haematologica
Journal of the Ferrata Storti Foundation

Pivekimab sunirine in blastic plasmacytoid dendritic cell neoplasm: assessing spatial response and unraveling resistance mechanisms

by Margaux Poussard, Morgane Boichut, Imane Belakri, Sabeha Biichle, Maxime Fredon, Blandine Cael, Xavier Roussel, Florian Renosi, Kris Van Moer, Bassam Janji, Franck Monnien, Cecile Boichut, Romain Boidot, Babacar Ndao, Olivier Adotevi, Francine Garnache-Ottou, Krystal Watkins, Sribalaji Lakshmikanthan and Fanny Angelot-Delettre

Received: July 10, 2025.
Accepted: January 26, 2026.

Citation: Margaux Poussard, Morgane Boichut, Imane Belakri, Sabeha Biichle, Maxime Fredon, Blandine Cael, Xavier Roussel, Florian Renosi, Kris Van Moer, Bassam Janji, Franck Monnien, Cecile Boichut, Romain Boidot, Babacar Ndao, Olivier Adotevi, Francine Garnache-Ottou, Krystal Watkins, Sribalaji Lakshmikanthan and Fanny Angelot-Delettre. Pivekimab sunirine in blastic plasmacytoid dendritic cell neoplasm: assessing spatial response and unraveling resistance mechanisms.
Haematologica. 2026 Feb 5. doi: 10.3324/haematol.2025.288663 [Epub ahead of print]

Pivekimab sunirine in blastic plasmacytoid dendritic cell neoplasm: assessing spatial response and unraveling resistance mechanisms

Margaux Poussard[1,2]*, Morgane Boichut[1]*, Imane Belakri[1], Sabeha Biichle[1], Maxime Fredon[3], Blandine Cael[1], Xavier Roussel[1,4], Florian Renosi[1,5], Kris Van Moer[2], Bassam Janji[2], Franck Monnien[6], Cecile Boichut[6], Romain Boidot[7], Babacar Ndao[1], Olivier Adotevi[1,8], Francine Garnache-Ottou[1,9], Kristal Watkins[10], Sribalaji Lakshmikanthan[10,] Fanny Angelot-Delettre[1#]

[1] Université Marie et Louis Pasteur, EFS, INSERM, UMR RIGHT, F-25000 Besançon, France
[2] Department of Cancer Research, Luxembourg Institute of Health, Luxembourg, L-1210, Luxembourg
[3] CARLA Biotherapeutics, F-25000 Besançon, France
[4] Service d'hematologie clinique, CHU Besançon, F-25000 Besançon, France
[5] Laboratoire d'onco-hematologie moléculaire, CHU Besancon, F-25000 Besançon, France
[6] Department of Pathology, CHU Besançon, Besançon, France
[7] Unit of Molecular Biology, Department of Biology and Pathology of Tumors, Georges-François Leclerc Anticancer Center, UNICANCER, F-21000 Dijon, France
[8] Service d'oncologie médicale, CHU Besancon, F-25000 Besançon, France
[9] Laboratoire d'immunologie cellulaire et hématologie, CHU Besancon, F-25000 Besançon, France
[10] ImmunoGen, Inc., Waltham, United States of America
*These authors contributed equally to this work

#Corresponding authors fanny.delettre@efs.sante.fr

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Funding Statement: This work was supported by ImmunoGen®.

Conflict of interest: The authors declare that they have no conflict of interest.

Patient Consent Statement: Not applicable. This study did not involve human participants.
Permission to Reproduce Material from Other Sources: Not Applicable
Clinical Trial Registration: Not applicable.

Acknowledgements: The authors would like to thank Dr Marine Abad for her help in analysing IHC and HES slides.

Author Contributions Statement: M.B. and M.P. performed the experiments. M.B., I.B. and M.P. analyzed data. M.P. and S.B. provided help with flow cytometry analysis. M.B., and M.P. performed statistical analysis. K.V.M. performed the western blot analysis. F.M., C.B., analyses the HES and IHC staining. B.J, X.R., S.B., M.F., F.R., F.G.O., O.A., and F.A.D. provided guidance and expertise in their respective areas of study. M.B., M.P., and F.A.D. wrote the manuscripts, and F.G.O. and O.A. commented on the manuscript. F.G.O. and F.A.D supervised the research. All authors provided input and edited and approval the final version of the manuscript.

Keywords: PVEK – BPDCN – Resistance – Spatial response

Short running title: PVEK in BPDCN

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive malignancy, which often manifests with skin lesions in addition to lymph node, blood, and bone marrow involvement[1]. Standard of care treatments for BPDCN patients are intense chemotherapy or a CD123 targeting drug, tagraxofusp (Elzonris®)[2]. Although many patients initially respond to standard-of-care therapies, most will eventually relapse[3]. Overexpression of CD123 is a hallmark of BPDCN thus making this antigen an attractive target[4]. Pivekimab sunirine (PVEK) is an antibody-drug-conjugate (ADC) comprised of a humanized anti-CD123 monoclonal antibody conjugated to a payload *via* a cleavable linker[4, 5]. PVEK is currently being tested for the treatment of BPDCN patients as monotherapy and, as a triple therapy in combination with azacitidine and venetoclax for the treatment of AML patients[6]. Preclinically, PVEK has demonstrated potent activity against BPDCN cells[4] and importantly, showed a favorable safety profile and promising efficacy in BPDCN patients[7]. However, resistance mechanisms are often described for targeted therapies in oncology, such as ADC. Among them, a downregulation of the expression of the targeted antigen has been reporter by Loganzo *et al.*, in HER2+ breast cancer for example[8]. Modification of transport proteins, such as upregulation of ABC transporters was shown to decrease ADC intracellular concentration, leading to a decrease of its cytotoxic action[9]. The goal of this study was to investigate spatial response to PVEK *in vivo* in order to identify potential sanctuary organs that could lead to relapse and further resistance development. All animal procedures were carried out in accordance with the guidelines for animal experimentation (Veterinary Services for Animal Health & Protection, issued by the Ministry for Agriculture, Paris, France). We also developed a resistant BPDCN model to PVEK to anticipate the potential mechanism involved in resistance to the ADC and prevent it from happening.

The efficacy and specificity of PVEK were first assessed *in vitro* by flow cytometry (FC) on a BPDCN cell line called GEN2.2 after 24h, 48h and 72h of treatment (**Supp Figure 1A-C**). GEN2.2 cell line presents a high expression of CD123 (**Figure 1A**) as well as a mutation of TP53 (**Supp Figure 1D**), known to be associated with a worse prognosis in AML[10] rendering this model a promising tool to evaluate our drug. The ADC presents a half eliminatory concentration value (EC50) between 1.3pM and 4pM after 72h of treatment (**Supp Figure 1C**). Shorter treatment times (24h and 48h) show a similar pattern, with a reduction of the viability in a time- and dose-dependant manner (**Supp Figure 1A-B**). The payload alone showed a lower potency against GEN2.2 (EC50 between 12pM and 36pM), while the non-targeting antibody failed to reduce the cell viability even at high concentrations and long-time of exposure (EC50 not reached). Previous studies in AML and ALL models also showed an *in vitro* efficacy of PVEK on CD123-expressing cell lines with IC50 in the picomolar range, similarly to our data[5, 11]. Treatment with PVEK *in vivo* in cell line xenograft model, using GEN2.2 cells, regardless the concentration used, showed a controlled tumor burden compared to the vehicle-treated group (**Supp Figure 1E**). Similarly, survival of mice treated with PVEK (both concentrations) was significantly increased compared to the vehicle group (median survival of 45 days and 17 days respectively) (**Supp Figure 1F**), as previously shown in different AML mice models[5].

To assess spatial response to pivekimab sunirine, we utilized a bioluminescent (BLI) GEN2.2-luc model. Tumor burden was significantly lower at day 16 for the treated group accompanied by a significant increased survival compared to the vehicle group (median survival of 42 day and 22 days respectively) (**Figure 1B-C**). On day 16 for vehicle-treated group and day 30 for PVEK-treated group, a separate set of mice (n=3 per group) was euthanized to harvest organs and assess tumor infiltration and response to PVEK treatment among each organ (**Figure 1E**). The vehicle group showed the presence of tumor cells in the bone marrow, spleen, lungs and liver. PVEK treated mice showed no sign of tumor cells in any of the organs in BLI evaluation. HES staining confirmed the elimination of tumor cells in the lungs and the liver while few remaining tumor cells were observed in the spleen and the bone marrow. These few cells could be resistant

cells and be the cause of relapses. That's why we went on to evaluate potential mechanisms of resistance to PVEK. Brain didn't show any sign of tumor infiltration by BLI evaluation for both groups (**Figure 1F**). However, HES staining revealed an infiltration of the brain tissue by the BPDCN cells in the vehicle-treated group. This infiltration was lower in the brain from the PVEK-treated group (**Figure 1F**). Very early on, the central nervous system (CNS) was considered a sanctuary for the persistence of BPDCN blasts. Indeed, in a retrospective study of 23 patients, Feuillard et al. described patients with CNS localization at diagnosis, but also 5 patients with CNS localization at relapse[12]. Further studies may be of interesting to determine whether PVEK can cross the blood-brain barrier and kill BPDCN cells in the brain or prevent relapses with this CNS localization.

To get deeper understanding of PVEK efficacy in BPDCN we have developed a resistant model from a BPDCN cell line. We cultured sensitive GEN2.2 cells with increasing concentration of PVEK. After 8 cycles of treatment (GEN2.2 R^{Dose8}) a reduction in sensitivity to PVEK was observed with reduction in viability of less than 40% only at the highest concentration (**Supp Figure 2A**). CD123 expression was assessed on these cells, and FC analysis showed a decrease in CD123 expression on resistant cells (**Supp Figure 2B**). In resistant cell line GEN2.2 R^{Dose8}, we sorted 3 fractions: cells expressing the most CD123, cells expressing the least CD123 and cells expressing moderate CD123. The fraction with the lowest expression was the most resistant, with 92±5.4% viability compared to the fraction with the highest expression with 63.5±13% viability, after treatment at the highest dose (3000 pM) (**Supp Figure 2C**). FC showed the different fraction, high, medium and low for CD123 expression (**Supp Figure 2D**). The Relative Fluorescence Intensity (RFI) of sensitive GEN2.2 was significantly higher (25.4) than 3 fractions of GEN2.2 R^{Dose8} cells (low: 1.7; medium: 2.6; high: 5.7) (**Supp Figure 2D**). The exposition of the cells to PVEK was continued and after 12 cycles of treatment (GEN2.2 R+) a complete insensitivity to PVEK was observed even at the highest concentrations (95.6± 1.6 % viability) (**Figure 2A**). The evaluation of the sensitivity of those cells to PVEK confirmed that we successfully established a resistant cell line to this ADC. CD123 expression of GEN2.2 R+ was close to isotype control and the RFI of this cell line is negative (1.5±0.3) compared to GEN2.2 (22.4±4.4) (**Figure 2B**). Significant decreased expression of CD123 was further confirmed by FC using another clone, which does not target the same area of the FC as PVEK (G4723A) (**Supp Figure 2E**). An intracellular staining by FC, of CD123 also showed a significant reduction of the RFI by GEN2.2R+ compared to GEN2.2 (0.9±0.0 and 46.2±0.0 respectively) (**Supp Figure 2F**).

To assess molecular mechanism contributing to the establishment of BPDCN resistance to PVEK, we performed a RNA sequencing of GEN2.2 and GEN2.2 R+ cell lines. Expectedly, we observed a clear segregation of these two entities (**Figure 3A**). We further found 2287 differentially genes expressed between GEN2.2 and GEN2.2 R+, of which 1491 and 796 are respectively upregulated and downregulated in GEN2.2 (**Figure 3B**). Our results showed an antigen loss (CD123) at protein levels but not RNA levels. Some studies have shown the acquisition of a mutation under treatment, leading to a lower affinity between the protein and the antibody[13]. Truncated forms of the antigen are another potential mechanism of resistance[14]. Further investigations are thus needed to confirm which mechanism could explain why the CD123 decrease observed at the protein level is not seen at RNA level. We also observed an up-regulation of ABCB1 expression which is part of the top 100 differentially expressed genes between GEN2.2 and GEN2.2 R+ (**Figure 3C and 3D**). The significant up-regulation of ABCB1 transporters was confirmed at mRNA level qPCR with significantly higher expression on GEN2.2 R+ compared to GEN2.2 (49.5±28 compared to 2.25±2.5 respectively) (**Figure 3E**) and further at the protein level by western blot (**Figure 3F**). Upregulation of ABC transporters expression was reported several times in cancer resistance to chemotherapies and ADC[9]. Yu et al. showed in a non-Hodgkin lymphoma model made resistant to ADC targeting CD22 and CD79b that upregulation of P-glycoprotein (P-

gp)/ABCB1 was the major driver of the resistance to the ADC payload[15]. They further showed that changing the payload while keeping the same targeted antigen could also reverse the resistance acquired by the tumor cells[15].

Overall, our data provide an additional BPDCN model showing efficacy of PVEK as well as detailed information for the first time of spatial response to this ADC. Our resistant model also help us to identify potential mechanism of resistance that could be developed by patients upon PVEK treatment. Further experiments are necessary to confirm the described mechanism in patients relapsing and also to evaluate strategies to overcome the resistance. Antigen loss and efflux pumps are known resistance mechanisms observed in ADCs and it is critical to monitor these components in PVEK clinical trials. Further experiments are will thus necessary to confirm the described mechanism to evaluate strategies to overcome the resistance.

References

1. Garnache-Ottou F, Vidal C, Biichle S, et al. How should we diagnose and treat blastic plasmacytoid dendritic cell neoplasm patients? Blood Adv. 2019;3(24):4238-4251.
2. Pemmaraju N, Sweet KL, Stein AS, et al. Long-Term Benefits of Tagraxofusp for Patients With Blastic Plasmacytoid Dendritic Cell Neoplasm. J Clin Oncol. 2022;40(26):3032-3036.
3. Guru Murthy GS, Pemmaraju N, Atallah E. Epidemiology and survival of blastic plasmacytoid dendritic cell neoplasm. Leuk Res. 2018;73:21-23.
4. Zhang Q, Cai T, Han L, et al. Pre-Clinical Efficacy of CD123-Targeting Antibody-Drug Conjugate IMGN632 in Blastic Plasmacytoid Dentritic Cell Neoplasm (BPDCN) Models. Blood. 2018;132(Suppl_1):3956.
5. Kovtun Y, Jones GE, Adams S, et al. A CD123-targeting antibody-drug conjugate, IMGN632, designed to eradicate AML while sparing normal bone marrow cells. Blood Adv. 2018;2(8):848-858.
6. Daver N, Montesinos P, Altman JK, et al. Pivekimab Sunirine (PVEK, IMGN632), a CD123-Targeting Antibody-Drug Conjugate, in Combination with Azacitidine and Venetoclax in Patients with Newly Diagnosed Acute Myeloid Leukemia. Blood. 2023;142(Supplement 1):2906.
7. Pemmaraju N, Martinelli G, Todisco E, et al. PB1828: CADENZA: A PIVOTAL STUDY OF PIVEKIMAB SUNIRINE (IMGN632) IN PATIENTS WITH UNTREATED/FRONTLINE BPDCN. Hemasphere. 2022;6(Suppl)1708-1709.
8. Loganzo F, Tan X, Sung M, et al. Tumor cells chronically treated with a trastuzumab-maytansinoid antibody-drug conjugate develop varied resistance mechanisms but respond to alternate treatments. Mol Cancer Ther. 2015;14(4):952-963.
9. Abelman RO, Wu B, Spring LM, Ellisen LW, Bardia A. Mechanisms of Resistance to Antibody-Drug Conjugates. Cancers (Basel). 2023;15(4):1278.
10. Wen XM, Xu ZJ, Jin Y, et al. Association Analyses of TP53 Mutation With Prognosis, Tumor Mutational Burden, and Immunological Features in Acute Myeloid Leukemia. Front Immunol. 2021;12:717527.
11. Angelova E, Audette C, Kovtun Y, et al. CD123 expression patterns and selective targeting with a CD123-targeted antibody-drug conjugate (IMGN632) in acute lymphoblastic leukemia. Haematologica. 2019;104(4):749-755.
12. Feuillard J, Jacob MC, Valensi F, et al. Clinical and biologic features of CD4(+)CD56(+) malignancies. Blood. 2002;99(5):1556-1563.
13. Coates JT, Sun S, Leshchiner I, et al. Parallel Genomic Alterations of Antigen and Payload Targets Mediate Polyclonal Acquired Clinical Resistance to Sacituzumab Govitecan in Triple-Negative Breast Cancer. Cancer Discov. 2021;11(10):2436-2445.
14. Scaltriti M, Rojo F, Ocaña A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst. 2007;99(8):628-638.
15. Yu SF, Zheng B, Go M, et al. A Novel Anti-CD22 Anthracycline-Based Antibody-Drug Conjugate (ADC) That Overcomes Resistance to Auristatin-Based ADCs. Clin Cancer Res. 2015;21(14):3298-3306.

Figures legends

Figure 1: In vitro and in vivo spatial efficacy of pivekimab sunirine (PVEK) on BPDCN model

(A) Representative flow cytometry (FC) histogram of CD123 staining on GEN2.2 cell lines.
(B) GEN2.2-luc-bearing mice were treated one a week for three weeks with PVEK (IV). Tumor burden was monitored weekly by BLI measurements. Black cross indicates dead mice during experiment.
(C) Mice were monitored daily until death, or euthanized if they showed signs of suffering in accordance with animal welfare guidelines. Kaplan-Meier survival curves for all groups were generated from GEN2.2-luc injection, using death of the animal as the terminal event. Comparison of survival between groups is shown, **p<0.002. Overall survival analysis of mice revealed significant gain of survival for pivekimab sunirine-treated mice compared with untreated mice (n=5 mice per group).
(D) BLI measurements was also performed on selected organs (spleen, bone marrow, liver, lung, kidney, and brain) after sacrifice at day 16 post GEN2.2-luc injection for vehicle-treated mice and at day 30 for PVEK-treated mice. HES staining was performed on these organs to confirm results found with BLI measurement.
(E) HES and IHC staining performed on brain collected at day 16 from vehicle-treated mice and at day 30 from PVEK-treated mice.

Figure 2: Phenotypic characterization of established BPDCN cell line resistant to PVEK.

(A) GEN2.2 cells were cultured in the presence of chronic PVEK exposure (addition every 72 h, increasing concentrations over time). Acquired resistance was subsequently assessed by FC after 72h of treatment with PVEK at different concentration (0.45 to 3000pM) (n=3). Resistant cells to PVEK are named as GEN2.2 R+.
(B) Representative FC histogram of CD123 staining on GEN2.2 cells and GEN2.2 R+ cells compared to isotype control (**left**) and all expriments of CD123 cell surface expression on GEN2.2 vs. GEN2.2 R+ (n=3, **p<0.002) (**right**).

Figure 3: Transcriptomic analysis of established BPDCN cell line resistant to PVEK reveals modified ABCB1 expression.

(A) Principal component analysis (PCA) of GEN2.2 (**blue**) and GEN2.2 R+ (**pink**).
(B) Differentially Expressed Genes (DEG) between GEN2.2 versus. GEN2.2 R+.
(C) Volcano plot of protein encoding genes in GEN2.2 versus GEN2.2 R+ showing the relationship between fold change (log2 fold change (FC); horizontal axis)) and significance (-log10 (p value); vertical axis), respectively. *ABCB1:* log2FC=5,69; adjusted p-value=$1.13x10^{-25}$.
(D) The list of top 100 DEG in GEN2.2 (**blue**) and GEN2.2 R+cells (**red**).
(E) The mRNA expression of *ABCB1* was assessed by qPCR on cells treated with PVEK. Results are reported as relative expression to GEN2.2. (n=3, *p<0.05)
(F) Western blot analysis realized with specific anti-ABCB1 transporters primary antibodies. **Upper panel:** Representative blot of 3 independent experiments. **Lower panel:** quantification of expression intensity of ABCB1 by Image J software. β-actin was used as a loading control. (n=3, ****p<0.0001).

Figure 1

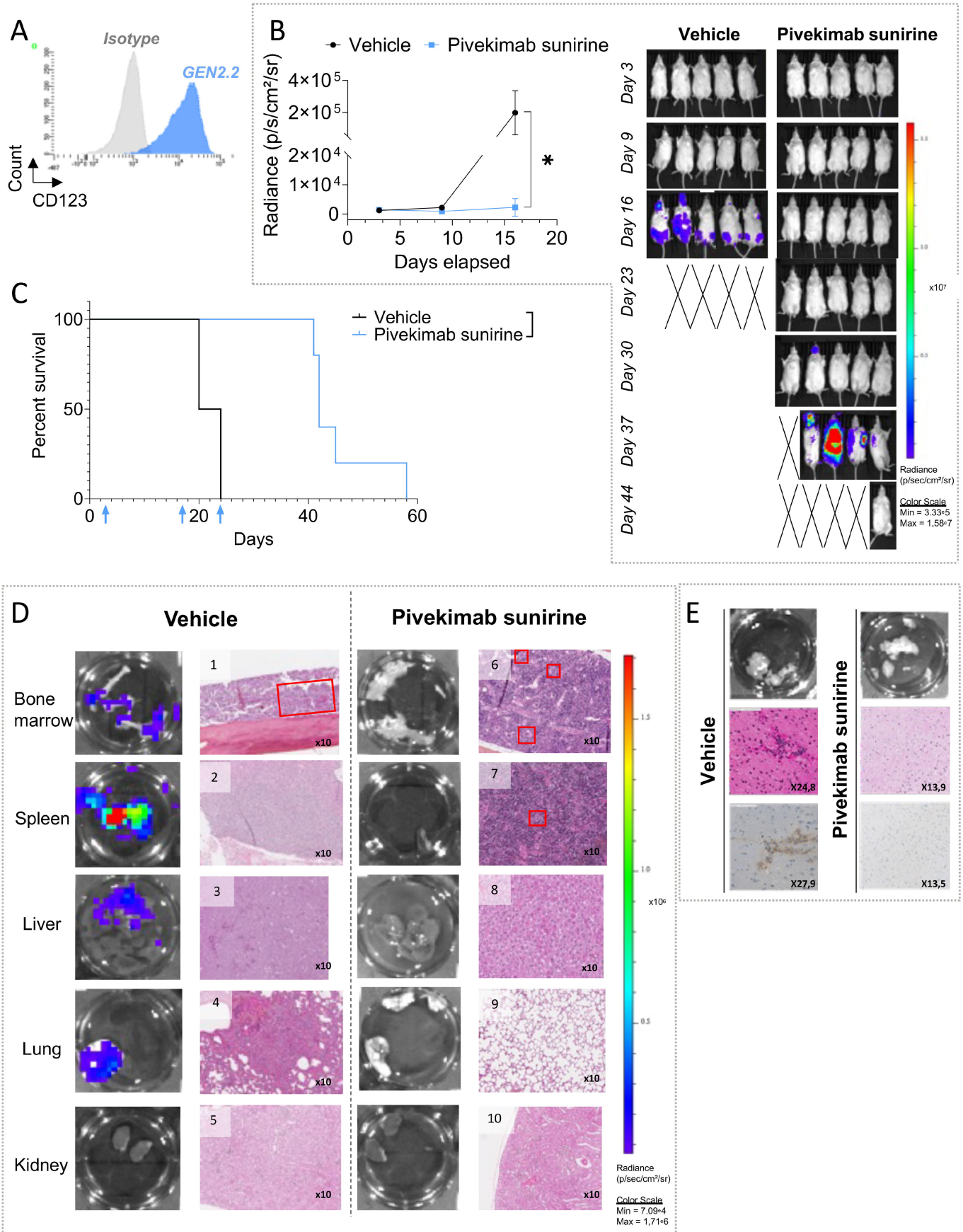
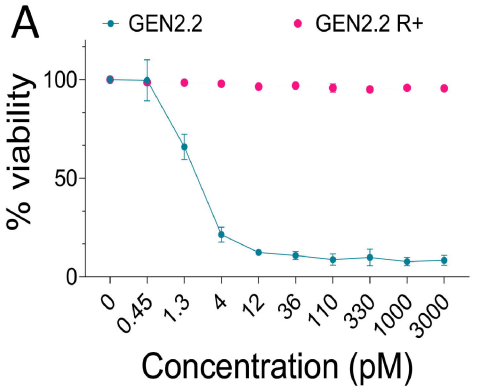

A
Isotype
GEN2.2
Count
CD123
B
Vehicle
Pivekimab sunirine
Radiance (p/s/cm²/sr)
4×10⁵
2×10⁵
2×10⁴
1×10⁴
0
0
5
10
15
20
Days elapsed
*
Vehicle
Pivekimab sunirine
Day 3
Day 9
Day 16
Day 23
Day 30
Day 37
Day 44
x10⁷
Radiance (p/sec/cm²/sr)
Color Scale
Min = 3.33e5
Max = 1,58e7
C
Percent survival
100
50
0
0
20
40
60
Days
Vehicle
Pivekimab sunirine
D
Vehicle
Pivekimab sunirine
Bone marrow
Spleen
Liver
Lung
Kidney
1
2
3
4
5
6
7
8
9
10
x10
x10⁶
Radiance (p/sec/cm²/sr)
Color Scale
Min = 7.09e4
Max = 1,71e6
E
Vehicle
Pivekimab sunirine
X24,8
X27,9
X13,9
X13,5

Figure 2


A
GEN2.2
GEN2.2 R+
% viability
100
50
0
0
0.45
1.3
4
12
36
110
330
1000
3000
Concentration (pM)

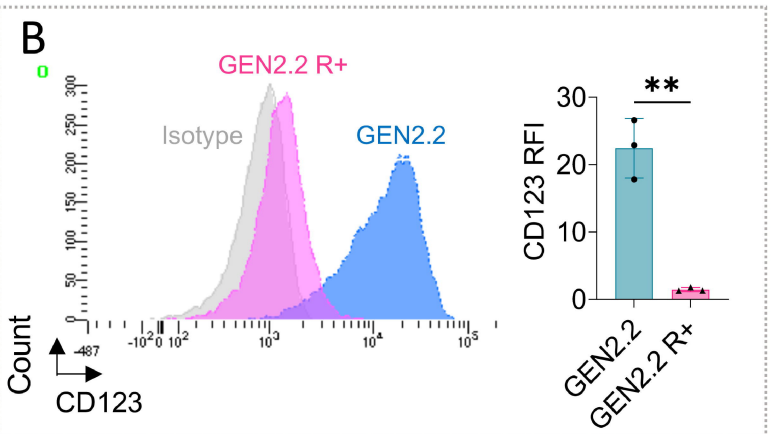
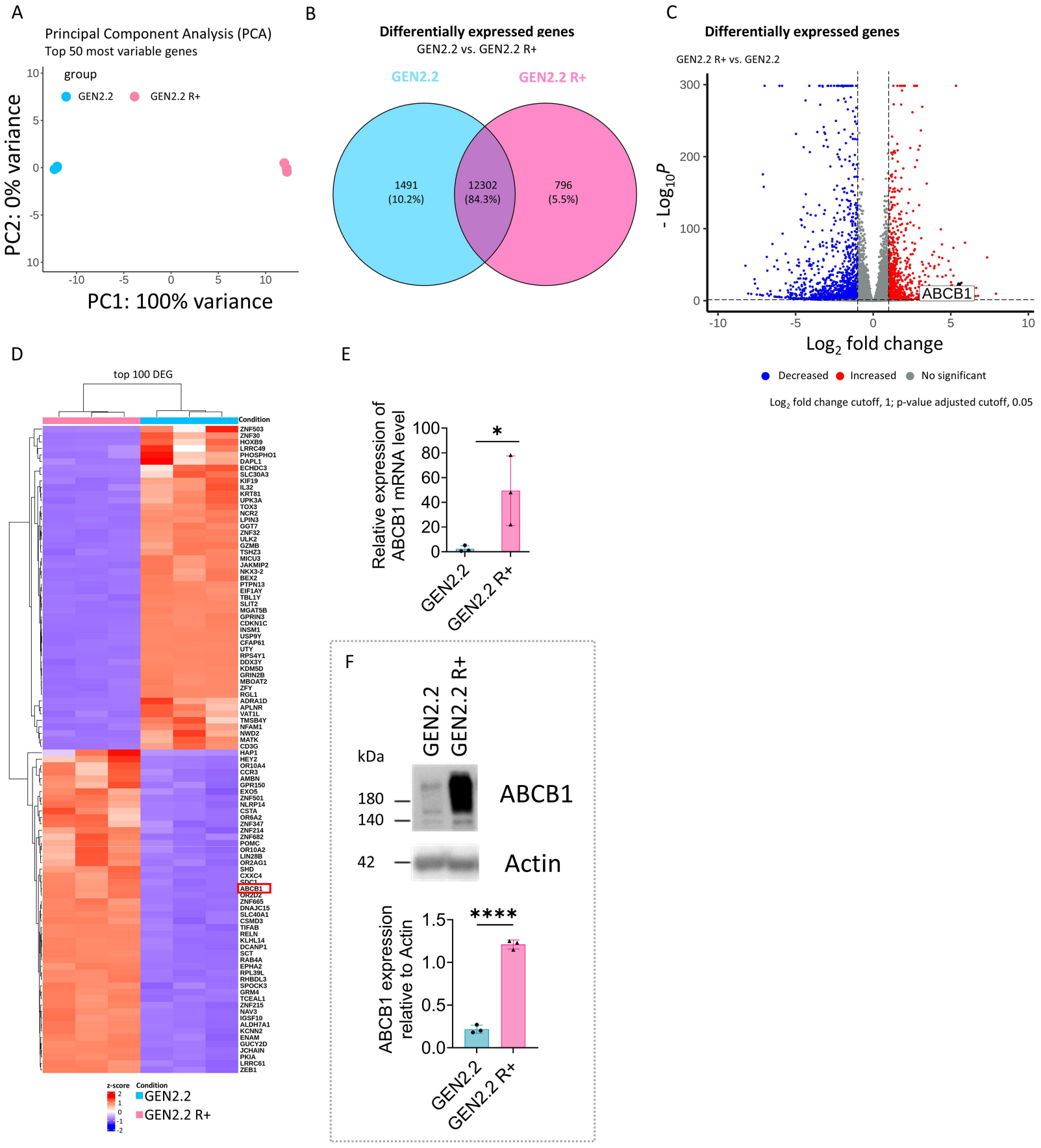
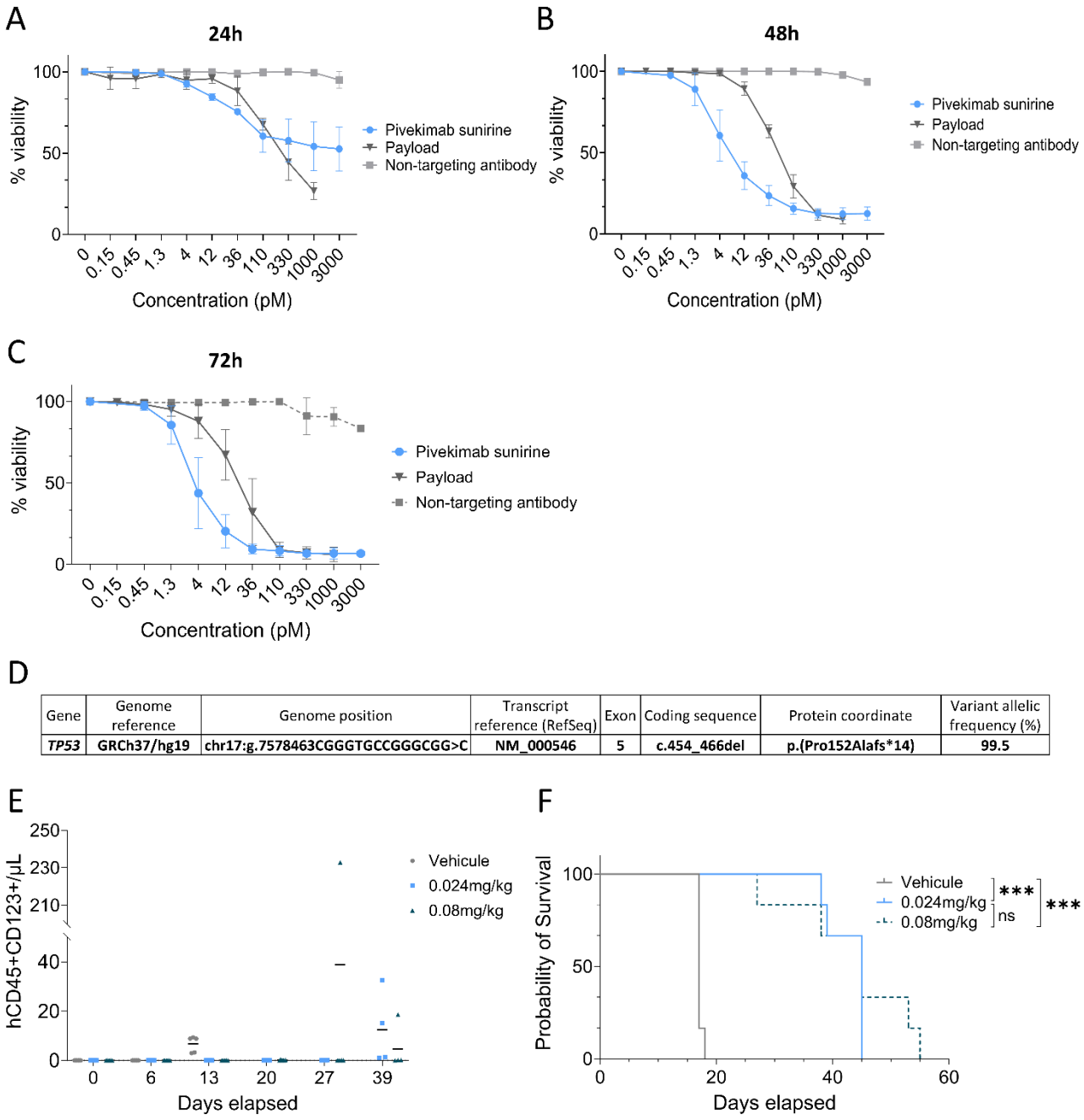

B
GEN2.2 R+
Isotype
GEN2.2
Count
CD123
CD123 RFI
**
30
20
10
0
GEN2.2
GEN2.2 R+

Figure 3


A
Principal Component Analysis (PCA)
Top 50 most variable genes
group
GEN2.2
GEN2.2 R+
PC2: 0% variance
PC1: 100% variance
B
Differentially expressed genes
GEN2.2 vs. GEN2.2 R+
GEN2.2
GEN2.2 R+
1491
(10.2%)
12302
(84.3%)
796
(5.5%)
C
Differentially expressed genes
GEN2.2 R+ vs. GEN2.2
- Log10P
Log2 fold change
ABCB1
Decreased
Increased
No significant
Log2 fold change cutoff, 1; p-value adjusted cutoff, 0.05
D
top 100 DEG
Condition
z-score
GEN2.2
GEN2.2 R+
E
Relative expression of ABCB1 mRNA level
*
GEN2.2
GEN2.2 R+
F
GEN2.2
GEN2.2 R+
kDa
180
140
ABCB1
42
Actin
ABCB1 expression relative to Actin
****
GEN2.2
GEN2.2 R+

Pivekimab sunirine in Blastic Plasmacytoid Dendritic Cell Neoplasm: Assessing Spatial Response and Unraveling Resistance Mechanisms

Supplementary Figure 1:



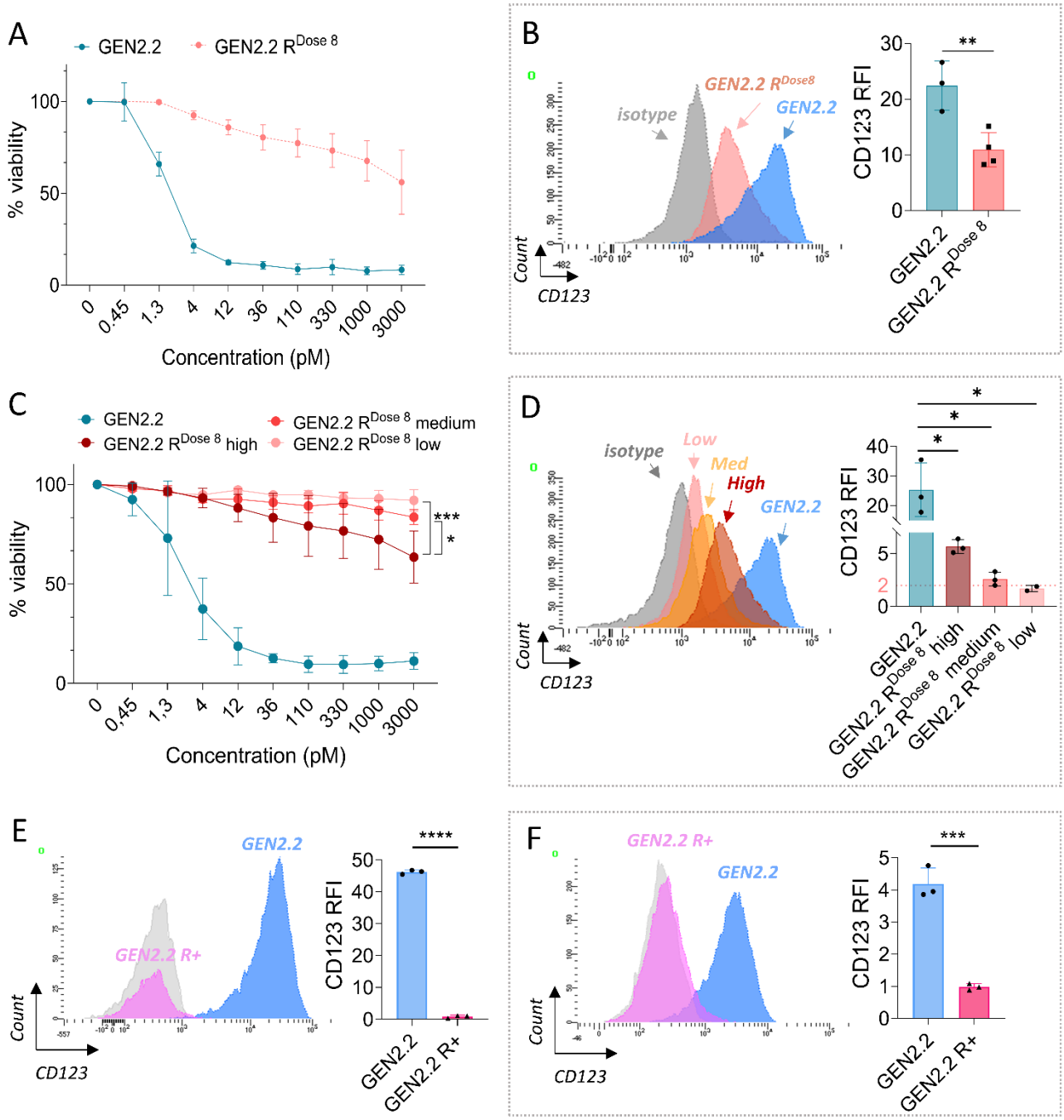
D

Gene	Genome reference	Genome position	Transcript reference (RefSeq)	Exon	Coding sequence	Protein coordinate	Variant allelic frequency (%)
TP53	**GRCh37/hg19**	**chr17:g.7578463CGGGTGCCGGGCGG>C**	**NM_000546**	**5**	**c.454_466del**	**p.(Pro152Alafs*14)**	**99.5**

(A-B-C) GEN2.2 cell line was treated with or without PVEK at different concentration ranging (from 0.45 to 3000pM) for 24h, 48h and 72h and cytotoxicity was analyzed by FC (n=3).
(D) Table displaying variant allelic frequency (%) of genes expressed by GEN2.2.
(E) Mean of $CD45^{+}/CD123^{+}$ cell counts in blood from mice injected with GEN2.2 during treatment with vehicle (grey) or PVEK (light blue: 0.024mg/kg; dark blue:0.08mg/kg).
(F) Mice were monitored daily until death, or euthanized if they showed signs of suffering in accordance with animal welfare guidelines. Kaplan-Meier survival curves for all groups were

generated starting from the day of GEN2.2 injection and using death of the animal as the terminal event. Comparison of survival between groups is shown, ***p<0.0002. Overall survival analysis of mice revealed significant gain of survival for PVEK-treated mice compared with untreated mice (n=6 mice per group).

Supplementary Figure 2:



(A) GEN2.2 and GEN2.2 R^{Dose8} cell lines were treated with or without PVEK at different concentration ranging (from 0.45 to 3000pM) for 72 hours and cytotoxicity was analyzed by FC (n=3).

(B) Evaluation of CD123 RFI by FC on GEN2.2 or GEN2.2 R$^{Dose\ 8}$ cell lines (n=3).

(C) GEN2.2 R^{Dose8} was sorted into 3 fractions based on CD123 expression (low, medium and high expression of CD123). GEN2.2 and the 3 fractions sorted from GEN2.2 R^{Dose8} (low, medium and

high) cell lines were treated with or without PVEK at different concentration ranging (from 0.45 to 3000pM) for 72 hours and cytotoxicity was analyzed by FC (n=3).

(D) Evaluation of CD123 RFI by FC on different GEN2.2 cell lines (GEN2.2, GEN2.2 R^{Dose8} low, medium and high) (n=3).

(E) Evaluation of CD123 RFI by FC on GEN2.2 and GEN2.2 R+ using 9F5 clone CD123 antibody (n=3) ($p<0.0001$).

(F) Evaluation of intracellular CD123 RFI by FC on GEN2.2 and GEN2.2 R+ using 6H6 clone CD123 antibody (n=3) ($p=0.0004$).