European Journal of Cancer (2012) 48, 432–440

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Imaging apoptosis with positron emission tomography: 'Bench to bedside' development of the caspase-3/7 specific radiotracer [^{18}F]ICMT-11

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Received 17 November 2011; accepted 21 November 2011
Available online 5 January 2012

KEYWORDS
Positron emission tomography
[^{18}F]ICMT-11
Caspase-3/7
Apoptosis
Annexin V
Cancer

Abstract The capacity to evade apoptosis has been defined as one of the hallmarks of cancer and, thus, effective anti-cancer therapy often induces apoptosis. A biomarker for imaging apoptosis could assist in monitoring the efficacy of a wide range of current and future therapeutics. Despite the potential, there are limited clinical examples of the use of positron emission tomography for imaging of apoptosis. [^{18}F]ICMT-11 is a novel reagent designed to non-invasively image caspase-3 activation and, hence, drug-induced apoptosis. Radiochemistry development of [^{18}F]ICMT-11 has been undertaken to improve specific radioactivity, reduce content of stable impurities, reduce synthesis time and enable automation for manufacture of multi-patient dose. Due to the promising mechanistic and safety profile of [^{18}F]ICMT-11, the radiotracer is transitioning to clinical development and has been selected as a candidate radiotracer by the QuIC-ConCePT consortium for further evaluation in pre-clinical models and humans. A successful outcome will allow use of the radiotracer as qualified method for evaluating the pharmaceutical industry's next generation therapeutics.

1. Introduction

Positron Emission Tomography (PET) is a sensitive functional imaging modality that provides dynamic and quantitative measurement of the physiological characteristics of tumours occurring at the molecular and cellular levels. The major current use of PET in the clinical management of patients is the staging of cancer. The

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[c] On behalf of the QuIC-ConCePT consortium. See Appendix A for consortium participants.

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doi:10.1016/j.ejca.2011.11.033

information is predominantly of value in assigning appropriate treatment and sparing patients from radical interventions such as surgery. PET can also be used to monitor the response of tumours to treatment with chemotherapy or radiotherapy. Information from scans performed during treatment could be used to change and adapt treatment regimens.[1] In addition to its routine clinical use, PET has particular strengths when used as a tool to aid the development of new drugs. Clinical trials of anticancer drugs currently follow a well-established route including initial evaluation in late stage cancer patients of safety, tolerability and pharmacokinetic/pharmacodynamic (phase I), followed by single-arm or randomised phase II trials in selected tumour types for the evaluation of antitumour activity of compounds as single agent or in combination, and finally phase III trials for regulatory approval.[2] This process, however, remains inefficient, slow and costly, in part because of the low success rate of the compounds being evaluated in the clinic.[3] In this context, PET has the potential to be used to study the pharmacokinetics of drugs or as an imaging biomarker – pre-treatment biomarker to aid in selection of study participants or post-treatment pharmacodynamic and early surrogate end-point.[4–10]

PET is a non-invasive imaging modality that measures biological processes including, but not limited to, blood flow, metabolism, cell surface receptor expression, angiogenesis, proliferation and apoptosis. It involves the administration of compounds of biological interest (including metabolic enzyme substrates, receptor ligands and small molecules) labelled with positron-emitting radioisotopes (carbon-11 or fluorine-18) that are formulated for intravenous injection. The flexibility of labelling virtually any molecule that targets a specific biological process, and the ability to image the spatial distribution of the labelled molecule over time, open up many potential uses for PET in cancer. In this context, signalling proteins and pathways that are associated with the hallmarks of cancer[11,12] (essential alterations that collectively define malignant growth) are particularly attractive for the development of PET radiotracers in oncology; many of the hallmarks are based on the accelerated intermediary metabolism (such as energy production, protein synthesis, and phospholipids synthesis) needed to support tumour growth. The gold standard PET radiotracer in oncology is the clinically approved glucose analogue [^{18}F]FDG ([^{18}F]-labelled 2-deoxy-glucose), the tissue uptake of which is generally considered to be indicative of viable cell density. The role of [^{18}F]FDG PET in monitoring and predicting chemotherapy efficacy has been widely reported over the last few years.[13,14] It is clear that, although [^{18}F]FDG is the mainstay PET radiotracer, it is not suited for all applications, and currently [^{18}F]FDG PET is not as commonly used for response evaluation as it is for detection and staging.[15] [^{18}F]FDG has important limitations, including the inability to detect small volume disease and less aggressive or less glycolytic tumours. Energy metabolism is associated with tumour growth, but also with a variety of other biological processes, including inflammation and tissue repair in response to damage. As cancer treatment becomes more targeted and individualised to patients and tumour characteristics, more specific PET radiopharmaceuticals will help guide treatment selection by quantifying the therapeutic target, identifying resistance factors and measuring early response to therapy.[15]

The development of novel PET radiotracers is, by extension, analogous to the drug development process and therefore requires a similar amount of input in resources, validation and final regulatory approval. It encompasses various critical stages including chemistry/radiochemistry development, *in vitro* and *in vivo* biological validation, preclinical assessment of biodistribution, metabolism and specific tumour retention, toxicology and dosimetry measurements, and application to regulatory bodies prior to clinical assessment and validation.[16,17] The present review article describes the development of the caspase-3/7-specific PET radiotracer [^{18}F]ICMT-11, selected by the QuIC-ConCePT consortium for evaluation as a non-invasive biomarker of tumour apoptosis.

2. Imaging apoptosis with PET

Apoptosis is an essential process for eliminating unwanted cells during embryonic development, growth, differentiation and maintenance of tissue homeostasis. Deregulation of apoptosis signalling pathways is, therefore, associated with various pathologies including autoimmunity, neurodegeneration, cardiac ischaemia and transplant rejection,[18] and the capacity to evade apoptosis has been defined as one of the hallmarks of cancer.[11,12] Moreover, because effective anti-cancer therapy, involving cytotoxic drugs, molecular targeted drug and specific apoptosis-targeting drugs,[19] often requires induction of tumour cell death through apoptosis, monitoring of this process could provide important predictive outcome information in the context of routine patient management and early clinical trials.[20,21] We envisage that a biomarker for apoptosis will assist in monitoring the efficacy of a wide range of current and future therapeutics.

Although defined for the first time on the basis of morphological changes (plasma membrane blebbing, cell shrinkage, chromatin condensation and formation of apoptotic bodies), the apoptotic process is also characterised by biochemical changes. Molecularly, apoptosis is activated *via* either the death receptors extrinsic pathway or the mitochondria-directed intrinsic pathway (induction phase), both of them leading to the execution phase involving the activation of effector caspases which

Table 1
Overview of PET radiotracers for apoptosis imaging.

Apoptotic biochemical features detected	Radiotracers	Characteristic and development stage	Limitations
Phosphatidylserine translocation on the extracellular surface of the plasma membrane	[^{18}F]-annexin V[24–26]	Annexin V is a 36 kDa protein that binds with high affinity to phosphatidylserine head groups. In preclinical development (the SPECT counterpart, [^{99}Tc]-annexin-V was evaluated in the clinical settings)	Slow delivery to the site of interest, slow clearance from tissues leading to high effective doses, lack of specificity (can detect necrosis, inflammation and platelet activation)
	[^{18}F]-C2A[27]	The C2A domain of synaptotagmin I can target apoptotic cells by binding to exposed anionic phospholipids. In preclinical development	Lack of specificity (can detect necrosis, inflammation and platelet activation)
Caspase-3 activation	[^{18}F]-WC-II-89 [^{18}F]-WC-IV-3[28–31] [^{18}F]ICMT-11[32,33]	Small molecules based on isatin sulfonamide moiety that bind with a high affinity to activated caspase-3. In preclinical development	Further specificity evaluations to be made
Collapse of the mitochondrial membrane potential	[^{18}F]-BnTP[34,35]	Voltage sensitive organic cations that accumulate in the mitochondrial matrix. In preclinical development	Potential efflux from the cells by multi-drug resistance proteins
Membrane imprint (loss of plasma membrane potential, acidification of the external plasma membrane leaflet and cytosol, activation of the membrane phospholipids scramblase system)	[^{18}F]-ML-10[36–38]	Small molecule (Aposense family) that detect apoptosis-related complex of cellular alterations, with consequent accumulation in cells. In phase I/II clinical development	Undefined target prompt to further specificity evaluations

cleave recognised substrates (e.g. DNA fragmentation and key structural proteins) to break-up cells programmed to die *via* this mechanism.[22] Another major biochemical feature of apoptosis involves the exposure of phosphatidylserine on the external surface of the plasma membrane, which allows phagocyte recognition of dying cells. Based on various biochemical events that characterise apoptosis, a number of positron emitting radiotracers have been developed to non-invasively detect this process, both in preclinical studies and in humans (Table 1 and Fig. 1). The expected requirements of a PET radiotracer for imaging apoptosis include high selectivity and specificity for apoptotic cells, high *in vivo* stability with rapid distribution and clearance from non-target tissues, adequate dosimetry and safety, as well as a rapid, robust and reproducible radiolabelling procedure.[23]

3. Chemical development and radiolabelling of [^{18}F]ICMT-11

3.1. Rationale of caspase-3 as an imaging target of apoptosis

In cancer, apoptosis is induced by a large variety of stimuli including cytotoxics, mechanism-based therapeutics and radiotherapy. Although those stimuli trigger different apoptotic signalling pathways, the molecular events in the execution phase of apoptosis are largely shared and involve the caspases, which constitute a family of cysteine proteases that cleave their substrate after specific tetrapeptide motifs. Within the caspase family, the effector caspases (caspases-3, -6 and -7) orchestrate the demolition phase of apoptosis that results in the controlled dismantling of a range of key structures within the cell and its subsequent disposal.[22] Moreover, one of the most noticeable and specific features of apoptosis is the degradation of the DNA into numerous fragments, often down to multiples of 200 base pairs, driven by the activation of caspase-3,[39] the central effector caspase, which makes it an attractive biomarker of apoptosis.

3.2. Chemical design and development of ICMT-11

[^{18}F]ICMT-11 was designed as a small molecule radiotracer with potential advantages including flexible structural modifications, facile radiolabelling and improved biodistribution and clearance profiles. The [^{18}F]ICMT-11 structural core is based on isatin sulfonamide, a chemical class known to have caspase inhibitory activity. Lee and co-workers identified a number of highly potent isatin sulfonamides as inhibitors of caspase-3/7.[40,41] The mechanism of action of this class of compounds is believed to involve the formation of an intracellular enzyme-inhibitor complex with caspase-3

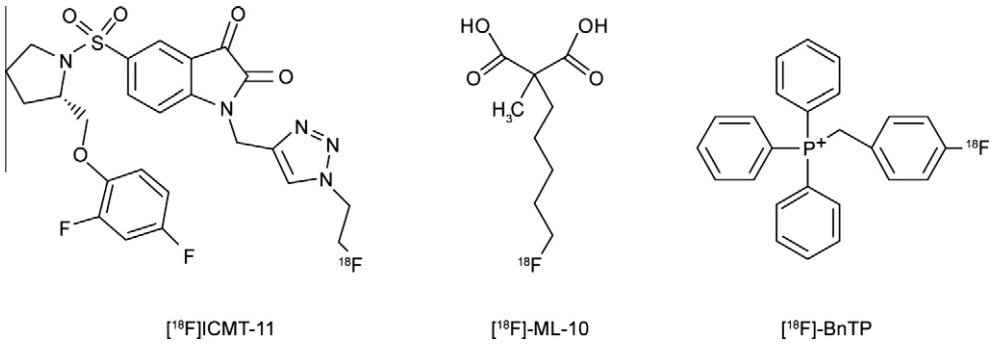

Fig. 1. Chemical structures of selected lead imaging agents being developed for PET assessment of apoptosis.

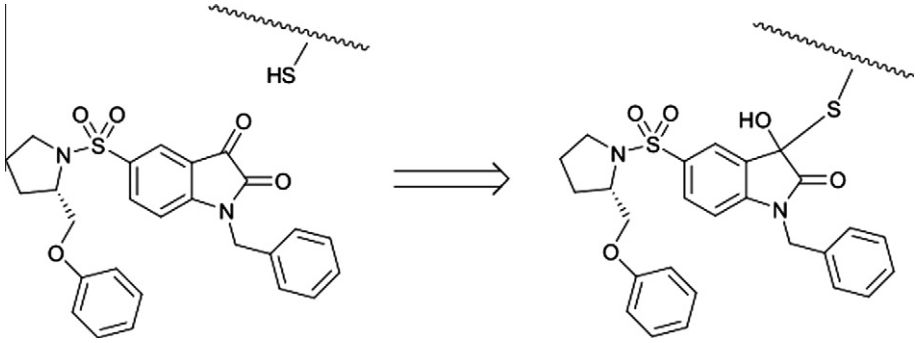

Fig. 2. Schematic indicating the mechanism of action of isatin sulfonamides. Activated caspases contain a catalytic site comprised of a nucleophilic cysteine and a histidine. Binding of the dicarbonyl functionality in the isatin sulphonamide to activated caspase-3 to form a thiohemiketal at C3 with the active cysteine constitutes the basis of its use in medical imaging.

through covalent binding to the enzyme active site. The dicarbonyl functionality of isatins is essential to its mechanism of action (Fig. 2). It binds to the cysteine residue of the active site forming a thiohemiketal *via* the electrophilic C-3 carbonyl of the isatin sulfonamide and the nucleophilic cysteine thiol functionality.[40,42] From the isatin 5-sulfonamide scaffold, a focused library of compounds was screened for activated caspase-3 inhibitory affinity and ICMT-11 emerged as the lead compound with subnanomolar affinity for activated caspase-3 (0.5 nM) and reduced lipophilicity.[33,43]

3.3. Radiochemistry development of [^{18}F]ICMT-11

Originally the radiolabelling of [^{18}F]ICMT-11 was achieved by a facile 'click chemistry' approach using an isatin sulphonamide alkyne precursor and 2-[^{18}F]fluoroethylazide resulting in modest specific activity.[33] Further validation and clinical translation of [^{18}F]ICMT-11 required a robust and reliable automated radiosynthesis procedure. To this aim, an initial attempt was made to improve the specific radioactivity using copper(I) stabilising bathophenanthroline disulphonate (BPDS) as catalyst together with acetal protection of the reactive isatin C-3 carbonyl; this led to significant reduction in the amount of alkyne precursor required (1/3 of original protocol), shortened the reaction time (1/2 of original protocol) and suppressed formation of stable by-product.[44] Finally an automated radiosynthesis of [^{18}F]ICMT-11 was developed using a protected tosylate precursor and displays high EOS yield in a shorter time with very high specific activity (Fig. 3). The automated procedure was developed and validated to clinically acceptable standards.

4. Biological characterisation of [^{18}F]ICMT-11 as a caspase-3-specific radiotracer for the detection of apoptosis

ICMT-11 has been shown to retain casapase-3 inhibitory activity and further experiments using cellular models of anticancer drug induced-apoptosis have highlighted the binding of [^{18}F]ICMT-11 in caspase-3 activated cells undergoing apoptosis.[32,33] The initial *in vivo* assessment of [^{18}F]ICMT-11 in small animals indicated favourable biodistribution of the radiotracer with rapid clearance from the blood and no indication of defluorination.[33] Moreover, the preclinical assessment of [^{18}F]ICMT-11 showed increased tumour retention of the radiotracer detected as early as 24 h post-treatment.

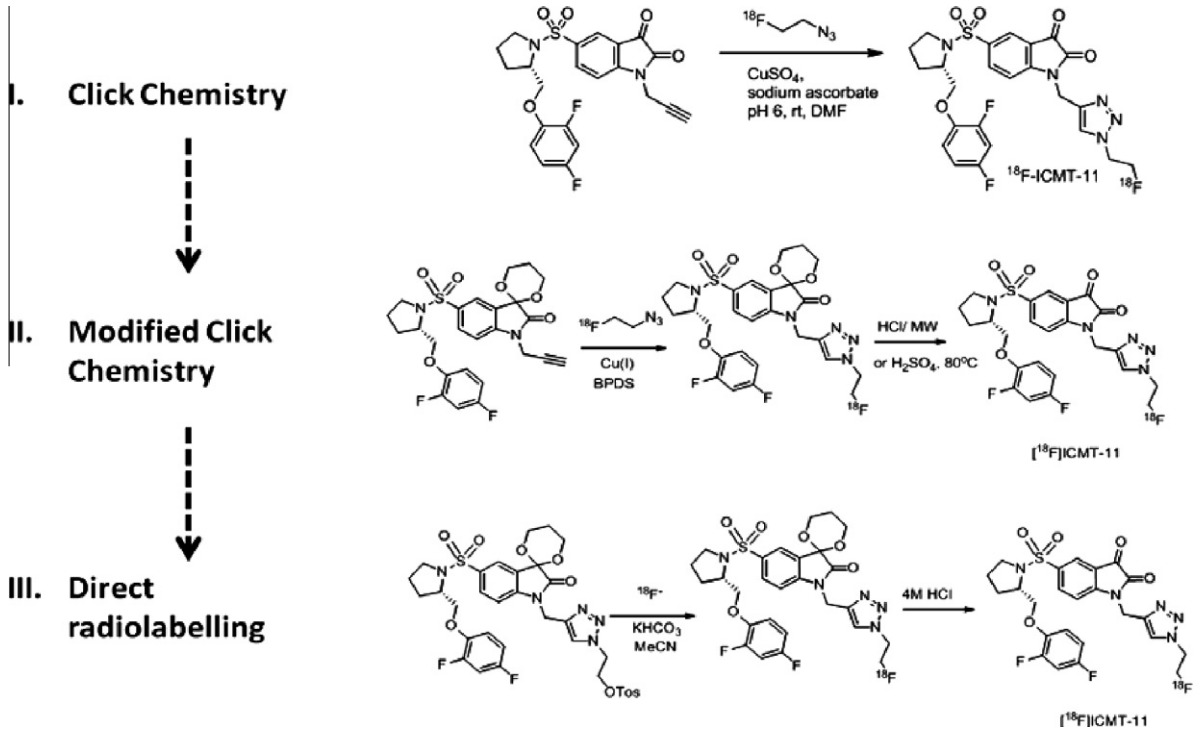

Fig. 3. Radiochemistry development of [^{18}F]ICMT-11 undertaken to improve specific radioactivity, reduce content of stable impurities, reduce synthesis time and enable automation for multi-patient dose synthesis. I. Initial click chemistry,[33] II. modified click chemistry[44] and III. Direct method.

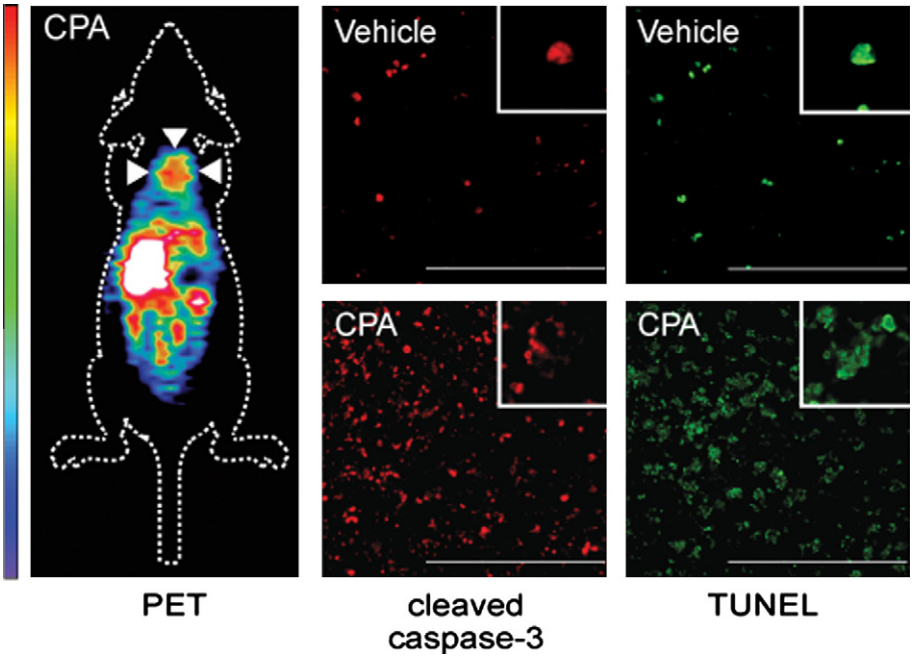

Fig. 4. PET Imaging of chemotherapy induced apoptosis with [^{18}F]ICMT-11. Comparison with cleaved caspase-3 immunohistochemistry (IHC). Left panel is a PET image of a 24 h cyclophosphamide (CPA)-treated mouse 60 min after injection of the radiotracer; arrowheads indicate the tumour. Middle and right panels are vehicle and CPA-treated lymphoma tumours processed for IHC staining of cleaved caspase-3 and DNA fragmentation (TUNEL), respectively.

Confirmatory studies indicated that [^{18}F]ICMT-11 tumour retention was associated with tumour apoptosis (Fig. 4).[32,44] The favourable biological validation of [^{18}F]ICMT-11 as a caspase-3 specific radiotracer to image tumour apoptosis prompted its clinical development.

Table 2
Clinical imaging of apoptosis.

	Radiotracers	Sample size	Disease group	Comments
Annexin				
Belhocine et al.[45]	^{99m}Tc-Annexin V	$n = 15$	Lymphoma lung cancer Breast cancer	Increased Annexin uptake 24–48 h after chemotherapy Increased uptake related to complete response/partial response (CR/PR) No uptake related to stable or progressive disease (SD/PD) Physiological uptake seen in salivary glands, liver, spleen, bone marrow, colon, kidneys & bladder Prevents imaging in abdomen
Kemerink et al.[46,46]	^{99m}Tc-HYNIC-annexin V		Healthy Volunteer (HV) study	Accumulation seen in kidneys, liver, red marrow, spleen and less uptake in bowel
Rottey et al.[47]			Biodistribution study with chemotherapy	Annexin uptake in normal tissues (spleen, bone marrow, kidneys and liver) does not change significantly with chemotherapy
Haas et al.[48]	^{99m}Tc-HYNIC-annexin V	$n = 11$	Follicular Lymphoma	Uptake increased after 4 Gy – correlated with clinical outcome and cytology
Kartachova et al.[49]	^{99m}Tc-HYNIC-annexin V	$n = 33$	Non small cell lung cancer (NSCLC) Lymphoma Leukaemia Head & Neck Squamous cell carcinoma (H&N SCC)	Increased uptake was associated with CR/PR after radiotherapy (RT)/chemotherapy Suggested that annexin V may be used as a predictive marker for early treatment response
Kartachova et al.[50]	^{99m}Tc-HYNIC-annexin V	$n = 16$	NSCLC	Increase in uptake post-treatment correlated with tumour response to platinum based CCT SD was associated with unchanged or slight decrease in uptake PD was associated with marked decrease in tumour uptake
Kartachova et al.[51]	^{99m}Tc-HYNIC-annexin V		NSCLC	Quantitative & visual assessment of post-treatment uptake correlated extremely well with RECIST response with good intraobserver reproducibility and interobserver variability
Hoebers et al.[52]	^{99m}Tc-HYNIC-annexin V	$n = 13$	H&N SCC	No correlation between uptake and patient outcome. Baseline necrosis in tumours is a confounding factor
Van de Wiele et al.[53]	^{99m}Tc-HYNIC-annexin V		H&N SCC	Annexin uptake correlated with TUNEL assay of resected surgical specimens
Theseus Imaging corporation started ph II/III studies using ApomateTM (^{99m}Tc-HYNIC-annexin V) but unit closed due to lack of Good Manufacturing Practise (GMP) production				
ML-10				
Hoglund *et al.*[38]	[^{18}F]-ML-10	$n = 8$	HV study	Favourable dosimetry, biodistribution, stability, and safety profile
Shirvan *et al.*[54]	[^{18}F]-ML-10	$n = 10$	Brain metastasis	ML-10 able to detect cell death induced by RT Uptake increased on 10th day after start of RT

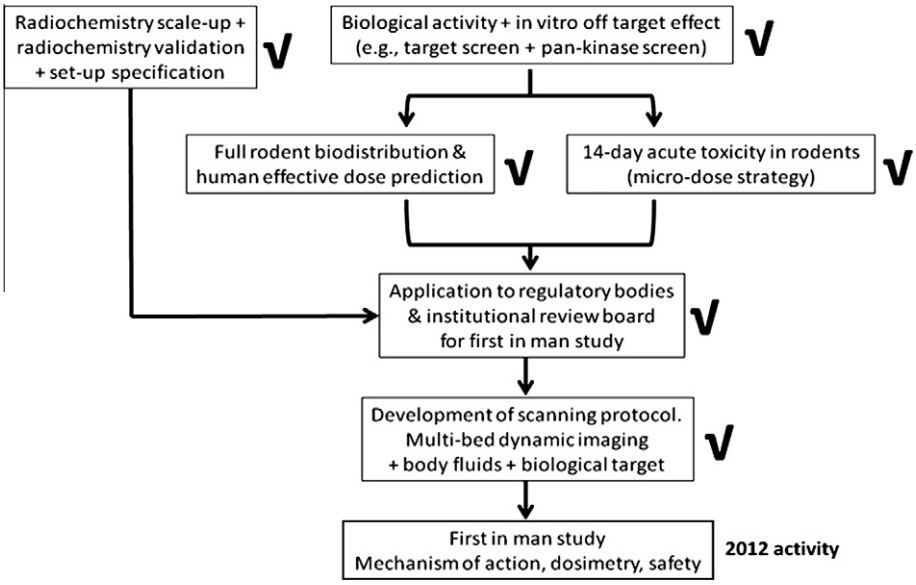

Fig. 5. Requirements for translation of [^{18}F]ICMT-11 into the clinic. Studies done to support first in man are highlighted (√).

5. Requirements for the translation of [^{18}F]ICMT-11 in clinical settings

There is paucity of data for clinical use of PET in the imaging of drug-induced apoptosis. Dosimetry and initial uptake studies of [^{18}F]-ML-10 following radiotherapy have been reported recently (Table 2).[38] Thus, [^{18}F]-ML-10 is the first in class PET imaging agent for drug-induced apoptosis to enter clinical trials. In contrast, mechanistic studies of apoptosis with radiolabelled annexin V using analogous single photon emission computed tomography (SPECT) have been extensively reported (Table 2). Other than the non-specific uptake of [^{99m}Tc]-HYNIC-annexin V, these clinical studies provided important learning outcomes that should be noted for future design of PET studies of apoptosis with positron emitting radiotracers. In general apoptosis following a variety of therapeutics evolved as an early event within 24–48 h that was associated with immunohistochemical staining of DNA oligomers (TUNEL). It is expected, for instance, that evolution of caspase-3 activation will occur earlier than phosphatidylserine translocation to the outer leaflet of cells – the molecular target for radiolabelled annexin V; this outcome means that one will require careful validation of the best time for use of caspase-3 imaging agents. Regarding association of [^{99m}Tc]-annexin V uptake with clinical outcome, there were mixed reports, with some studies indicating association and others reporting lack of a correlation. This aspect may be due in part to the timing of the apoptosis process and needs careful validation.

[^{18}F]ICMT-11 is transitioning into human studies. Key studies performed to date are highlighted in Fig. 5. Biological activity comprised of *in vitro* screen with different caspase enzymes, *in vitro* uptake studies, metabolic stability assays *in vivo* and *in vitro*, and *in vivo* imaging studies.[32,33,44] Rat dosimetry studies for prediction of human effective dose were performed according to the ICRP Publication-30.[55] Rat toxicity testing was performed in accordance to EMEA guidance: CPMP/ICH/286/95 published July 2008 with a safety window of 2000-fold the equivalent in humans. Notably, the radiotracer demonstrated suitable rodent dosimetry and safety profiles. Part of the success could be attributed to robust radiochemistry leading to low levels of stable impurities. First in man studies of the radiotracer are scheduled for early 2012 to precede further mechanistic studies by the QuIC-ConCePT consortium. The qualification of [^{18}F]ICMT-11 (developed by an academic consortium member) by QuIC-ConCePT consortium (comprising of 12 European academics institutions and 8 major pharmaceutical companies) *via* an open-innovation principle demonstrates how European academics and industrial partners can collaborate to develop tools for assessing the next generation therapeutics.

6. Conclusions

There are limited clinical examples of radiotracers for use by PET. [^{18}F]ICMT-11 is a novel reagent designed to image caspase-3 activation and hence drug-induced apoptosis. Due to the promising mechanistic and safety profile of [^{18}F]ICMT-11, the radiotracer is transitioning to clinical development. [^{18}F]ICMT-11 has been selected as a candidate radiotracer by the QuIC-ConCePT consortium for further evaluation in preclinical models and humans. A successful outcome will permit use of this reagent as an 'off-the-shelf' apoptosis radiotracer for evaluation of the pharmaceutical industry's next generation therapeutics.

Funding source

The author's work is funded by the Cancer Research UK-Engineering and Physical Sciences Research Council Grant C2536/A10337 and the UK Medical Research Council Grant U1200.005.00001.01.

Conflict of interest statement

None declared.

Acknowledgements

The authors are funded by the Cancer Research UK & Engineering and Physical Sciences Research Council, in association with the Medical Research Council and Department of Health (England) Grant (C2536/A10337) and the UK Medical Research Council Grant (U.1200.02.005.00001.01).

The Authors are members of the QuIC-ConCePT project partly funded by the EFPIA companies and the Innovative Medicine Initiative Joint Undertaking (IMI JU) under grant agreement number 115151.

Appendix A

QuIC-ConCePT Consortium participants include: AstraZeneca, European Organisation for Research and Treatment of Cancer (EORTC), Cancer Research UK, University of Manchester, Westfälische Wilhelms-Universität Münster, Radboud University Nijmegen Medical Center, Institut National de la Santé et de la Recherche Médical, Stichting Maastricht Radiation Oncology 'Maastro Clinic', VUmc Amsterdam, King's College London, Universitair Ziekenhuis Antwerpen, Institute of Cancer Research – Royal Cancer Hospital, Erasmus Universitair Medisch Centrum Rotterdam, Imperial College of Science Technology and Medicine, Keosys S.A.S., Eidgenössische Technische Hochschule Zürich, Amgen NV, Eli Lilly and Company Ltd., GlaxoSmithKline Research & Development Limited, Merck KGa, Pfizer Limited, F.Hoffmann – La Roche Ltd., Sanofi–Aventis Research and Development.

References

1. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med* 2009;**50**(Suppl 1):122S–50S.
2. Carden CP, Banerji U, Kaye SB, Workman P, de Bono JS. From darkness to light with biomarkers in early clinical trials of cancer drugs. *Clin Pharmacol Ther* 2009;**85**(2):131–3.
3. Kamb A, Wee S, Lengauer C. Why is cancer drug discovery so difficult? *Nat Rev Drug Discovery* 2007;**6**(2):115–20.
4. Aboagye E. The use of imaging to accelerate drug development. *Drug Discovery Today* 2002;**7**(13):706–7.
5. Aboagye EO. Imaging in drug development. *Clin Adv Hematol Oncol* 2006;**4**(12):902–4.
6. Contractor KB, Aboagye EO. Monitoring predominantly cytostatic treatment response with 18F-FDG PET. *J Nucl Med* 2009;**50**(Suppl. 1):97S–105S.
7. Gupta N, Price PM, Aboagye EO. PET for in vivo pharmacokinetic and pharmacodynamic measurements. *Eur J Cancer* 2002;**38**(16):2094–107.
8. Rosso L, Brock CS, Gallo JM, et al. A new model for prediction of drug distribution in tumor and normal tissues: pharmacokinetics of temozolomide in glioma patients. *Cancer Res* 2009;**69**(1):120–7.
9. Saleem A, Yap J, Osman S, et al. Modulation of fluorouracil tissue pharmacokinetics by eniluracil: in-vivo imaging of drug action. *Lancet* 2000;**355**(9221):2125–31.
10. Workman P, Aboagye EO, Chung YL, et al. Minimally invasive pharmacokinetic and pharmacodynamic technologies in hypothesis-testing clinical trials of innovative therapies. *J Natl Cancer Inst* 2006;**98**(9):580–98.
11. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;**100**(1):57–70.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;**144**(5):646–74.
13. Juweid ME, Cheson BD. Positron-emission tomography and assessment of cancer therapy. *N Engl J Med* 2006;**354**(5):496–507.
14. Weber WA, Wieder H. Monitoring chemotherapy and radiotherapy of solid tumors. *Eur J Nucl Med Mol Imaging* 2006;**33**(Suppl):27–37.
15. Mankoff DA, Eary JF, Link JM, et al. Tumor-specific positron emission tomography imaging in patients: [18F] fluorodeoxyglucose and beyond. *Clin Cancer Res* 2007;**13**(12):3460–9.
16. Sharma R, Aboagye E. Development of radiotracers for oncology – the interface with pharmacology. *Br J Pharmacol* 2011;**163**(8):1565–85.
17. Aboagye EO. The future of imaging: developing the tools for monitoring response to therapy in oncology: the 2009 Sir James MacKenzie Davidson Memorial lecture. *Br J Radiol* 2010;**83**(994):814–22.
18. Reed JC. Apoptosis-based therapies. *Nat Rev Drug Discovery* 2002;**1**(2):111–21.
19. Storey S. Targeting apoptosis: selected anticancer strategies. *Nat Rev Drug Discov* 2008;**7**(12):971–2.
20. Chang J, Ormerod M, Powles TJ, et al. Apoptosis and proliferation as predictors of chemotherapy response in patients with breast carcinoma. *Cancer* 2000;**89**(11):2145–52.
21. Dubray B, Breton C, Delic J, et al. In vitro radiation-induced apoptosis and early response to low-dose radiotherapy in non-Hodgkin's lymphomas. *Radiother Oncol* 1998;**46**(2):185–91.
22. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008;**9**(3):231–41.
23. Reshef A, Shirvan A, Akselrod-Ballin A, Wall A, Ziv I. Small-molecule biomarkers for clinical PET imaging of apoptosis. *J Nucl Med* 2010;**51**(6):837–40.
24. Yagle KJ, Eary JF, Tait JF, et al. Evaluation of 18F-annexin V as a PET imaging agent in an animal model of apoptosis. *J Nucl Med* 2005;**46**(4):658–66.
25. Toretsky J, Levenson A, Weinberg IN, et al. Preparation of F-18 labeled annexin V: a potential PET radiopharmaceutical for imaging cell death. *Nucl Med Biol* 2004;**31**(6):747–52.
26. Grierson JR, Yagle KJ, Eary JF, et al. Production of [F-18]fluoroannexin for imaging apoptosis with PET. *Bioconjugate Chem* 2004;**15**(2):373–9.
27. Wang F, Fang W, Zhang MR, et al. Evaluation of chemotherapy response in VX2 rabbit lung cancer with 18F-labeled C2A domain of synaptotagmin I. *J Nucl Med* 2011;**52**(4):592–9.
28. Zhou D, Chu W, Chen DL, et al. [18F]- and [11C]-labeled N-benzyl-isatin sulfonamide analogues as PET tracers for apoptosis: synthesis, radiolabeling mechanism, and in vivo imaging study of apoptosis in Fas-treated mice using [11C]WC-98. *Org Biomol Chem* 2009;**7**(7):1337–48.

29. Faust A, Wagner S, Law MP, et al. The nonpeptidyl caspase binding radioligand (S)-1-(4-(2-[18F]Fluoroethoxy)-benzyl)-5-[1-(2-methoxymethylpyrrolidinyl)s ulfonyl]isatin ([18F]CbR) as potential positron emission tomography-compatible apoptosis imaging agent. *Q J Nucl Med Mol Imaging* 2007;**51**(1):67–73.
30. Podichetty AK, Faust A, Kopka K, et al. Fluorinated isatin derivatives. Part 1: synthesis of new N-substituted (S)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins as potent caspase-3 and -7 inhibitors. *Bioorg Med Chem* 2009;**17**(7):2680–8.
31. Podichetty AK, Wagner S, Schroer S, et al. Fluorinated isatin derivatives. Part 2. New N-substituted 5-pyrrolidinylsulfonyl isatins as potential tools for molecular imaging of caspases in apoptosis. *J Med Chem* 2009;**52**(11):3484–95.
32. Nguyen QD, Smith G, Glaser M, et al. Positron emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [18F]-labeled isatin sulfonamide. *Proc Nat Acad Sci U.S.A.* 2009;**106**(38):16375–80.
33. Smith G, Glaser M, Perumal M, et al. Design, synthesis, and biological characterization of a caspase 3/7 selective isatin labeled with 2-[18F]fluoroethylazide. *J Med Chem* 2008;**51**(24):8057–67.
34. Madar I, Ravert H, Nelkin B, et al. Characterization of membrane potential-dependent uptake of the novel PET tracer 18F-fluorobenzyl triphenylphosphonium cation. *Eur J Nucl Med Mol Imaging* 2007;**34**(12):2057–65.
35. Madar I, Huang Y, Ravert H, et al. Detection and quantification of the evolution dynamics of apoptosis using the PET voltage sensor 18F-fluorobenzyl triphenyl phosphonium. *J Nucl Med* 2009;**50**(5):774–80.
36. Cohen A, Shirvan A, Levin G, et al. From the Gla domain to a novel small-molecule detector of apoptosis. *Cell Res* 2009;**19**(5):625–37.
37. Reshef A, Shirvan A, Waterhouse RN, et al. Molecular imaging of neurovascular cell death in experimental cerebral stroke by PET. *J Nucl Med* 2008;**49**(9):1520–8.
38. Hoglund J, Shirvan A, Antoni G, et al. 18F-ML-10, a PET tracer for apoptosis: first human study. *J Nucl Med* 2011;**52**(5):720–5.
39. Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 1999;**6**(2):99–104.
40. Lee D, Long SA, Adams JL, et al. Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality. *J Biol Chem* 2000;**275**(21):16007–14.
41. Lee D, Long SA, Murray JH, et al. Potent and selective nonpeptide inhibitors of caspases 3 and 7. *J Med Chem* 2001;**44**(12):2015–26.
42. Aulabaugh A, Kapoor B, Huang X, et al. Biochemical and biophysical characterization of inhibitor binding to caspase-3 reveals induced asymmetry. *Biochemistry* 2007;**46**(33):9462–71.
43. Smith G, Nguyen QD, Aboagye EO. Translational imaging of apoptosis. *Anticancer Agents Med Chem* 2009;**9**(9):958–67.
44. Glaser M, Goggi J, Smith G, et al. Improved radiosynthesis of the apoptosis marker 18F-ICMT11 including biological evaluation. *Bioorg Med Chem Lett* 2011;**21**(23):6945–9.
45. Belhocine T, Steinmetz N, Hustinx R, et al. Increased uptake of the apoptosis-imaging agent (99m)Tc recombinant human Annexin V in human tumors after one course of chemotherapy as a predictor of tumor response and patient prognosis. *Clin Cancer Res* 2002;**8**(9):2766–74.
46. Kemerink GJ, Liu X, Kieffer D, et al. Safety, biodistribution, and dosimetry of 99mTc-HYNIC-annexin V, a novel human recombinant annexin V for human application. *J Nucl Med* 2003;**44**(6):947–52.
47. Rottey S, Van den Bossche B, Slegers G, Van Belle S, van de Wiele C. Influence of chemotherapy on the biodistribution of [99mTc]hydrazinonicotinamide annexin V in cancer patients. *Q J Nucl Med Mol Imaging* 2009;**53**(2):127–32.
48. Haas RL, de Jong D, Valdes Olmos RA, et al. In vivo imaging of radiation-induced apoptosis in follicular lymphoma patients. *Int J Radiat Oncol Biol Phys* 2004;**59**(3):782–7.
49. Kartachova M, Haas RL, Olmos RA, et al. In vivo imaging of apoptosis by 99mTc-Annexin V scintigraphy: visual analysis in relation to treatment response. *Radiother Oncol* 2004;**72**(3):333–9.
50. Kartachova M, van Zandwijk N, Burgers S, et al. Prognostic significance of 99mTc Hynic-rh-annexin V scintigraphy during platinum-based chemotherapy in advanced lung cancer. *J Clin Oncol* 2007;**25**(18):2534–9.
51. Kartachova MS, Valdes Olmos RA, Haas RL, et al. 99mTc-HYNIC-rh-annexin-V scintigraphy: visual and quantitative evaluation of early treatment-induced apoptosis to predict treatment outcome. *Nucl Med Commun* 2008;**29**(1):39–44.
52. Hoebers FJ, Kartachova M, de Bois J, et al. 99mTc Hynic-rh-Annexin V scintigraphy for in vivo imaging of apoptosis in patients with head and neck cancer treated with chemoradiotherapy. *Eur J Nucl Med Mol Imaging* 2008;**35**(3):509–18.
53. van de Wiele C, Lahorte C, Vermeersch H, et al. Quantitative tumor apoptosis imaging using technetium-99m-HYNIC annexin V single photon emission computed tomography. *J Clin Oncol* 2003;**21**(18):3483–7.
54. Shirvan A. Apoptosis imaging with PET-18F-ML-10 for early assessment of response of brain metastasis to radiotherapy. *J Nucl Med* 2009;**50**(Suppl. 2):118P.
55. International Commission on Radiological Protection. Limits for intakes of radionuclides by workers. ICRP Publication. New York: Pergamon Press;1979:30.