SUPPLEMENTAL DATA

Method for Partial Volume Effect Correction in Quantitative MicroPET-CT Imaging

Measured activity values within TEP images were corrected to take into account the signal lost due to the partial volume effect. Briefly, a ratio of activity values obtained by CT segmentation and PET manual segmentation was previously determined on 20 images 90 min after injection. The standard deviation between each ratio measurement was less than 5 % and was then considered as constant. Afterwards, activity values measured in CT-based ROI were multiplied by this ratio to estimate the total activity within the total skeleton.

Compartmental Model detailed description

Progenitors: P

$$\frac{dC_P(t)}{dt} = \left(\varphi_{P-P}(t) - \varphi_{P-M} - \Omega_{\mathrm{P}-\emptyset}(t)\right)C_P(t) \qquad \text{Supplemental Eq. 1}$$

Where $\varphi_{P-P}(t)$ represents the self-renewal function of the progenitor cells compartment, φ_{P-M} is the differentiation rate constant from progenitors to megakaryocytes.

$\Omega_{\mathrm{P}-\emptyset}(t)$ is the death rate due to radiation dose. The function $\Omega_{\mathrm{P}-\emptyset}(t)$ is directly derived from the linear quadratic formula to describe the response of cells after irradiation. The survival fraction of cells after irradiation can be expressed as follows:

$$\ln\left(\frac{C_P(t)}{C_{P_0}}\right) = -\alpha D(t) - \beta D(t)^2, \qquad \text{Supplemental Eq. 2}$$

Where $C_P(t)$ is the number of surviving cells at time t, C_{P_0} is the initial cell population. α and β are radiosensitive coefficients of non-repairable damage per Gy and repairable damage per Gy^2 respectively for an electrons radiation source of 300 keV.

The radiation dose rate $\dot{D}(t)$ at time t varies with time due to the physical decay and as a result of the radionuclide biodistribution in organs. The absorbed dose $D(t)$ at time t is derived from the following equation**:**

$$D(t) = \int_0^t \dot{D}(\tau)d\tau. \qquad \text{Supplemental Eq. 3}$$

By derivation of the Supplemental Equation 3 we obtained a new differential equation governing the progenitors surviving fraction with time:

$$\frac{dC_P(t)}{dt} = -\left(\alpha\dot{D}(t) + 2\beta\dot{D}(t)D(t)\right)C_P(t) \qquad \text{Supplemental Eq. 4}$$

thus, one can express $\Omega_{\mathrm{P}-\emptyset}$ as follows:

$$\Omega_{\mathrm{P}-\emptyset}(t) = \alpha\dot{D}(t) + 2\beta\dot{D}(t)D(t)\mathbf{.} \qquad \text{Supplemental Eq. 5}$$

$\dot{D}(t)$ changes as the ^{18}FNa uptake in bone. So we can rewrite:

$$\dot{D}(t) = C_B(t)S(BM \leftarrow B) \qquad \text{Supplemental Eq. 6}$$

where $C_B(t)$ is the contents of the compartments representing the cortical bone. $S(BM \leftarrow B)$ is the S-value of ^{18}F for a C57BL/6 mouse of 22 g, the target organ is the BM, the source is the bone.

The absorbed dose D(t) at time t must be explicitly expressed in the model.

$$D(t) = \int_0^t \dot{D}(t)d\tau = \int_0^t S(BM \leftarrow B)C_B(\tau)d\tau.$$ Supplemental Eq. 7

The variable $\varphi_{P-P}(t)$ gathers several terms:

$$\varphi_{P-P}(t) = \varphi_{renew}(t) \,.F(t)$$ Supplemental Eq. 8

where F(t) characterizes the main effect of the stromal microenvironment. It is a regulation factor for progenitor cell self-renewal, which is proportional to the number of stromal cells at time t:

$$F(t) = \varepsilon \frac{C_S(t)}{C_{S_0}} + \epsilon$$ Supplemental Eq. 9

where ε and ϵ are constant and C_{S_0} is the initial population of stromal cells.

$\varphi_{renew}(t)$ represents the variation of the self-renewal rate of the progenitors. It is assumed that this rate can increase twice its initial value φ_{renew_0} when progenitors undergo depletion. $\varphi_{renew}(t)$ is a direct feedback that affects progenitors:

$$\varphi_{renew}(t) = \frac{\varphi_{renew\,0}}{0.5+\omega\left(3C_P(t)/38C_{P_0}+15C_M(t)/38C_{M_0}+\left(20C_{Pl}(t)/38C_{Pl_0}\right)\right)^{1.2}}$$ day^{-1}

Supplemental Eq. 10

where ω is an arbitrary constant.

Finally,

$$\frac{dC_P(t)}{dt} = \left(\varphi_{P-P}(t) - \varphi_{P-M} - \Omega_{P-\emptyset}(t)\right)C_P(t)$$

$$= \left(\varphi_{renew}(t).F(t) - \varphi_{P-M} - \left(\alpha\dot{D}(t) + 2\beta\dot{D}(t)D(t)\right)\right)C_P(t)$$

$$= \left[\left(\frac{\varphi_{renew_0}}{0.5+\omega\left(3C_P(t)/38C_{P_0}+15C_M(t)/38C_{M_0}+\left(20C_{Pl}(t)/38C_{Pl_0}\right)^{1.2}\right)} \cdot\left(\varepsilon\frac{C_S(t)}{C_{S_0}}+\epsilon\right)\right)-\right.$$

$$\varphi P-M- \qquad \alpha CB(t)SBM\leftarrow B-2\beta CB(t)SBM\leftarrow B0tSBM\leftarrow BCB\tau d\tau CP(t)$$

Supplemental Eq. 11A

For the erythrocyte model, this Supplemental Equation 11A equals:

$$\frac{dC_P(t)}{dt} = \left(\varphi_{P-P}(t) - \varphi_{P-Reti} - \Omega_{\mathrm{P}-\emptyset}(t)\right)C_P(t)$$

$$= \left(\varphi_{renew}(t).F(t) - \varphi_{P-Reti} - \left(\alpha\dot{D}(t) + 2\beta\dot{D}(t)D(t)\right)\right)C_P(t)$$

$$= \left[\left(\frac{\varphi_{renew_0}}{1+\omega\left(5C_P(t)/86C_{P_0}+1C_M(t)/86C_{M_0}+\frac{80}{86}\left(C_{Pl}(t)/C_{Pl_0}\right)^5\right)} \cdot\left(\varepsilon\frac{C_S(t)}{C_{S_0}}+\epsilon\right)\right)-\varphi_{P-M}-\right.$$

$\alpha CB(t)SBM\leftarrow B-2\beta CB(t)SBM\leftarrow B0tSBM\leftarrow BCB\tau d\tau CP(t)$ Supplemental Eq. 11B

φ_{P-M} represents the differentiation rate of progenitor cells. At each progenitor cell doubling, one cell was assumed to enter in the megakaryocyte compartment, and one progenitor cell was assumed to self renew. Therefore, $\varphi_{P-M} = \frac{\ln(2)}{1}$ day.

Megakaryocytes: M

$$\frac{dC_M(t)}{dt} = -\varphi_{M-Pl}\nu(t)C_M(t) + \varphi_{P-M}\delta(t)C_P(t)$$ Supplemental Eq. 12

φ_{P-M} and φ_{M-Pl} are differentiation rate constants for progenitors to megakaryocytes and for megakaryocytes to platelets respectively. It is assumed that under normal conditions when a progenitor cell divides, a

new progenitor cell is created (self-renewal) and a megakaryocyte is formed (differentiation). In other words, one cell stays in the progenitor compartment and the other one enters into the precursor one. Therefore, $\varphi_{P-M} = \varphi_{renew_0}$day^{-1}.

For the erythrocyte model $\varphi_{P-Reti} = \frac{\varphi_{renew_0}}{1.6} = \frac{2.4}{1.6} = 1.5$

The mathematical description of the complicated process of nucleus duplication in megakaryocytes, which eventually determines the megakaryocyte ploidy and the number of thrombocytes produced, is substituted by introducing a quantity, namely, the coefficient of megakaryocyte ploidy $\delta(t)$. When the number of blood thrombocytes is reduced ($C_{Pl}(t) < C_{Pl_0}$, where C_{Pl_0} is the initial number of platelets), the average ploidy $\wp(C_{Pl}(t))$ increases: $\wp\ (C_{Pl}(t)) > \wp\ (C_{Pl_0})$, where $\wp\ (C_{Pl}(t))$ is the ploidy at time t and $\wp\ (C_{Pl_0})$ is the ploidy in normal conditions. The ratio of $\wp\ (C_{Pl}(t))$ to $\wp\ (C_{Pl0})$ is the ploidy coefficient $\delta(t)$:

$$\delta(t) = \frac{1}{0.5+0.5\frac{C_{Pl}(t)}{C_{Pl_0}}} \qquad \text{Supplemental Eq. 13}$$

The quantity $\nu(t)$ is the maturation time factor:

$$\nu(t) = \frac{1}{0.33+0.66\frac{C_M(t)}{C_{M_0}}} \qquad \text{Supplemental Eq. 14}$$

Concerning the erythrocyte model:

Reticulocytes spend 2-3 days maturing in bone marrow before being released in the circulation in most species. However, we assume that when erythrocytes are depleted, reticulocytes can pass from the marrow to blood immediately (2) with a shortening maturation time. Therefore we set $\varphi_{Reti-Ery} = \frac{1.5}{\left(\frac{C_{Ery}(t)}{C_{Ery_0}}\right)^8}$ in which the value in exponent allows to increase the value of $\varphi_{Reti-Ery}$ rapidly even for a small depletion.

Platelets:

$$\frac{dC_{Pl}(t)}{dt} = -\varphi_{Pl-\emptyset}C_{Pl}(t) + \varphi_{M-Pl}\nu(t)C_M(t) \qquad \text{Supplemental Eq. 15}$$

$\varphi_{Pl-\emptyset}$ and φ_{M-Pl} are death rate constants for platelets and differentiation rate for megakaryocytes to platelets respectively.

Before irradiation, the hematopoietic system is assumed to be in steady state as platelets destruction is balanced by platelets production.

In the literature, it is reported that the lifespan of mouse thrombocytes is approximately 4-5 days (3) and the corresponding rate constant for platelets destruction is reported to be $\varphi_{Pl-\emptyset} = \frac{ln(2)}{2.5}$ day^{-1}.

The maturation of megakaryocytes is reported to be about 2.5 days. Shen set the rate constant to $\varphi_{M-Pl} = \frac{ln(2)}{0.9}$ day^{-1} as seven mature megakaryocytes are generated to release platelets. This value was adapted and set to $\varphi_{M-Pl} = \frac{ln(2)}{2}$ day^{-1}.

For the erythrocyte model, the estimated lifespan of erythrocyte in mice ranges from 41 to 52 days. The corresponding rate constant adapted from Smirnova (4) was set to $\varphi_{Ery-\emptyset} = \frac{ln(2)}{17.3}$ day^{-1}.

Compartmental Model for the Stromal Environment.

Normal stromal: S

Although stromal cells are not directly part of the hematopoietic cells, stroma is indirectly involved in hematopoiesis and supports progenitor cell growth.

The normal stroma is governed by the following differential equation:

$\frac{dC_S(t)}{dt} = (\varphi_{S-S}(t) - \Omega_{S-\emptyset}(t) - \Omega_{S-IS}(t))C_S(t) + \varphi_{IS-S}C_{IS}(t)$ Supplemental Eq. 16

Where $C_S(t)$ represents the number of normal stromal cells at time t. The three parameters $\varphi_{S-S}(t), \Omega_{S-\emptyset}(t) \text{ and } \Omega_{S-IS}(t)$ represent the proliferation rate, the death rate due to radiation and the damage rate of normal stromal cells respectively.

$\varphi_{S-S}(t)$ is defined as the carrying capacity formula for normal stroma:

$$\varphi_{S-S}(t) = \left(\frac{ln(2)}{2.83+(2.83C_S(t)/C_{S_0})}\right) * \left(1 - \frac{C_S(t)}{C_{S_0}}\right) \quad \text{Supplemental Eq. 17}$$

Two phenomena are in correlation. Firstly, when the quantity $C_S(t)$ is equal to its initial value C_{S_0}, $\varphi_{S-S}(t)$ is nul. The stromal cells stop renewing themselves because they have reached their maximal amount C_{S_0}. The rate of proliferation of the stroma can change depending on the number of stromal cells and can vary up to twice the normal value (2). Since no data are available for stroma irradiation with electron, radiosensitivity values are taken from X-rays irradiation. This simplification is due to the fact that the normal stroma is represented by only one compartment. In reality, cells are still renewing. In the same time when $C_S(t) = C_{S_0}$ the quantity $\left(\frac{ln(2)}{2.83+(2.83C_S(t)/C_{S_0})}\right)$ is at its lowest value, which is defined as the self-renewal capacity under normal conditions for the stroma. The second one allows the stromal cells for self-renewal once their number is lower than their initial value. The lower the quantity $C_S(t)$, the higher $\varphi_{S-S}(t)$ is achieved and the higher cell renewal capacity is. The more $C_S(t)$ is far from its original value, the more it will tend to return quickly until it reaches the maximal carrying capacity C_{S_0}.

In this study, the main effect of the stromal microenvironment on hematopoiesis was simplified as a regulation factor for progenitor cell self-

renewal. This effect is taken into account directly in the term $\varphi_{P-P}(t)$ by the factor F(t), which regulates the rate constant of progenitor cell self-renewal, and was assumed to be proportional to the number of normal stromal cells at time t.

The term $\Omega_{\mathrm{S}-\emptyset}(t)$ allows for describing the radiation effect on stromal cells leading to death

$$\Omega_{S-\emptyset}(t) = \sigma_{S-\emptyset}\dot{D}(t) \qquad \text{Supplemental Eq. 18}$$

Because the stroma is defined as one compartment, we apply a global parameter of radiosensitivity $\sigma_{S-\emptyset}$. $\dot{D}(t)$ is the dose rate as defined earlier (5). Finally,

$$\Omega_{S-\emptyset}(t) = \sigma_{S-\emptyset}\dot{D}(t) = \sigma_{S-\emptyset}C_{Bl}(t)S(BM \leftarrow Bl) \qquad \text{Supplemental Eq. 19}$$

We can apply the same reasoning for the term $\Omega_{\mathrm{S}-\mathrm{IS}}(t)$ which describes the radiation effect on stromal cells leading to injured stromal cells.

$$\Omega_{S-IS}(t) = \sigma_{S-IS}\dot{D}(t) = \sigma_{S-\emptyset}C_{B}(t)S(BM \leftarrow B) \qquad \text{Supplemental Eq. 20}$$

Injured Stroma: IS

$$\frac{dC_{IS}(t)}{dt} = -(\varphi_{IS-S} + \Omega_{\mathrm{IS}-\emptyset}(t))C_S(t) + \Omega_{\mathrm{S}-\mathrm{IS}}(t)C_S(t) \qquad \text{Supplemental Eq. 21A}$$

Where $C_{IS}(t)$ represents the number of injured stromal cells at time t, φ_{IS-S}, $\Omega_{\mathrm{IS}-\emptyset}(t)$ are the repair rate of sublethal injury, and death rate due to the irradiation of injured stromal cells respectively.

$$\Omega_{\mathrm{IS}-\emptyset}(t) = \sigma_{IS-\emptyset}\dot{D}(t) = \sigma_{IS-\emptyset}\big(C_B(t)S(BM \leftarrow B)\big) \qquad \text{Supplemental Eq. 22A}$$

$\Omega_{IS-\emptyset}(t)$ describes the radiation effect on injured stromal cells leading to death with $\sigma_{IS-\emptyset}$ a radiosensitivity parameter of the injured stroma. Injured stromal cells can repair the irradiation induced damages.

Finally the term φ_{IS-S} expresses the capacity of repair of the injured stroma. φ_{IS-S} is the rate constant from injured stroma to normal stroma and represents the flux of damaged cells which have successfully repaired.

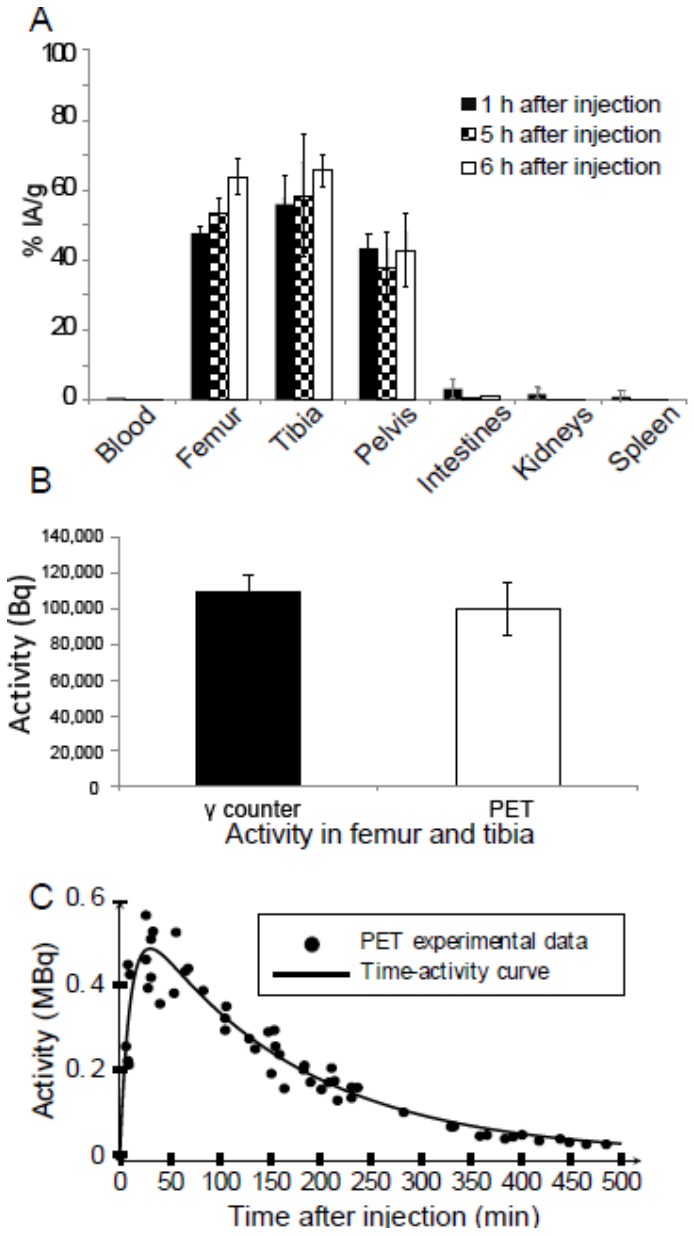
Results of Activity Quantification and Dosimetry

^{18}FNa uptake within organs of interest was studied by γ-counter measurements (Supplemental Figure 1A). A high blood clearance was observed with only 0.7 %IA/g remaining in blood at 1 h post injection (pi). Similar observations were made for kidneys, spleen and intestines with uptake inferior to 2 %AI/g. Measured activity within BM was negligible and close to background. In contrast, the uptake was important at 1 h pi for the femur, tibia, and pelvis (46, 54 and 42 %IA/g respectively). This bone accumulation was confirmed by PET imaging (Figure 2), which also showed that ^{18}FNa was excreted through urine in the first hours after injection. Calculations showed that the BM irradiation by the bladder was negligible. A bladder containing 50 %IA, considering no further excretion, would contribute only to 0.7 mGy/MBq within BM. These results confirmed that the skeleton could be considered as the principal source organ for BM absorbed dose calculations.

To determine the cumulated activity in bones, $\widetilde{A}_{Bone}$, 46 PET images were used to establish the time-activity curve. PET quantification was compared to the activities measured in femurs and tibias using the γ-counter for 3 mice (Supplemental Figure 1B). Histograms showed that the activity quantified on PET images with the developed segmentation protocol gave similar results as compared to the gold standard method. Therefore, PET

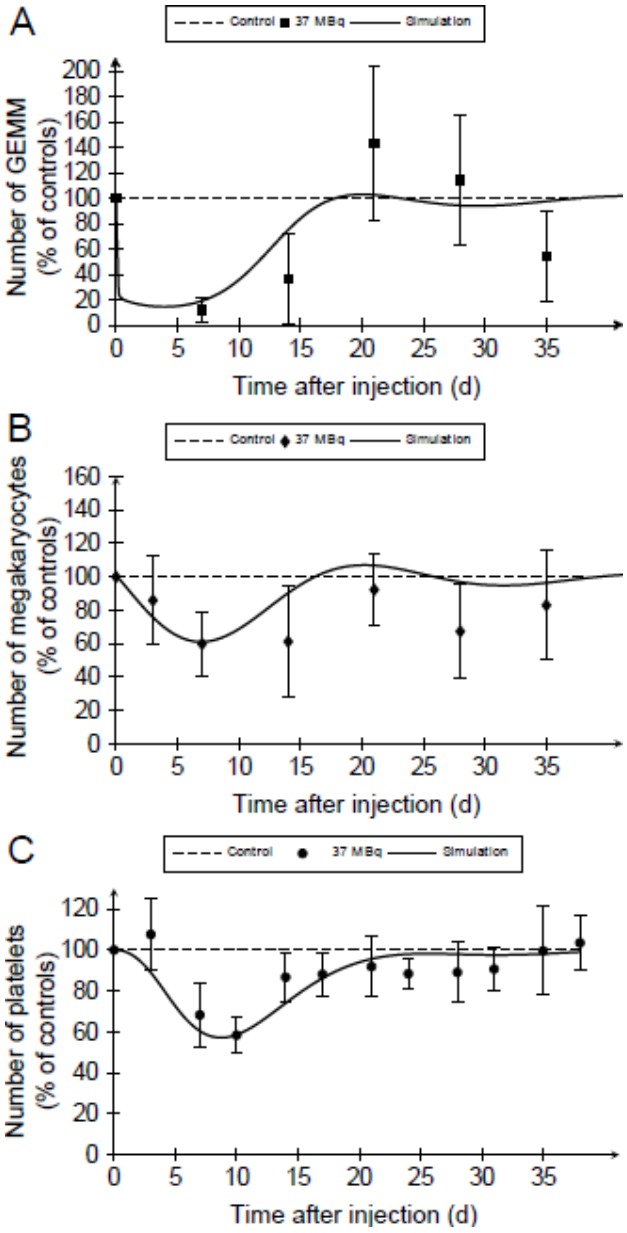
images were used to quantify the activity within bones for all mice. The time-activity curve within bones was obtained from a group of mice where each point corresponded to one individual mouse (Supplemental Figure 1C). The fitted curve was in good agreement with experimental data. The calculated mean absorbed dose was therefore 57 mGy/MBq to the whole BM. Because some cell analyses were performed using cells extracted from femur BM, the absorbed dose within the femur BM was also calculated: 79 mGy/MBq. As a consequence, 37 and 60 MBq injected activities of ^{18}FNa were calculated to deliver respectively 2.1 and 3.4 Gy to the whole BM and 2.9 and 4.7 Gy to the femoral BM.

Rate constants of the adapted biodistribution compartment model were calculated from time-activity curves of the entire BM for blood cells simulations and of the femur BM for BM cells ones. They were representative of the ^{18}FNa biodistribution in mice and are described in Supplemental Table 1.

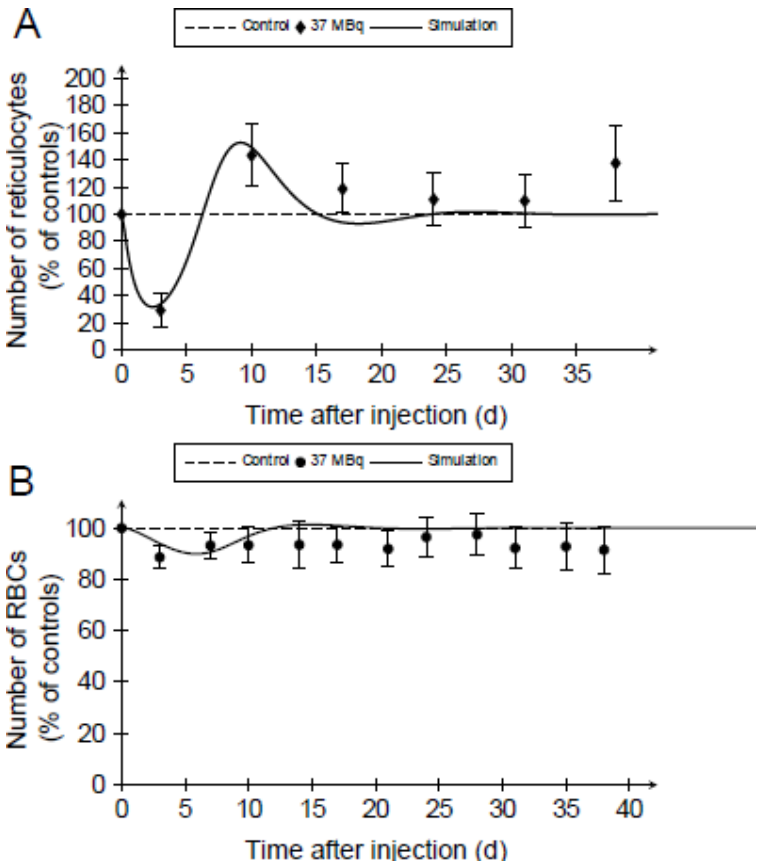


SUPPLEMENTAL FIGURE 1: Assessment of ^{18}FNa biodistribution to determine the activity-time curve. (A) Fluoride uptake was evaluated by γ-counting at 1, 5 and 6 h pi (n=4 per analysis). Measurements were corrected for background and decay. Results are expressed as percentages of injected activity per gram of tissue (%IA/g). (B) Quantification by PET imaging and tissue counting were compared on femur+tibia (n=3). Results for A to C are expressed as mean +/- SD. (C) Time activity curve normalized for 1 MBq of ^{18}FNa injected within the whole skeleton is shown. Each data point represents one measurement on one

mouse by PET imaging. The solid line corresponds to the fitted time-activity curve.



SUPPLEMENTAL FIGURE 2: As compared to experimental measurements (dots), compartmental model simulations (solid lines) predict thrombopoieitic cell kinetics after injection of 37 MBq of ^{18}FNa. BM cells were isolated from a femur after irradiation and cultured for GEMM progenitor colonies counting (A). Megakaryocyte number per mm^2 within the femur was determined by histology (B). PLTs were counted in the peripheral blood (C).



SUPPLEMENTAL FIGURE 3: Compartmental model simulations (solid lines) of the erythroid cells after BM irradiation are representative of experimental conditions (dots). Reticulocytes (A) and RBCs (B) numbers in blood were determined after injection of 37 MBq of ^{18}FNa.

SUPPLEMENTAL TABLE 1: Parameter values of the model

Definition	Parameter/Quantity	Thrombopoiesis value	Source	Erythropoiesis value	Source
Differentiation	φ_{P-Pr}	$\frac{\ln(2)}{1} day^{-1}$	Shen	$\frac{\ln(2)}{4.6} day^{-1}$	Smirnova
Maturation	φ_{Pr-Pl}	$\frac{ln(2)}{2} day^{-1}$	Adapted	$\frac{\ln(2)}{0.6} day^{-1}$	Smirnova
Life span	$\varphi_{Pl-\emptyset}$	$\frac{ln(2)}{2.5} day^{-1}$	Shen	$\frac{\ln(2)}{17} day^{-1}$	Smirnova
Repair of stroma	φ_{IS-S}	23.3 day^{-1}	Jones	23.3 day^{-1}	Jones
Initial values	C_{P_0}	40		2.6	
Initial values	C_{M_0}	36		3.6	
Initial values	C_{Pl_0}	100		100	
Initial values	C_{S_0}	100		100	
Initial values	C_{IS_0}	0		0	
Initial values	C_{PB_0}	A(0) (MBq)		A(0) (MBq)	
Initial values	C_{B_0}	0		0	
Initial values	C_{EcF_0}	0		0	
Initial values	C_{BEcF_0}	0		0	
Initial values	C_{Bl_0}	0		0	
Radiobiological constant	α	0.35 Gy^{-1}	Hendry	0.65 Gy^{-1}	Hendry
Radiobiological constant	β	0.04 Gy^{-2}	Hendry	0.07 Gy^{-2}	Hendry
Rate of stroma Damage by radiation	$\sigma_{S-\emptyset}$	0.347	Jones	0.347	Jones
Rate of injured stroma damage by	$\sigma_{IS-\emptyset}$	0.832	Jones	0.832	Jones

radiation					
Rate of injured stroma	σ_{S-IS}	0.513	Jones	0.513	Jones
S factor	S(BM←B)	0.891$\frac{Gy}{MBq.day}$	Larsson		
S factor femur to BM femur	S(BMF←F)	26.687$\frac{Gy}{MBq.day}$	Larsson		
S factor tibia to BM tibia	S(BMT←T)	14.41$\frac{Gy}{MBq.day}$	Larsson		
Feedback influence of stroma	ε	$\frac{250}{499}$	Shen	/	
Feedback influence of stroma	ϵ	$\frac{249}{499}$	Shen	/	
Constant	ω	0.5	Shen	0.6	Smirnova
Initial value	φ_{renew_0}	ln(2) day^{-1}	Jones	ln(2)/0.28	
Normal ploidy coefficient	δ_0	1	Smirnova	/	
Normal maturation time factor	ν_0	1	Smirnova	/	
Rate constant	λ_{PB-EcF}	14.1367 min	Experimental	14.1367 min	Experimental
Rate constant	$\lambda_{PB-BEcF}$	0.4384 min	Experimental	0.4384 min	Experimental
Rate constant	λ_{PB-Bl}	0.33 min	Experimental	0.33 min	Experimental
Rate constant	λ_{EcF-PB}	2.3815 min	Experimental	2.3815 min	Experimental
Rate constant	$\lambda_{BEcF-PB}$	0.064 min	Experimental	0.064 min	Experimental
Rate constant	λ_{Bl-PB}	0.0454 min	Experimental	0.0454 min	Experimental
Rate constant	λ_{BEcF-B}	2.3962 min	Experimental	2.3962 min	Experimental
Rate constant	λ_{B-BEcF}	0.0002 min	Experimental	0.0002 min	Experimental
Rate constant	$\lambda_{Bl-\emptyset}$	0.150 min	Experimental	0.150 min	Experimental

Supplemental References

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