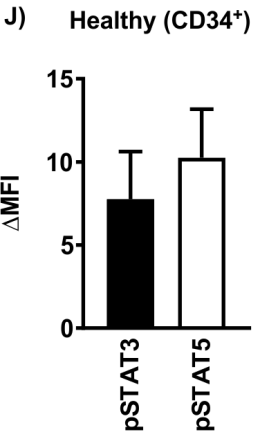


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Figure 2

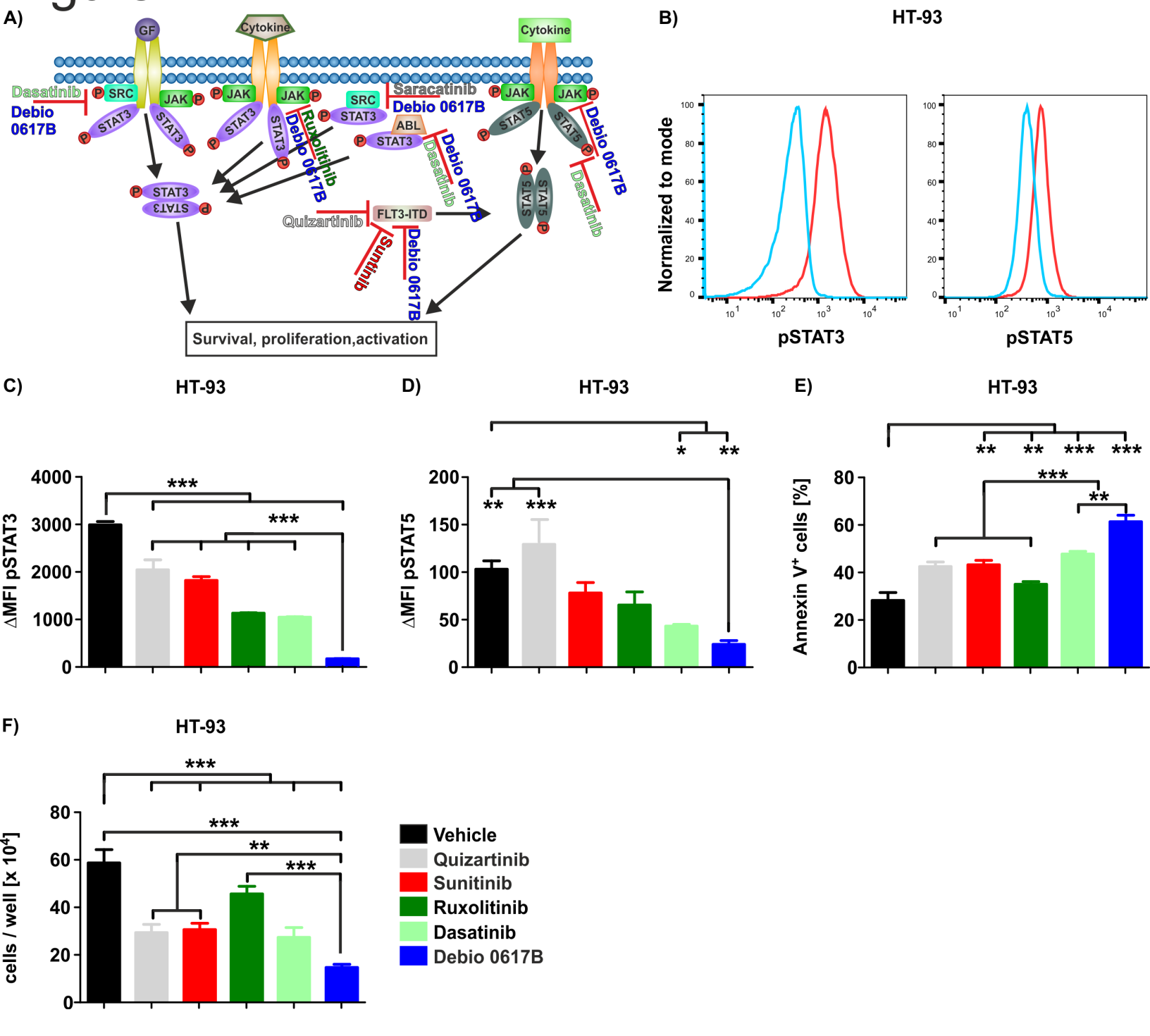

Figure 3

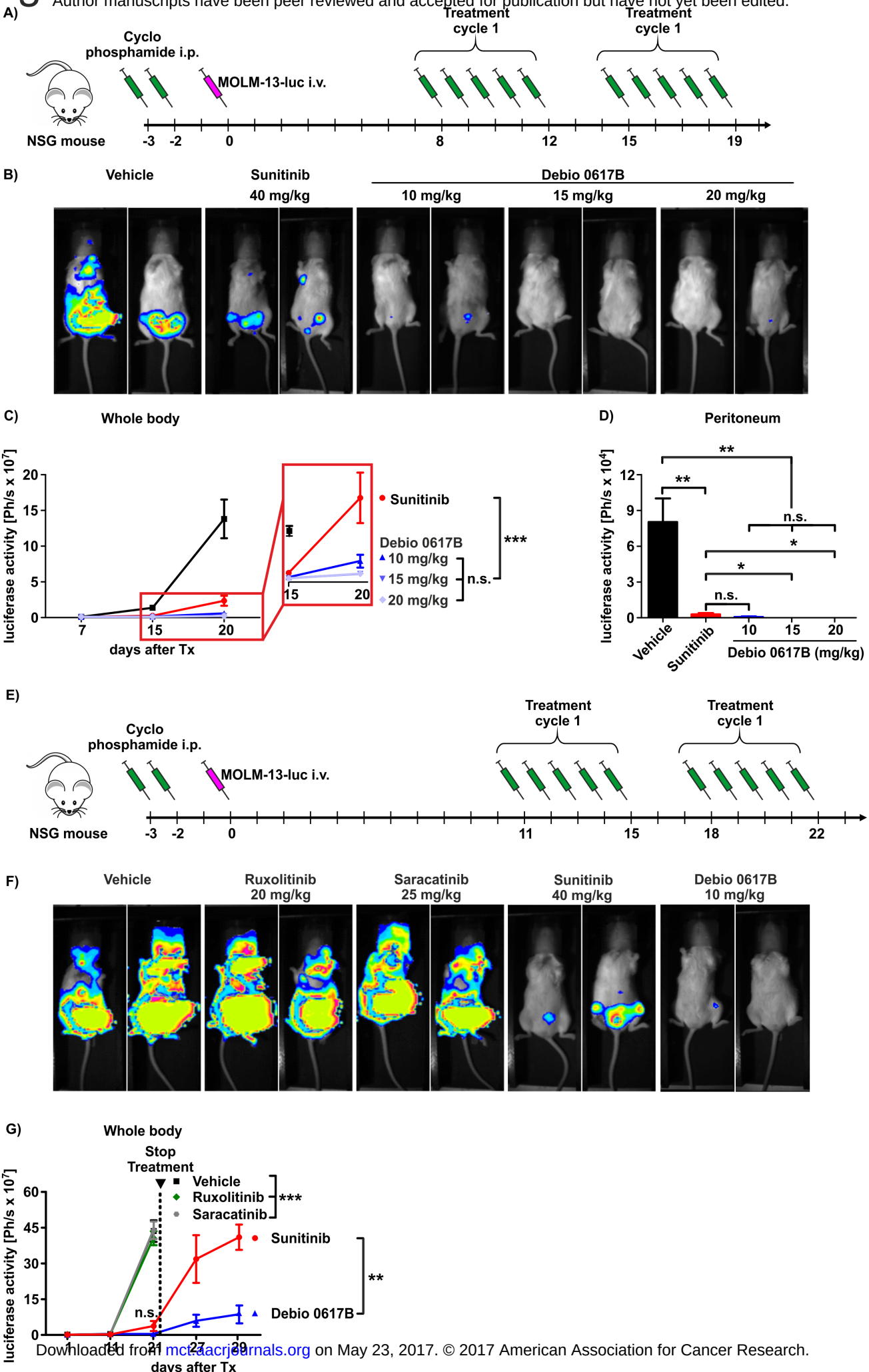

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Figure 4

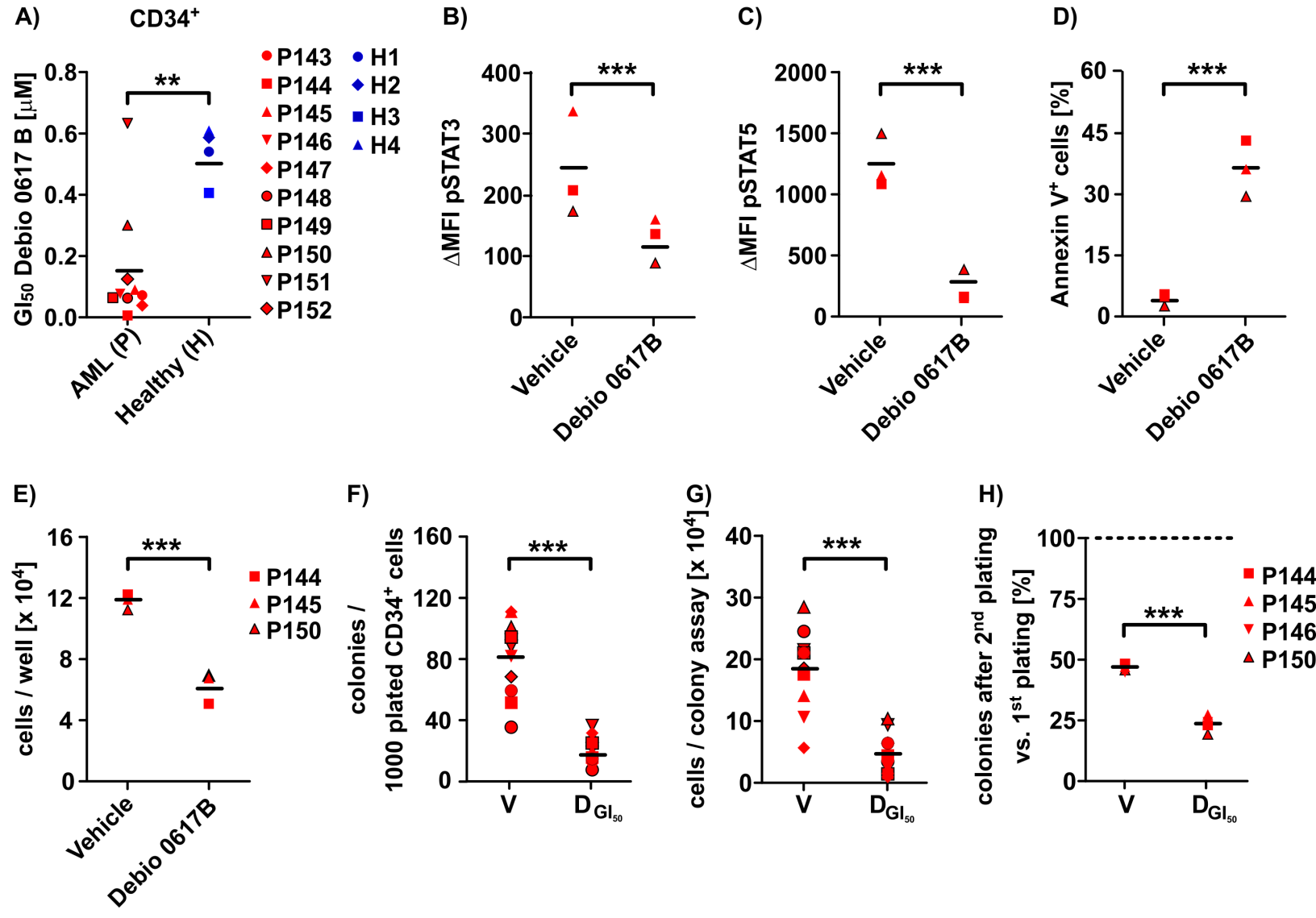

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Figure 5

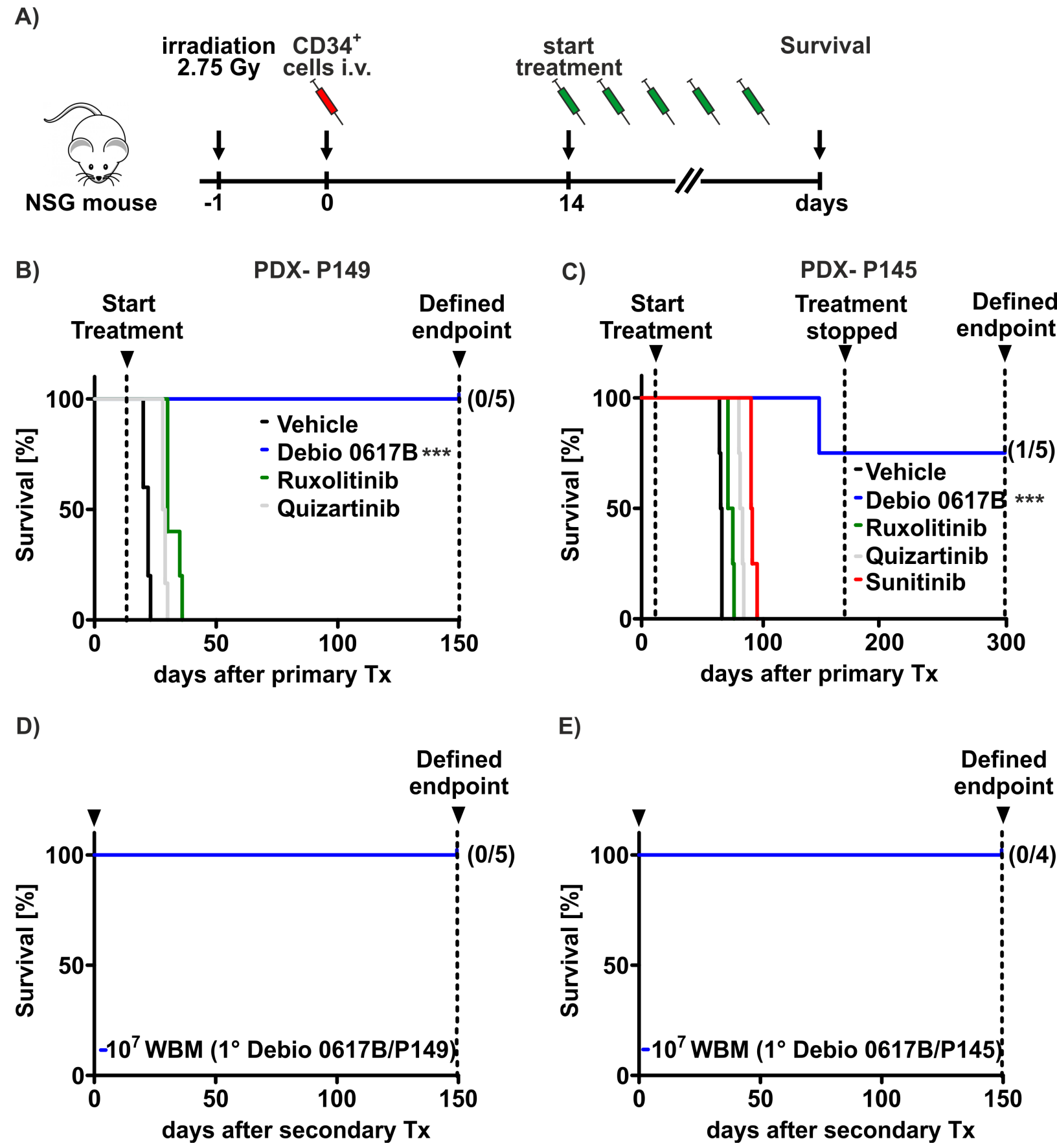

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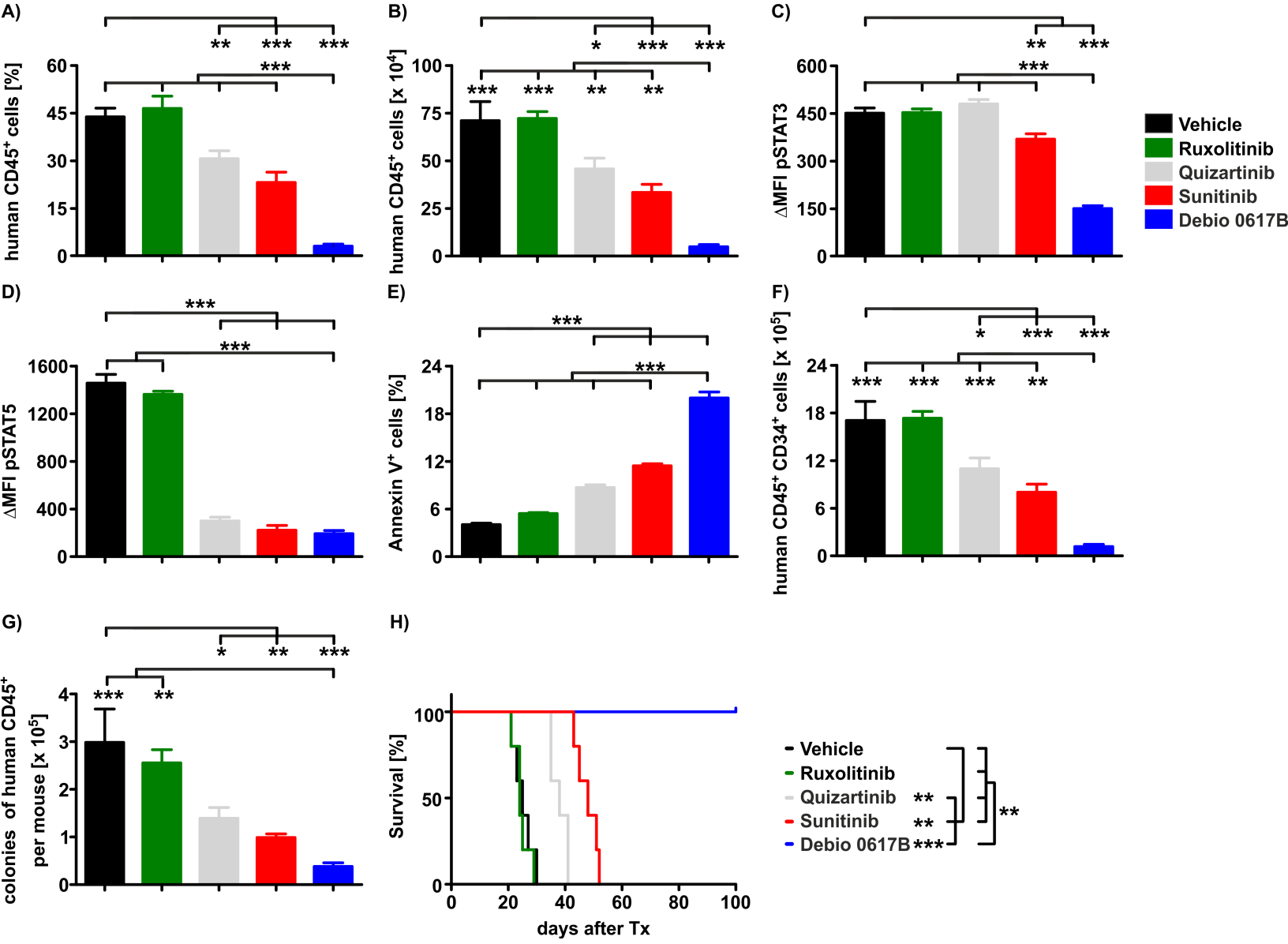
Figure 6



AACR American Association for Cancer Research

Molecular Cancer Therapeutics

The multi-kinase inhibitor Debio 0617B reduces maintenance and self-renewal of primary human AML CD34+ stem/progenitor cells.

Maximilien Murone, Ramin Radpour, Antoine Attinger, et al.

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