European Journal of Nuclear Medicine and Molecular Imaging (2019) 46:1902–1910
https://doi.org/10.1007/s00259-019-04389-2

ORIGINAL ARTICLE

Characteristics and outcomes of therapy-related myeloid neoplasms after peptide receptor radionuclide/chemoradionuclide therapy (PRRT/PRCRT) for metastatic neuroendocrine neoplasia: a single-institution series

Isaac Goncalves[1] · Kate Burbury[1] · Michael Michael[2,3,4] · Amir Iravani[2,5] · Aravind S. Ravi Kumar[2,3,5] · Tim Akhurst[2,3,5] · Ing S. Tiong[1] · Piers Blombery[1] · Michael S. Hofman[2,3,5] · David Westerman[1] · Rodney J. Hicks[2,3,5] · Grace Kong[2,3,5]

Received: 11 March 2019 / Accepted: 31 May 2019 / Published online: 11 June 2019

Abstract

Purpose Peptide receptor radionuclide/chemoradionuclide therapy (PRRT/PRCRT) is an effective therapy for metastatic neuroendocrine neoplasia (NEN), but therapy-related myeloid neoplasms (t-MN) remain of concern. The study reviewed the clinicopathological features and outcomes of patients who developed t-MN.

Methods Retrospective analysis of all patients diagnosed with t-MN by 2016 WHO classification, from a cohort of 521 patients who received PRRT/PRCRT over a 12-year period. Molecular next-generation sequencing using an in-house 26-gene panel was performed.

Results Twenty-five of 521 (4.8%) patients were diagnosed with t-MN, including six acute myeloid leukaemia (AML) and 19 myelodysplastic syndrome (MDS). The median time from first cycle PRRT/PRCRT to diagnosis of t-MN was 26 months (range 4–91). Twenty-two of 25 (88%) patients had grade 1–2 pancreatic or small bowel NEN with moderate metastatic liver burden. Six patients (24%) had prior chemotherapy. Median number of PRRT cycles = 5 (22/25 (88%) with concomitant radiosensitising chemotherapy). All 25 patients achieved disease stabilisation (68%) or partial response (32%) on RECIST 1.1 at 3 months post-PRRT. At t-MN diagnosis, all patients presented with thrombocytopenia (median nadir 33×10^9/L, range 3–75) and 17 (68%) remained NEN progression-free. Marrow genetic analysis revealed unfavourable karyotype in 16/25 (66%) patients with tumour protein 53 (TP53) mutation in nine (36%). Azacitidine therapy was utilised in ten eligible patients, while four received induction chemotherapy for AML. The median overall survival from first PRRT was 62 months (19–94), but from t-MN diagnosis was only 13 months (1–56), with death due primarily to haematological disease progression.

Conclusions The diagnosis of t-MN after PRRT/PRCRT is an infrequent but serious complication with poor overall survival. Most patients present with thrombocytopenia; unfavourable genetic mutations have a poor response to t-MN treatment. Prospective data are needed to explore potential pre-existing genetic factors and predictive biomarkers to minimise the risk of t-MN.

Keywords ^{177}Lu-DOTATATE · Pepetide receptor radionuclide therapy · Neuroendocrine neoplasm · Therapy-related myeloid neoplasm · Myelodysplasia · Acute myeloid leukaemia

This article is part of the Topical Collection on Oncology – Digestive Tract.

✉ Isaac Goncalves
isaac.goncalves@petermac.org

[1] Department of Haematology, Peter MacCallum Cancer Centre, Melbourne 3000, Australia

[2] Nuclear Medicine Department, Peter MacCallum Cancer Centre, Melbourne, Australia

[3] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia

[4] Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

[5] Neuroendocrine Tumour Service, Peter MacCallum Cancer Centre, Melbourne, Australia

Introduction

Peptide receptor radionuclide therapy (PRRT) using ^{177}Lu-DOTA-octreotate (^{177}Lu-DOTATATE) is becoming more widely used in patients with inoperable (metastatic) neuroendocrine neoplasia (NEN). Multiple single-institution studies have shown consistent efficacy results comparing favourably with chemotherapy, and substantially higher responses than targeted agents, such as everolimus and sunitinib in delaying disease progression [1]. More recently, the first prospective randomised controlled NETTER-1 trial has confirmed the superiority of ^{177}Lu-DOTATATE compared to high dose (60 mg) octreotide long-acting repeatable (LAR) in progressive grade 1–2 inoperable midgut NEN (median progression-free survival not reached vs 8.4 months respectively), with interim analysis also suggesting an overall survival benefit [2].

Generally, PRRT is well tolerated with limited acute and medium-term toxicity profiles. The end-organs at greatest risk are renal and marrow. While co-administration of amino acids with PRRT have resulted in less renal toxicity, there is ongoing concern of short and long-term toxicity to the bone marrow. A number of recent studies have reported clinically significant acute cytopenia, particularly thrombocytopenia, and the longer-term development of haematological neoplasms such as myelodysplastic syndrome (MDS) and acute leukaemia (AL). Subacute grade 3–4 haematological toxicity has been reported to occur in up to 11% of patients, whilst for MDS and AL, the reported incidence rates range from 1 to 5.4% [3–5].

The development of a haematopoietic neoplasm after PRRT may be broadly categorised as a therapy-related myeloid neoplasm (t-MN) (2016 WHO classification) [6], comprising patients with acute myeloid leukaemia (AML) and MDS who were exposed to cytotoxic or radiation therapy for an unrelated malignancy. t-MN remains a feared adverse complication of cytotoxic therapy due to a limited response to available therapies and substantially reduced survival compared to de-novo myeloid neoplasms. Genetics remain an important prognostic factor and patients with t-MN often have notable unfavourable cytogenetic aberrations, in particular a high frequency of tumour protein 53 (*TP53*) pathway mutations [7–9]. Whether PRRT-related t-MN demonstrates similar genetic aberrations has not yet been ascertained.

The impact of PRRT-induced haematological toxicity can be multi-layered. The development of significant cytopenia often delays or limits further PRRT and other cytotoxic therapies, impacting NEN therapy efficacy and patient outcomes. Significant haematological toxicity, particularly from t-MN, results in substantial adverse consequences for the patient, due to infection and thrombohaemorrhagic risk, requirement for blood products, impaired quality of life and increased hospitalisation, as well as inferior survival.

Peter MacCallum Cancer Centre, an ENETS centre of excellence, represents a specialised referral centre for the management of NEN and delivery of PRRT. For almost two decades we have combined PRRT with radiosensitising chemotherapy, initially with infusional 5-fluorouracil [10], and subsequently with capecitabine [11, 12]. We have also used the combination of capecitabine and temozolomide (CAPTEM) in a protocol adapted from that reported by Claringbold and colleagues [13]. We call this approach peptide receptor chemoradionuclide therapy (PRCRT). We report the clinical features, therapy utilised and outcomes among a cohort of patients who developed t-MN and describe the associated morphological and genetic marrow abnormalities.

Methods

Patients were selected for PRRT/PRCRT as per the inclusion criteria published in previous studies [1]. Between October 2005 and June 2017, 521 individuals with gastroenteropancreatic (GEP), bronchial or unknown primary site NEN were treated with Lu-177-based PRRT/PRCRT protocols at Peter MacCallum Cancer Centre in Melbourne, Australia, and patient records were retrospectively reviewed. Haematological toxicity was evaluated as ≥ grade 2 neutropenia and/or thrombocytopenia that persisted for more than 6 weeks post PRRT. Haematological toxicity was graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [14]. T-MN was defined according to the revised World Health Organisation 2016 diagnostic criteria [6]. Cytogenetic analyses of bone marrow aspirate samples were performed using conventional G-banded chromosome analysis. DNA sequencing analysis of bone marrow aspirate samples were performed using a custom-designed, next-generation sequencing of a 26-gene myeloid amplicon panel as described previously [15].

Each patient's tumour burden was quantified by using whole-body molecular tumour volume (MTV) in millilitres using functional imaging, either ^{68}Ga-DOTATATE- PET/CT or ^{111}In-Octreotide SPECT/CT. The MTV was measured on the ^{68}Ga-DOTATATE PET/CT using a semi-automated threshold on MIM Encore (MIM 6.7; MIM Software, Cleveland, OH). The threshold used was based on similar recommendations for defining metabolic tumour volume with ^{18}F-FDG from the PERCIST recommendations. The threshold used was based on liver uptake and was defined as the 1.5 × liver SUV_{mean} + 2SD. If there was no ^{68}Ga-DOTATATE PET/CT available, the disease volume was quantified using a visually selected threshold on the ^{111}In-Octreotide SPECT/CT. The proportion of whole-body disease burden that was in the liver and bones was also calculated by creating subregions of disease in the liver or bones.

Median overall survival (OS) was estimated using Kaplan-Meier analysis measured from first PRRT and also from the time of t-MN diagnosis to death from any cause. Statistical analyses were performed using SPSS version 19 software (IBM Corp., Armonk, NY). NEN progression was defined by evidence of symptomatic relapse (for those treated for symptomatic disease), or biochemical recurrence and imaging progression on molecular imaging (SSTR ± FDG) or RECIST 1.1 criteria. MDS and AML treatment response were categorised according to the International Working Group (IWG) standardisation of response criteria [16–18].

Results

Patient characteristics and PRRT/PRCRT treatment parameters

Overall, 25 of 521 (4.8%) consecutive patients developed definite therapy-related myeloid neoplasms (t-MN) based on definitive marrow morphology and/or cytogenetic/gene sequencing confirmation of definitive clonal abnormality (WHO Classification 2016).

Baseline patient characteristics of the 25 patients are shown in Table 1. Overall, 15 (60%) were female with a median age 64 years (range 38–81). Twelve (48%) had primary pancreatic NEN, ten (40%) primary small bowel NEN and most were grade 1 (Ki67 < 2%) or 2 (Ki67 3–20%) tumour differentiation [9 (36%) and 11 (44%) patients, respectively]. The metastatic disease burden analysis found that 22 (88%) patients had <20 NEN metastatic lesions. Both quantitation of disease burden and evaluation of metastasis distribution were available in 19/25 patients using ^{68}Ga-DOTATATE PET or ^{111}In-Octreotide SPECT scans. Twelve patients (48%) showed high NEN disease burden, defined as MTV > 200 ml. The median MTV for the cohort was 256 ml (range 10–1278). The liver was the dominant site of metastasis in 16 patients, with >60% of total tumour burden. In contrast, osseous metastatic volume was low, with only four patients demonstrating osseous uptake and contributing <2% of total MTV.

Patient treatment parameters are shown in Table 2. Prior to PRRT/PRCRT, six (24%) patients had prior chemotherapy, most commonly carboplatin/etoposide, while 18 patients (72%) had received only somatostatin analogues. Nearly half (n = 12, 48%) had undergone prior surgical removal of the primary NEN. The most common indication for initiating PRRT was radiological disease progression [23 (92%)], while for 2 (8%) patients, the indication was for uncontrolled hormone-related symptoms. The median number of ^{177}Lu-DOTATATE PRRT cycles was five (range 2–9), and median cumulative radionuclide administered activity was 40.5 GBq (range 24.4–74.8). Fourteen (56%) patients received ^{90}Y-DOTA-Octreotate as part of the PRRT treatment course with a median of 1 cycle (range 1–3). The majority, 22 patients (88%), received concomitant radiosensitising chemotherapy, most commonly 5-fluorouracil (5-FU continuous infusion) (11, 44%) or capecitabine (7, 28%) as per our institutional protocols. Four patients (16%) had capecitabine in combination with temozolomide.

Table 1 Patient characteristics

Characteristic	Patients No. (%)
Age at first treatment	
Median	64
Range	38–81
Gender	
Male	10 (40)
Female	15 (60)
Primary site	
Pancreatic	12 (48)
Small bowel	10 (40)
Large bowel	0 (0)
Bronchial	1 (4)
Unknown	1 (4)
Non-pancreatic	1 (4)
Grade of tumour differentiation	
Grade 1 (Ki67 index <2%)	9 (36)
Grade 2 (Ki67 index 3–20%)	11 (44)
Grade 3 (Ki67 index >20%)	2 (8)
Unknown	3 (12)
No. of lesions	
<5	11 (44)
5–20	11 (44)
>20	3 (12)
Volume of tumour burden[a]	
High whole-body FTV (>200 ml)	12 (48)
Low whole-body FTV (<200 ml)	7 (28)
Dominant site of metastasis	
Liver	16 (64)
Osseous	0 (0)
Other	3 (12)

[a] The patient's tumour burden was quantified by using whole-body functional tumour volume (FTV) in millilitres using molecular imaging, either Ga-68 octreotate PET/CT or In-111 Octreotide SPECT/CT. The threshold of 200 ml is based on liver uptake and was defined as 1.5 × liver SUV_{mean} + 2SD. The proportion of whole-body disease burden that was in the liver and osseous was also calculated by creating subregions of disease in the liver or bones

PRRT/PRCRT objective response

As shown in Table 2, somatostatin receptor molecular imaging response at 3 months post induction ^{177}Lu-DOTATATE PRRT/PRCRT completion showed that 18/25 (72%) achieved

Table 2 Patient treatment parameters and response assessment

Prior chemotherapy, no. (%)	
None	19 (76)
Carboplatin/etoposide	4 (16)
Doxorubicin	2 (8)
Prior non-chemotherapy, no. (%)	
None	7 (28)
Everolimus	0 (0)
Somatostatin analogues	18 (72)
Surgery, no. (%)	
Removal of primary	12 (48)
Indication of PRRT induction, no. (%)	
Progressive disease	23 (92)
Uncontrolled hormone-secretory symptoms	2 (8)
PRRT cycles and dose	
Number of patients prescribed ^{177}LuTate (%)	25 (100)
Median number of ^{177}LuTate cycles (range)	5 (2–9)
Number of patients concomitantly prescribed ^{90}Ytate (%)	14 (56)
Median number of ^{90}Ytate cycles (range)	1 (1-3)
Number of patients concomitantly prescribed ^{111}InTate (%)	3 (12)
Median cumulative ^{177}LuTate administered activity (total), GBq	40.5
Median cumulative ^{90}Ytate administered activity (Total), GBq	3.1
Radiosensitising chemotherapy	
No. (%)	22 (88)
Median no. of cycles	4
5FU, no. (%)	11 (44)
Median no. of cycles	4
Capecitabine, no. (%)	7 (28)
Median no. of cycles	2.5
Capecitabine + temozolomide, no. (%)	4 (16)
Median no. of cycles	4
Response assessment (3 months), no. (%)	
Molecular imaging response (SSTR ± FDG)	
Partial response	18 (72)
Stable disease	7 (28)
Complete response	0 (0)
Progressive disease	0 (0)
CT RECIST 1.1	
Partial response	8 (32)
Stable disease	17 (68)
Complete response	0 (0)
Progressive disease	0 (0)
Biochemical (reduction of CgA from baseline)	
<25%	11 (44)
25–50%	3 (12)
>50%	10 (40)

a partial response, 7/25 (28%) disease stabilisation, but none achieved a complete response (0%) or had progressive disease (0%). Response assessment by RECIST 1.1 criteria showed that 8/25 (32%) achieved a partial response, 17/25 (68%) disease stabilisation. Again, none had a complete response or progressive disease. Biochemical response (CgA) demonstrated that 13/24 (54%) patients achieved a decrease of >25% from baseline level and one patient had no follow-up result.

Haematologic and genetic analysis

Analysis of haematological parameters demonstrated that all 25 patients had persisting (>3 months) grade 2–4 thrombocytopenia. Thirteen (52%) patients had concurrent neutropenia (grade 2–4). Median platelet nadir was 33×10^9/L (range 3–75×10^9/L). The time to development of persisting cytopenia from C1 of PRRT/PRCRT was a median of 16 months (range 1–85 months). The onset in 11/ 25 (44%) occurred during induction PRRT/PRCRT and after completion in 14/ 25 (56%). Thrombocytopenia or neutropenia limited further ^{177}Lu-DOTATATE or other cytotoxic treatments in 9/25 (36%) patients.

The median time from cycle one of PRRT/PRCRT to definitive diagnosis of t-MN was 26 months (range 4–91). At this time, 17 (68%) patients were free from NEN progression but four (16%) patients had NEN metastasis demonstrated within the diagnostic bone marrow biopsy. Nineteen patients (3.6% of entire cohort) developed MDS and six patients (1.2% of entire cohort) developed AML. No patient developed acute lymphoblastic leukaemia. Of the 19 patients with MDS, four had MDS with single-lineage dysplasia, ten MDS with multi-lineage dysplasia (MDS-MLD) and five had MDS with excess blasts (MDS-EB) (Table 3). Of these, three patients (one MDS-EB and two MDS-MLD) progressed to AML. All six patients diagnosed with de novo AML demonstrated multi-lineage dysplasia. Karyotype analyses are outlined in Table 4. Eight patients had a complex karyotype (≥3 chromosomal abnormalities), nine had 1-2 chromosomal abnormalities, six had a normal diploid karyotype and two were not assessable. Abnormalities involving chromosome 5 and/or 7 were the most common finding (13/25 patients, 52%). Sequence variant analysis of genes recurrently mutated in myeloid malignancy was performed using a next-generation sequencing myeloid amplicon gene panel. Mutations in *TP53* were the most frequent (nine patients) mutations detected but no mutations detected in seven patients (see Table 4). Other mutations found included Ten-eleven translocation 2 (*TET2*), and U2 small nuclear RNA auxiliary factor 1 (*U2AF1*). Seven patients had no DNA material for analysis.

Table 3 Haematological toxicity and bone marrow diagnosis

	Patients No. (%)
Cytopenia (≥ grade 2)	
Thrombocytopenia	25 (100)
Grade 2	8 (32)
Grade 3	11 (44)
Grade 4	6 (24)
Neutropenia	13 (52)
Grade 2/3	11 (44)
Grade 4	2 (8)
NEN bone marrow metastasis	4 (16)
Myelodysplastic syndrome	19
MDS with single-lineage dysplasia	4
MDS with multi-lineage dysplasia	10
MDS with excess blasts	5
Acute myeloid leukaemia	6

Haematological toxicity was graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [14]

Treatment and outcome of patients with t-MN

Treatment for t-MN was highly variable and included; high-dose cytarabine containing anti-AML regimes (four patients with AML); azacitidine (five patients with intermediate-2 or high risk MDS according to the international prognostic scoring system (IPSS) and five patients with AML); allogeneic bone marrow transplant (one patient with MDS-EB); supportive therapy [6 patients with MDS (transfusions, growth factor therapy e.g. Erythropoietin)]; and observation-only (six patients with MDS). Median number of azacitidine cycles administered was 5.5 (range 1–21). Of the 13 patients who received treatment for t-MN, best-response assessment according to the international working group (IWG) criteria was able to be performed in nine patients; three achieved complete response CR (one MDS, two AML), three achieved partial response/stable disease (two MDS, one AML) and three showed progressive disease (three AML). The four patients who could not be assessed had progressive disease and died before response assessment could be formally performed.

Overall survival and follow-up

Median follow-up for all patients was 51 months (range 19–94) from C1 of PRRT/PRCRT. The median OS from C1 PRRT/PRCRT was 62 months (range 19–94). The median OS from diagnosis of t-MN was 13 months (range 1–56), specifically, from diagnosis of AML, 7 months (range 2–19) and MDS 13.5 months (range 1–56). The cause of death in the MDS cohort (11 of 19 deaths, 58%) was as follows: three sepsis, five MDS progression, two NEN progression and one unknown. Cause of death in the AML cohort (4 of 6 deaths, 67%) was as follows: three AML progression and one NEN progression. Five patients achieved long-term benefit with t-MN therapy, two patients with AML survived >18 months following induction chemotherapy, and three patients with MDS had successful long-term azacitidine treatment >18 months. Of the patients who remain alive (10/25, 40%), the majority are low-risk MDS and undergoing surveillance or supportive therapy.

Table 4 Characteristics, genetic mutations and outcomes of 25 t-MN patients

Age/sex	t-MN	NEN progression	Karyotype	Mutation	Treatment	OS^	Cause of death
61F	MDS-MLD	No	Normal	No mutation	Supportive	13	Sepsis
81M	MDS-MLD	No	Normal	TP53	Supportive	8	UNK
63M	MDS-MLD	No	Normal	TET2	Supportive	13+	
70F	MDS-MLD	No	Normal	Not available	Supportive	28+	
67F	MDS-SLD	Yes	Normal	No mutation	Supportive	8+	
38F	MDS-SLD	No	Normal	No mutation	Supportive	24+	
64F	MDS MLD	No	45, XX, −7	Not available	Supportive	22+	
77M	MDS-MLD	Yes	46, XX, −13q	TP53	Supportive	4	NEN
63M	MDS-MLD	Yes	47, XY, +Y	No mutation	Supportive	14	NEN
76F	MDS-MLD	No	45, XX, dic(7;17)	TP53	Azacitidine	26	AML
74F	MDS-SLD	Yes	46, XX, −7p	Not available	Supportive	30+	
73F	MDS-SLD	No	46, XX, −7, +r	No mutation	Supportive	1	Sepsis
63F	MDS-MLD	Yes	46, XX, +1, der(1;7)	Not available	Supportive	6	MDS
66F	MDS-MLD	No	49, XX, +1, der(1;7), +8, +13, +21	No mutation	Induction	56	AML
69F	MDS-EB2	Yes	44, XX, dic(5;17), −7, −12, +r, +mar1	TP53	Azacitidine	27	MDS
58M	MDS-EB1	No	47, XY, −3q, −6, −7q, −12, +17p, +18q, +mar1, +mar2	Not available	BMT	12+	
64M	MDS-EB1	No	45, XY, +3p, +6p, −7q, +8, −13q, −15, t(5;18), −21, +mar1	TP53	Azacitidine	4	Sepsis
53M	MDS-EB2	No	43–47, XY, −2, −3, −5, −5q +8, +9, −12, −13, +r, and dic(7;17)	TP53	Azacitidine	18+	
64M	MDS-EB2	No	80–88, XYY, hypotetraploid, including −5, −7, −17, +17p	TP53	Azacitidine	3	AML
59F	AML	No	Not available	Not available	Azacitidine	13	AML
72M	AML	Yes	Not available	TP53	Azacitidine	2	AML
72M	AML	No	46, XY, FISH MYC amplification	TET2/U2AF1	Induction	19+	
53F	AML	No	46, XX, −5q, −7q, −8, +13, +mar1	Not available	Azacitidine	5	NEN
47F	AML	No	46, XX, t(11;17), MLL-rearrangement at 11q23	No mutation	Induction*	7	AML
74F	AML	Yes	46, XX, −5q, +11, −18, +20, +r	TP53	Azacitidine	8+	

MDS-SLD myelodysplasia and single-lineage dysplasia, *MDS-MLD* multilineage dysplasia, *MDS-EB1* excess blasts-1, *MDS-EB2* excess blasts-2, *AML* acute myeloid leukaemia, *Mar* markers chromosomes of unknown origin, *dic* dicentric, *r* ring chromosome, *TP53* tumour protein 53, *FISH* fluorescence in situ hybridisation, *TET2* ten-eleven translocation 2, *U2AF1* U2 small nuclear RNA auxiliary factor 1, *BMT* allogeneic bone marrow transplant

*Azacitidine prior to induction. + Alive. ^OS from t-MN diagnosis

Discussion

PRRT is a highly effective treatment for patients with inoperable somatostatin receptor positive NEN, which is increasingly utilised as a standard therapy in these patients. However, although significant marrow toxicity, including development of MDS or leukaemia, appears relatively uncommon, these are of clinical importance [19]. In this retrospective single-centre study of 521 patients receiving PRRT or PRCRT for metastatic NEN, over a 12-year period (2005–2017), we found an incidence of t-MN of 4.8%. These findings are comparable to other retrospective studies. Using PRRT, Bodei et al., reported an MDS/acute leukaemia incidence of 3.5% in a cohort of 807 NEN patients treated with ^{177}Lu-DOTATATE or ^{90}Y-octreotide PRRT [4] and Bergsma et al., recently demonstrated in a cohort of 274 GEP NEN patients treated with ^{177}Lu-DOTATATE, a 4% incidence of bone marrow neoplasm or failure [20].

While the survival data from published studies using ^{177}Lu-DOTATATE PRRT are largely based on uncontrolled, retrospective analyses, most report median OS in the range of 46 to 60 months [1], which is substantially longer than in most series of other available therapies. The first randomised controlled trial (NETTER-1 trial), which confirmed the superiority of ^{177}Lu-DOTATATE compared to high dose LAR, showed median progression-free survival not reached (at 31 months) in the ^{177}Lu-DOTATATE group and interim analysis suggesting a long-term survival benefit [2]. A key observation from our study was the poor overall survival in patients after diagnosis of t-MN following PRRT/PRCRT. The median OS from t-MN diagnosis was 13 months. However, the median overall survival from C1 PRRT/PRCRT in our cohort

was 62 months, similar to that reported for those receiving PRRT without this complication. This outcome must be considered in the context that the majority of patients were treated for progressive disease and the remaining patients for uncontrolled symptoms impairing quality of life. Overall the cohort had several adverse prognostic features including relatively high disease burden and >50% had G2/3 disease. Unfortunately, at the time of t-MN diagnosis, most patients were NEN progression-free and would likely have lived for a significantly longer period had they not developed t-MN. Therefore, defining the level of risk and predictive factors of developing t-MN, to guide better patient selection for safer delivery of PRRT/PRCRT remain critical issues. This supports our approach with limiting this treatment to patients with objective evidence of progression, except in patients with a high disease burden and biologically aggressive disease with a high-anticipated and imminent likelihood of progression. These include, G2 NEN with ^{18}F-flurodeoxyglucose (FDG)-avid disease or well differentiated G3 NEN [21].

In our cohort, patients diagnosed with t-MN after PRRT/PRCRT typically had (i) moderate metastatic burden mostly in the liver but, somewhat surprisingly, with relatively small volume osseous disease; (ii) minimal exposure to prior chemotherapy; (iii) only modest numbers of PRRT cycles (median 5); (iv) a favourable response to PRRT/PRCRT; (v) predominant thrombocytopenia (G2–4) which persisted for >3 months; and (v) unfavourable cytogenetic and genetics mutations. Using a regression analysis model, Bodei et al., showed that prior chemotherapy exposure, including myelotoxic chemotherapy, platelet toxicity grade and duration of PRRT were significant risk factors associated with MDS or acute leukaemia. However, these factors predicted only 15–30% of their patients [4]. Furthermore, Bergsma et al. retrospectively analysed potential risk factors for predicting marrow dysfunction, specifically, cumulative administered activity of ^{177}Lu-DOTATATE and estimated bone marrow dose, but no significant risk factor was identified [20]. The lack of definite treatment associated factors raises the possibility of pre-existing biological or genetic susceptibility as a contributing factor. The high rate of disease control in our patients raises the possibility that these individuals may have enhanced radiosensitivity, possibly related to deficiencies in DNA-repair that could predispose them to both neuroendocrine tumour and t-MN. Mutations in genes associated with DNA-repair have been described in pancreatic neuroendocrine tumours [22]. Clearly, well-structured prospective studies are needed to specifically explore the risk factors predicting t-MN after PRRT/PRCRT and assessing both clinical and biomarker factors, to estimate their predictive capacity as well as potentially defining a mechanism of action.

In our study, we highlight that T-MN after PRRT/PRCRT share key features characteristic of other t-MN, including; a favourable response to the primary cancer treatment, unfavourable genetic aberrations (chromosome 5 and/or 7 and p53 mutation), limited response to available therapies and poor survival. Therefore, a critical issue is how to optimally treat patients who develop t-MN after PRRT/PRCRT, and to our knowledge, our study is the first to describe the treatments utilised and patient outcomes. In our cohort, azacitidine was the most common utilised treatment for eligible patients, while only a small number of patients received induction chemotherapy. The poor response to therapy in our patients is likely due to two significant factors. First, none of the patients were in complete remission from NEN, and this limited deliverability of higher intensity therapy. Second, most of these patients had an unfavourable karyotype and *TP53* mutations, which are well established poor prognosis factors in myeloid neoplasms [23, 24]. Similarly to other t-MN, our findings demonstrate that single-agent azacitidine may not be effective treatment for t-MN after PRRT/PRCRT and raise the possibility of azacitidine-based combinations as a more effective therapy option. Studies of azacitidine combinations with novel agents (e.g. lenalidomide, vorinostat or venetoclax) have been investigated in patients with AML unfit for intensive therapy and have shown improved outcomes [25–27].

Limitations

This study suffers from the potential limitation of being a retrospective case-finding series. However, the vast majority of patients in our PRRT/PRCRT program remain under continuing surveillance by our NET Service. Those who are managed in a shared-care model with external referring clinicians provided regular laboratory and clinical updates on their patients. It is, however, possible that additional cases of t-MN may not have been recognised. The use of variable PRRT/PRCRT treatment protocols, which had evolved over the years with increasing experience and evidence, limited our ability to determine specific aspects of the treatment that might predispose to t-MN. Given a minority of the whole cohort received CAPTEM chemotherapy, the presence of four cases raises the possibility that the concurrent use of an alkylating agent may constitute a particular risk factor, but this will be better assessed in an ongoing prospective study being performed in Australia. The lack of control arm and small numbers limited the ability to analyse predictive and prognostic factors for development of t-MN.

Conclusion

The diagnosis of t-MN after PRRT/PRCRT is an infrequent but serious long-term complication and carries a poor prognosis. Most patients present with thrombocytopenia, unfavourable genetic mutations and a poor response to t-MN treatment. However, this must be balanced against the high

efficacy and durable responses to PRRT in these same patients, particularly given the majority of this cohort had prior progressive disease and adverse prognostic features. Importantly, their overall survival from the initiation of PRRT/PRCRT was similar to that reported for those receiving PRRT without this complication. Our results raise the possibility of pre-existing biological or genetic susceptibility to radiation as a contributing factor both for tumour response and development of t-MN. Prospective data are needed to explore potential genetic factors, predictive biomarkers, define mechanism of action, and to improve treatment for t-MN.

Acknowledgements IG, KB, MM, RJH, and GK designed the study and wrote the manuscript. IG, KB, MM, IST, AI, ARK, TA, and GK identified cases and collected data. IG, PB, IST, AI, ARK, TA, MSH, GK performed data analysis. All authors contributed to and approved the final manuscript.

Funding No funding source involved.

Compliance with ethical standards

Conflict of interest RJH holds shares in Telix Pharmaceuticals on behalf of the Peter MacCallum Cancer Centre. MSH reports personal fees and non-financial support from Ipsen and Sanofi Genzyme, personal fees and other from Endocyte, outside the submitted work. All remaining authors declare no competing interests.

Ethical approval The study was approved by the Peter MacCallum Cancer Centre ethics committee as a retrospective audit with approval of waiver for patient consent (HREC Project number 18/61R). All patients had previously provided written, informed consent for PRRT/PRCRT under existing compassionate use guidelines. This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Hicks RJ, Kwekkeboom DJ, Krenning E, Bodei L, Grozinsky-Glasberg S, Arnold R, et al. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Neoplasia: peptide receptor radionuclide therapy with radiolabeled somatostatin analogues. Neuroendocrinology. 2017;105:295–309. https://doi.org/10.1159/000475526.
2. Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 trial of (177)Lu-Dotatate for midgut neuroendocrine tumors. N Engl J Med. 2017;376:125–35. https://doi.org/10.1056/NEJMoa1607427.
3. Bergsma H, Konijnenberg MW, Kam BL, Teunissen JJ, Kooij PP, de Herder WW, et al. Subacute haematotoxicity after PRRT with (177)Lu-DOTA-octreotate: prognostic factors, incidence and course. Eur J Nucl Med Mol Imaging. 2016;43:453–63. https://doi.org/10.1007/s00259-015-3193-4.
4. Bodei L, Kidd M, Paganelli G, Grana CM, Drozdov I, Cremonesi M, 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. 2015;42:5–19. https://doi.org/10.1007/s00259-014-2893-5.
5. Kesavan M, Turner JH. Myelotoxicity of peptide receptor radionuclide therapy of neuroendocrine tumors: a decade of experience. Cancer Biother Radiopharm. 2016;31:189–98. https://doi.org/10.1089/cbr.2016.2035.
6. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405. https://doi.org/10.1182/blood-2016-03-643544.
7. Kayser S, Dohner K, Krauter J, Kohne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117:2137–45. https://doi.org/10.1182/blood-2010-08-301713.
8. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477–87. https://doi.org/10.1056/NEJMoa1409405.
9. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–98. https://doi.org/10.1056/NEJMoa1408617.
10. Kong G, Johnston V, Ramdave S, Lau E, Rischin D, Hicks RJ. High-administered activity In-111 octreotide therapy with concomitant radiosensitizing 5FU chemotherapy for treatment of neuroendocrine tumors: preliminary experience. Cancer Biother Radiopharm. 2009;24:527–33. https://doi.org/10.1089/cbr.2009.0644.
11. Hubble D, Kong G, Michael M, Johnson V, Ramdave S, Hicks RJ. 177Lu-octreotate, alone or with radiosensitising chemotherapy, is safe in neuroendocrine tumour patients previously treated with high-activity 111In-octreotide. Eur J Nucl Med Mol Imaging. 2010;37:1869–75. https://doi.org/10.1007/s00259-010-1483-4.
12. Kong G, Thompson M, Collins M, Herschtal A, Hofman MS, Johnston V, et al. Assessment of predictors of response and long-term survival of patients with neuroendocrine tumour treated with peptide receptor chemoradionuclide therapy (PRCRT). Eur J Nucl Med Mol Imaging. 2014;41:1831–44. https://doi.org/10.1007/s00259-014-2788-5.
13. Claringbold PG, Price RA, Turner JH. Phase I-II study of radiopeptide 177Lu-octreotate in combination with capecitabine and temozolomide in advanced low-grade neuroendocrine tumors. Cancer Biother Radiopharm. 2012;27:561–9. https://doi.org/10.1089/cbr.2012.1276.
14. Program CTE. Common Terminology Criteria for Adverse Events v4.0 (CTCAE). https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40. 2010.
15. Kenealy M, Hertzberg M, Benson W, Taylor K, Cunningham I, Stevenson W, et al. Azacitidine with or without lenalidomide in higher risk myelodysplastic syndrome & low blast acute myeloid leukemia. Haematologica. 2018. https://doi.org/10.3324/haematol.2018.201152.
16. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000;96:3671–4.
17. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21:4642–9. https://doi.org/10.1200/JCO.2003.04.036.

18. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006;108:419–25. https://doi.org/10.1182/blood-2005-10-4149.
19. Bodei L, Modlin IM, Luster M, Forrer F, Cremonesi M, Hicks RJ, et al. Myeloid neoplasms after chemotherapy and PRRT: myth and reality. Endocr Relat Cancer. 2016;23:C1–7. https://doi.org/10.1530/ERC-16-0258.
20. Bergsma H, van Lom K, Raaijmakers M, Konijnenberg M, Kam B, Teunissen JJM, et al. Persistent hematologic dysfunction after peptide receptor radionuclide therapy with (177)Lu-DOTATATE: incidence, course, and predicting factors in patients with gastroenteropancreatic neuroendocrine tumors. J Nucl Med. 2018;59:452–8. https://doi.org/10.2967/jnumed.117.189712.
21. Thang SP, Lung MS, Kong G, Hofman MS, Callahan J, Michael M, et al. Peptide receptor radionuclide therapy (PRRT) in European Neuroendocrine Tumour Society (ENETS) grade 3 (G3) neuroendocrine neoplasia (NEN)—a single-institution retrospective analysis. Eur J Nucl Med Mol Imaging. 2018;45:262–77. https://doi.org/10.1007/s00259-017-3821-2.
22. Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature. 2017;543:65–71. https://doi.org/10.1038/nature21063.
23. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079–88.
24. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998;92:2322–33.
25. Montalban-Bravo G, Huang X, Naqvi K, Jabbour E, Borthakur G, DiNardo CD, et al. A clinical trial for patients with acute myeloid leukemia or myelodysplastic syndromes not eligible for standard clinical trials. Leukemia. 2017;31:318–24. https://doi.org/10.1038/leu.2016.303.
26. Pollyea DA, Zehnder J, Coutre S, Gotlib JR, Gallegos L, Abdel-Wahab O, et al. Sequential azacitidine plus lenalidomide combination for elderly patients with untreated acute myeloid leukemia. Haematologica. 2013;98:591–6. https://doi.org/10.3324/haematol.2012.076414.
27. DiNardo C, Pollyea D, Pratz K, Thirman MJ, Letai A, Frattini M, et al. A phase 1b study of venetoclax (ABT-199/GDC-0199) in combination with decitabine or azacitidine in treatment-naive patients with acute myelogenous leukemia who are >= to 65 years and not eligible for standard induction therapy. Blood. 2015;126:327.