[^{11}C]Methionine emerges as a new biomarker for tracking active myeloma lesions

Multiple myeloma (MM) accounts for approximately 1% of all cancers and around 10% of haematological malignancies (Siegel *et al*, 2013). The disease is of remarkable heterogeneity both from a clinical as well as from a genetic perspective (Manier *et al*, 2017). The utility of molecular imaging using positron emission tomography (PET) with the radiolabelled glucose analogue [^{18}F]-2‘-deoxy-2‘-fluoro-D-glucose (FDG) for diagnosis, staging and response assessment has been demonstrated by several studies (Durie *et al*, 2002; Bartel *et al*, 2009; Kumar *et al*, 2016). However, glucose metabolism does not fully allow for a refined capturing of tumour biology with diffuse bone marrow involvement and low metabolic activity of myeloma reducing sensitivity (Terpos *et al*, 2011). Due to the rapid uptake and metabolic incorporation of radiolabelled amino acids into newly synthesized immunoglobulins, *L*-methyl-[^{11}C] methionine (MET) has emerged as a promising alternative imaging agent (Dankerl *et al*, 2007). We previously reported a significantly higher retention of MET in myeloma cell lines and patient-derived CD138^{+}-plasma cells and the feasibility of monitoring early treatment response with MET *in vitro* and *in vivo* (Luckerath *et al*, 2013, 2015). Recently, in an initial analysis MET clearly outperformed FDG as a more accurate marker of tumour burden and disease activity (Lapa *et al*, 2016).

Ten patients (6 males, 4 females, mean age, 59 $\pm$ 10 years) with biopsy-proven multiple myeloma underwent both FDG- and MET-PET/CT. The patients enrolled are part of a prospective study approved by the local ethics committee of the University of Würzburg. All patients gave written informed consent to sequential FDG- and MET-PET/computed tomography (CT) imaging. MET was administered under the conditions of the pharmaceutical law (German Medicinal Products Act, AMG §13 2b) according to German law and in accordance with the responsible regulatory bodies (Regierung von Oberfranken).

All patients presented with serologically active disease as assessed by free serum light chains (FLC) and/or serum (or urine) M protein. Interestingly, the corresponding bone marrow (BM) biopsies from the iliac crest revealed only minor monoclonal plasma cell infiltration with a median of 15% (range, 0–40%). Patients‘ characteristics are summarized in Table I.

PET/CT was performed after injection of 304 $\pm$ 31 MBq FDG and 653 $\pm$ 143 MBq MET and analysed as previously described (Lapa *et al*, 2016). Imaging results were compared with LAT1 (CD98, SLC7A5) and GLUT1 (SLC2A1) expression as well as the proliferation indices (Ki67) of the myeloma cells as assessed by standard immunohistochemistry on trephine biopsies. The following antibodies were used: CD98 (H-300, sc-9160: Santa Cruz Biotechnology, Dallas, TX), pretreated in citrate buffer pH 7·0, 1:50 dilution; Glut1 (SPM498, AM505-5M; BioGenex Laboratories, Freemont, CA), citrate buffer pH 6·0, ready to use; Ki67 (MIB-1, M7240; Dako, Glostrup, Denmark), citrate buffer pH 6·0, 1:800 dilution).

MET-PET/CT was positive in all patients whereas FDG did not reveal active disease in a single case (Figure S1). MET-positive, FDG-negative extramedullary disease was identified in a single case. On a lesion basis, MET detected more than 20 focal lesions (FL) in 1 patient; 7/10 had >50 FL detected. The remaining 3/10 subjects had less than 20 FL.

One patient with lambda light chain (LC) MM and no secretory activity in serum and urine had disseminated intramedullary myeloma exclusively detected by MET-PET. He was managed with watchful waiting and underwent follow-up FDG-PET 3 months after the initial examination when he presented with disseminated disease, which was now also detectable by serum parameters and FDG-PET/CT (Fig 1).

Analysis of monoclonal plasma cells in bone marrow aspirates yielded only sparse to moderate, distinctly patchy infiltrates with Ki67 levels between 10% and 20%. Immunohistochemical analysis confirmed intense expression of both GLUT1 and moderately strong expression of LAT1 (as the major routes of transport of FDG and MET into the myeloma cell, respectively) in all myeloma samples with little variation between cases. However, all patients were FDG-PET negative, whereas MET-PET imaging revealed hypermetabolic multiple focal lesions in all cases. The reason for this finding is not entirely clear. It can be speculated that the patchy pattern of monoclonal plasma cells encountered in these

Table I. Patients' characteristics.

Patient	Sex	Age (years)	Myeloma type	Disease duration (months)	Serum M protein (g/l)	FLC (mg/l)	BM involvement (%)
1	M	68	IgG κ	30	29·2	20·4 (κ)	10–15
2	M	56	IgA κ	32	44·7	0·5 (κ)	40
3	M	63	LC λ	1	–	444 (λ)	15
4	M	62	IgG κ	199	13·0	14 015 (κ)	20
5	F	53	LC κ	122	–	99 (κ)	15
6	F	63	LC λ	23	–	1876 (λ)	10
7	M	48	LC λ	10	–	37·2	0
8	F	53	LC λ	11	–	3396 (λ)	20
9	M	64	LC κ	54	–	1183 (κ)	25
10	F	62	IgG κ	39	1·7	955 (κ)	10

BM, bone marrow; disease duration, defined as the time from intial diagnosis to time point of imaging; F, female; FLC, free serum light chains; LC, light chain; M, male.

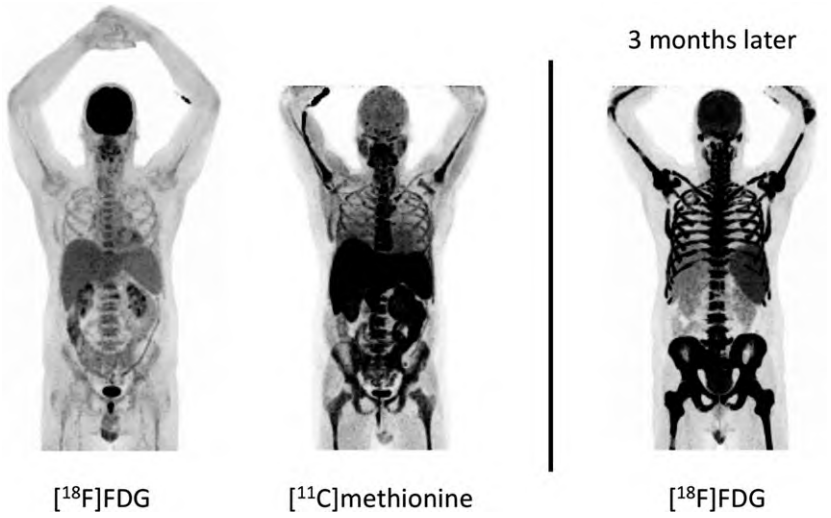

Fig 1. MET-PET preceding FDG-PET results in a patient with IgG λ multiple myeloma (Patient 7). At first imaging, positron emission tomography/computed tomography (PET/CT) with [^{18}F]-2'-deoxy-2'-fluoro-D-glucose (FDG) was unremarkable whereas *L*-methyl-[^{11}C] methionine (MET) detected numerous lesions throughout the skeleton. Three months later, the patient presented with full-blown relapse of the disease now easily distinguished in FDG-PET.

patients favours sampling bias by random bone marrow biopsy. In particular, all patients presented with only modest monoclonal plasma cell infiltration in the iliac crest biopsy samples, which were missed by FDG-PET. In contrast, MET seems to be more sensitive, most likely due to high demand for amino acids in serologically active myeloma. Given that the uptake mechanism of MET in the malignant plasma cells has not yet been fully illuminated, potentially other transport systems including LAT2, system A or ASC might have contributed to amino acid uptake and thereby favoured the stronger signal of MET-PET. More research has to be done to elucidate the underlying biological implications of amino acid transport in different MM subtypes.

This study further highlights the potential superiority of the amino acid MET for staging MM. In this small cohort of patients with serologically active disease, MET-PET/CT detected viable myeloma in all patients and proved its suitability as non-invasive read-out of tumour burden. Of note, all patients would have been missed by sole FDG-PET/CT, the current functional imaging reference standard. In one patient, changes in MET-PET preceded those detected in FDG-PET by 3 months. It might therefore prove its value for very early assessment of therapy response. However, this additional value has yet to be demonstrated.

Interestingly, all patients in our study presented with non-proportionally high serological markers as compared to only modest bone marrow findings. Given that random bone marrow biopsies from the iliac crest are prone to sampling error and the heterogeneity of disease, whole-body PET can also aid in determining the "correct" sites for taking biopsies. This might prove even more useful given the availability of other non-FDG radiotracers, including markers of lipid metabolism (e.g. [^{11}C]-choline, [^{11}C]-acetate) or cell membrane receptor expression, such as C-X-C motif chemokine receptor (CXCR4), which can aid in further understanding of myeloma biology complexity. Further research to investigate the differences and prognostic implications in myeloma biology is highly warranted.

Disclosure

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Different findings of [^{18}F]FDG and [^{11}C]methionine PET/CT in a patient with MM Ig G κ, bone marrow infiltration of 20% and high serologic disease activity (serum free κ light chains, 14 015 mg/l; patient #4).

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