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Analysis of ^{177}Lu-octreotate therapy-induced DNA damage in peripheral blood lymphocytes of patients with neuroendocrine tumors

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ABSTRACT

Ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) can lead to cell death, genome instability and carcinogenesis. Immunofluorescence detection of phosphorylated histone variant H2AX (γ-H2AX) is a reliable and sensitive technique to monitor external beam IR-induced DSBs in peripheral blood lymphocytes (PBL). Here, we investigated whether γ-H2AX could be used as an in vivo marker to assess normal tissue toxicity after extended internal irradiation with ^{177}Lu-DOTA-octreotate peptide receptor radionuclide therapy (LuTate PRRT) of neuroendocrine tumors.

Methods: We analyzed the kinetics of γ-H2AX foci in PBL of 11 patients undergoing PRRT. The number of γ-H2AX foci was determined before and up to 72h after treatment. These values were compared with the estimated absorbed dose to blood, spleen, bone marrow and tumor and with subsequent PBL reduction.

Results: Decrease in ^{177}Lu activity in blood with time followed a bi-exponential kinetic pattern, with approximately 90% of circulating activity in blood cleared within 2h. Absorbed dose to blood but not to spleen or bone marrow, correlated with the administered ^{177}Lu activity. PRRT increased γ-H2AX foci in lymphocytes in all patients, relative to pre-therapy values. The

response varied significantly between patients, but the average number of foci indicated a general trend towards increase at 0.5-4h with subsequent decrease by 24-72h post-treatment. The peak foci number correlated with the absorbed dose to tumor and bone marrow and the extent of PBL reduction.

Conclusion: γ-H2AX can be exploited in the LuTate PRRT as a biomarker of PBL cytotoxicity. Long-term follow-up studies investigating whether elevated residual γ-H2AX values are associated with acute myelotoxicity and secondary blood malignancy may be worthwhile.

Key Words: ^{177}Lu-octreotate, PRRT, normal tissue toxicity, DNA damage, γ-H2AX

INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) using radiolabeled somatostatin analogues that bind to somatostatin receptor 2 has proven effective in treating patients with metastatic or inoperable neuroendocrine tumors (NET) (*1*,*2*). One of the most promising radiolabelled somatostatin analogues for tumor targeting therapy is [^{177}Lu-DOTA^{0}, Tyr^{3}]octreotate (LuTate) (*1*,*3*).

PRRT is a safe treatment, but mild hematologic toxicity is frequently observed. Repeated administration of PRRT increases the accumulated dose to normal tissues and thereby increases the risk of accumulation of unrepaired DNA damage that can lead to genetic instability and, potentially, to carcinogenesis. In addition to acute myelotoxicity, myelodysplastic syndrome (MDS) has been reported in 4 out of 504 patients in one series (*4*,*5*). To estimate the effect of PRRT and limit the toxicity of such treatment, organ dosimetry based on radioisotope biodistribution and kinetics is desirable. However, the image-based dosimetry findings alone have not been very useful for predicting development of blood disorders in individual patients (*4*). Furthermore, there is no consensus on an appropriate radiobiological model for short-range, low dose-rate radiopeptides. Studying DNA damage at the single-cell level could provide additional insight into the mechanisms of PRRT-induced toxicity and

be useful in combination with organ dosimetry to determine or predict the biological consequences of radiation dose in vivo.

Phosphorylated histone H2AX (γ-H2AX), a biomarker of radiation-induced DNA double-strand breaks (DSBs) (*6*), is commonly used to monitor DNA damage and repair in a wide range of applications including radiotherapy (*7,8*). External ionizing radiation (IR) induces γ-H2AX foci in a dose-dependent manner. The maximum size and number are observed at 30min post-IR and subsequently decline to pre-IR levels within several hours, due to DSB repair. The direct response to radiation and sensitivity of the assay have provided a basis for the adoption of γ-H2AX as a "biodosimeter" to evaluate exposure of peripheral blood lymphocytes (PBL) after external body irradiation (*9-13*). Recent publications evaluated the γ-H2AX assay as an attractive functional assay to identify radiosensitive individuals with early or chronic radiation-induced normal tissue toxicity, and for a personalized therapy (*7,14,15*).

The kinetics of radiopharmaceutical-induced DNA damage is more complex than that induced by external IR, reflecting the nature of radionuclide emissions, biodistribution of the agent within cells and organs, and the time course of radiation delivery determined by physical decay characteristics of the

radionuclide and tissue clearance kinetics. Current data on the use of γ-H2AX as a biomarker of toxicity and as a biodosimeter after systemic administration of radiopharmaceuticals, come primarily from studies investigating ^{131}I-therapy in patients with thyroid cancer (*16,17*) and ^{18}F-FDG PET/CT imaging (*18*). These studies recommended γ-H2AX as a biomarker of DNA damage after internal irradiation. However, since the spatial distribution of ionization events on a microscopic scale is related to the chemical form of the radiopharmaceutical and the energy of the beta-particles (affecting the proportion of DNA single strand breaks and DSBs), the use of γ-H2AX should be validated for specific radiopharmaceuticals.

This study aimed to assess DNA damage in PBL at multiple time-points after LuTate PRRT, and to correlate the number of γ-H2AX foci with the estimated absorbed radiation dose to blood, spleen, bone marrow and tumor. We assessed the impact of tumor volume and whether nadir lymphocyte counts were related to the level of DSBs. We investigated whether γ-H2AX could be used as a biodosimeter to estimate the absorbed dose or predict normal tissue toxicity to PRRT.

MATERIALS AND METHODS

Patient Treatment

Eleven patients enrolled in the study (5 females and 6 males) with a mean age of 56 years (Range 32-65 years) had NET and underwent one or more PRRT cycles (Table 1). This trial was approved by the Peter MacCallum Cancer Centre Human Ethics Committee. All patients signed a consent form. The standard induction course of PRRT comprised 3-5 cycles of LuTate therapy given at 6-9 weekly intervals. LuTate was given in combination with an infusion of amino acids (25g of arginine and 25g of lysine in 1L over 4h) for renal protection. Nine patients received infusions of 5-fluorouracil (5-FU) (200mg/m^2/24h) 1-3 days prior to LuTate injection and continuing for a total of 14 days as a radiosensitising agent (*1*).

Description of blood collection and processing, dosimetry and data analysis is found in Supplemental Data.

RESULTS

Dosimetry

Patients received an administered activity of 6500-10000 MBq^{177}Lu resulting in a mean cumulative absorbed dose to blood (42±3.9 mGy, median, 42 mGy; range 22-57 mGy), to bone marrow (250±21 mGy, median, 210 mGy; range

170-410 mGy), to spleen (3700±650 mGy, median, 3300 mGy; range 1700-10000 mGy), and to tumor (22000±12000 mGy, median, 20000 mGy; range 6000-46000 mGy) (Table 2).

Initial LuTate uptake, typically seen at 4h on SPECT/CT scans in spleen, kidneys and bladder, cleared substantially after 24h (Supplemental Fig.1). Consequently, primarily the tumor was detectable at 72h.

The time-activity curve of LuTate in the blood (Fig.1) for the study series followed a bi-exponential function. The clearance halftime for the first exponent was 0.72h with 89±2.6% of LuTate clearing within 2h. The remaining radioactivity was then gradually cleared from the blood reaching <1% at 72h with a clearance halftime of 35.7h. The high LuTate uptake in kidneys and bladder at early time-points and clearance at 24h usually indicate that this radiotracer is cleared primarily by renal excretion.

Kinetics of LuTate Therapy-Induced γ-H2AX Foci in PBL

Individual data for each patient revealed that the pattern of γ-H2AX kinetics varied greatly between patients (Fig.2). Additionally, differences between cycles were observed within the same patients (Fig.2B). A peak of γ-H2AX

foci/cell was observed at different time-points in similar PRRT cycles. Conversely, similar patterns of γ-H2AX foci kinetics were seen in some patients who received different numbers of PRRT cycles. In the 10min-72h interval following therapy, all patients showed an excess γ-H2AX foci/cell (defined as the number of foci/cell at each time-point minus the number of foci/cell at baseline). However, in some cases, the peak number of excess foci/cell was very low, e.g. in patients P2.3 (0.3 foci/cell above baseline), P3.4 (0.2 foci/cell above baseline) and P6.1 (0.1 foci/cell above baseline). Interestingly, in patients P4.1 and P11.6 the peak number of excess foci/cell was detected only at 72h post-LuTate administration.

The pre-treatment level of γ-H2AX foci/cell ranged between 0.06 and 0.75 with an average of 0.28±0.06 foci/cell. Administration of the radiosensitising agent 5-FU prior to PRRT (in 12 out of 16 analyzed treatment cycles) increased the baseline value 2-fold (0.15±0.04 without 5-FU and 0.33±0.08 with 5-FU, P=0.049). In patient P2, 5-FU was not given at cycle 2, but this PRRT cycle induced a γ-H2AX response similar to cycle 5, with 5-FU. The low γ-H2AX response was observed in patients P4.1, P6.1 and P11.6 without 5-FU administration. However, these events may not be related, since a similar low level response was observed also in patient P2.3 and P3.3-4 who received 5-FU.

The average numbers of γ-H2AX foci/cell increased rapidly in the first 30min after LuTate administration (Fig.3A) and gradually increased in the following 2h where the average numbers of γ-H2AX foci reached a peak. Subsequently, the numbers of foci gradually returned close to baseline at 24h. Similarly, the percentage of cells with multiple foci increased up to 2h (2h *vs.* baseline, *P* value=0.0103) before decreasing at later time-points (Fig.3B).

We simulated the γ-H2AX kinetics in PBL assuming that the major source of DNA damage in PBL comes from LuTate irradiation in blood (red line in Fig.3C). The simulated kinetics for gradual accumulation of the dose (black line in Fig.3C) to a total value of 42 mGy (average blood dose) is based on the course of LuTate elimination from the blood (Fig.1). For comparison, we included the simulated γ-H2AX kinetics for a single 42 mGy dose at the start of the 80h-period (blue line in Fig.3C). Compared to a single instantaneous dose, the simulation of the isotope decay scenario with protracted dose delivery provides the better prediction of the time course of experimental data for accumulation and decay of γ-H2AX foci. However, the overall predicted level of foci is less than experimental values.

PRRT Toxicity on PBL

The number of total PBL was assessed before each PRRT cycle and 2 weeks after treatment (Table 3). Early toxicity was observed in all patients. The average PBL reduction from pre-treatment to 2 weeks after the first PRRT cycle was 51% (n=4; range 33-63%). The extent of reduction generally decreased for successive treatment cycles; for example 42% for the 3rd cycle (n=6; 20-56%), down to 12% for 6 cycles (one case only). However, the two cases for the 5th cycle were "outliers" for this trend (average of 45% reduction). The number of PBL increased between cycles, but did not return to the levels observed prior to the first treatment indicating that there was only a partial recovery after each cycle. The overall reduction of PBL from the start of treatment to 2 weeks after completion of multiple cycles of PRRT was 68% (average of 12 patients; 34–88%).

Relationships between Foci Count, Dosimetry and PBL reduction

We investigated the correlation between various γ-H2AX foci endpoints with absorbed doses in blood at 2 and 72h, or absorbed doses in spleen, bone marrow and tumor. The endpoints were the peak number of excess foci/cell within either 10min-4h, or 24-72h after PRRT, or over the total observation interval. The analysis revealed no clear correlation for the majority of

parameters (Supplemental Fig.2) including the dose in blood at 2h post-LuTate administration (Fig.4A). As radioactivity clears rapidly (Fig.1), this short circulation time would be temporally loaded towards early radiation exposure. Therefore, the absorbed dose to blood was expected to correlate with the peak of damage to lymphocytes around this time. However, no correlation was found. This applies for all data points involved as well as for correlation for different cycles within individual patient. The best correlative trend was found between peak number of excess foci/cell and tumor dose (Fig.4B, with the exception of one outlier, patient P6.1 who exhibited the lowest γ-H2AX response) and bone marrow dose (Fig.4C). Again, with P6.1 exception, a positive correlative trend was observed between peak number of excess foci/cell in the 10min-4h interval and fraction of lymphocyte reduction within the corresponding cycle (Fig.4D). Absorbed dose to blood but not to spleen or bone marrow, correlated with administered ^{177}Lu activity (Supplemental Fig.2A).

DISCUSSION

Numerous pre-clinical and clinical studies have reported the linear radiation dose response of the γ-H2AX assay (*9-13,19-22*). Clinical studies have demonstrated that the stochastic γ-H2AX foci values after external radiation

exposure depend on the dose, exposed area of the body, duration/fractionation of the exposure, and time of analysis. In cancer patients treated with 3D conformal radiotherapy for a variety of tumour types, a linear correlation was established between the integrated total body radiation dose and γ-H2AX foci per lymphocyte (*13*), making it possible to estimate the applied integral body dose. Moreover, the γ-H2AX-based measurements in exposed PBL were in concordance with physical dosimetry of IMRT and 3D treatment modalities of prostate cancer (*23*). Therefore, the assay demonstrates characteristics of a reliable biodosimeter for external beam radiotherapy (*7*).

This study represents the first exploration of the γ-H2AX assay in PBL for PRRT with ^{177}Lu octreotate. Despite some inter- and intra-individual variability, the average number of γ-H2AX foci for the whole study group increased rapidly within the first 30min after PRRT, and more slowly from 30min to 2h. Repair mechanisms prevail over DNA DSB formation between 2-24h post-treatment, resulting in progressive disappearance of γ-H2AX foci. PRRT-induced lymphopenia has been reported previously (*24*). Since circulating lymphocytes are traversed by radiation tracks from ^{177}Lu, the absorbed dose to blood would be most likely to correlate with DNA damage to PBL. Indeed we found that lymphocyte counts dramatically reduced following

the first cycle of LuTate in all patients. Importantly, the peak number of excess foci/cell in the 10min-4h interval correlated with subsequent PBL reduction.

It is unlikely that lymphocyte depletion was due solely to direct irradiation since the absorbed dose in blood is 20- to 50-fold lower (mGy range) than the dose needed to achieve 10-20% cell death (≥1Gy). Indeed, no correlation between peak number of excess foci/cell and absorbed dose in blood was found. Moreover, while the kinetics of foci formation followed the trend predicted by the simulation based on gradual accumulation of absorbed dose in blood, the predicted level of γ-H2AX foci was substantially less than the experimental values. These results indicate that the dose in blood only partially account for the induction of γ-H2AX foci in lymphocytes. Based on the correlation studies, the tumor dose also contributes to foci formation in lymphocytes. This is consistent with larger doses to tumors by at least one order of magnitude compared to other organs (see Table 2). The peak number of excess foci/cell also correlated with the dose to bone marrow suggesting its possible contribution to the damage in PBL.

The residual number of foci in several patients 24-72h post-treatment generally did not decrease as much as predicted by the simulation. Possible explanations

include inefficient DNA repair and/or mobilization of lymphocytes with LuTate-induced DNA damage from high-uptake tissues (spleen, bone marrow and tumor) into the circulation. It may be possible to improve the correlation between dose in blood and PBL γ-H2AX response by incorporating residence time of these lymphocytes into high-uptake tissues in the calculations. The absorbed dose to bone marrow can affect the genomic stability of progenitor cells and their progeny. Individual radiation sensitivity influences large variability in clinical response to radiotherapy, as well as their cellular radiosensitivity measured by the γ-H2AX assay in PBL (*7*,*14*,*15*). There is also an ongoing debate on the mechanisms of the radiation-induced bystander effect (*25*) which may contribute to an elevated appearance of dose to circulating lymphocytes through reactive oxygen and nitrogen species and immune response.

Our findings are in line with previous studies showing similar patterns of γ-H2AX foci kinetics after a single dose of ^{131}I therapy in patients with differentiated thyroid cancer (*16*,*17*), or after injection of ^{18}F-FDG in patients with various malignant or benign diseases (*18*). Interestingly, radiopharmaceuticals with different biodistributions, pharmacokinetics and dose rates showed similar patterns of DNA DSBs induction and repair.

However, the peak values were found earlier with diagnostic dose of ^{18}F-FDG (30min) (*18*) compared to therapeutic doses of LuTate or ^{131}I (2h for both radiopharmaceuticals) (*17*). This is most likely due to the shorter physical half-life of ^{18}F (109.8min) than ^{177}Lu (6.7 days) and ^{131}I (8 days).

One limitation of our study is that patients received varied administered activities of LuTate and some did not receive 5-FU. Administration of 5-FU before PRRT significantly increased the background level of γ-H2AX foci. This is consistent with studies showing that 5-FU causes DNA damage in various cancer types (*26*,*27*) and in lymphocytes (*28*). In the latter study, elevated DNA damage remained for 80 days after completion of treatment. Therefore, intra-patient variability in the level of γ-H2AX foci reported here could come from variations in the pharmacokinetic parameters possibly due to the different administered activities between cycles and/or from the inclusion of 5-FU in the therapy regimen. Since clinical studies at our and other institutions have shown that adding 5-FU to LuTate may be beneficial to NET patients (*1*,*29*) and that dosimetry to tumor and normal tissues may relate to tumor burden (*3*), it may be worthwhile reassessing the utility of γ-H2AX foci in a standardised dosimetry-based trial of LuTate and 5-FU.

Stem and progenitors cells residing in lymphoid tissues (bone marrow, spleen) are also exposed to radiation. The absorbed doses to these organs and induction of DNA damage may be of concern given that MDS has been associated with prior anti-cancer therapy and abnormal DNA repair (*30*). In particular, increased γ-H2AX levels in bone marrow correlated with poor overall survival in MDS patients (*31*). MDS can progress to acute myeloid leukemia, a "genomic instability disorder". In our study, patients were followed up for 1 year. No serious adverse myelotoxic events related to LuTate treatment were recorded apart from one patient, who died from leukaemia during the follow-up period. However, this patient had an elevated white cell count prior to PRRT and review of the peripheral blood film suggested that this condition preceded LuTate PRRT. Whether kinetics of γ-H2AX induction and repair correlate with the risk of secondary hematologic malignancy in LuTate-treated patients, remains to be confirmed with longer-term follow-up studies.

CONCLUSION

The complexity of PRRT precludes a simple correlation between physical dosimetry and clinical endpoints with the γ-H2AX response in PBL. Nevertheless a correlation between decrease in PBL and peak γ-H2AX foci numbers was found. The absorbed dose to blood, bone marrow and tumor can account for the induction of γ-H2AX foci in PBL. Overall, our results warrant a

larger clinical study of elevated γ-H2AX levels and a risk of lymphopenia, MDS and secondary malignancy in LuTate-treated patients.

ACKNOWLEDGMENTS

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REFERENCES

1. Barber TW, Hofman MS, Thomson BN, Hicks RJ. The potential for induction peptide receptor chemoradionuclide therapy to render inoperable pancreatic and duodenal neuroendocrine tumours resectable. *Eur J Surg Oncol.* 2012;38:64-71.

2. Modlin IM, Moss SF, Oberg K, et al. Gastrointestinal neuroendocrine (carcinoid) tumours: current diagnosis and management. *Med J Aust.* 2010;193:46-52.

3. Beauregard JM, Hofman MS, Kong G, Hicks RJ. The tumour sink effect on the biodistribution of 68Ga-DOTA-octreotate: implications for peptide receptor radionuclide therapy. *Eur J Nucl Med Mol Imaging.* 2012;39:50-56.

4. Bodei L, Kidd M, Paganelli G, et al. Long-Term tolerability of PRRT in 807 Patients with Neuroendocrine Tumours: the Value and Limitations of Clinical Factors. *Eur J Nucl Med Mol Imaging.* 2014;Oct 2.

5. Kwekkeboom DJ, de Herder WW, Kam BL, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA. *J Clin Oncol.* 2008;26:2124-2130.

6. Bonner WM, Redon CE, Dickey JS, et al. GammaH2AX and cancer. *Nat Rev Cancer.* 2008;8:957-967.

7. Ivashkevich A, Redon CE, Nakamura AJ, Martin RF, Martin OA. Use of the gamma-H2AX assay to monitor DNA damage and repair in translational cancer research. *Cancer Lett.* 2012;327:123-133.

8. Redon CE, Weyemi U, Parekh PR, Huang D, Burrell AS, Bonner WM. gamma-H2AX and other histone post-translational modifications in the clinic. *Biochim Biophys Acta.* 2012;1819:743-756.

9. Ghardi M, Moreels M, Chatelain B, Chatelain C, Baatout S. Radiation-induced double strand breaks and subsequent apoptotic DNA fragmentation in human peripheral blood mononuclear cells. *Int J Mol Med.* 2012;29:769-780.

10. Redon CE, Dickey JS, Bonner WM, Sedelnikova OA. gamma-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin. *Adv Space Res.* 2009;43:1171-1178.

11. Redon CE, Dickey JS, Nakamura AJ, et al. Tumors induce complex DNA damage in distant proliferative tissues in vivo. *Proc Natl Acad Sci U S A.* 2010;107:17992-17997.

12. Redon CE, Nakamura AJ, Sordet O, et al. γ-H2AX detection in peripheral blood lymphocytes, splenocytes, bone marrow, xenografts, and skin. *Methods Mol Biol.* 2011;682:249-270.

13. Sak A, Grehl S, Erichsen P, et al. gamma-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment. *Int J Radiat Biol.* 2007;83:639-652.

14. Adams G, Martin OA, Roos DE, et al. Enhanced intrinsic radiosensitivity after treatment with stereotactic radiosurgery for an acoustic neuroma. *Radiother Oncol.* 2012;103:410-414.

15. Van Oorschot B, Oei AL, Nuijens AC, et al. Decay of gamma-H2AX foci correlates with potentially lethal damage repair and P53 status in human colorectal carcinoma cells. *Cell Mol Biol Lett.* 2014;19:37-51.

16. Doai M, Watanabe N, Takahashi T, et al. Sensitive immunodetection of radiotoxicity after iodine-131 therapy for thyroid cancer using gamma-H2AX foci of DNA damage in lymphocytes. *Ann Nucl Med.* 2013;27:233-238.

17. Lassmann M, Hanscheid H, Gassen D, et al. In vivo formation of gamma-H2AX and 53BP1 DNA repair foci in blood cells after radioiodine therapy of differentiated thyroid cancer. *J Nucl Med.* 2010;51:1318-1325.

18. May MS, Brand M, Wuest W, et al. Induction and repair of DNA double-strand breaks in blood lymphocytes of patients undergoing (1)(8)F-FDG PET/CT examinations. *Eur J Nucl Med Mol Imaging.* 2012;39:1712-1719.

19. Andrievski A, Wilkins RC. The response of gamma-H2AX in human lymphocytes and lymphocytes subsets measured in whole blood cultures. *Int J Radiat Biol.* 2009;85:369-376.

20. MacPhail SH, Banath JP, Yu TY, Chu EH, Lambur H, Olive PL. Expression of phosphorylated histone H2AX in cultured cell lines following exposure to X-rays. *Int J Radiat Biol.* 2003;79:351-358.

21. Rothkamm K, Horn S. gamma-H2AX as protein biomarker for radiation exposure. *Ann Ist Super Sanita.* 2009;45:265-271.

22. Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A.* 2003;100:5057-5062.

23. Zwicker F, Swartman B, Sterzing F, et al. Biological in-vivo measurement of dose distribution in patients' lymphocytes by gamma-H2AX immunofluorescence staining: 3D conformal- vs. step-and-shoot IMRT of the prostate gland. *Radiat Oncol.* 2011;6:62.

24. Sierra ML, Agazzi A, Bodei L, et al. Lymphocytic toxicity in patients after peptide-receptor radionuclide therapy (PRRT) with 177Lu-DOTATATE and 90Y-DOTATOC. *Cancer Biother Radiopharm.* 2009;24:659-665.

25. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. *Nat Rev Cancer.* 2009;9:351-360.

26. Adamsen BL, Kravik KL, De Angelis PM. DNA damage signaling in response to 5-fluorouracil in three colorectal cancer cell lines with different mismatch repair and TP53 status. *Int J Oncol.* 2011;39:673-682.

27. Ikeda M, Kurose A, Takatori E, et al. DNA damage detected with γH2AX in endometrioid adenocarcinoma cell lines. *Int J Oncol.* 2010;36:1081-1088.

28. Sánchez-Suárez P, Ostrosky-Wegman P, Gallegos-Hernández F, et al. DNA damage in peripheral blood lymphocytes in patients during combined chemotherapy for breast cancer. *Mutat Res.* 2008;640:8-15.

29. Claringbold PG, Brayshaw PA, Price RA, Turner JH. Phase II study of radiopeptide 177Lu-octreotate and capecitabine therapy of progressive disseminated neuroendocrine tumours. *Eur J Nucl Med Mol Imaging.* 2011;38:302–311.

30. Zhou T, Hasty P, Walter CA, Bishop AJ, Scott LM, Rebel VI. Myelodysplastic syndrome: an inability to appropriately respond to damaged DNA? *Exp Hematol.* 2013;41:665-674.

31. Kefala M, Papageorgiou SG, Kontos CK, et al. Increased expression of phosphorylated NBS1, a key molecule of the DNA damage response machinery, is an adverse prognostic factor in patients with de novo myelodysplastic syndromes. *Leuk Res.* 2013;37:1576-1582.

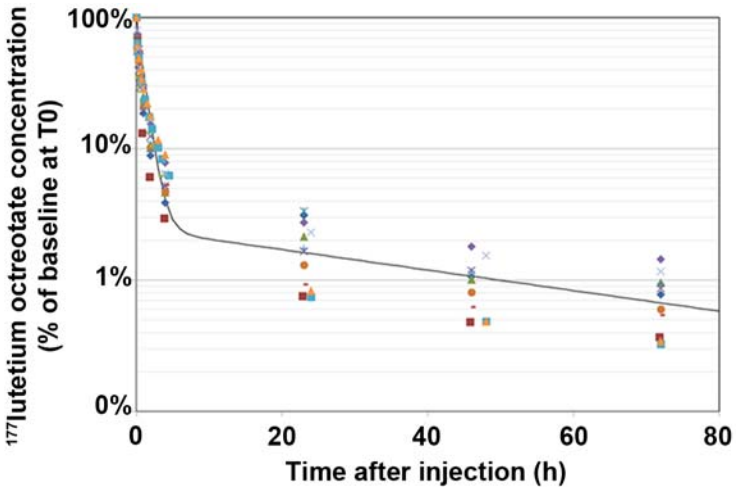

FIGURE 1. LuTate time-activity curve in blood up to 72h post-treatment. Each point represents a single measurement of radioactivity in the blood of 11 patients at indicated times. The solid line is the bi-exponential fit of LuTate disappearance from blood.

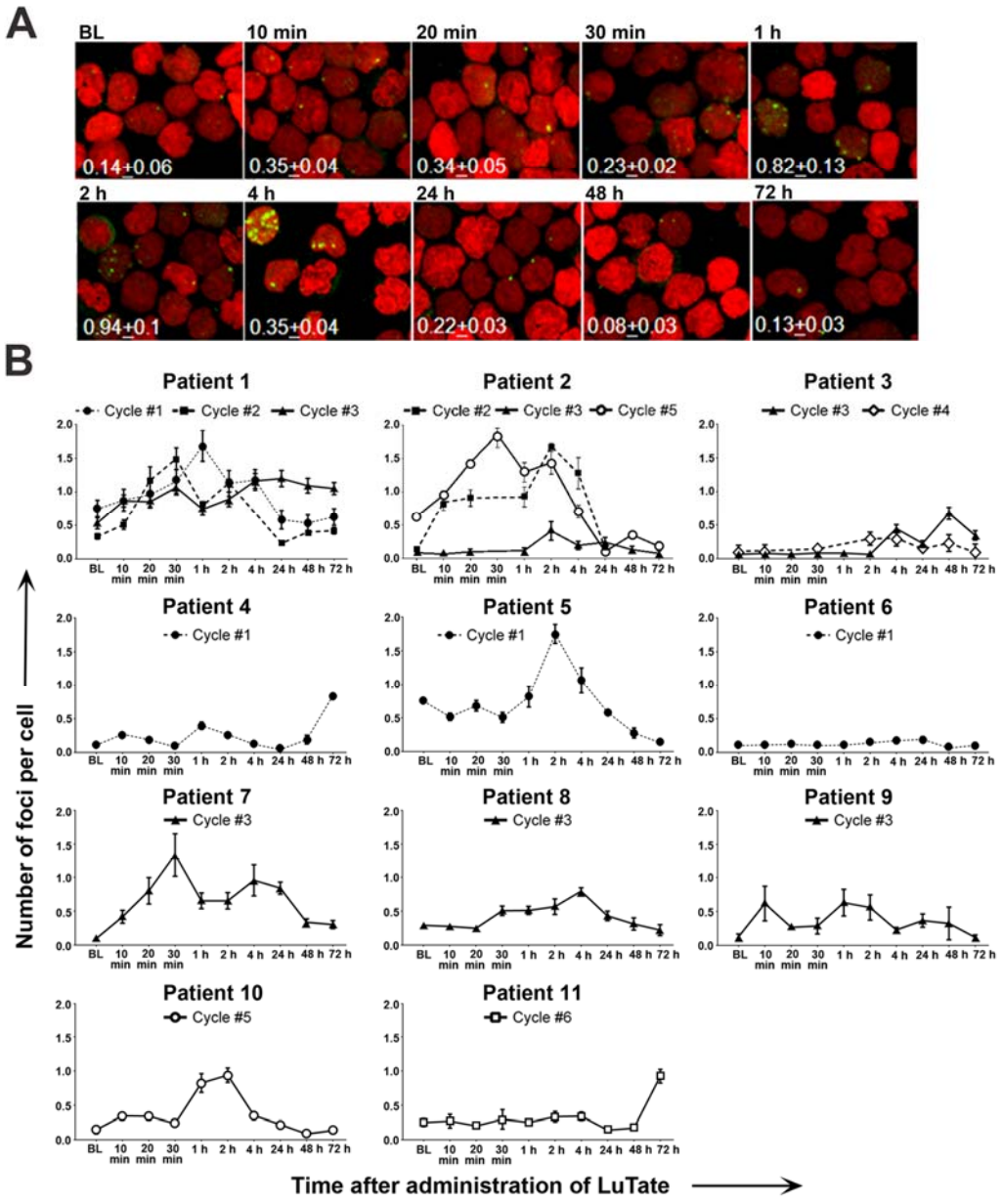

FIGURE 2. Kinetics of γ-H2AX foci formation in PBL. (A) Representative confocal images of γ-H2AX foci (green) and nuclei (red, DNA) of patient P10.5 after administration of 8362MBq LuTate. Values correspond to average number of foci/cell±SEM. **(B)** Number of foci/cell in individual patients measured at

baseline (BL) before therapy and 10, 20, 30min, 1, 2, 4, 24, 48 and 72h after LuTate administration in one or more cycles of therapy.

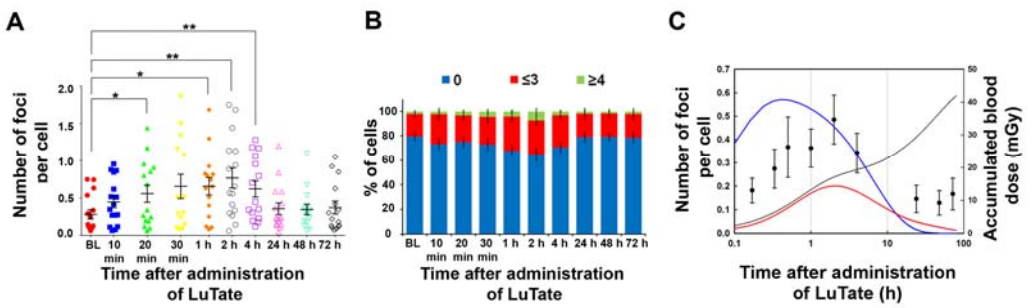

FIGURE 3. Analysis of γ-H2AX foci formation in PBL. (A) Number of foci/cell as versus time after PRRT for individual patient. The black horizontal bar and error bars represent the mean±SEM of 14-16 samples. Baseline versus other time-points, **P*<0.05, ***P*<0.01, Mann-Whitney test. **(B)** Fraction of cells with no foci (blue bars), ≤3 foci (red bars) and ≥4 foci (green bars), mean±SEM, n=14-16). **(C)** Simulation of the number of foci/cell as a function of time after PRRT. Symbols represent average experimental values across all patients ±SD. Simulation was implemented for a radiation dose of 42mGy delivered as a pulse (blue line) or accumulated (red line) within 80h interval. Accumulated dose was calculated according to LuTate kinetics in blood (black line).

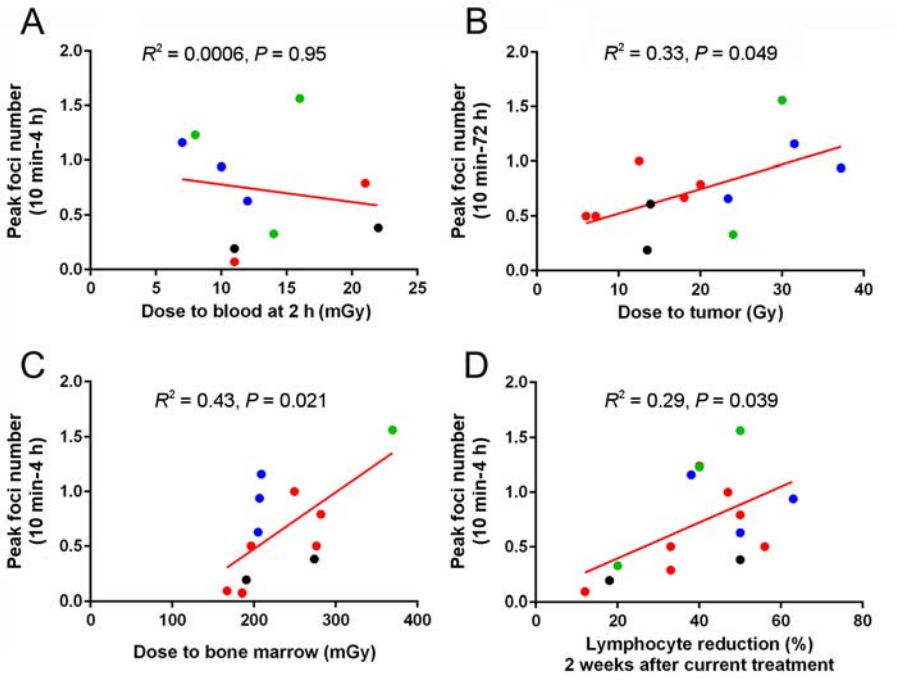

FIGURE 4. Correlation studies. (A) Peak number of excess foci/cell in 10min-4h interval versus dose to blood at 2h. **(B)** Peak number of excess foci/cell in 24-72h interval versus tumor dose. **(C)** Peak number of foci/cell in 10min-4h interval versus dose to bone marrow. **(D)** Peak number of excess foci/cell in 10min-4h interval versus lymphocyte reduction 2 weeks after treatment. Data for different treatment cycles of the same patient are shown as blue (P1), green (P2) and black (P3) symbols; other data points are shown as red symbols. Red lines represent linear regression for all points.

Table 1. Demographics and characteristics of patients.

Patient Blood ID	Sex	Age at treatment	Body Weight (kg)	Diagnosis	Primary site	Treatments before PRRT	5-FU in combination with PRRT
P1.1	M	46	98	Neuroendocrine	Pancreas	Chemotherapy	+
P1.2	M	47	96	Neuroendocrine	Pancreas	Chemotherapy	+
P1.3	M	47	100.4	Neuroendocrine	Pancreas	Chemotherapy	+
P2.2	M	62	82.3	Carcinoid	Small Bowel	Nil	-
P2.3	M	62	82	Carcinoid	Small Bowel	Nil	+
P2.5	M	62	80.1	Carcinoid	Small Bowel	Nil	+
P3.3	F	46	133	Neuroendocrine	Small Bowel	Nil	+
P3.4	F	46	130	Neuroendocrine	Small Bowel	Nil	+
P4.1	M	55	77	Carcinoid	Bowel	Surgery	-
P5.1	F	52	79	Neuroendocrine	Small Bowel	Chemotherapy/Sandostatin	+
P6.1	M	57	81	Neuroendocrine	Pancreas	Sandostatin	-
P7.3	F	49	58.6	Islet Cell	Pancreas	Surgery	+
P8.3	M	32	85	Neuroendocrine	Pancreas	Surgery	+
P9.3	F	46	77	Glucagonoma	Pancreas	Chemotherapy/Sandostatin /Surgery	+
P10.5	F	65	83	Gastrinoma	Duodenum	Chemotherapy	+
P11.6	M	60	114	Carcinoid	Unknown	Chemotherapy/Sandostatin	-

Patients are numbered from P1 to P11 followed by treatment cycle number. Sandostatin=Long-acting octreotide

Table 2. Dosimetry

Patient blood ID	Octreotate-avid tumor volume at treatment (cm^3)	Administered activity (MBq)	Cumulative absorbed dose to blood at 72h (mGy)	Cumulative absorbed dose to blood at 2h (mGy)	Absorbed dose to spleen (mGy)	Absorbed dose to bone marrow (mGy)	Absorbed dose to tumor (mGy)*
P1.1	640	9000	40	10	2800	210	37000
P1.2	700	7000	22	7	3600	210	31000
P1.3	720	8000	33	12	2000	200	23000
P2.2	3100	10000	57	16	3200	370	30000
P2.3	3300	7900	52	14	3400	410	24000
P2.5	3000	7800	37	8	ND	ND	ND
P3.3	420	9300	55	22	3300	270	14000
P3.4	240	6500	24	11	1700	190	13000
P4.1	94	7100	ND	ND	ND	ND	ND
P5.1	130	6900	ND	ND	1700	250	12000
P6.1	1200	7700	45	11	10000	190	46000
P7.3	ND	ND	ND	17	ND	ND	ND
P8.3	29	8600	ND	ND	6100	200	6000
P9.3	30	6800	ND	ND	3400	280	7000
P10.5	110	8400	52	21	5100	280	20000
P11.6	900	6700	ND	ND	1900	170	18000

*For multiple lesions, the lesion with the highest value was used. ND=Not Determined

Table 3. Lymphocyte toxicity after PRRT

Patient blood ID	**Baseline PBL count before 1st treatment (cells×10^3/mm^3)**	**PBL counts before indicated treatment* (cells×10^3/mm^3)**	**PBL count 2 weeks after indicated treatment* (cells×10^3/mm^3)**	**PBL reduction (%) between before indicated treatment and 2 weeks after indicated treatment**	**PBL reduction (%) between baseline and 2 weeks after indicated treatment**
P1.1	1.6	1.6	0.6	63	63
P1.2	1.6	0.8	0.5	38	69
P1.3	1.6	1.0	0.5	50	69
P2.2	1.4	1.0	0.5	50	64
P2.3	1.4	0.5	0.4	20	71
P2.5	1.4	0.5	0.3	40	79
P3.3	2.9	1.8	0.9	50	69
P3.4	2.9	1.1	0.9	18	69
P4.1	1.2	1.2	0.8	33	33
P5.1	1.5	1.5	0.8	47	47
P6.1	2.3	2.3	0.9	61	61
P7.3	2.5	0.5	0.3	40	88
P8.3	1.7	0.9	0.6	33	65
P9.3	1.3	0.9	0.4	56	70
P10.5	1.9	1.2	0.6	50	68
P11.6	2.3	1.7	1.5	12	34

*"Indicated treatment" refers to treatment cycle number denoted by the last digit in the Patient blood ID.