Biomarker identification of radionuclide therapy-induced radiation responses.

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This is a pilot study to validate the possibility of determining the effect of PRRT with 177Lu-DOTATATE on transcriptional regulation and DNA damage induction in PBMCs and how this is related to the radiation dose.We will also want to explore if we...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Neoplastic and ectopic endocrinopathies
Study type	Observational invasive

Summary

ID

NL-OMON51484

Source
ToetsingOnline

Brief title
Radio-Marker

Condition

- Neoplastic and ectopic endocrinopathies
- Endocrine neoplasms malignant and unspecified

Synonym
Neuroendocrine tumor

Research involving
Human

Sponsors and support

Primary sponsor: Moleculaire genetica
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: 177Lu-DOTATATE, ctDNA, DNA damage, PRRT, transcriptional regulation

Outcome measures

Primary outcome

This is a pilot study to validate the possibility of determining the effect of

PRRT with 177Lu-DOTATATE on transcriptional regulation and DNA damage

induction in PBMCs and how this is related to the radiation dose.

Secondary outcome

To see if it is possible to detect ctDNA in NET patients.

Exploratory objectives

- Evaluate the effect of PRRT on ctDNA levels

- Discover genetic biomarkers of PRRT-induced NET damage by methylation

sequencing.

Study description

Background summary

PRRT with 177Lu-DOTATATE is a form of internal radiation treatment for patients with NETs to reduce tumor growth and stabilize disease. Unfortunately, objective response rates are limited and fewer than 1% of the patients can achieve complete response following PRRT. Administering a higher cumulative dose than currently applied might induce more toxicity in healthy tissues and probably will be detrimental to patients. To be able to eventually improve this therapy it is necessary to define its underlying molecular mechanisms, such as the cellular response of healthy cells to the radiation. A better understanding of these responses, such as transcriptional- and DNA damage responses, could contribute to identification of biomarkers for toxicity and/or efficacy prediction. This was previously shown for external beam radiation in which they identified radiation responsive genes in ex vivo irradiated blood cells. These

genes were also up- or downregulated following in vivo exposure to total-body irradiation of patients. Besides identification of novel radiation biomarkers, this shows that ex vivo data can provide good prediction of in vivo exposures.

PRRT induces different types of DNA damage in both cancer cells and healthy tissue, of which double-strand breaks (DSBs) are the most cytotoxic. Radiation-induced foci (RIF) at the site of a DSB and can be visualized using the biomarkers γ-H2AX and 53BP1. Different studies have shown a good correlation between radiation dose to the blood and DSB level detection in peripheral blood mononuclear cells (PBMCs) for various PRRT-like treatments. Since a blood draw is a relatively non-invasive manner, it is an ideal way to provide this information .

In PBMCs of 16 patients treated with 177Lu-DOTATATE the average number of RIF per cell increased in the first hours after treatment and significantly deceased 5 hours after administration of the radiopharmaceutical. This illustrates the effect of ionizing radiation on PBMCs after PRRT as a function of the absorbed dose to the blood and provides a clear understanding of the correlation between the average number of RIF per cell and the absorbed dose to the blood. Furthermore, this enables the use of the RIF assay as an in vivo dosimeter.

Schumann et al. looked into DNA damage and repair in PBMCs of patients treated with [131I]NaI. They show that DNA repair after internal radiation with low-dose rates follows similar patterns as ex vivo irradiation with high-dose rates. Since lutetium-177 and Iodine-131 are both beta-emitters, DSB analysis in PBMCs seem to be an ideal method to analyze dose response mechanisms with PRRT as well.

Exposure to ionizing radiation (IR) leads to complex cellular responses including changes in gene expression which can differ between individuals. It is shown that in vivo exposure to x-ray showed a transcriptional radiation induced response after 24 hours for the genes APOBEC3H and FDXR, together with a strong dose-dependent response in blood irradiated ex vivo. The expression of the gene FDXR was significantly up-regulated 24 hours after radiotherapy and no significant difference was seen between in vivo and ex vivo irradiated blood. This indicates the possibility to identify radiation response biomarkers in PMBCs.

Altogether, these results confirm that ex vivo irradiation can mimic the in vivo transcriptional regulation and DNA damage, and these events can be measured in PBMCs. Our study for PRRT with 177Lu-DOTATATE can teach us more about the radiation effects and might give us information on biomarkers for effectiveness and toxicity. Therefore, we will analyze the effects of PRRT with 177Lu-DOTATATE on transcriptional regulation in white blood cells and how is this regulation related to radiation dose and DNA damage induction.

Next to healthy cells, we will also investigate the tumor cell response to PRRT using ctDNA. We want to explore if it is possible to detect ctDNA in patients with NET as currently only one group has reported on this biomarker in NET. Since limited is known about this. Similar to what has been observed in other malignancies, we expect that the detection of ctDNA can provide information about therapeutic response, assessment of tumor burden and disease progression. ctDNA contains genetic information from tumor cells in multiple regions (primary tumor and metastases), making it thus more representative than a single-lesion tissue biopsy to assess the tumor DNA. In addition, blood draw is less invasive compared to a tissue biopsy. The presence of ctDNA in blood of NET patients has been associated with poor prognosis in small, heterogeneous patient populations. In addition, ctDNA analysis is a promising tool to monitor treatment response. ctDNA presence in the blood is a result of cancer cell death and can therefore provide information on the radiation response. Indeed, tumor irradiation led to temporarily amplification of the release of ctDNA in lung cancer mouse models treated with external beam radiation (7). This indicates that changes in ctDNA levels early after radiotherapy may predict treatment outcome and allow clinicians to modify the therapy if needed (14). Indeed, Azad et al. showed in a group of 45 patients with esophageal cancer who received chemoradiotherapy that the presence of ctDNA was associated with tumor progression, formation of distant metastases and shorter disease-specific survival times.

Various studies have been performed in which potential genetic abbreviations in the ctDNA can be detected. ctDNA analysis research for NETs so far, however, has been hampered by the absence of a highly recurrent genetic variation in this population (such as TP53). The whole genome data generated in NET patients in the CPCT study were recently analyzed in depth, showing that it is still a challenge to identify a (set of) markers which can be detected in all NET subtypes. Alternatively, methylation sequencing could provide an opportunity to look at epigenetic marks in a larger part of the tumor genome compared to mutation-based approaches, and these marks in general have a higher penetrance throughout the tumor. Anticancer treatments can influence these methylation patterns and could thus be used to monitor treatment response. Liao et al. investigated 41 patient with hepatocellulair carcinoma (HCC) before and after surgery. In 8 of those patients, they successfully analyzed presented tumor-associated mutations in ERT, CTNNB1 and TP53 genes in ctDNA. Patients with mutations in ctDNA were more likely to relapse. A prospective clinical trial evaluated ctDNA methylation markers (WIF1 and NPY) in 805 patients with colorectal carcinoma (CRC) postoperatively. The 2-year disease-free survival rate was significantly lower in the ctDNA-positive group (64%) than in the ctDNA-negative group (82%). In 87 patients with breast cancer treated with neoadjuvant chemotherapy, methylated ctDNA was detected based on hypermethylation of the RASSF1A promoter. Methylated ctDNA levels were significantly correlated with the extent of residual tumor burden. Altogether, next to PBMCs, ctDNA could be a way to monitor PRRT response in a minimal

invasive manner.

Study objective

This is a pilot study to validate the possibility of determining the effect of PRRT with 177Lu-DOTATATE on transcriptional regulation and DNA damage induction in PBMCs and how this is related to the radiation dose.
We will also want to explore if we can detect ctDNA in NET patients to investigate the effect of PRRT to ctDNA.

Study design

This concerns a prospective pilot study in patients.
Twenty subjects with advanced NETs and an indication for PRRT will be prospectively enrolled following informed consent. For purposes of this study, all subjects will undergo venous blood sampling at four time points. Blood samples will undergo isolation of cell-free DNA for ctDNA analysis.

Study subjects will undergo 4 venipunctures for blood collection at different time-points surrounding the first cycle of PRRT. Those time-points are:
- At baseline.
- 4h hours after the administration of PRRT
- 24 hours after the administration of PRRT
- Before the 2nd cycle of PRRT.

In our routine practice PRRT is given in approximately 8 week cycles.
Study subjects will have completed the study after collection of the 4th sample.

DNA damage and transcriptional profiles in PBMCs will be analyzed and correlated to the radiation dose found in the blood. Moreover, ctDNA levels and methylation profiles will be analyzed and correlated to the radiation dose found in the blood.
For PBMC analysis, blood will be collected at the 4 time points. Cell free ctDNA will be isolated from blood collected at baseline and before the 2nd cycle of PRRT. For all analyses, laboratory findings will be correlated to clinical outcome of the patients.
Patient inclusion and samples collection will take place at the ENETS Center of Excellence, Erasmus Medical Center, Rotterdam, The Netherlands.

Radioactive dose will be determined by measuring blood samples in the gamma-counter. Gamma-counter measurements will be performed as technical triplicate per sample.

DNA damage will be assessed by immunofluorescent stainings and microscopic detection of γ-H2AX and 53BP1 RIF. RIF numbers of at least 50 cells from at least 4 fields of view per blood sample will be quantified using an automated quantification macro in ImageJ.

Transcriptional profiling will be assessed by nanopore sequencing. RNA isolation, sequencing, analysis and qPCR validation will be done at Radiation Effects Department of UK Health Security in Oxfordshire, United Kingdom. Validation of the identified differentially expressed genes will be performed in triplicate by qPCR. For sequencing, we will use an unique in-house analysis method using a Snakemake pipeline. RIF and sequencing analysis will be performed on blinded samples to perform unbiased analy

For the assessment of circulating biomarkers of the tumor cells we will measure ctDNA levels in the blood before PRRT and 8 weeks after the first cycle. DNA methylation sequencing method will be used to analyze the ctDNA. The MeD-seq assay will be used for genome-wide DNA methylation profiling on cell-free DNA (cfDNA).

Study burden and risks

The action being done consists of blood samples at four time points during admission for the PRRT. The burden and risks are both low since no interventions are done and a blood sample is a minor burden.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

- Patient with an advanced of metastatic, well-differentiated gastroenteropancreatic neuroendocrine tumor .
- Indication for treatment with PRRT with 7.4 GBq 177Lu-DOTATATE by the multidisciplinary team.
- Age >= 18 years.

Exclusion criteria

- Failure to obtain informed concent.
- Patient received ionizing radiation for imaging purposes within one week prior to PRRT or ionizing radiation for therapeutic purposes within 3 months prior to PRRT.
- Previous treatment with PRRT.
- Indication to receive a lower activity of PRRT than 7.4 GBq.

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	12-01-2023
Enrollment:	25

Type:	Actual

Ethics review

Approved WMO Date:	23-11-2022
Application type:	First submission
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)
Approved WMO Date:	02-03-2023
Application type:	Amendment
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)
Approved WMO Date:	21-06-2024
Application type:	Amendment
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
ClinicalTrials.gov	NCT05513469
CCMO	NL80190.078.22