^{68}Ga-labelled RGD PET/CT imaging of angiogenesis

From bench to bedside

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The development of therapies targeting specific molecular pathways is for both oncologic and non-oncologic diseases subject of extensive research. With an expanding number of these available therapies, molecular imaging may support in selecting patients that can benefit from these therapies. One potential molecular imaging target is $\alpha_v\beta_3$ integrin, expressed on activated endothelial cells required in the formation of new blood vessels. Integrin $\alpha_v\beta_3$ expression is a hallmark of angiogenesis and is induced in response to microenvironmental or genetic changes.
In this thesis, an overview of the latest developments in radionuclide labelled peptides targeting $\alpha_v\beta_3$ integrins was given, followed by a preclinical study comparing the four most promising multimeric ^{68}Ga-labelled RGD peptides. The second part of the thesis described the feasibility of [^{68}Ga]Ga-DOTA-E-[c(RGDfK)]2 (^{68}Ga-RGD$_2$) PET/CT in patients with oral squamous cell carcinomas (OSCC) and peripheral arteriovenous malformations (AVMs).

Comparing multimeric ^{68}Ga-labelled RGD peptides

A variety of radiolabelled RGD peptides for imaging $\alpha_v\beta_3$ integrins have been studied to improve tracer characteristics, such as binding affinity, biodistribution, and pharmacokinetics. Chemical modifications like pharmacokinetic modifiers and chelators have demonstrated to influence the *in vivo* behavior of the radiolabelled RGD-based tracers. It was furthermore shown that cyclisation of linear RGD peptides and multimerisation improved the binding affinity of RGD ligands for $\alpha_v\beta_3$ integrin. We compared the $\alpha_v\beta_3$ targeting characteristics of four multimeric ^{68}Ga-labelled RGD peptides in two different xenograft models under the same conditions using an identical experimental setup for each radiotracer. The four tracers all showed a fast blood clearance and excretion via the kidneys. Although tumour uptake was highest for the trimeric compounds, their enhanced uptake in the tumour as compared to the dimeric compound did not improve imaging quality when considering enhanced tumour–to–normal tissue ratios.

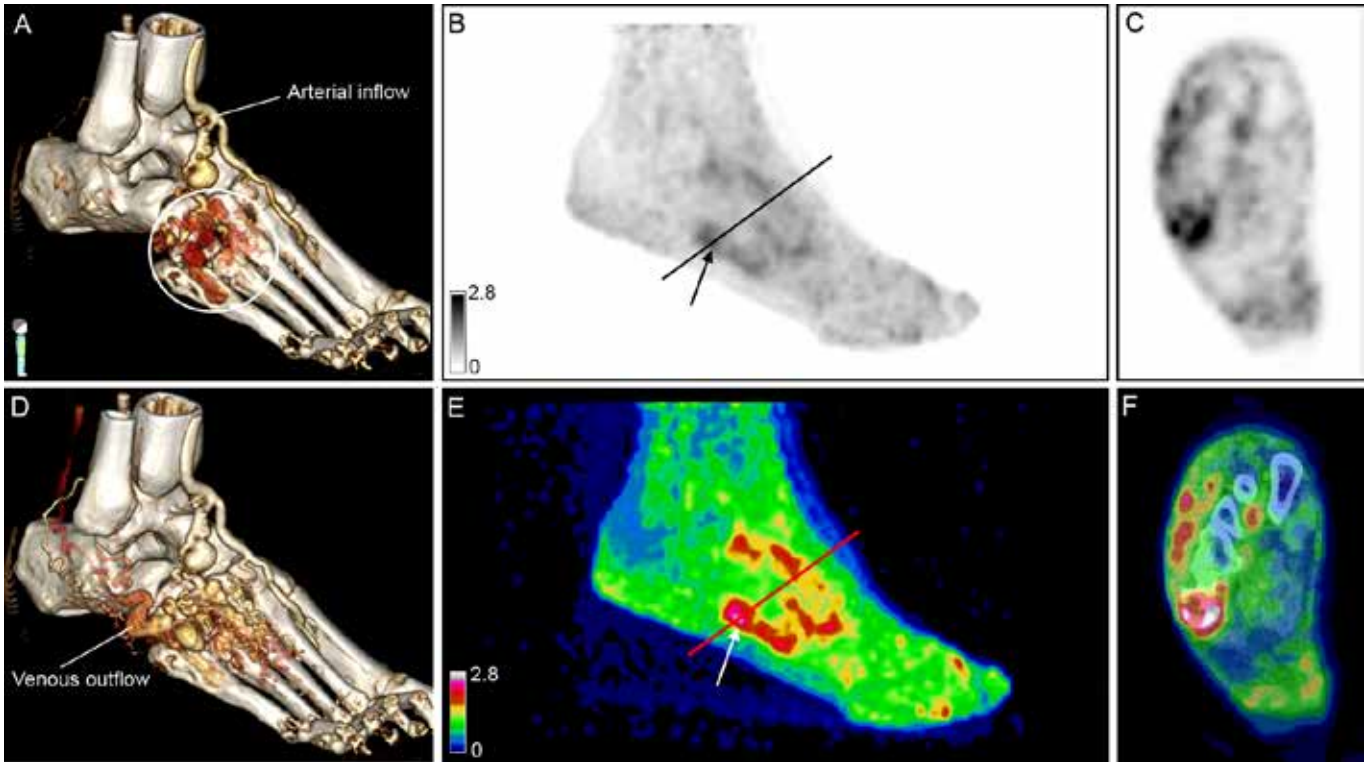
^{68}Ga-RGD$_2$ PET/CT in OSCC

The preclinical studies using ^{68}Ga-RGD$_2$ have shown promising results in terms of angiogenesis targeting specificity, efficacy, and tumour-to-background ratios xenografts. Furthermore, it was demonstrated that $\alpha_v\beta_3$ integrins are solely expressed on the neovasculature of head and neck squamous cell carcinoma (HNSCC) tumours and not on tumour cells themselves, suggesting that ^{68}Ga-RGD$_2$ PETCT is a suitable technique to image angiogenesis.
In the first clinical study, the biodistribution and kinetics of ^{68}Ga-RGD$_2$ was studied in ten patients with OSCC who were scheduled for resection of their primary tumour and possible metastases. A ^{68}Ga-RGD$_2$ PET/CT scan was performed shortly before surgery. All patients underwent dynamic or static PET/CT imaging. Heterogeneous radiotracer accumulation was observed in all tumours SUV$_{max}$ ranged between 4.0 and 12.7). While tracer accumulation in tumour tissue plateaued at ten minutes after injection, uptake in background tissue did not change over time, resulting in sufficient tumour-to-background tissue ratios.

The results of this study indicate that visualizing the $\alpha_v\beta_3$ integrin expression pattern in OSCC with ^{68}Ga-RGD$_2$ PET/CT imaging is feasible. Although not tested, it may also provide information on the level of new blood vessel formation in other subtypes of HNSCC, providing more insight in the tumour microenvironment of HNSCC tumours. This information may eventually be used to assess the response to (anti-angiogenic) therapies.

^{68}Ga-RGD$_2$ PET/CT in AVM

An abnormal structure and function of the microvasculature is seen not only in solid tumours like OSCC, but



Images of a 44-years-old man previously treated with multiple embolisations for a large symptomatic AVM of the foot. (A and D) 3D reconstructions of 4D-CTA demonstrate arterial input flow with nidus indicated by white circle (A) and venous outflow (D). (B-F) ^{68}Ga-RGD$_2$ PET shows tracer accumulation within lesion (arrows), but with minimal uptake in the treated part of the fore foot. A few persistent foci of enhanced activity are seen around toes (C/F). The arterial and venous flow in this location are not shown in A or D.

also in many other types of diseases, including cardiovascular diseases and (chronic) inflammatory conditions. Where these diseases are related to inflammatory-induced angiogenesis, an abnormal formation of blood vessels can also be caused by genetic mutations in the signalling pathways regulating angiogenesis, as observed in vascular malformations. In recent years, anti-angiogenic drugs have been proposed for the treatment of vascular malformations, after it had been revealed that angiogenesis was implicated in the pathogenesis of this type of disease. Molecular imaging techniques assessing the process of angiogenesis in vascular malformations could therefore contribute to an improved understanding of the pathophysiology and may also contribute to new advances in the treatment strategy of this disease.

First, the $\alpha_v\beta_3$ integrin expression patterns in peripheral arteriovenous malformations and other malformations were investigated. It was shown that $\alpha_v\beta_3$ integrin is predominantly expressed in endothelial cells of the blood vessels present in the nidus of vascular malformations. This immunohistological study was followed by a clinical feasibility study in which ten patients with peripheral AVMs confined to the subcutaneous and/or muscle tissue, and scheduled for a percutaneous or transarterial embolisation treatment with ethanol, underwent an ^{68}Ga-RGD$_2$ PET/CT scan before, in between, or at the end of embolisation treatment. All nidus could be clearly visualised on ^{68}Ga-RGD$_2$ PET/CT, due to low uptake pattern in background tissue. Although the number of patients included was relatively small, we made some interesting observations as potential future applications of the use of ^{68}Ga-RGD$_2$ PET/CT in peripheral AVMs. ^{68}Ga-RGD$_2$ PET/CT identified additional foci of integrin $\alpha_v\beta_3$ expression as compared to conventional angiographic imaging and revealed a clear nidus in a complex AVM that was not revealed on conventional imaging. These ^{68}Ga-RGD$_2$ PET/CT findings may possibly have implications for treatment planning in those patients without a clear nidus visible on angiography, or in patients with a complex and/or large size AVM. In addition, ^{68}Ga-RGD$_2$ PET/CT imaging may also guide the

application of novel anti-angiogenic drugs in vascular malformations on which research currently is focusing, but this needs further investigation.

Conclusions

Both the preclinical and clinical observations indicate that ^{68}Ga-RGD$_2$ is a potential imaging biomarker for specific diagnostic application of imaging $\alpha_v\beta_3$ integrin expression using PET/CT. However, for successful clinical implementation it should be investigated whether radiotracer uptake in the target correlates with clinical outcome, and validate whether ^{68}Ga-RGD$_2$ accumulation truly reflects $\alpha_v\beta_3$ integrin expression on activated endothelial cells during angiogenesis. The encouraging results from the clinical studies that have been described in this thesis have stimulated further research in both fields. Ongoing and future clinical trials will show how ^{68}Ga-RGD$_2$ PET/CT can contribute to treatment personalisation.

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