Journal of Nuclear Medicine, published on December 7, 2017 as doi:10.2967/jnumed.117.200527

Invited Perspective

ImmunoPET in pontine glioma : more than meets the eye ?

Wim J.G. Oyen, MD, PhD[1]

Chris Jones, PhD[2,3]

The Institute of Cancer Research

Divisions of Radiotherapy and Imaging[1], Molecular Pathology[2] and Cancer Therapeutics[3]

London, U.K.

For correspondence:

Prof. Wim J.G. Oyen, MD, PhD

The Institute of Cancer Research

Division of Radiotherapy and Imaging

15 Cotswold Road

Sutton, SM2 5 NG

U.K.

e-mail : wim.oyen@icr.ac.uk

Overexpression of a membrane-associated target on tumor or immune cells or of a factor in the tumor microenvironment is a major determinant if a patient can be treated with a monoclonal antibody against such target. Target expression is often judged on small tissue biopsies, which does not take into account that there may be significant intra- and intertumoral heterogeneity of expression. This is particularly true of diffuse intrinsic pontine glioma (DIPG), a paediatric glial tumour of the brainstem for which surgical resection is not possible. Significant heterogeneity in genetic mutations has been shown in studies studying the diffuse spread of these tumours across whole brain autopsy specimens [1;2]. Besides gene mutations affecting target expression, tumor targeting of monoclonal antibodies is a driven by a complex set of factors, resulting in heterogeneity of tumoral uptake of monoclonal antibodies. The affinity of the antibody for its target is relevant, but also target accessibility which is associated e.g. with tumor perfusion and intratumoral pressure are of pivotal importance for an antibody to even reach its target. Earlier work on tumor targeting of the anti-Carbonic Anhydrase IX monoclonal antibody girentuximab has shown large variability of targeting of the radiolabeled antibody to the tumor antigen within resected primary renal cell cancer specimens, using immunoSPECT [3]. More recently, ImmunoPET studies in breast cancer patients by Gebhart et al. have shown, that large differences in tumor targeting of trastuzumab can be observed between patients, but also between metastases in an individual patient [4]. An additional strength of immunoPET is the assessment of the accessibility of a target beyond expression in multidrug treatment regimens. Desar et al. showed that treatment of patients with metastatic renal cell cancer with sorafenib resulted in a significant decrease of radiolabeled bevacizumab uptake in the tumor. Although ex-vivo assessment showed that expression of VEGF-A, the target for bevacuzimab, remained intact, the destruction of the tumor neovasculature prevented bevacuzimab to bind to its target [5].

In the report by Veldhuijzen van Zanten et al. in this issue of The Journal of Nuclear Medicine [6], direct histologic validation of imaging results in a child with DIPG became possible, because the patient participated in a protocol with Zr-89-bevacizumab immunoPET only days prior to her untimely death and because the parents subsequently consented to an autopsy. Despite being a case report of a single patient, the availability of a recent MRI scan, a Zr-89-

bevacizumab PET/CT, and post-mortem extensive immunohistochemistry in combination with Zr-89 tissue counting within a very short period of time resulted in a unique opportunity to assess the in-vivo imaging results without significant bias of due to treatment effects or tumor progression.

The 70% lower in-vivo uptake of Zr-bevacizumab in small tumors is an important observation. Thus, the well-known caveats of partial volume effects in small lesions (resulting in underestimation of tumoral Zr-89 bevacizumab uptake) as well the persisting blood pool activity (resulting in overestimation of tumoral Zr-89 bevacizumab uptake) do result in a net underestimation of tumor uptake, which needs to factored in when decisions on drug doses are derived from imaging results. Obviously when using small, fast clearing radiolabeled molecules the impact of underestimated tumor targeting due to partial volume effects will be even higher.

It must be appreciated that bevacizumab binds to VEGF, which is a soluble target with a relatively short half life. Thus, underestimation of VEGF content in tissue samples may have occurred due to wash-out, resulting in low, nonspecific findings. Using In-111-bevacuzimab immunoSPECT in patients with colorectal liver metastases, also a lack of correlation was reported between the level of anti-VEGF antibody accumulation and the level of VEGF-A expression in the tissue as determined by in situ hybridization and ELISA [7]. It was concluded that that this may be due to the inability to visualize the soluble $VEGF_{121}$ isoform. It was also postulated that enhanced vascular permeability in tumors may play a role in nonspecific bevacuzimab targeting . Nevertheless, Veldhuijzen van Zanten et al. did observe higher Zr-89-bevacizumab targeting to areas of microvascular proliferation. Although this may indicate an additional target of bevacizumab, it may very well be that both the Zr-89-bevacizumab targeting and the microvascular proliferation are the result of high local VEGF levels in-vivo. It is notable however that this observation did not explain all areas of heterogeneous uptake across the specimen, and therefore further exploration of the microenvironment in which DIPG cells growth and infiltrate are warranted. Recent elegant studies have shown a remarkable co-option of normal neuronal signalling by DIPG cells [8,9], highlighting the complexity of cell-cell interactions during tumour progression. This particular case was also unusual given it's apparent lack of the highly prevalent histone H3 K27M mutation. What these H3 wild-type

tumours represent within the pathogenesis of DIPG is not clear, and extension of the present insightful studies across further tumours within the newly recognised diagnostic entity of 'diffuse midline glioma with H3K27 mutation' will be extremely valuable.

In conclusion, extensive ex-vivo verification of non-invasive imaging-based assessment of target expression reveals important additional information, underpinning the strengths and challenges of molecular imaging as a tool for "in-vivo immunohistochemistry". This especially holds true for conditions where obtaining tissue is not straightfoward, such as brain tumours. Veldhuijzen van Zanten et al. elegantly demonstrated that the untimely death of a very young patient with pontine glioma did result in unique and important scientific information.

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