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Comparison of *In vivo* [18F]fluoro-desoxyglucose and [18F]fluoro-thymidine Positron Emission Tomography for disease monitoring in a mouse model of higher-risk myelodysplastic syndrome

Laure Sarda-Mantel[1,2], Panhong Gou[3], Fortune Hontonnou[2,3], Benoit Hosten[2,4,5], Nicolas Vignal[1,2], Carine San[2,4], Caren Brumpt[6], Pierre Fenaux[7,8], Christine Chomienne[3], Stephane Giraudier[3,9], Rose Ann Padua[3]

[1] Nuclear Medicine Department, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris (APHP), 2 rue Ambroise Paré, 75010 Paris, France. Laure.sarda-mantel@aphp.fr
[2]Claude Kellershohn Unit, Saint-Louis Research Institute, Paris University, 1 avenue Claude Vellefaux, 75010 Paris, France
[3]Inserm UMR-S1131, Saint-Louis Research Institute, 1 avenue Claude Vellefaux, 75010 Paris, France
[4] Radiopharmacy Department, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris (APHP), 1 avenue Claude Vellefaux, 75010 Paris, France
[5] Pharmacy Faculty, Paris University, 4 avenue de l'observatoire, 75006 Paris, France
[6]Hematology Laboratory, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris (APHP), 2 rue Ambroise Paré, 75010 Paris, France
[7]Inserm UMR-S944, Saint-Louis Research Institute, 1 avenue Claude Vellefaux, 75010 Paris, France
[8]Hematology Seniors Department, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris (APHP), 1 avenue Claude Vellefaux, 75010 Paris, France
[9]Hematology, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris (APHP), 1 avenue Claude Vellefaux, 75010 Paris, France

Correspondance to Pr L. Sarda-Mantel : laure.sarda-mantel@aphp.fr

ABSTRACT

Higher-risk myelodysplastic syndrome (HR-MDS) has a poor prognosis in the absence of efficient therapy. The evaluation of new therapies in animal models of HR-MDS is hampered by the absence of accurate *in vivo* biomarkers of the disease. In this study we compared [18F]Fluoro-desoxyglucose Positron Emission Tomography (FDG-PET) and [18F]Fluoro-thymidine (FLT)-PET imaging for disease follow-up in a triple transgenic *MMTVtTA/TetoBCL-2/MRP8NRASD12* mouse model of HR-MDS. Normal control FVB/N mice (G1,n=9) and HR-MDS mice (G2,n=12) underwent both FDG- and FLT-PET procedures at 2-day intervals, on a dedicated small animal device. Blood cell counting, BCL-2 and Mac-1hi/Gr-1lo expression measurements in blood were performed before each PET procedure. Visually, PET images of G2 mice demonstrated homogeneous FDG uptake in the whole skeleton similar to that observed in G1 mice, and abnormal FLT hot spots in bone marrow not observed in G1 mice. The intensity of FLT hot spots in bone marrow was higher in 3-months old G2 mice than in 2-months old G2 mice, concordant with a higher percentage of cells expressing Mac-1hi/Gr-1lo and lower platelets counts. We conclude that FLT-PET/CT imaging is a more valuable surrogate non-invasive quantitative marker of HR-MDS bone marrow involvement than FDG-PET/CT in our mouse model of HR-MDS.

Keywords: Myelodysplastic syndrome, Positron Emission Tomography, [18F]fluoro-thymidine, small animal imaging

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INTRODUCTION

Myelodysplastic syndromes (MDS) is an orphan disease of myeloid stem cells. It is characterized by ineffective hematopoiesis leading to life threatening blood cytopenias, and a variable risk of progression to acute myeloid leukemia (AML).[1] In high risk (HR) MDS (defined by an international score, IPSS), the poor median overall survival is only 15 months, except in the small proportion of cases (15%) who benefit from allogeneic stem cell transplantation. A large phase III clinical trial showed that azacitidine (AZA), a hypomethylating agent, significantly increased median survival of HR-MDS to about 24 months. However, 50% of the patients do not respond to AZA, and no drug has demonstrated clear efficacy after AZA failure.[2,3] HR-MDS thus remains an unmet medical need of the elderly. Allogeneic stem-cell transplantation remains, with few exceptions, the only curative treatment of higher-risk myelodysplastic syndromes, with prolonged disease-free survival of 35–50%. However, only about 15% of HR-MDS patients may receive allogeneic SCT, due to the median age of the population (about 70 years) and the absence of an HLA matched donor, justifying the development of other therapeutic approaches.

In MDS, ineffective haemopoiesis results from the increased susceptibility of clonal myeloid progenitors to apoptosis, which leads to cytopenias despite a generally hypercellular marrow. Progression to AML is thought to result from a subsequent shift from apoptosis to proliferation of these clonal progenitors. Expansion of minor subclones can also contribute to transformation to AML.

Positron Emission Tomography (PET) imaging using [^{18}F]fluoro-desoxyglucose (FDG) has proven to be highly efficient for early assessment of therapeutic response in glucose-avid malignant diseases, especially in lymphomas. FDG-PET seems useful for the detection and therapeutic follow-up of extra-medullary lesions in AML. But FDG uptake in bone marrow (BM) is weak.[4] Conversely, few but promising data have been reported on the use of PET imaging with [^{18}F]Fluoro-thymidine (FLT), for the detection and therapeutic monitoring of BM disease in patients with MDS or AML.[5-7] FLT uptake in tissues is correlated with exogenous thymidine uptake and incorporation in DNA during the S mitotic phase.[8] FLT-PET imaging has been used for therapeutic evaluations in several malignant diseases. It demonstrated good correlation with proliferative indexes on histology, and to TK1 expression in cells.[8]

An animal model of HR-MDS and AML progression was created in Inserm Unit 1131, using mutant NRAS and overexpression of BCL-2,[9] known to be poor prognostic indicators of the human diseases.[10] This model was used to test anti-BCl2 agents ABT737 and ABT199, as well as anti-MEK and anti-p53 agents. However such evaluations are hampered by the absence of accurate *in vivo* biomarkers to follow the therapeutic effects of the treatments, BM biopsy, although feasible, is not easy in mice. Currently, the only valuable end-point in these studies is survival duration.

In this study, our aim was to evaluate the ability of FDG- and FLT-PET to monitor BM involvement in our triple transgenic *MMTVtTA/TetoBCL-2/MRP8NRASD12* mouse model of HR-MDS.

RESULTS

We investigated 2 groups of mice (FVB/N strain): normal FVB/N mice (G1, n=9), and triple transgenic mice (G2, HR-MDS, n=12). Mice of G1 aged 2-3 months underwent FDG- and

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FLT-PET/CT once. HR-MDS mice of G2 underwent FDG- and FLT-PET/CT twice: at the age of 2 months, then 1 month later at 3 months of age. For each imaging set, FDG-PET/CT was performed 48h before or after FLT-PET/CT. For all mice, just before FLT-PET/CT imaging, peripheral blood samples were taken for peripheral blood (PB) cell counts, and for BCL-2 and Mac-1hi/Gr-1lo (blast cells) expression measurements by flow cytometry as described previously.[9] After the last imaging procedure, all mice were sacrificed for BM cytological analysis.

Biological data

The results of PB parameters in G1 and G2 are reported in Table 1. As expected, BCL-2 and Mac-1hi/Gr-1lo expressions are increased in G2 vs G1. White Blood cells (WBC) counts are higher in HR-MDS G2 than in G1 mice, and higher in 3-months G2 mice than in 2-months G2 mice. Platelet and Hb counts were lower in G2 than in G1 mice, and decreased between 2 months and 3 months in G2 mice. These latter data are consistent with disease progression.

Cytological data

No blast was observed in PB of all mice. In femoral BM, the percentage of blasts was 4.2±2.2% in FVB/N G1 mice, and 16.3±9.3 in 3 month-old G2 HR-MDS mice ($p<0.04$ vs G1). Dystrophic megakaryocytes were seen in the BM of G2 mice, confirming the diagnosis of MDS. Cell counts in 3-month-old G2 mice were evocative of infection (numerous immature granulocytes and only few erythroblasts).

***Table 1**: Peripheral Blood parameters, FLT and FDG quantification on PET/CT images, in normal FVB/N mice (G1) and in 2 months-old then 3 months-old triple transgenic HR-MDS mice (G2).*

	G1 normal N=9	**G2 2mo HR-MDS N=12**	**G2 3mo HR-MDS N=12**	**G2 2mo versus G1 (*p*)**	**G2 3mo versus G1 (*p*)**	**G2 3mo versus G2 2mo (*p*)**
WBC x 10^3/µl	5.13±1.18	10.39±3.08	16.67±8.56	*<0.03*	*<0.03*	*<0.02*
RBC x 10^3/µl	9.49±0.18	8.65±0.41	7.88±2.58	*<0.02*	*NS*	*NS*
Platelets x 10^3/µl	1055±221	917±198	681±199	*NS*	*<0.04*	*<0.007*
Hb (g/dL)	16.40±0.14	15.27±0.81	13.17±4.48	*NS*	*NS*	*NS*
BCL-2 %	1.8±1.2	20.5±4.9	25.9±12.0	*<0.0002*	*<0.006*	*NS*
Mac-1hi/Gr-1lo %	4.3±2.4	28.9±14.5	30.1±13.62	*NS*	*<0.03*	*NS*
FLT-PET TBR in femoral BM	1.06±0.16	2.02±0.94	3.13±2.32	*<0.002*	*<0.0003*	*= 0.05*

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FDG-PET max %ID/g in femoral BM	3.98±1.11	16.50±3.68	12.27±3.58	*<0.007*	*<0.02*	*<0.05*

PB parameters: Number of WBC, Red Blood Cells, and platelets per ml, hemoglobin (Hb) level, percentage of cells expressing BCL-2, percentage of cells expressing Mac-1hi/Gr-1lo in PB.

FLT and FDG quantification on PET/CT images: TBR = the intensity of abnormal hot spots in femoral bone marrow determined on FLT-PET/CT images; max %ID/g in femoral bone marrow = maximal percentage of FDG injected dose per gram in femoral bone marrow on FDG-PET/CT images.

FLT-PET/CT data

FLT radiosynthesis was achieved in 48 min with a non-corrected yield of 11.5-20.7%. Radiochemical purity was > 99%. Specific activity was 267-1567 GBq/µmol and volumic activity was 1-1.5 GBq/mL. The elution time of FLT was 8.8 min.

Visual analysis of FDG-PET/CT images demonstrated homogeneous FDG uptake in the whole skeleton in all G1 and G2 mice (Figure 1A). Conversely, as compared to FLT-PET/CT images obtained in normal control G1 mice, FLT-PET/CT images of all G2 mice revealed abnormal unilateral or bilateral asymmetric hot spots in femoral diaphysis, in spine and/or in humeral bones (Figure 1B). The abnormalities observed in G2 HR-MDS mice became more intense and/or extensive at the age of 3 months than at the age of 2 months.

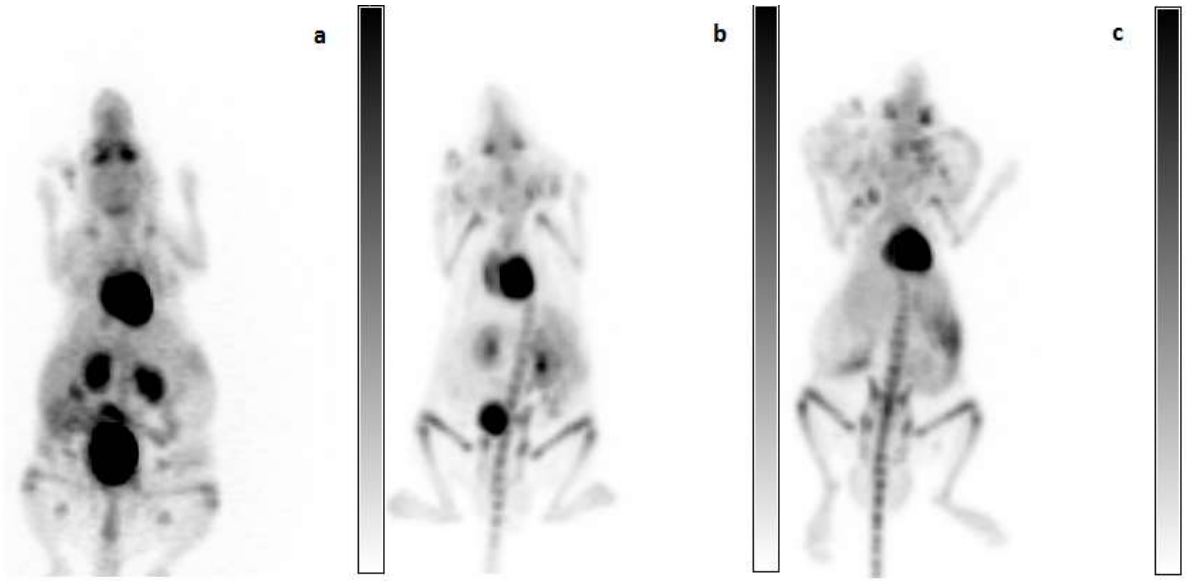

***Figure 1A**: FDG-PET images (whole-body volumes) of a normal FVB/N (G1) mouse (a) and of a HR-MDS (G2) mouse at 2 months of age (b) then 3 months of age (c).*

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Visually, all three FDG-PET scans show homogeneous FDG uptake in the whole-skeletal bone marrow.

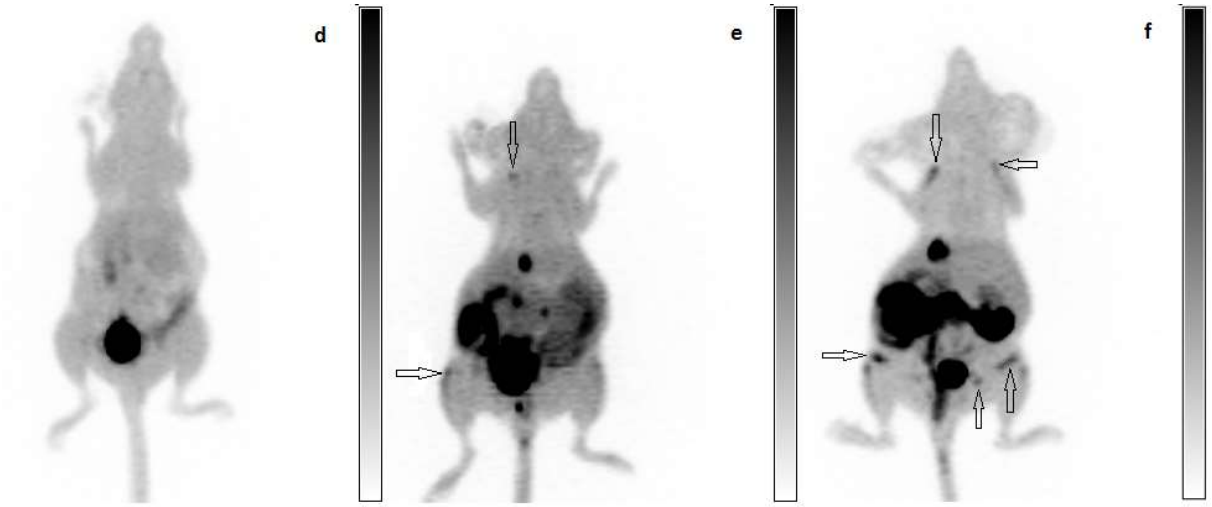

***Figure 1B**: FLT-PET images (whole-body volumes) of the same normal FVB/N G1 mouse (d) and G2 HR-MDS mouse (e,f) than in Figure 1A.*
Clear abnormal hot spots are seen in the humerus and the femurs in the HR-MDS mouse (e,f) as compared to the control mouse (d). These abnormalities increase in extent and intensity between the age of 2 (e) and 3 (f) months (arrowed).

The results of FLT- and FDG-PET quantifications obtained in the two groups of mice are reported in Table 1 and Figure 2. On FLT-PET/CT, the femoral BM-to-background bloodpool activity ratio was higher in G2 than in G1 mice, and higher in 3-months old G2 mice than in 2-months old G2 mice. FDG max %ID/g in femoral bone marrow was higher in G2 than G1 mice, but decreased between the age of 2 and 3 months in G2 mice.

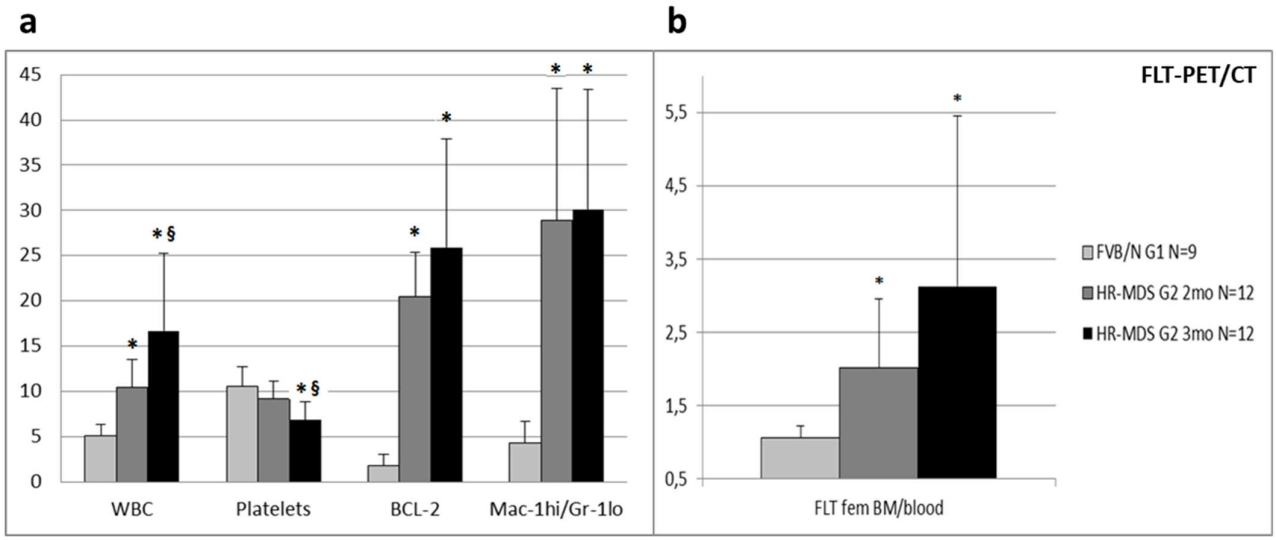

***Figure 2:** Graphs comparing quantitative PET/CT and PB parameters in G1 and G2.*
(a) PB parameters in G1 and G2 (number of WBC x10^3/ µl of blood, number of platelets x10^5/

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µl of blood, % of blood cells expressing BCL2, % of blood cells expressing Mac-1hi/Gr-1lo). (b) FLT-PET/CT quantification: femoral BM-to-background (bloodpool) activity ratio *on FLT-PET/CT images of G1 and G2.*
**: significant difference ($p<0.05$) between G2 and G1. §: significant difference between 3-month old G2 mice and 2-month old G2 mice.*

DISCUSSION

In our triple transgenic model of HR-MDS, both FDG-PET/CT and FLT-PET/CT were performed and compared to normal control group of mice. FDG-PET/CT and FLT-PET/CT scans demonstrated different features. FDG-PET/CT showed homogeneous increased FDG uptake in the whole skeleton BM in HR-MDS mice as compared to controls and decreased with age. FLT-PET/CT showed focal abnormalities in one or several bones of HR-MDS mice, the intensity of which increased with age, together with BCL-2 and Mac-1hi/Gr-1lo peripheral blood expression as well as with PB WBC counts and decreased blood platelet counts consistent with disease progression between 2 and 3 months.

To our knowledge, this is the first study reporting a direct comparison between FDG and FLT-PET/CT in HR-MDS disease. FDG and FLT-PET/CT were previously compared in a tumor-bearing mouse model of human chronic myeloid leukemia (K562 cell line).[11] The authors reported increased FLT uptake in the tumors, with a bone marrow-to-background activity ratio of 5.39±0.42 at 30min post-IV, 4.88±0.43 at 1h and 3.81±0.38 at 2h post-IV, whereas FDG-PET/CT scans were negative. Our results show completely different patterns of BM uptake between FLT and FDG in HR-MDS mice.

FDG uptake in BM of our HR-MDS mice was always homogeneous. FDG uptake in MDS was previously described in few clinical case reports: focal abnormalities[12,13] or diffuse increased FDG uptake in the whole skeleton[14] were retrieved. The most important series using FDG-PET/CT in leukemic diseases is that of Li H. et al in 35 patients suspected of acute leukemia relapse. In this study, only 3 of 16 patients showed focal BM FDG abnormalities, all with positive BM biopsy; 24 showed diffuse increased FDG uptake in BM, among which 14 had positive BM biopsy and 10 negative BM biopsy. Overall, visual analysis achieved a successful diagnosis only in 68.6% (24/35) of patients[14]. In our mice, FDG uptake in BM decreased between the age of 2 and 3 months, whereas all peripheral blood parameters demonstrated more severe disease at 3 months than at 2 months: WBC (including blasts) counts, expression of BCL2 and of Mac-1hi/Gr-1lo increased, whereas platelet counts decreased. This suggests that FDG uptake in bone marrow may not be an accurate marker of HR-MDS.

FLT-PET allows *in vivo* quantification of cell proliferation and has been suggested for the diagnosis and follow-up of patients with myelodysplastic disorders. Conversely, FLT abnormalities in HR-MDS mice were always focal, and increased in intensity with age. This suggests that: firstly, FLT-PET/CT scans are easier to interpret (normal or abnormal) than FDG-PET/CT scans; secondly, FLT is a valuable quantitative marker of HR-MDS disease. Concordantly to our data in mice, Agool et al reported increased FLT uptake in epiphyses and expansion in diaphysis of peripheral bones in patients with MDS.[5,6]

The question of whether blast or normal hematopoietic cells accumulate FLT is still unclear. Authors agree that both types of cells can proliferate in MDS and acute leukemia. Moreover, it seems that not all the blasts proliferate at the same time.[15]

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Our data suggest that FLT-PET/CT is a better marker of HR-MDS disease than FDG-PET/CT in our mouse model of HR-MDS. If this proves to be the same in HR-MDS patients, it may allow to avoid or to guide BM biopsy in some cases and to monitor response to therapy. To this regard, Vanderhoeka et al suggested the potential value of FLT-PET for *in vivo* chemotherapeutic monitoring in AML patients.[7] FLT uptake in BM was absent in patients with complete remission, whereas significant uptake was still observed in patients with resistant disease, including one with aplastic BM (complete remission) on post-therapy BM biopsy.

MATERIALS and METHODS

Animal models

All mice lines were maintained in Friend leukemia Virus B (FVB/N) strain from the National Institutes of Health.

The triple transgenic MMTVtTA/TetoBCL-2/MRP8NRASD12 murine model of HR-MDS was previously validated in the lab.[9,16,17] *MMTVtTA/TetoBCL-2/MRP8NRASD12* were obtained by crossing MMTVtTA/TBCL-2 mice with mice harboring mutant NRASD12 gene under the regulation of the myeloid promoter MRP8. The mice were genotyped as previously described[9] The mice develop an MDS-like disease with around 15% BM blasts with a dysplasia, some liver invasion and a relatively indolent disease with a long latency period before they die. Normal FVB/N mice have 0 to 4% of blasts in BM.[9,17]

All animal experiments were performed in accordance with European guidelines for care of laboratory animals (2010/63/EU) and were approved by the Animal Ethics Committee of Paris Nord.

FLT-PET/CT and FDG-PET/CT

[^{18}F]fluoro-thymidine (FLT) radiosynthesis

FLT was synthetized in the UCK lab on the All-In-One® (Trasis©, Ans, Belgium) radiosynthesis automate by nucleophilic substitution (90°C, 5min) of nosylated precursor (3-N-boc-5-ODMTr-3-O-nosyl-lyxothymidine), after activation of fluorine- 8 by $TBAHCO_3$ (100°C, 10min). Further steps are a deprotection of the product by acid hydrolysis (1,5mL HCL 2M, 95°C, 5min) then neutralisation (2,4mL NaOH 1M, 40°C). After neutralisation the solution was passed through 2 cartridges (IC-H, Alumina), then the purification was performed using CLHP column Akzo Nobel Kromasil® C18 10µm 250*10mm, in a solution H2O/ethanol (90/10 v/v) at 6 mL/min. The chemical purity, the radiochemical purity (RCP) and specific activity were determined by analytic CLHP (Akzo Nobel Kromasil® column (C18 5µm 250*4,6mm), with an H20/acetonitrile gradient starting from 90/10 to 5/95 at 1 mL/min. The identity of the labeled compound [^{18}F]FLT was confirmed by co-injection with a non-radioactive standard of FLT. The FLT concentration in the radioactive sample was obtained using the UV-peak area ratio between the radioactive product and the standard solution.

PET-CT acquisitions

PET/CT imaging was performed as previously described[18] using Inveon PET/CT scanner (Siemens Medical Solutions) designed for small laboratory animals.

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Mice were anesthetized (isoflurane/oxygen, 2.5% for induction at 0.8-1.5 L/min and 1.5% at 0.4-0.8 L/min thereafter) during injection of FLT or FDG (7-10 MBq) in a volume of 0.15 mL via the tail vein, and during PET/CT acquisitions. For FLT-PET/CT, mice were kept on standby for 1h after FLT injection, then were re-anesthetized and placed in the PET camera in prone position under isoflurane anesthesia and respiratory monitoring for a 15 min-duration static PET acquisition followed by a 10min-duration CT acquisition for attenuation correction of PET images and anatomic localization of PET signals. For FDG-PET/CT, mice were kept anesthetized during 60min after radiotracer injection (to avoid muscular uptake) under a heat lamp to avoid FDG uptake in brown fat tissues, then placed in the PET camera for a static acquisition of 10 min-duration then a CT acquisition of 10min-duration. The spatial resolution of Inveon PET device was 1.4 mm full-width at half-maximum (FWHM) at the centre of the field of view. Images were reconstructed using a 2-D ordered subset expectation maximization (Fourier rebinning/2-D OSEM) method including corrections for scanner dead time, scatter radiations and randoms.

PET-CT analysis

Qualitative visual analysis of whole-body FLT and FDG uptakes in axial and peripheral skeleton, liver and spleen were performed. Then FLT or FDG uptake was quantified in volumes of interest manually drawn on PET/CT images (IRW 4,2 software, Siemens). The software calculates the percentage of injected dose (Bq) per gram of tissue (%ID/g) in each voxel of the volumes of interest drawn on the images. The max %ID/g values obtained in the volumes of interest were considered for quantitative analysis. For FLT-PET/CT images, max %ID/g obtained in femoral BM was divided by max %ID/ml obtained in the blood (volume of interest drawn on the cardiac area), in order to calculate a bone marrow-to-background (bloodpool) activity ratio. For FDG-PET/CT images, only the max %ID/g obtained in femoral BM was considered. Indeed the bone marrow-to-background (bloodpool) activity ratio could not be calculated on FDG-PET/CT images because of high normal FDG uptake in the myocardium impairing the drawing of blood volumes of interest.

Ex-vivo analyses

On the day of FLT-PET/CT imaging, PB samples were taken for red blood cell, leukocyte, and platelet counting, hemoglobin (Hb) dosage and for BCL-2 and Mac-1hi/Gr-1lo (as a marker of primitive cells) expression measurements. After the last set of PET/CT imaging the mice were sacrificed, femoral BM was flushed, and PB and femoral BM were smeared on slides for cytological analysis.

May Grunwald Giemsa stainings were performed on PB and femoral BM smears and examined by a cytologist of Lariboisière Hospital with an Olympus Bx41 microscope. The cell morphology was studied at magnification 100X, 500X and 1000X, according to the WHO 2016 criteria. The percentage of blasts was determined on the BM smears by counting 200 cells at magnification 1000X.

Statistical analysis

Statistical analysis was performed using Graphpad prism 5 version 5.0 software. Kruskal Wallis and Wilcoxon signed-rank test was used to compare PB cell counts, BCL2 and Mac-1hi/Gr-1lo expressions in blood, % of blasts on BM smears, FDG-max %ID/g and FLT-bone marrow –to-background activity ratios between groups of mice. A significance value of $p<0.05$ was used.

CONCLUSION

In our triple transgenic HR-MDS mouse model, FLT-PET/CT imaging demonstrated focal BM abnormalities, with intensity increasing with age concordantly with PB parameters that evidenced more severe disease at the age of 3 months than at the age of 2 months. Whereas FDG-PET/CT imaging demonstrated homogeneous increased FDG uptake in the whole skeleton's BM decreasing with age, thus not correlating with PB parameters. These results suggest that FLT-PET/CT imaging is a more valuable surrogate non-invasive quantitative marker of HR-MDS BM involvement than FDG-PET/CT in our mouse model. It remains to be determined whether this finding may be the same in HR-MDS patients.

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Conflict of interest: The authors have no relevant conflict of interest.

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REFERENCES

1. Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet.* 2014 ; 383: 2239-52.

2. Garcia-Manero G and Fenaux P. Hypomethylating agents and other novel strategies in myelodysplastic syndromes. *J Clin Oncol.* 2011; 29: 516-523.

3. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol.* 2009; 10: 223-232.

4. Cribe AS, Steenhof M, Marcher CW, Petersen H, Frederiksen H, Friis LS. Extramedullary disease in patients with acute myeloid leukemia assessed by 18F-FDG PET. *Eur J Haematol.* 2013; 90: 273-8.

5. Agool A, Schot BW, Jager PL, Vellenga E. 18F-FLT PET in hematologic disorders: a novel technique to analyze the bone marrow compartment. *J Nucl Med.* 2006; 47: 1592-8.

6. Agool A, Glaudemans AW, Boersma HH, Dierckx RA, Vellenga E, Slart RH. Radionuclide imaging of bone marrow disorders. *Eur J Nucl Med Mol Imaging*. 2011; 38: 166-78.

7. Vanderhoek M, Juckett MB, Perlman SB, Nickles RJ, Jeraj R. Early assessment of treatment response in patients with AML using [(18)F]FLT PET imaging. *Leuk Res.* 2011; 35: 310-6.

8. McKinley ET, Ayers GD, Smith RA, Saleh SA, Zhao P, Washington MK, et al. Limits of

[18F]-FLT PET as a biomarker of proliferation in oncology. *PLoS One.* 2013; 8: e58938.

9. Omidvar N, Kogan S, Beurlet S, le Pogam C, Janin A, West R et al. BCL-2 and mutant NRAS interact physically and functionally in a mouse model of progressive myelodysplasia. *Cancer Research.* 2007**;** 67: 11657-67.

10. Padua RA, Guinn BA, Al-Sabah AI, Smith M, Taylor C, Pettersson T, et al. RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. *Leukemia.* 1998; 12: 887-892.

14. Lu L, Jiang L, Guan H, Gao Y, Lu H. Imaging proliferation in human leukemia-tumor bearing mice with (18)F-FLT: Comparison with (18)F-FDG PET. *Hell J Nucl Med.* 2012; 15: 206-9.

11. Valls L**,** Badve C, Avril S, Herrmann K, Faulhaber P, O'Donnell J, et al. FDG-PET imaging in hematological malignancies. *Blood Rev.* 2016; 30: 317-31.

12. Liu F, Cao Q. Transformation of myelodysplastic syndrome to acute myeloid leukemia: A case with whole-body 2-[F18] fluoro-2-deoxy-D-glucose positron emission tomography. *Indian J Nucl Med.* 2011; 26: 104-6.

13. Inoue K, Okada K, Harigae H, Taki Y, Goto R, Kinomura S, et al. Diffuse bone marrow uptake on F-18 FDG PET in patients with myelodysplastic syndromes. *Clin Nucl Med.* 2006; 31: 721-3.

14. Li H, Xu C, Xin B, Zheng C, Zhao Y, Hao K, Wang Q, et al. (18)F-FDG PET/CT Radiomic analysis with Machine Learning for Identifying Bone Marrow Involvement in the Patients with Suspected Relapsed Acute Leukemia. *Theranostics.* 2019; 9: 4730-4739.

15. Clarkson B, Ohkita T, Ota K, Fried J. Studies of cellular proliferation in human leukemia. I. Estimation of growth rates of leukemic and normal hematopoietic cells in two adults with acute leukemia given single injections of tritiated thymidine. *J Clin Invest.* 1967; 46: 506-29.

16. Beurlet S, Omidvar N, Gorombei P, Krief P, Le Pogam C, Setterblad S, et al. BCL-2 inhibition with ABT-737 prolongs survival in an NRAS/BCL-2 mouse model of AML by targeting primitive LSK and progenitor cells. *Blood.* 2013; 122: 2864-2876.

17. Guerenne L, Beurlet S, Said M, Gorombei P, Le Pogam C, Guidez F et al. GEP analysis validates high risk MDS and acute myeloid leukemia post MDS mice models and highlights novel dysregulated pathways. *J Hematol Oncol*. 2016; 9: 5.

18. Rizzo-Padoin N, Chaussard M, Vignal N, Kotula E, Tsoupko-Sitnikov V, Hontonnou F, et al. [18F]MEL050 as a melanin-targeted PET tracer: fully automated radiosynthesis and comparison to 18F-FDG for the detection of pigmented melanoma in mice primary subcutaneous tumors and