The road to clinical PET tracers must be paved with good intentions

SRSTT Radiopharmaconnect[1]

[1]Radiopharmaconnect

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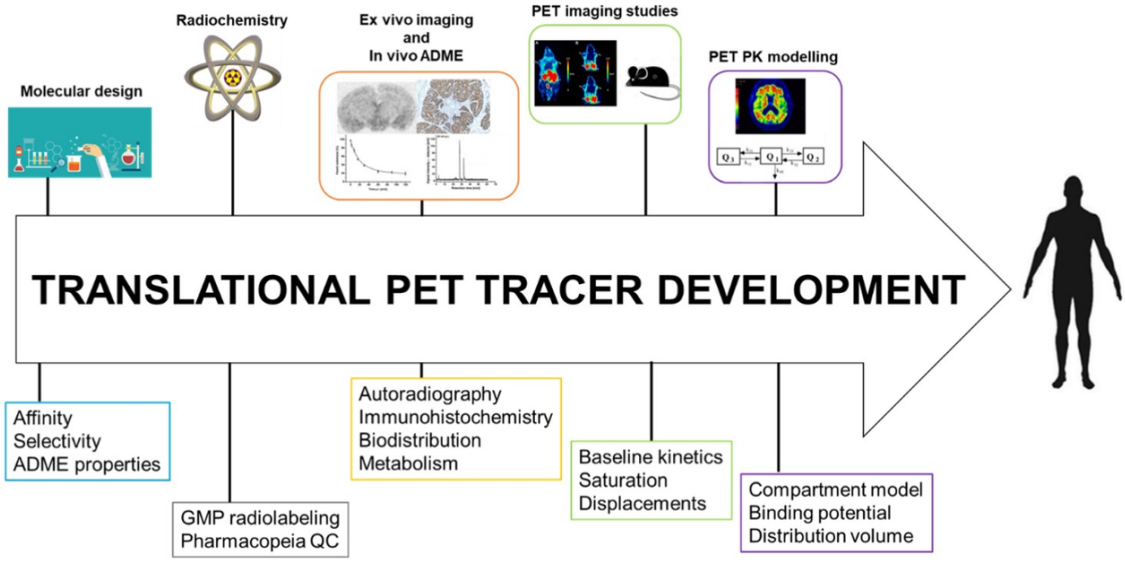
Translational development of PET tracers from bench to clinical applications involves different expertise and requires the determination of numerous parameters to minimize pitfalls

Herein we present which are, to our minds, the important steps to follow when developing a PET tracer aiming at clinical applications. A quick overview of pharmacokinetic modelling using PET imaging to quantify radiotracer binding to its target will also be discussed.



1. Designing the optimal molecule: structure-activity relationships[1]

To image a relevant biological target (receptors, transporters, enzymes, etc.) with PET, the first goal is to design the optimal molecule. Upon the preparation of libraries of molecules (from small organic molecules to biologics like proteins or antibodies), *in vitro* assays are conducted to assess key properties and select the best lead. When possible, a computational modelling approach can be realized to refine the appropriate molecular scaffolds targeted.

The **affinity** of the molecule for its target, usually in the nanomolar range for a promising PET tracer, depending on the abundance of the biological target *in vivo*, is one of the most important parameters.

The **selectivity** over other structurally close biological entities is also relevant to overcome off-target issues. It is calculated as a ratio of affinities.

Absorption Distribution Metabolism Excretion (ADME) profiles include several parameters that can give insights to the *in vivo* pharmacokinetic of the molecule. The **lipophilicity** (logP or logD), the **permeability to membrane** or the **affinity and selectivity regarding transporters of xenobiotics** such as the P-glycoprotein (P-gp, ABCB1) are relevant examples for intracellular or interface-protected targets.

The quantity of tracer reaching its target is also determinant to optimize the target quantification. **Binding to plasma proteins, stability in plasma** and **metabolic stability** are therefore key parameters to assess.

2. Radiolabelling: the manufacturing perspective

Prior to any of the above, when designing a molecule for PET, one must anticipate the radiolabelling feasibility to incorporate a positron-emitting atom (typically carbon, fluorine, gallium or zirconium) into a suitable precursor. Carbon-11 is usually introduced by methylation on a heteroatom whereas fluorine-18 is often grafted on scaffolds by aliphatic or aromatic nucleophilic substitution, preferentially on electron-poor cores. Chelates like NOTA or DFO are used for gallium or zirconium complexation respectively. In a clinical application prospective, the radiotracer production must be automated, reproducible and **comply with the Good Manufacturing Procedures (GMP)**.[2]

The quality control of the radiotracer including measurements of the chemical identity, the chemical and radiochemical and radionuclide purities, calculation of the molar activity, control of the pH and the visual, the determination of residual solvents, sterility and endotoxins tests must be **compliant with the Pharmacopeia guidelines** of the country.[3]

3. *In vitro / Ex vivo* imaging: a glance at the radiotracer potential[4]

The first evaluation of a radiotracer can be performed on cells or tissues expressing the biological target. **Autoradiography**, ideally on human tissues, is a powerful tool to observe the binding of the tracer in the biological environment. **Immunohistochemistry** (IHC) can confirm the presence of the target using a specific antibody. Overlapping autoradiography and IHC provides a map for the specificity of the tracer.

Autoradiography can be semi-quantitative when using a region of reference (where the tracer does not bind) and used to compare tracers. Non-specific and off-target specific bindings can be determined by **saturation**

experiments where pre-incubation with the non-radioactive tracer itself or non-radioactive references for the biological target respectively is applied.

4. *In vivo* ADME: pre-pharmacokinetic studies

I*n vivo* biodistribution, usually in rodents, is realized by quantitative whole body PET imaging for dynamic assessment of the radiotracer repartition over time. Biodistribution can also be performed by withdrawing and counting organs after i.v. injection. Typically, organs of elimination (liver, kidney, intestines, bladder) are investigated together with the heart, lungs and the brain, blood, muscles and bones. Organs containing the biological target are of course included.

In vivo metabolism is a key factor for the interpretation of further PET imaging studies. Metabolism leads to the formation of non-radioactive and radioactive metabolites. The latter, called radiometabolites, potentially induce a background signal complicating the interpretation and quantification of PET images. Determining the **radiochemical purity of the PET signal,** *i.e* the proportion of parent compound in the organ of interest, is highly relevant. For that matter, the extracted organ is grinded for radio-HPLC analysis.

The determination of the **radiotracer parent fraction** in the blood is probably the keystone of PET imaging, at least for translational development. After *i.v.* injection of the radiotracer, extracted blood and plasma samples (preferentially at the arterial level) are gamma-counted and the percentage of the radioactivity attributed to the radiotracer is assessed by analytical methods (radio-HPLC, solid phase extraction, thin layer chromatography). This information gives access to the metabolite-corrected arterial input function of the radiotracer that represents the plasma kinetics of the radiotracer over time.[5]

5. PET imaging studies: Where do we start?

First, the **baseline kinetics** of the radiotracer within the organs of interest is realized to estimate the distribution and retention of the tracer. Then, it is recommended to determine the *in vivo* specific binding of the tracer through **pre-saturation** and **displacement** experiments.

Pre-saturation experiments consist in injecting a high dose (typically around 10^3 times the quantity of tracer depending on the target concentration and the toxicity of the compound) of the unlabelled tracer before the radiotracer (from minutes to hours, depending on the compound kinetics). The cold will saturate the specific binding of the radiotracer leading to the visualization of the "free radiotracer" (unbound fraction) and its remaining binding: the non-specific binding.

Displacement studies consist in injecting a high dose of an unlabelled compound highly specific for the target after the radiotracer injection, preferably at equilibrium. The compound will displace the radiotracer from the target, leaving only the non-specific binding, and potential off-target(s) (other specific binding). These experiments can be multiplied with as many specific compound as necessary if several off targets bindings are suspected.

After these preliminary experiments, it is time to perform the tissue pharmacokinetic modelling of the radiotracer to determine a wide range of key parameters describing the **influx**, the **efflux**, the **binding affinity** and **target density** of the radiotracer within the organ of interest.

Dynamic PET imaging gives access to the **time-activity curves (TACs)** for each investigated regions, namely **volumes of interest (VOI)**, that correspond to the evolution of the radioactive concentration over

time within these VOI. The TACs can be expressed as raw data (kBq/cc) or normalized by:

- the injected dose (ID): TACs expressed as the **percentage of injected dose (%ID/cc)**

- the ID and the patient weight: TACs expressed in **standardized uptake values (SUV, dimensionless ratio)**

6. PET PK modelling[6]

PK modelling is performed through the analysis of the radiotracer TACs, for each VOI, regarding the plasma kinetics. Modelling allows for the estimation of parameters such as the **binding potential (BP)** (ratio between the **density of targets (B_{max})** and the **affinity** of the radiotracer) or the **distribution volume ratio (DVR)** (ratio of the radiotracer concentration between the tissue and the plasma at the equilibrium of distribution). Modelling can be compartmental or non-compartmental depending on the radiotracer properties and the pathophysiological context.

6.1. Compartmental models

Compartmental modelling consists in studying of the kinetics of a tracer by dividing the organism into compartments and by estimating the exchanges between them. The choice of compartments is not necessarily motivated by anatomical criteria. A compartment represents an organ or a tissue that behaves the same way towards the radiotracer. Thus, a compartment must be seen as a step of binding of the tracer to a target (compartment with free tracer in a tissue, compartment with tracer non-specifically bound to plasma membranes, tracer specifically bound to the target ...). The kinetics of the tracer are described by differential equations of the first order, with constants that reflect the exchange rates between the compartments. Usually one or two tissue compartments are used. Compartmental modelling critically requires the metabolite-corrected arterial input function.

6.2. Non-compartmental models

Compartmental models may be difficult to apply in practice due to the needed blood sampling. Some alternatives, less informative, are nevertheless possible under certain conditions. Among these possibilities are the methods with **reference regions**, to overcome the measurement of the arterial input function, and the **graphical methods**, to overcome any compartmental configuration.

Reference Region Methods

A method with a reference region uses a region of the same nature than the one of interest, but devoid of the target. For instance, the *pons,* which does not express GABA receptors, is usable as a reference region for the study of brain GABA receptors.

The radiotracer kinetic within the reference region is used as an input to estimate the **BP**. These methods are extremely relevant in clinics due to their ease of implementation.

Graphical methods

A graphical analysis, such as the Logan plot, is a method based on linear regression to estimate the PK of radiotracer that binds to the target. It is a simplified method to estimate the **equilibrium distribution volume (V_T)** of the radiotracer in various tissue regions, by integrating the radioactive concentrations in the tissues and in the plasma. The V_T has the same biologic meaning as the **DVR**, and serve has a comparison parameter to determine the distribution profile of the radiotracer. These methods still require the arterial input function.

7. Conclusion

Translational development of a PET radiotracer is a long road from designing the molecule to evaluating the radiotracer *ex vivo* and *in vivo*. Most of these parameters mentioned above are of crucial importance to minimize pitfalls when translating to humans.

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(6) Bentourkia, M. 'hamed; Zaidi, H. Tracer Kinetic Modeling in PET. *PET Clin.* **2 (2)**, 267–277 (2007).

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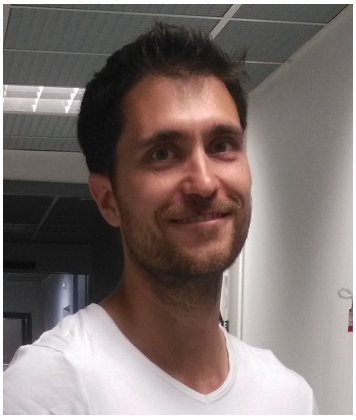

Fabien received his Ph.D. in chemistry from the University of Orléans in 2011 for his work on original contrast agents for MRI and optical imaging. He then completed two post-doctoral fellowships at Molecular Neuroimaging LLC in New Haven CT and at the University of Rouen working on original molecules for PET and MRI respectively. Fabien has been working at the Service Hospitalier Frédéric Joliot (SHFJ) of the CEA in Orsay since 2013 under the supervision of Dr. Bertrand Kuhnast. His main research activities focus on the translational development of PET radiotracers and the discovery of new carbon-11 radiolabelling methodologies.

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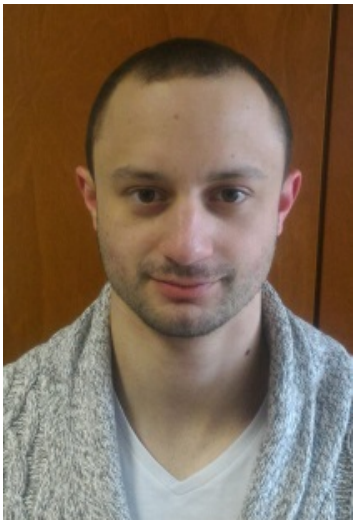

Sylvain received his Ph.D. in molecular imaging from the University of Paris in 2017 working on the variability of response to opioids using PET imaging, with a focus on neuroinflammation. He also completed in 2017 his Pharm.D. in Radiopharmacy at the University of Paris and has collaborated with the Service Hospitalier Frédéric Joliot (SHFJ) of the CEA since 2013 under the supervision of Dr. Nicolas Tournier. He is currently assistant professor in Pharmacokinetics/Radiopharmacy at the University of Paris and pharmacist at Necker Hospital. His research activities are focused on the understanding of the drugs tissue PK/PD relationship with PET.