In vivo Targeting MEK and TNK2/SRC pathways in PTPN11 driven leukemia

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- **Combining MEK and TNK2/SRC inhibitors has therapeutic potential in PTPN11 mutant JMML and AML**

Abstract:

PTPN11 encodes for a tyrosine phosphatase implicated in the pathogenesis of hematologic malignancies such as Juvenile Myelomonocytic Leukemia (JMML), Acute Myeloid Leukemia (AML), and Acute Lymphoblastic Leukemia (ALL). Since activating mutations of PTPN11 increase proliferative signaling and cell survival through the RAS/MAPK proliferative pathway there is significant interest in using MEK inhibitors for clinical benefit. Yet, single agent clinical activity has been minimal. Previously, we showed that PTPN11 is further activated by upstream tyrosine kinases TNK2/SRC, and that PTPN11-mutant JMML and AML cells are sensitive to TNK2 inhibition using dasatinib. In these studies, we adopted a genetically engineered mouse model of PTPN11 driven leukemia using the mouse strain 129S/Sv-*Ptpn11*^{tm6Bgn}/Mmucd crossed with B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J. The F1 progeny expressing Ptpn11^{D61Y} within hematopoietic cells destined along the granulocyte-monocyte progenitor lineage developed a myeloproliferative disorder. Cohorts of animals treated with combination of dasatinib and trametinib had a significant effect in mitigating disease parameters compared to single agents. Further, a primary patient-derived xenograft model using a myeloid leukemia with PTPN11 F71L also displayed improved response to combination. Collectively, these studies point to combined therapies targeting MEK and TNK2/SRC as a promising therapeutic potential for PTPN11-mutant leukemias.

Introduction

PTPN11 is a ubiquitous protein tyrosine phosphatase that activates many signaling pathways including RAS/MAPK[1]. Disease-associated gain-of-function mutations in PTPN11 have been identified as the most common driver of juvenile myelomonocytic leukemia (JMML), found in 35% of cases[2,3]. Similar mutations in PTPN11 are present in ~5-10% of acute myeloid leukemia (AML) cases, and ~5% of B-cell acute lymphoblastic leukemia (ALL)[2-7]. While in other cancers, mutations in RAS/MAPK pathway may be a secondary event, JMML is driven by

initiating mutations in the RAS/MAPK pathway[3,8], making this disease an ideal model for targeting this pathway for treatment. JMML remains a difficult to treat disease with allogeneic stem cell transplant as the only known cure with a 50% failure rate at 2 years post-transplant[9]. These current clinical outcomes make development of new therapies imperative.

Recent studies targeting RAS/MAPK activity through MEK inhibition have been validated in multiple diseases[10]. Further, preclinical studies have validated MEK inhibition in models of JMML and in patient derived xenografts [11,12]. This has led to a national phase II study of relapsed JMML using trametinib, a potent and selective MEK inhibitor (NCT03190915, ADVL1521). Although some responses were seen, alternate approaches appear to be necessary. Epigenetic signatures have further been hypothesized to represent another mechanism underpinning the pathogenesis of JMML[13-16] leading to a strong rationale for combining trametinib with hypomethylating agents[12] in the ongoing clinical trial (NCT05849662, TACL2020-004).

We previously described a patient with JMML who had responded to treatment with the multikinase inhibitor, dasatinib[17]. These studies identified a novel mechanism of action whereby Tyrosine Kinase Non-receptor 2 (TNK2) increases activity and dependence in PTPN11 mutated leukemias[17]. TNK2 is a cytoplasmic tyrosine kinase that is overexpressed in many solid tumors[18]. Although no current TNK2 selective inhibitor has been developed for clinical use, the multikinase inhibitor dasatinib has been shown to inhibit TNK2[19] suggesting that targeting TNK2 activity may have therapeutic effect in JMML. Further, other studies suggested a potential therapeutic effect for dasatinib in regulating SRC activity in the proliferation signal in JMML[20]. Thus, we proceeded to test whether combination of dasatinib with trametinib may have therapeutic effect.

To test this mechanism, we adopted a mouse model of PTPN11 driven JMML. A previous transgenic mouse model by Neel *et al* was developed as a conditional knock-in of Ptpn11^{D61Y} in the mouse strain 129S/Sv-*Ptpn11*^{tm6Bgn}/Mmucd (carrying a *loxP*-neo-*loxP*-STOP-*loxP* cassette between exons 2 and 3 of *Ptpn11* and inserting a D61Y point mutation)[21]. In our model, we crossed this strain with B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J[22] (expressing Cre in myeloid cells at the M lysozyme locus) to produce a progeny that express Ptpn11^{D61Y} mostly within myeloid hematopoietic progenitor cells. Herein, we describe the *in vivo* effects using this Ptpn11^{D61Y} mouse model as well as a primary AML patient-derived xenograft treated with prolonged exposure to combination of dasatinib and trametinib with potential benefit.

Methods

Patient Samples

All clinical specimens were collected with informed consent on a protocol approved by the Oregon Health & Science University (OHSU) Institutional Review Board (IRB #4422).

Transgenic mouse colonies

All murine studies were performed in accordance with OHSU IACUC (IP00000179), maintained in barrier conditions and monitored through the Department of Comparative Medicine at OHSU. We obtained the mouse strain 129S/Sv-*Ptpn11*^{tm6Bgn}/Mmucd (MMRRC at UC Davis) that carries a *loxP*-neo-*loxP*-STOP-*loxP* cassette between exons 2 and 3 of *Ptpn11* and inserts a D61Y point mutation[21]. These animals were then crossed with B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J[22] (Jackson Laboratories) that express the Cre recombinase under the control of the endogenous *Lyz2* promoter/enhancer elements. Thereby, the F1 progeny express Ptpn11^{D61Y} within hematopoietic cells destined along the granulocyte-monocyte progenitor lineage. Validation of the F1 progeny genotype was performed as previously described through TransnetYX[21]. Animals were monitored monthly with peripheral blood counts using Element HT5 veterinary hematology analyzer (Heska). NOD.*Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}Tg(CMVIL3,CSF2,KITLG) 1Eav/MloySzJ (NSGS)[23] (Jackson Laboratories) mice were used for primary AML xenograft experiments.

AML patient derived Xenograft

Six-week-old NSGS females were sub-lethally irradiated with 150cGy followed 24 hours later by tail vein injection of approximately 0.6x10^6 mononuclear cells per animal. The AML sample underwent human CD3 depletion using dynabead CD3 depletion kit (Invitrogen) prior to injection. Animals were monitored for engraftment starting 4 weeks from injection with blood draws. Peripheral blood was monitored for engraftment by flow cytometry using a cocktail of human CD45-FITC (Milteny), human CD33-APC (BD Phamingen), human CD3-PE (Invitrogen), and mouse CD45-erpCP Cy5.5 (BD Biosciences) and analyzed on an LSRFortessa

at the OHSU Knight Cancer Institute Flow Cytometry Core. Engraftment was determined when animals had approximately 1% human CD33/human CD45 chimerism prior to initiation of treatment.

In vivo drug treatment

Drug dosing was performed as previously described for dasatinib (40mg/kg/dose[24]) and trametinib (1mg/kg/dose[25]). Dasatinib (LC labs) was compounded in 80nM Sodium Citrate, pH 3.1 at 8mg/ml while trametinib (LC labs) was first dissolved in dimethylsulfoxide (DMSO) at 40mg/ml and then compounded in 0.5% hydroxypropylmethylcellulose/0.2% Tween 80 to a final concentration of 0.2mg/ml. Animals were treated once daily by oral gavage either by vehicle or by drug and were monitored for disease by peripheral blood counts as well as behavior and well-being.

Statistical analysis

Mean values ± SEM are shown unless otherwise stated. One-way ANOVA or two-tailed student's *t*-test were used for comparisons. Statistical analysis was conducted using GraphPad Prism Version 6.0. Sample sizes of approximately five animals per cohort were consistent with those reported in similar studies and provide sufficient power to detect changes with the appropriate statistical analysis.

Results and Discussion

Ptpn11^{D61Y} animals develop a myeloproliferative neoplasm.

129S/Sv-*Ptpn11^{tm6Bgn}*/Mmucd crossed with B6.129P2-*Lyz2$^{tm1(cre)Ifo}$*/J produce healthy pups with approximately twenty-five percent of the litter expressing Ptpn11^{D61Y}. Consistent with the original characterization of the Ptpn11^{D61Y} strain[21], the Ptpn11^{D61Y} progeny eventually develop a myeloproferative disorder within eight months of age (Figure 1A). This disorder was manifested by quite pronounced splenomegaly greater than 10x larger than WT and hepatomegaly 3x larger than WT (average control spleen weight of 85mg compared to Ptpn11 spleen weight of 2298mg, p=0.0002, Figure 1B). Peripheral blood examination identified a leukocytosis with absolute

neutrophilia, monocytosis and a lymphocytosis with a relative decrease in percent of lymphocytes. (Figure 1C). Although hemoglobin and platelets were both decreased the mean platelet volume and polychromasia would suggest a consumptive process (Figure 1D).

In vivo sensitivity to MEK and TNK2/SRC inhibition

To next test *in vivo* response to drugs, cohorts of Ptpn11^{D61Y} animals at 9 months of age began treatment with respective vehicles, dasatinib, trametinib, or combination daily five days per week by oral gavage for 3 months. The animals tolerated the treatment well with no change in weight, appearance, or activity. After three months of treatment, due to significant leukocytosis in the control group, the animals were sacrificed for disease evaluation. At the time of harvest there was a significant effect of the combination treatment in mitigating disease parameters compared to single agents (Figure 2A). Both single agents significantly reduced the total WBC, neutrophils and monocytes while the combination had the most dramatic effect on blood leukocytes (average WBC of 75.5x10^6 /mm^3 in control animals versus 6.1x10^6/mm^3 in combination treated animals, p=0.0009). Further, the hemoglobin (average control with 9.9gm/dL compared to combination with 14gm/dL, p<0.0001) and platelets (average control with 216x10^6/mm^3 compared to 1962x10^6/mm^3, p=0.006) showed a significant increase with values similar to their WT littermates. Further, there was a significant effect on spleen size with the 3 months of treatment as compared to the control group (average control spleen weight of 1814mg compared to the combination treated of 72mg comparable to WT spleen size, p=0.0046).

Our previous studies suggest that PTPN11 mutant AML may also respond to combined inhibition[17]. We currently have a biorepository (BEAT AML, local IRB4422) that has an extensive deposit of primary AML cells that have undergone genomic and functional interrogation[26,27]. Within this biorepository we identified a sample of Acute Monoblastic Leukemia with normal cytogenetics but a complex molecular pattern including a PTPN11 p.P71L mutation (variant allele frequency of 0.42). This sample was injected into cohorts of NSGS mice and monitored for human chimerism in peripheral blood. Once animals showed some evidence of human chimerism of approximately 1 percent, cohorts began drug treatment daily for five days each week. Animals continued treatment until evidence of ≥25% human leukemic cells in the blood or moribund features. The study completed after 3 months of

treatment and the remaining animals were harvested. At the end of 3 months or whenever animals met criteria for harvest, disease status was evaluated by spleen weight. There was a significant survival advantage in the animals treated in combination as compared to single agent or vehicle control (Figure 2B). It was further noted that at the end of 3 months, the animals treated with combination had a statistically improved disease status with the combination (average control spleen weight of 211mg compared to combination treated of 98mg, $p=0.0417$).

In these studies we have validated the concept of combination therapy using dasatinib and trametinib for decreasing disease burden in both a mouse model of JMML as well as a patient derived xenograft of AML carrying a PTPN11 activating mutation. Since both trametinib and dasatinib as single agents and combined with cytotoxic chemotherapy are well tolerated, it is our hope that our findings can rapidly translate to clinical testing.

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Author contributions: BHC conceived, designed, performed the experiments, and co-wrote the manuscript. KTK assisted in the study design and performed experiments. J.W.T. conceived, assisted in the design, and co-wrote the manuscript.

Conflicts of interest disclosure: BHC and KTK have no competing conflicts of interest. Research support for JWT is received from Acerta, Agios, Aptose, Array, AstraZeneca, Constellation, Genentech, Gilead, Incyte, Janssen, Kronos, Meryx, Petra, Schrodinger, Seattle Genetics, Syros, Takeda, and Tolero and serves on the advisory board for Recludix Pharma.

Data and materials availability:

All other data that support the findings of this study are available from the corresponding author upon reasonable request.

References:

1. Chan G, Cheung LS, Yang W, et al. Essential role for Ptpn11 in survival of hematopoietic stem and progenitor cells. *Blood*. 2011;117(16):4253-4261.
2. Kratz CP, Niemeyer CM, Castleberry RP, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. *Blood*. 2005;106(6):2183-2185.
3. Stieglitz E, Taylor-Weiner AN, Chang TY, et al. The genomic landscape of juvenile myelomonocytic leukemia. *Nat Genet*. 2015;47(11):1326-1333.
4. Tartaglia M, Martinelli S, Cazzaniga G, et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. *Blood*. 2004;104(2):307-313.
5. Schneeberger VE, Luetteke N, Ren Y, et al. SHP2E76K mutant promotes lung tumorigenesis in transgenic mice. *Carcinogenesis*. 2014;35(8):1717-1725.
6. Chen L, Chen W, Mysliwski M, et al. Mutated Ptpn11 alters leukemic stem cell frequency and reduces the sensitivity of acute myeloid leukemia cells to Mcl1 inhibition. *Leukemia*. 2015;29(6):1290-1300.
7. Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074.
8. Loh ML, Vattikuti S, Schubbert S, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. *Blood*. 2004;103(6):2325-2331.
9. Yoshida N, Doisaki S, Kojima S. Current management of juvenile myelomonocytic leukemia and the impact of RAS mutations. *Paediatr Drugs*. 2012;14(3):157-163.
10. Gouda MA, Subbiah V. Precision oncology for BRAF-mutant cancers with BRAF and MEK inhibitors: from melanoma to tissue-agnostic therapy. *ESMO Open*. 2023;8(2):100788.
11. Lee AQ, Konishi H, Ijiri M, et al. Therapeutic efficacy of RAS inhibitor trametinib using a juvenile myelomonocytic leukemia patient-derived xenograft model. *Pediatr Hematol Oncol*. 2024:1-9.
12. Pasupuleti SK, Chao K, Ramdas B, et al. Potential clinical use of azacitidine and MEK inhibitor combination therapy in PTPN11-mutated juvenile myelomonocytic leukemia. *Mol Ther*. 2023;31(4):986-1001.
13. Krombholz CF, Gallego-Villar L, Sahoo SS, et al. Azacitidine is effective for targeting leukemia-initiating cells in juvenile myelomonocytic leukemia. *Leukemia*. 2019;33(7):1805-1810.
14. Stieglitz E, Mazor T, Olshen AB, et al. Genome-wide DNA methylation is predictive of outcome in juvenile myelomonocytic leukemia. *Nat Commun*. 2017;8(1):2127.
15. Murakami N, Okuno Y, Yoshida K, et al. Integrated molecular profiling of juvenile myelomonocytic leukemia. *Blood*. 2018.
16. Lipka DB, Witte T, Toth R, et al. RAS-pathway mutation patterns define epigenetic subclasses in juvenile myelomonocytic leukemia. *Nat Commun*. 2017;8(1):2126.
17. Jenkins C, Luty SB, Maxson JE, et al. Synthetic lethality of TNK2 inhibition in PTPN11-mutant leukemia. *Sci Signal*. 2018;11(539).
18. Mahajan K, Mahajan NP. ACK1/TNK2 tyrosine kinase: molecular signaling and evolving role in cancers. *Oncogene*. 2015;34(32):4162-4167.
19. Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2011;29(11):1046-1051.
20. Bunda S, Kang MW, Sybingco SS, et al. Inhibition of SRC corrects GM-CSF hypersensitivity that underlies juvenile myelomonocytic leukemia. *Cancer Res*. 2013;73(8):2540-2550.
21. Chan G, Kalaitzidis D, Usenko T, et al. Leukemogenic Ptpn11 causes fatal myeloproliferative disorder via cell-autonomous effects on multiple stages of hematopoiesis. *Blood*. 2009;113(18):4414-4424.
22. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Forster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res*. 1999;8(4):265-277.
23. Wunderlich M, Chou FS, Link KA, et al. AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. *Leukemia*. 2010;24(10):1785-1788.

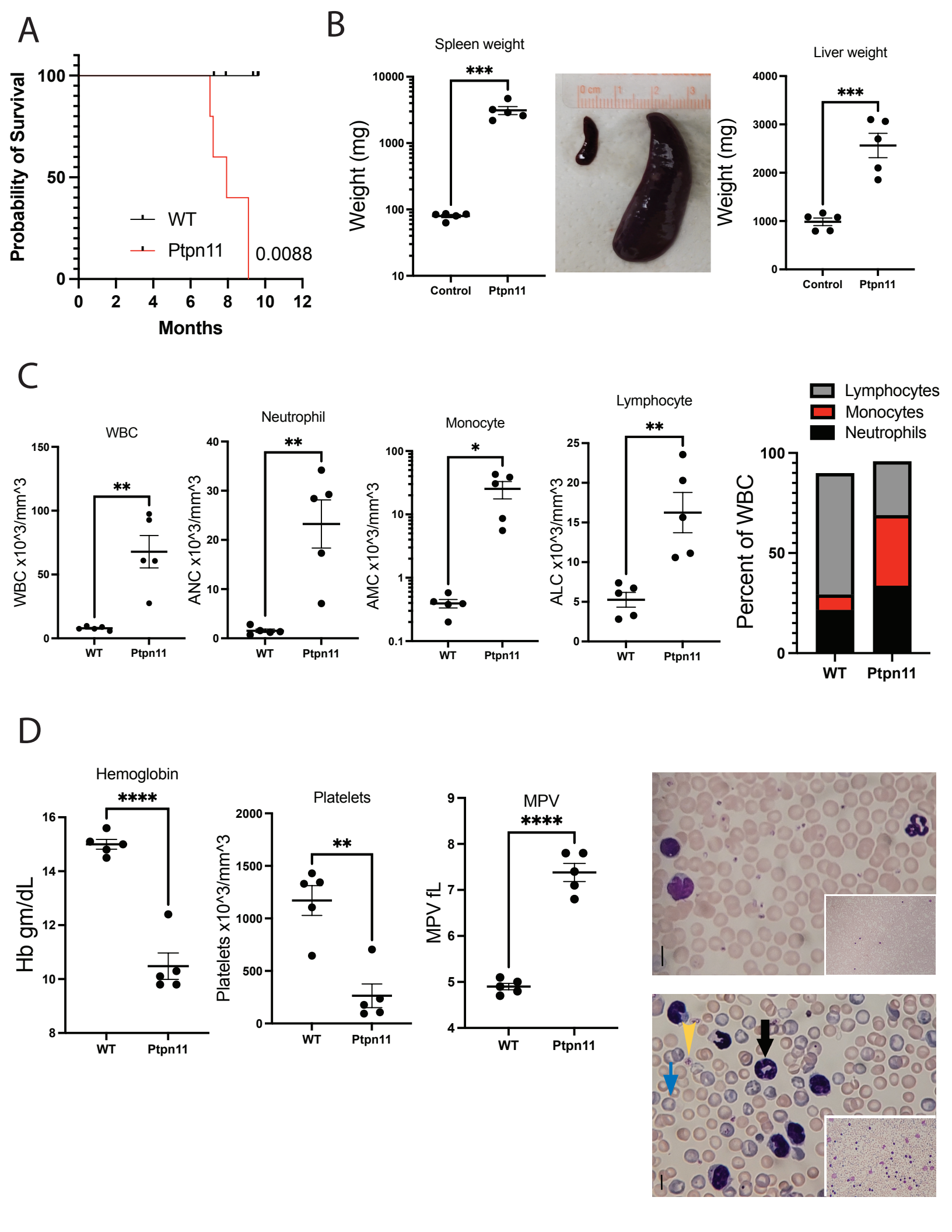
24. Kolb EA, Gorlick R, Houghton PJ, et al. Initial testing of dasatinib by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2008;50(6):1198-1206.
25. Bridgeman VL, Wan E, Foo S, et al. Preclinical Evidence That Trametinib Enhances the Response to Antiangiogenic Tyrosine Kinase Inhibitors in Renal Cell Carcinoma. *Mol Cancer Ther*. 2016;15(1):172-183.
26. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;562(7728):526-531.
27. Bottomly D, Long N, Schultz AR, et al. Integrative analysis of drug response and clinical outcome in acute myeloid leukemia. *Cancer Cell*. 2022;40(8):850-864 e859.

Figure Legends

Figure 1. ***Ptpn11^{D61Y} mice develop a myeloproliferative disorder.*** **A)** Survival of cohorts of Ptpn11 wildtype (WT) and Ptpn11^{D61Y} (Ptpn11) littermates. A cohort of five Ptpn11 animals and five age matched WT counterparts were followed over time. Ptpn11 animals developed signs of disease within 8 months compared to their WT counterparts (p=0.0088, Log-Rank). **B)** Spleen and liver weights at sacrifice of Ptpn11 animals compared to their age matched WT counterparts. The average spleen weight of Ptpn11 animals was 2300mg compared to WT animals of 85mg (p=0.0002***) with representative image. The average liver weight of Ptpn11 animals was 2500mg compared to WT animals of 980mg (p=0.0003***). **C)** Peripheral blood counts, including white blood cell counts (WBC), neutrophils, monocytes, and lymphocytes. The total WBC (mean 67.8 v 7.9 x10^3/mm^3, p=0.0015**), absolute neutrophil (23.3 v 1.5 x10^3/mm^3, p=0.0022**), monocyte (25.3 v 0.4 x10^3/mm^3, p=0.012*), and lymphocyte (16.2 v 5.5 x10^3/mm^3, p=0.0036**) counts were elevated in the Ptpn11 animals. **D)** Hemoglobin and platelets at the time of harvest. Ptpn11 animal showed a decrease in hemoglobin (10 v 15 gm/dL, p<0.0001****) and platelets (263 v 1200 x10^3/mm^3, p=0.0011**) with an elevation in mean platelet volume (MPV) (7.4 v 4.9 fL, p<0.0001). Photomicrographs of GIEMSA stained peripheral blood at 10x (inset) and 40x power. Upper panel of WT blood compared to lower panel of Ptpn11. The peripheral smear of Ptpn11 animals display increased WBC including neutrophils (black arrow), RBC's consistent with polychromasia (blue arrow), and a decrease in platelets (yellow arrow), suggestive of a consumptive process. Black line denotes 0.01mm. Images were captured with a Leica DMLS microscope and captured on Axiocam digital camera. Statistical comparisons were performed with a student's t-test.

Figure 2. ***in vivo response to combination therapy*****. A)** Ptpn11^{D61Y} mice treated with trametinib and dasatinib. Cohorts of five nine-month-old animals were treated with vehicle (Control), dasatinib (40mg/kg/day), trametinib (1mg/kg/day), or combination (40mg/kg/day dasatinib/1mg/kg/day trametinib). After 3 months of treatment, animals were sacrificed and assessed for disease status. Metrics included, blood counts with WBC, neutrophils, monocytes, lymphocytes, relative percentage of the total WBC, hemoglobin, platelets, and spleen weight at the time of harvest. Statistical comparisons were performed with a student's t-test. *p<0.05, **p<0.01, ***p<0.001, p<0.0001. **B**) Primary AML sample with PTPN11 F71L mutation treated with trametinib and dasatinib. A primary AML sample (15-892) carrying a P71L PTPN11 mutation was engrafted into cohorts of five NSGS mice. Upon signs of engraftment with peripheral blood chimerisms of 0.1 to 3% human CD33/CD45, animals initiated treatment with vehicle (Control), dasatinib (40mg/kg/day), trametinib (1mg/kg/day), or combination (40mg/kg/day dasatinib/1mg/kg/day trametinib). Survival was monitored for either ≥25%

human CD33/CD45 in the peripheral blood or the animal became moribund. Sacrificed animals were assessed for disease status by spleen weight. By day 98 of treatment, remaining animals were sacrificed to evaluate the disease status with the spleen weight. Statistical comparisons were performed with a Log-rank test for survival comparison ($p<0.05$ comparing control to das/tram) and student's t-test. * $p<0.05$.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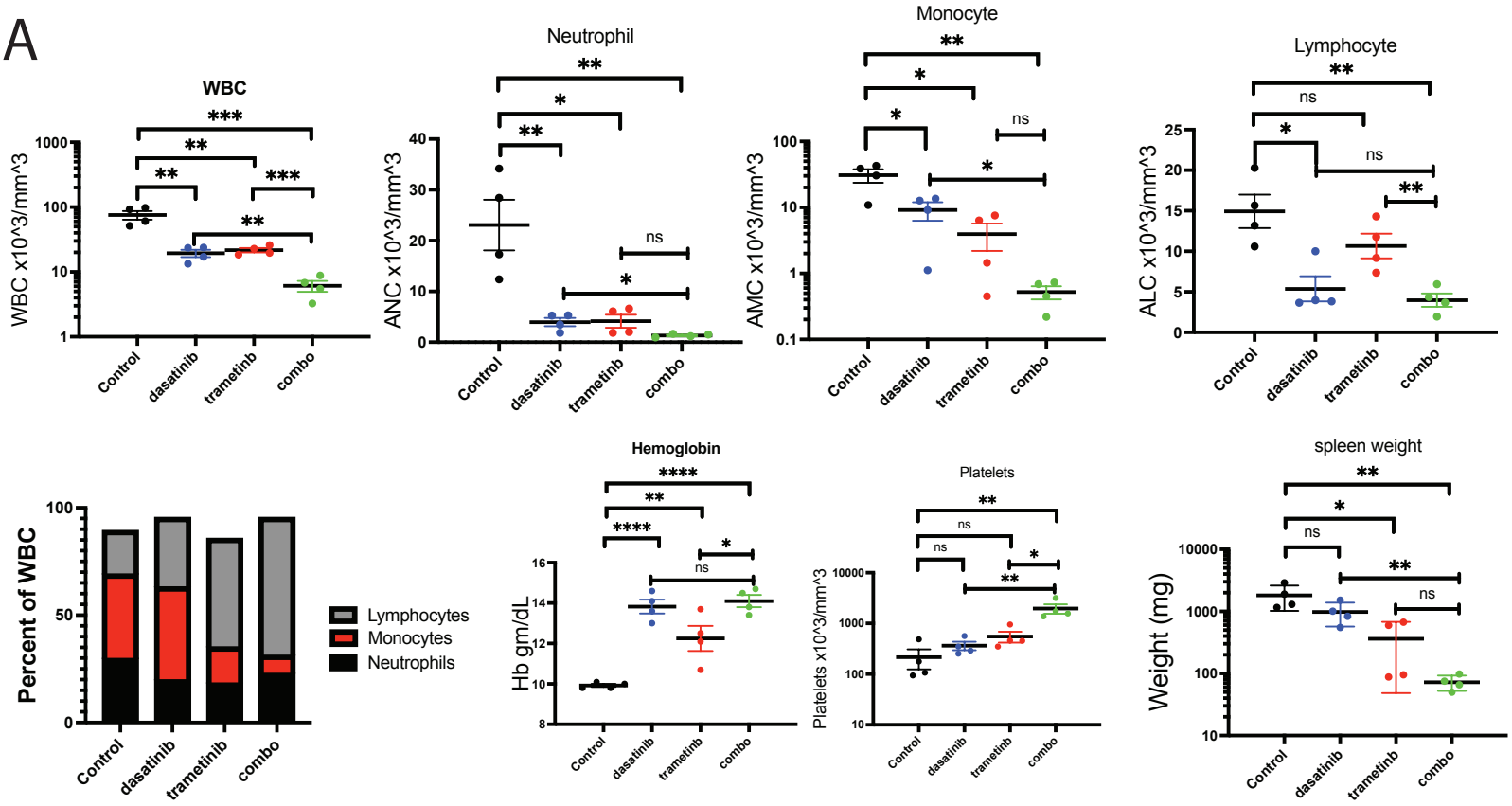
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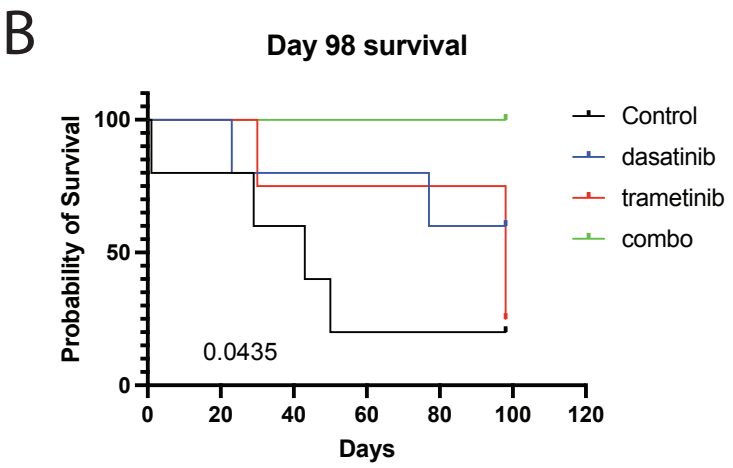
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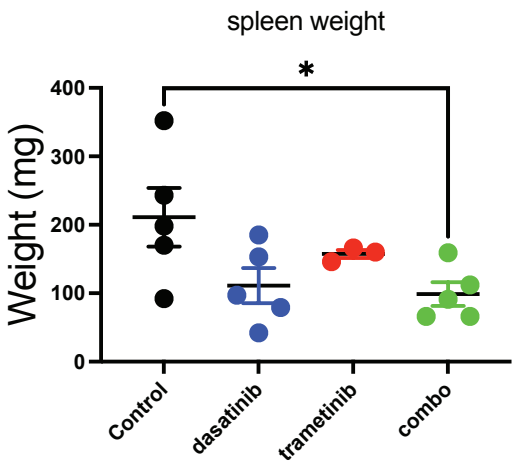
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