Potential influence of concomitant chemotherapy on CXCR4 expression in receptor directed endoradiotherapy

C-X-C-motif chemokine receptor 4 (CXCR4) is overexpressed on the cell surface of various tumour entities, including lymphoma, multiple myeloma (MM) and acute lymphoblastic leukaemia (ALL). Recently, the feasibility of CXCR4-directed imaging and therapy has been demonstrated (Demmer *et al*, 2011; Gourni *et al*, 2011; Philipp-Abbrederis *et al*, 2015; Wester *et al*, 2015; Herrmann *et al*, 2016; Lapa *et al*, 2017a,b).

Besides chemokine receptor-overexpressing tumour cells, CXCR4-directed endoradiotherapy (ERT) with a ^{177}Lu- or ^{90}Y-labelled CXCR4-directed therapeutic ligand (Pentixather) also targets CXCR4-positive haematological stem and progenitor cells, resulting in myeloablation and requiring subsequent stem cell transplantation (SCT). Pre-therapeutic work-up comprises diagnostic CXCR4-directed positron emission tomography (PET) imaging as well as dosimetry in order to estimate the organ radiation doses and the achievable tumour doses. Problems with the timing of autologous SCT occasionally extend the interval between initial CXCR4-PET and ERT. As most ERT patients suffer from advanced stages of their disease, concomitant or bridging chemotherapy to prevent tumour progression is often needed. However, little is known about the effects of such treatment on receptor expression.

Three highly pre-treated patients (1 male, 2 females; aged, 32, 62 and 75 years) with relapsed refractory MM (patient 1), diffuse large B cell lymphoma (DLBCL; patient 2) and ALL (patient 3) were considered (previous therapies are described in Data S1). Given the lack of alternative treatments, CXCR4-directed treatment was offered on a compassionate use basis (German Drug Act, §13 2b) as part of the conditioning regimen prior to SCT. The institutional ethics committee approved each individual treatment and all subjects gave written informed consent prior to therapy. Patient characteristics are depicted in Table SI.

Initial CXCR4-expression was confirmed in all patients using [^{68}Ga]Pentixafor PET/computed tomography (CT) (Lapa *et al*, 2017b) and immunohistochemical analysis (Data S1 and Figure S1). A pre-therapy dosimetry study was performed in all three patients after intravenous injection of approximately 200 MBq of [^{177}Lu]Pentixather, the therapeutic counterpart to Pentixafor, as recently described (Herrmann *et al*, 2016).

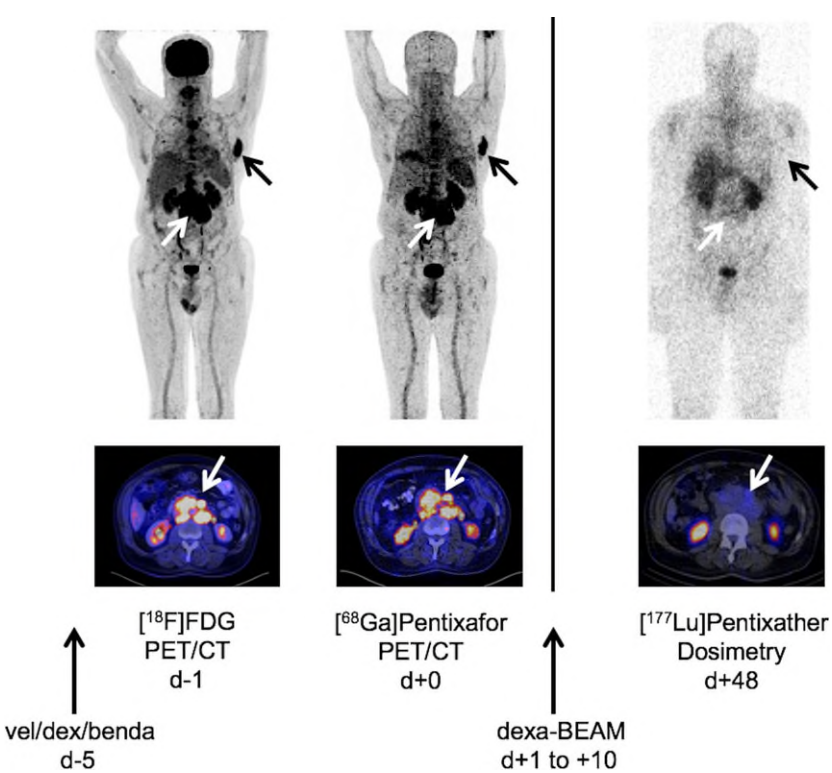

Fig 1. Example of therapy-induced CXCR4 downregulation in a patient with advanced multiple myeloma (MM) with extramedullary disease (patient 1). Display of CXCR4-expression as detected by [^{68}Ga]Pentixafor positron emission tomography/computed tomography (PET/CT) (maximum intensity projections), planar whole-body images ([^{177}Lu]Pentixather dosimetry) as well as representative transaxial fused PET/CT and single photon emission tomography (SPECT)/CT slices. The patient initially presented 5 days after one cycle of bortezomib/dexamethasone/bendamustine with highly CXCR4^{+} intra- (left scapula, black arrows) and extramedullary MM (retroperitoneal lymph nodes, white arrows), received one cycle of dexa-BEAM (total of 5 days) 1 day later and showed a pronounced receptor downregulation 7 weeks later.

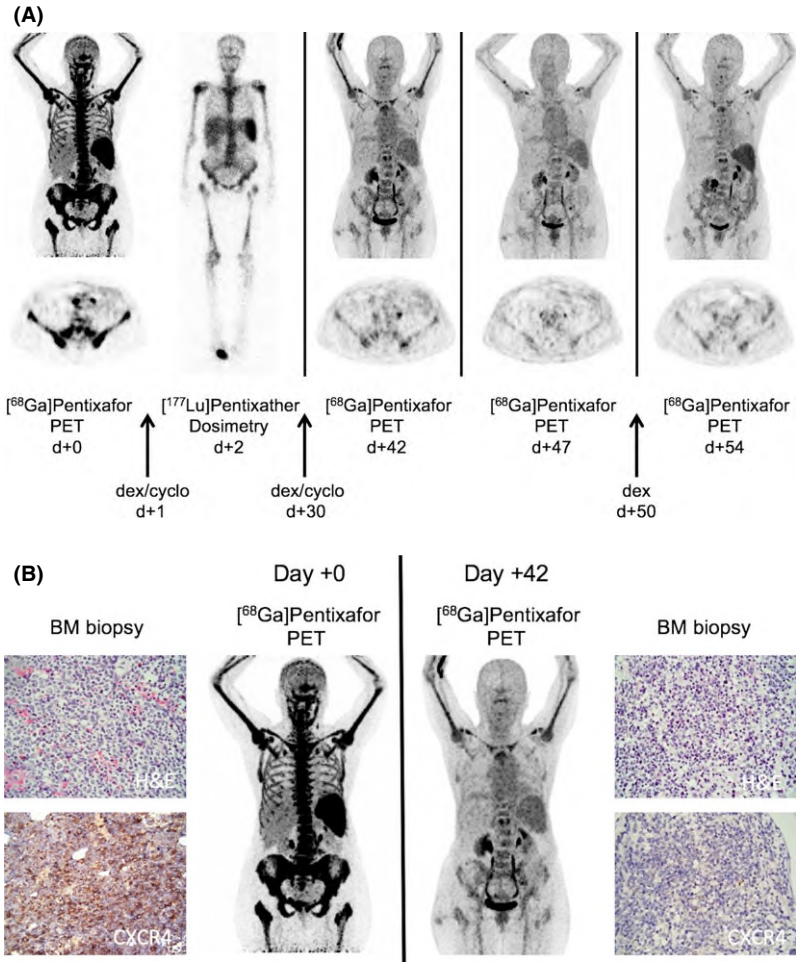

Fig 2. Example of therapy-induced CXCR4 downregulation in a patient with relapsed acute lymphoblastic leukaemia with diffuse bone marrow and splenic involvement (patient 3). (A) Display of CXCR4-expression as detected by [^{68}Ga]Pentixafor positron emission tomography/computed tomography (PET/CT) (maximum intensity projections), planar whole-body images ([^{177}Lu]Pentixather dosimetry) as well as representative transaxial PET slices. Due to difficulties in stem cell donor acquisition, intermittent low-dose dexamethasone/cyclophosphamide became mandatory for disease control. However, treatment resulted in pronounced downregulation of CXCR4 (both imaging- and histologically-proven) despite viable leukaemia within the bone marrow. Neither spontaneous (day +47) nor short-term high-dose dexamethasone-induced (day +54) CXCR4 re-upregulation could be observed at repeated PET imaging. (B) Correlation of [^{68}Ga]Pentixafor-PET/CT with bone marrow biopsy. Display of maximum intensity projections of [^{68}Ga]Pentixafor-PET/CT on day +0 and day +42 as well as the correlating haematoxyln-eosin staining and immunohistochemistry (IHC) for CXCR4 of respective bone marrow biopsy samples (magnification: 600×). Significant loss of [^{68}Ga]Pentixafor uptake from 80% to 0% positivity could be confirmed by IHC despite a still considerable amount of vital tumour cells (subtotal infiltration *versus* 70% viable leukaemia cells).

In Patient 1, disseminated intramedullary and extramedullary disease was detected by both [^{18}F]fluorodeoxyglucose and [^{68}Ga]Pentixafor-PET/CT (Fig 1). Pre-therapeutic dosimetry was postponed after initial strong receptor positivity due to logistic reasons. Instead, the patient received one cycle of dexa-BEAM (dexamethasone, carmustine, etoposide, cytarabine, melphalan), which resulted in a stable disease. At the time of presentation for dosimetry (day +48), serum myeloma levels were still at pre-treatment values. Surprisingly, no relevant CXCR4 expression could be demonstrated during dosimetry, thus precluding ERT (Fig 1).

In Patient 2, peri-diagnostic administration of concomitant chemotherapy (necessitated by the aggressiveness of disease) with one course of cyclophosphamide (200 mg/m^2, days −4 to −1) and cytarabine on days +4 and +5 (200 mg total) resulted in reduced receptor expression (30% of lymphoma cells weakly expressing CXCR4; Figure S2) which only partially recovered after a treatment-free period of 14 days. According to a second dosimetry (day +14), achievable tumour doses as low as 7·5 Gy were estimated, rendering CXCR4-directed treatment ineffective (Figure S3).

Patient 3 presented with intense [^{68}Ga]Pentixafor accumulation in the spleen, lymph node manifestations and throughout the skeleton (Fig 2A). Chemokine receptor expression was confirmed by bone marrow biopsy with 80% of leukaemic cells strongly expressing CXCR4. Due to favourable tracer kinetics, pre-therapeutic dosimetry returned achievable tumour doses of 140 Gy. Unfortunately, search for a suitable stem cell donor postponed ERT. Bridging chemotherapy with dexamethasone and cyclophosphamide (200 mg/m^2) resulted in pronounced downregulation of CXCR4 by both imaging- and histology, despite viable leukaemia in the bone marrow (Fig 2). In this patient, CXCR4-directed PET/CT was repeated to check for spontaneous (day +47) or short-term high-dose dexamethasone (day +54)-induced CXCR4 re-upregulation (dexamethasone had been shown to upregulate CXCR4 expression *in vitro* (Figure S4). However, no receptor recovery could be recorded. Consequently, ERT could not be performed (Fig 2B).

This is the first report on altered CXCR4 surface expression as a response to bridging therapy in patients with haematological malignancies. Although the treatment regimens did not result in significant tumour cell kill, a downregulating effect on CXCR4 cell surface expression was observed, preventing receptor-directed ERT. Given that all patients who are referred for ERT assessment suffer from advanced, refractory disease, CXCR4-directed therapy is an option to re-induce responses. ERT poses high logistic

challenges, so that bridging therapy is sometimes needed. In other cases, concomitant therapy to enhance tumour cell kill is offered. To our surprise, bridging chemotherapy eventually led to a markedly reduced cell surface CXCR4 expression. Of note, receptor downregulation could be observed at different intervals between chemotherapy administration and CXCR4 imaging, ranging from 4 days to 6 weeks. Interestingly, receptor expression seemed to recover in the DLBCL patient 2 weeks after therapy, whereas a more profound down-regulation was observed in Patient 3 (with ALL), thus highlighting heterogeneity in CXCR4 kinetics.

In general, CXCR4 expression is regulated on the transcriptional and translational level as well as by receptor cycling, i.e. dynamic change of CXCR4 localization on the cell surface versus intracellular compartments. Chemotherapy has been reported to alter CXCR4 expression *in vitro* (up- and down-regulation; Kim *et al*, 2009). The mechanisms involved seem to vary between cell lines and drugs tested, exhibit different kinetics and often include a combination of regulatory levels (e.g. transcriptional regulation and change in subcellular localization; Sison *et al*, 2013). We have noticed dynamic surface receptor expression in previous studies including small cell lung cancer patients (Lapa *et al*, 2016), however, these patients received combined radiochemotherapy and CXCR4 disappearance was interpreted as tumour response.

The highly dynamic regulation of this chemokine receptor is in contrast to the more stable expression of somatostatin receptor and other receptors addressed in nuclear medicine. Given the complexity of CXCR4 regulation, we can only speculate about the effects of bridging chemotherapy on CXCR4 levels in the presented cases. Future studies will need to further investigate therapy-induced down- and – preferably – up-regulation of CXCR4. Potentially, a sequential combination with chemotherapeutic agents might lead to improved efficacy of CXCR4-directed ERT.

In summary, CXCR4 expression on the tumour cell surface seems to be influenced by concomitant therapeutic interventions and might impact on receptor-directed endoradiotherapy.

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Authorship contributions

CL, KL, HH, AKB, HE, HJW, KMK and AS: conception and design. CL, AS, KH and SKi: development of methodology. CL, KL, SKi, GUG and KMK: acquisition of data. CL, KL, ASt, SK, AS, HH and KMK: analysis and interpretation of data. All authors: writing, review and/or revision of the manuscript. AKB, HE, KH, SK, AR, HJW and SKi: administrative, technical, or material support. HE; AKB, KMK, AR and AS: supervision.

Disclosure of conflicts of interest

HJW is a founder and shareholder of Scintomics; SK is CEO of Scintomics. All other 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:

Data S1. Supplementary material.

Table SI. Patient's characteristics.

Fig S1. Immunohistochemical controls for CXCR4.

Fig S2. Example of therapy-induced CXCR4 downregulation in a patient with advanced diffuse large B-cell lymphoma (patient #2).

Fig S3. Example of therapy-induced CXCR4 downregulation in a patient with advanced diffuse large B-cell lymphoma (patient #2).

Fig S4. Dexamethasone-induced up-regulation of CXCR4 in two myeloma cell lines.

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