Editorial

To Scan or Not To Scan, It Is a Question of Timing: Technetium-99m-Annexin V Radionuclide Imaging Assessment of Treatment Efficacy after One Course of Chemotherapy

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Doctor Belhocine and his group have conducted a seminal Phase I study correlating ^{99m}Tc-rh-annexin[2] V imaging with clinical outcome in patients with late-stage lung cancer and lymphoma within 1–3 days after chemotherapy (1). The conclusions that can be drawn from this study are quite important and include: (*a*) annexin V imaging can be performed serially and safely; (*b*) significant posttreatment increases in annexin V uptake above pretreatment levels predict at least a partial response to chemotherapy; (*c*) the increases in annexin V uptake appear to be specific and is not seen even in large necrotic tumors in which one would be concerned with nonspecific localization of the tracer both at baseline and posttreatment; and (*d*) the increase in annexin V uptake appears to be heavily dependent on the exact time after the start of chemotherapy. Of all these issues, the most interesting and the most difficult is the determination of when best to scan after the start of chemotherapy.

The peak(s) annexin V uptake (*i.e.*, PS expression) found in tumor models varies from 1 to more than 20 h after a single injection of a proapoptotic drug (2). Compounding the problem is the lack of significant redistribution of ^{99m}Tc-annexin V after 30 min following i.v. administration observed in animals. If the window of time in which peak(s) activity occurs is only a matter of hours, then the timing of annexin V imaging after a given therapy will become of paramount importance.

Although there are no definitive human or animal model data at this time, there are some clues as to when to perform annexin V imaging after the initiation of chemotherapy. We have seen a 2- to 3-fold increase in annexin V uptake as early as 1 h, lasting approximately 90 min, after a single injection of high-dose cyclophosphamide (150 mg/kg i.p.) in BALB/c mice bearing luciferase-expressing BCL-1 syngeneic murine lymphoma.[3] This early transient increase in annexin V uptake paradoxically was not accompanied by the loss of BCL-1 lymphoma cells, which did, however, eventually occur some 20–22 h later as confirmed by serial optical imaging.

The significance of this extremely early peak in annexin V uptake prior to tumor cell loss and shrinkage is unclear but may relate: (*a*) to low levels of apoptosis of cells at a particularly sensitive phase of the cell cycle or (*b*) to a preapoptotic "stressing" of tumor cells causing a transient expression of PS that may or may not be predictive of the commitment of a cell to apoptosis later on (3). Further evidence of early increases in annexin V uptake has been provided by Dumont *et al.* (4), who found marked PS expression within 30 min of occlusion/reperfusion injury in the beating murine heart with fluorescent-labeled annexin V microscopy. D'Arceuil *et al.* (5) were also able to demonstrate significant increases in PS expression in the brains of neonatal rabbits 2 h after relatively mild degrees of global ischemic reperfusion injury.

A second peak of annexin V uptake (PS expression) would also be expected to occur hours later just prior to the loss of the bulk of tumor cells due to apoptosis, in a similar fashion as observed with previous investigations (2, 6). Another concern is the response of sensitive host tissues to chemotherapy observed with annexin V radionuclide imaging of the spleen and bone marrow in normal rats after a single dose of cyclophosphamide (7). With high-dose cyclophosphamide, rats demonstrated a 2- to 3-fold rise in annexin V uptake in these organs as early as 8 h after treatment, lasting approximately 2 days. Lower doses of cyclophosphamide delayed the time to peak annexin V uptake and the magnitude and length of PS expression in the spleen and bone marrow. In Belhocine's series of human subjects, however, there appeared to be no significant increases in annexin V uptake in normal tissues (1). It is, therefore, unclear whether the chemotherapeutic effects seen in normal rodents will be an issue for clinical imaging with ^{99m}Tc-annexin V.

In summary, the evidence to date suggests that there are at least two peaks of annexin V uptake, one occurring early within hours of the initiation of chemotherapy and another later on, probably 24–72 h after the completion of treatment. Until more definitive data are acquired, it will be necessary to design clinical trials with ^{99m}Tc-annexin V so that early imaging [within the first day of therapy (immediate) followed up by scanning 24 h later (delayed)] and late imaging with a repeat injection of ^{99m}Tc-annexin V [radionuclide imaging both immediately and 24 h later after completion of a dose of chemotherapy] in addition to both baseline immediate and 24-h delay images. The suggested protocol will require three separate injections of

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[2] The abbreviations used are: ^{99m}Tc, technetium-99m; PS, phosphatidylserine.

[3] Unpublished personal data.

tracer as well as six radionuclide scans [*i.e.*, three single photon emission computed tomography with whole body scans (immediately) and three whole body scans (24 h delay)]. Whereas this protocol maybe difficult to carry out in a Phase II trial, it has the benefit of imaging over the potentially critical phases of annexin V uptake after chemotherapy.

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