APOPTOSIS IMAGING IN ONCOLOGY: DEATH OR ALIVE?

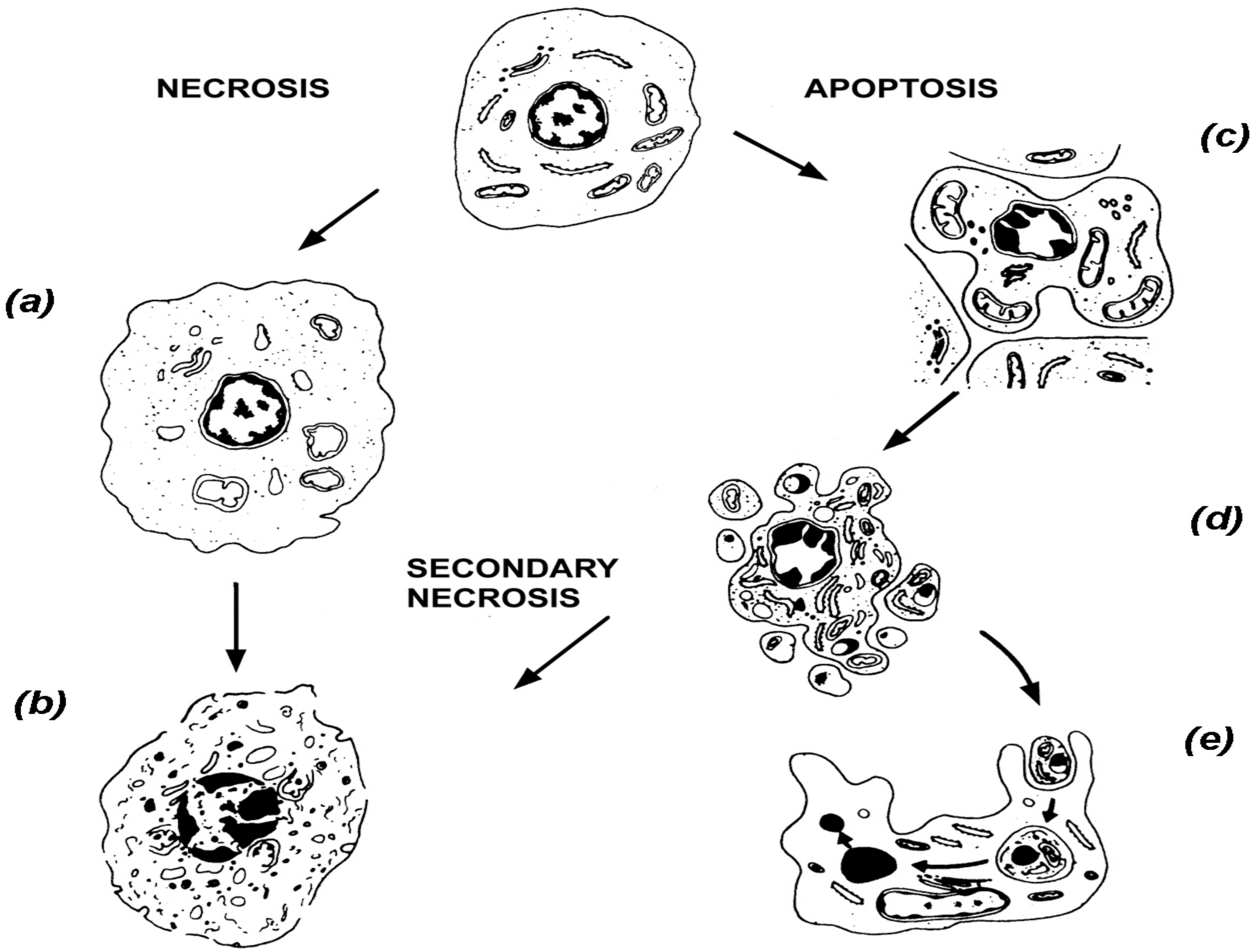
Olivier Gheysens, M.D., PhD
Department of Nuclear Medicine
University Hospitals Leuven, Belgium

Christophe Van de Wiele, M.D., PhD.,
Department of Nuclear Medicine,
University Hospital Ghent, Belgium

ISORBE, Pretoria, March 21-23, 2013

APOPTOSIS IMAGING IN ONCOLOGY

- **Basics**
- Caspase-3 activation imaging
- PS-expression imaging
  - Biodistribution and basic studies
  - Monitoring chemotherapy
  - Monitoring radiotherapy
- Future prospects


NECROSIS
APOPTOSIS
(c)
(a)
(d)
SECONDARY
NECROSIS
(b)
(e)

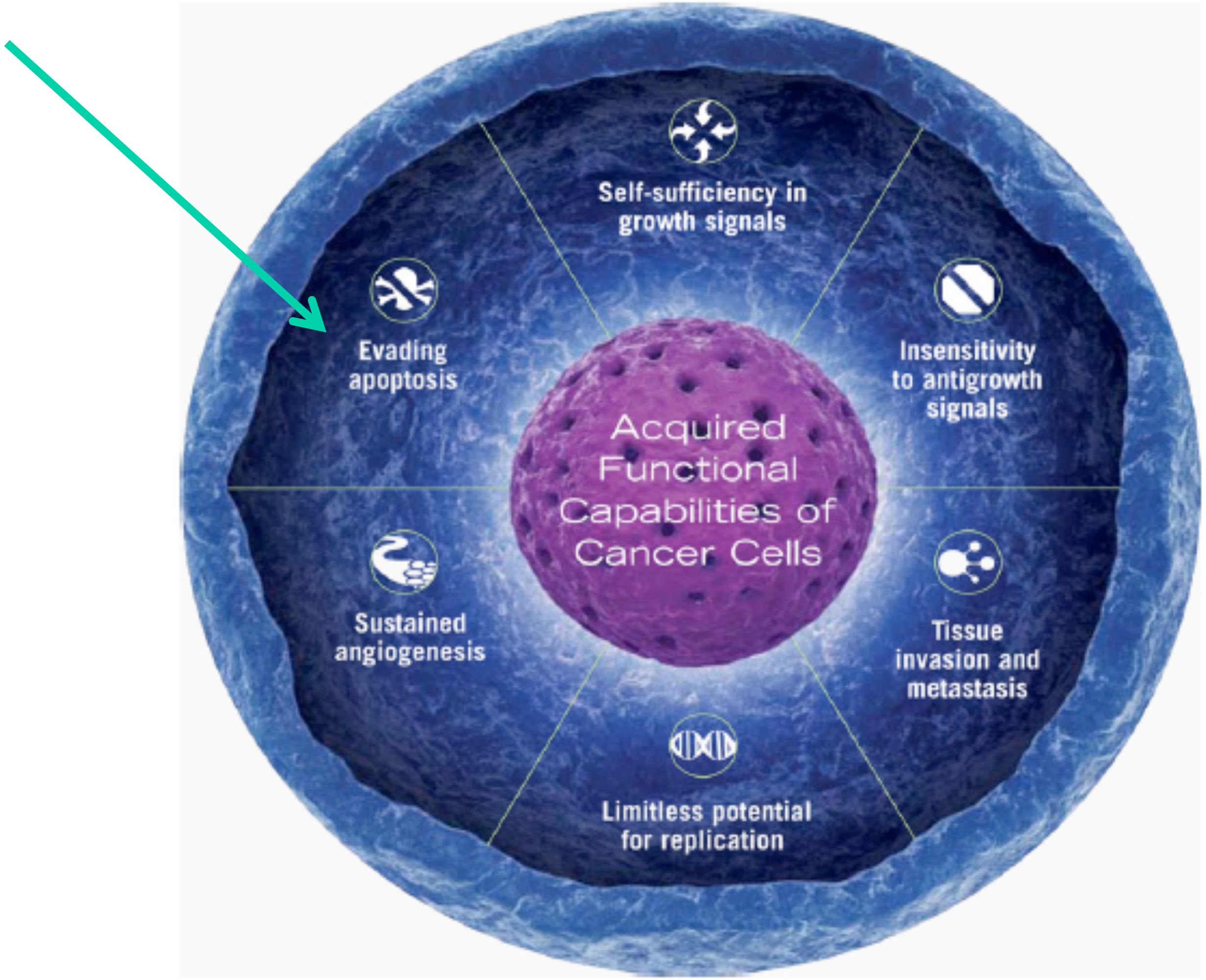
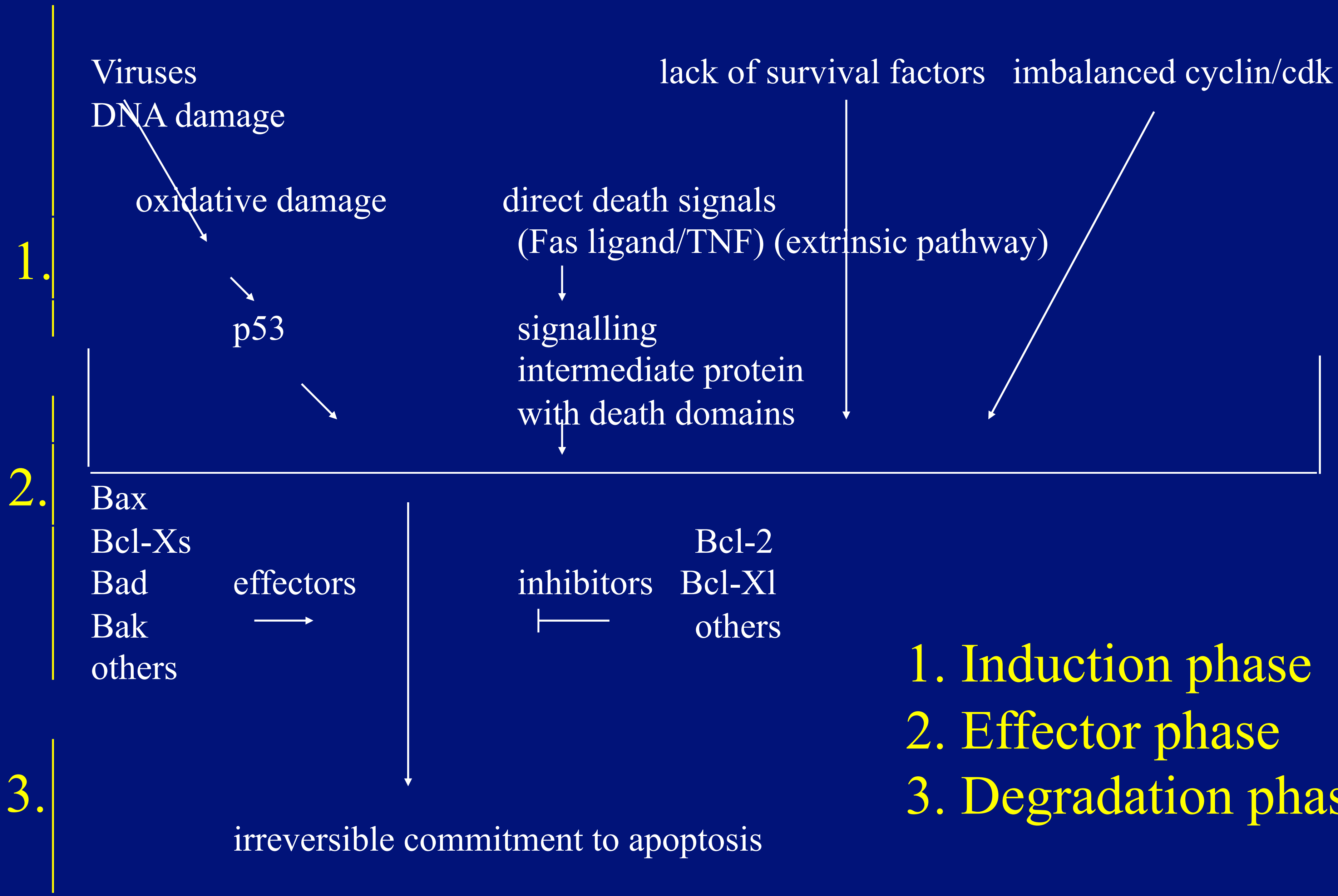
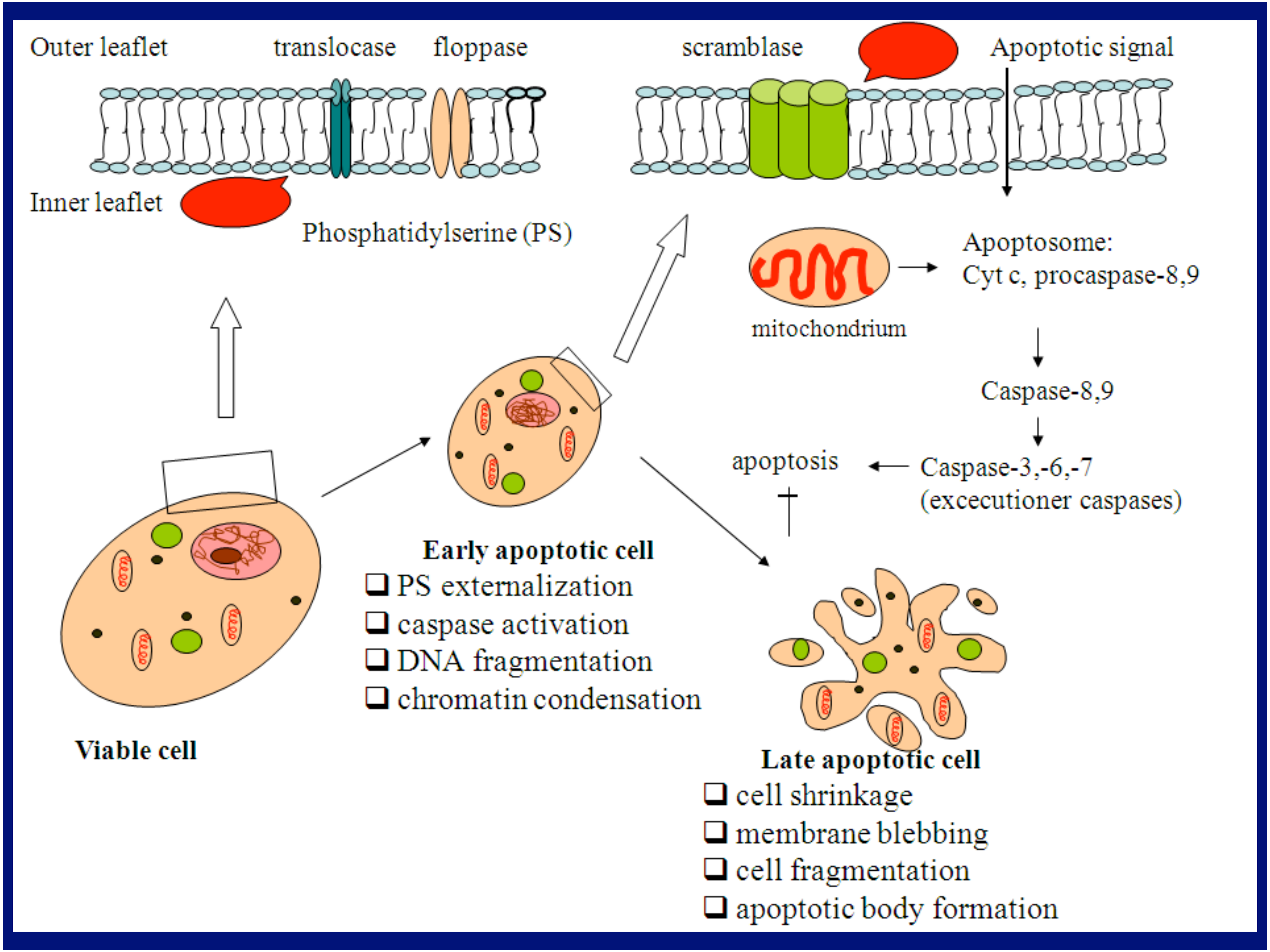


Fig. 1 The 6 hallmarks of cancer unifying common phenotypes that cancer cells take on to survive and metastasize (Hannahan D and Weinberg A).

APOPTOSIS PROCESS



1. Induction phase
2. Effector phase
3. Degradation phase


Outer leaflet
translocase
floppase
scramblase
Apoptotic signal
Inner leaflet
Phosphatidylserine (PS)
Apoptosome:
Cyt c, procaspase-8,9
mitochondrium
Caspase-8,9
apoptosis
Caspase-3,-6,-7
(excecutioner caspases)
Early apoptotic cell
❑ PS externalization
❑ caspase activation
❑ DNA fragmentation
❑ chromatin condensation
Viable cell
Late apoptotic cell
❑ cell shrinkage
❑ membrane blebbing
❑ cell fragmentation
❑ apoptotic body formation

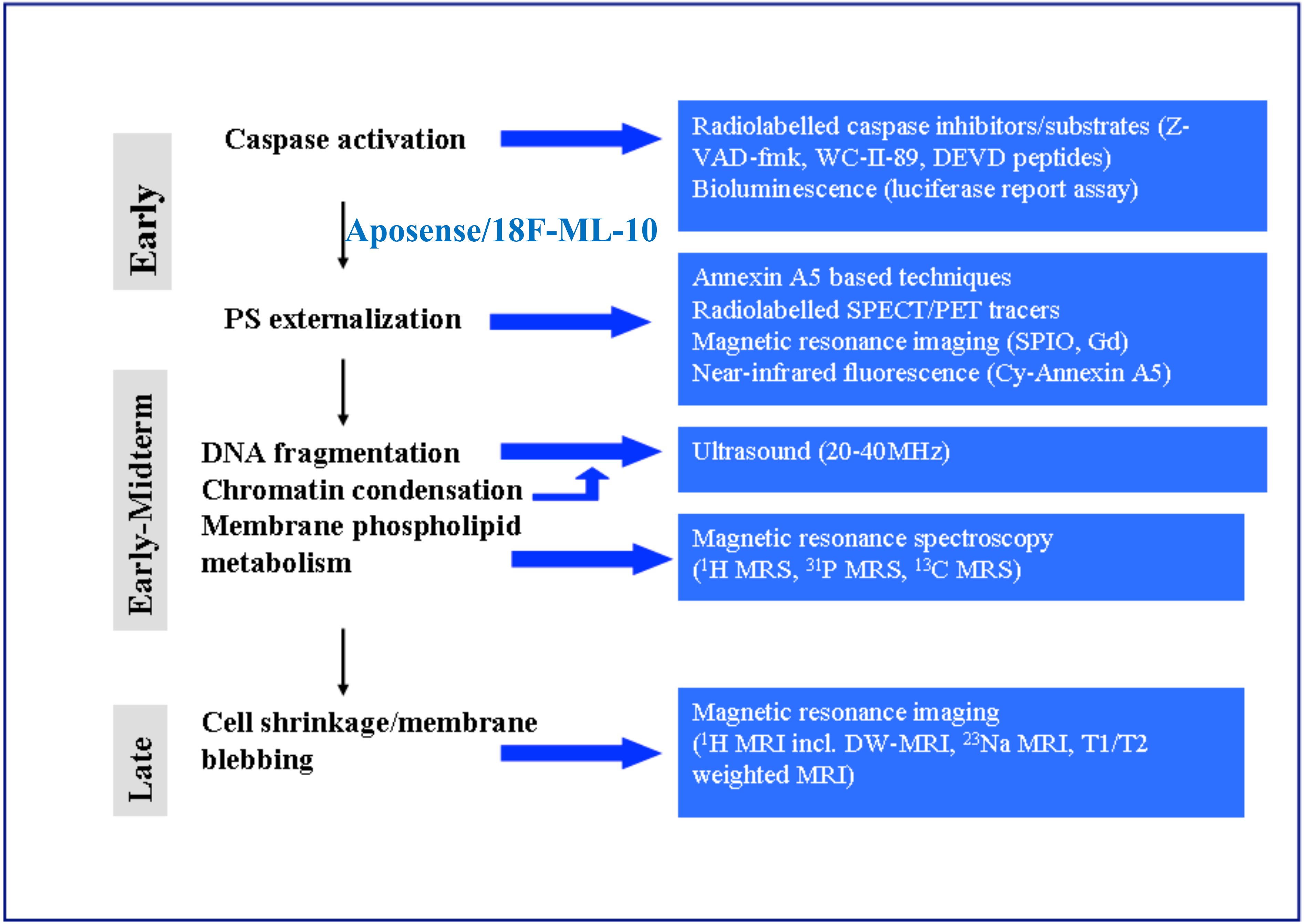

Early
Early-Midterm
Late
Caspase activation
Aposense/18F-ML-10
PS externalization
DNA fragmentation
Chromatin condensation
Membrane phospholipid metabolism
Cell shrinkage/membrane blebbing
Radiolabelled caspase inhibitors/substrates (Z-VAD-fmk, WC-II-89, DEVD peptides)
Bioluminescence (luciferase report assay)
Annexin A5 based techniques
Radiolabelled SPECT/PET tracers
Magnetic resonance imaging (SPIO, Gd)
Near-infrared fluorescence (Cy-Annexin A5)
Ultrasound (20-40MHz)
Magnetic resonance spectroscopy
(^{1}H MRS, ^{31}P MRS, ^{13}C MRS)
Magnetic resonance imaging
(^{1}H MRI incl. DW-MRI, ^{23}Na MRI, T1/T2 weighted MRI)

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Table 1

List of radioligands imaging early apoptotic events: Caspase Activation

Radioligand	Imaging Modality	Type of Study	Reference
^{131}I (Z-VAD-fmk)	SPECT	*In vitro*	[9]
^{18}F pyrrolidinylsulfonyl isatin	PET	Biodistribution in nude mice	[10]
^{18}F-WC-II-89	PET	Biodistribution in rats + Cycloheximide induced liver apoptosis	[11, 12]
^{11}C-WC-98	PET	Cycloheximide induced liver apoptosis	[13]
^{18}F-WC-IV-3	PET	Cycloheximide induced liver apoptosis	[13]
^{131}I-DEVD peptides	SPECT	*In vitro*	[14]
Recombinant luciferase reporter molecule	BLI	*In vitro* + D54-bearing nude mice treated with TRAIL	[15]
ANLucBCLuc	BLI	*In vitro* + D54-bearing nude mice treated with temozolomide, perifosine and irradiation	[16]

BLI = bioluminescence imaging; PET = positron emission tomography; SPECT = single-photon emission computed tomography; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand; Z-VAD-fmk = benzyloxylcarbonyl-Val-Ala-DL-Asp (O-methyl)-fluoromethyl ketone

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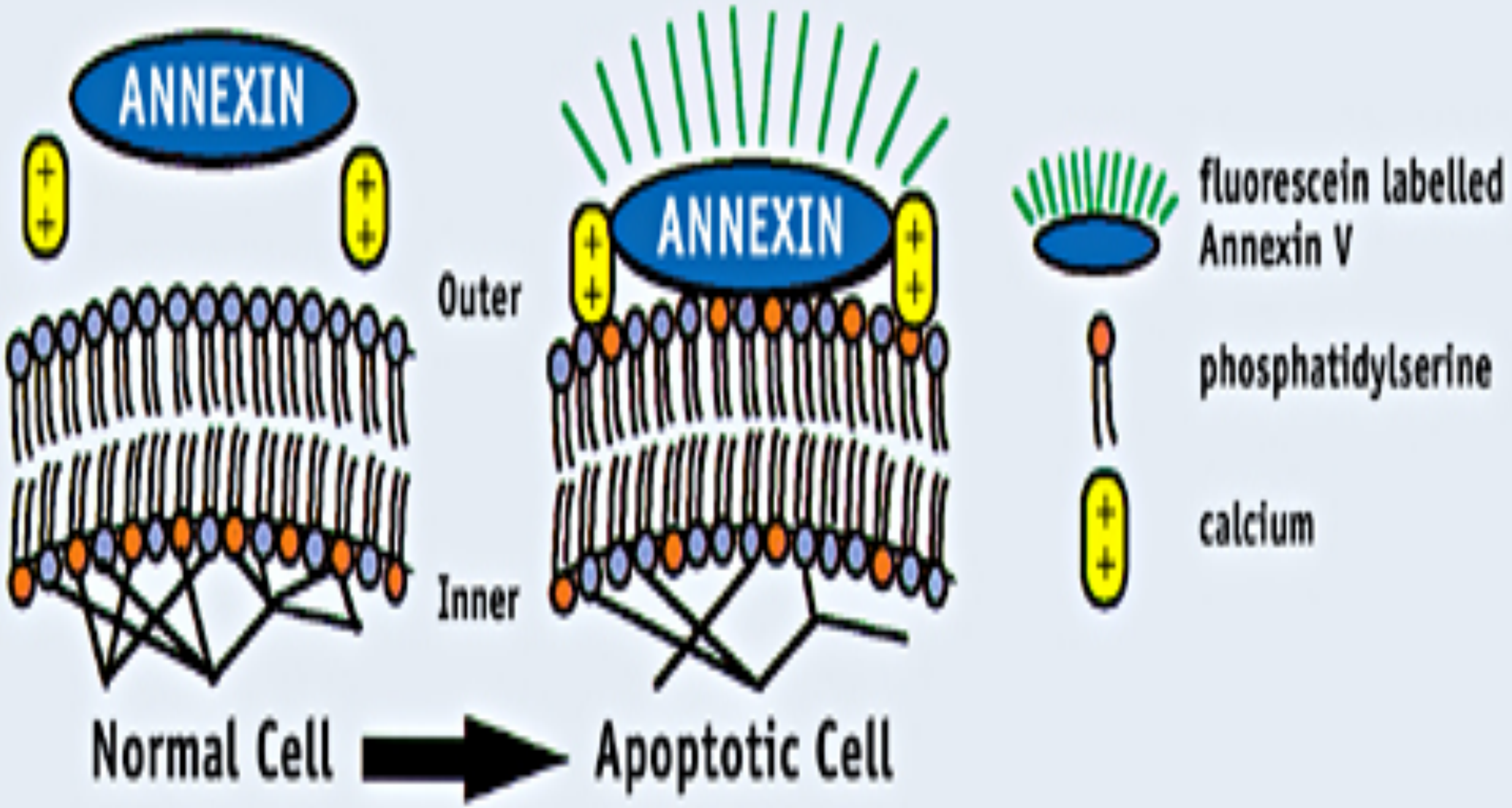
ANNEXIN
Outer
ANNEXIN
fluorescein labelled
Annexin V
phosphatidylserine
calcium
Inner
Normal Cell
Apoptotic Cell

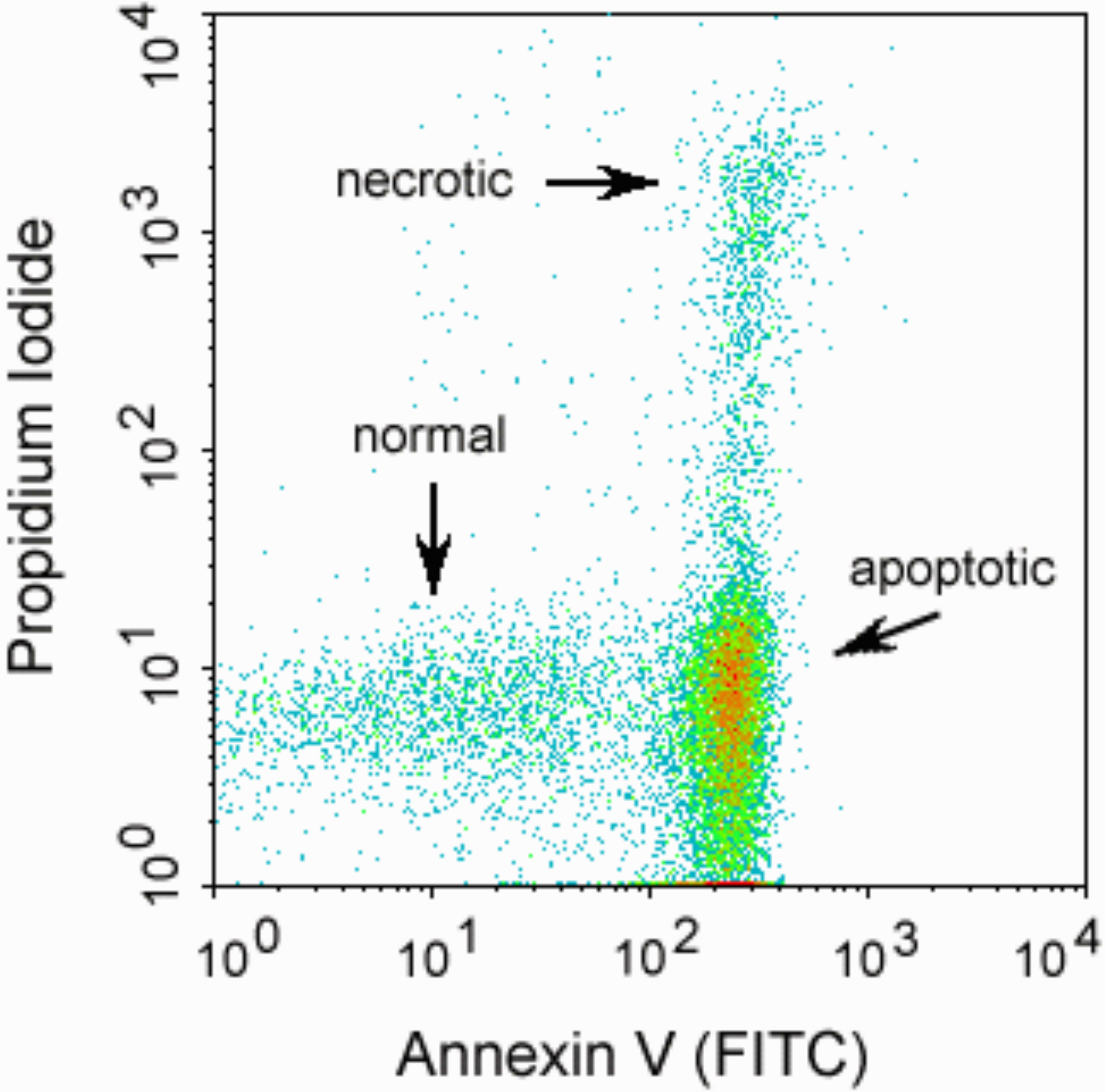
necrotic
normal
apoptotic
Propidium Iodide
10^0
10^1
10^2
10^3
10^4
Annexin V (FITC)

Table 2

List of Radioligands imaging phospholipid reorganization: PS externalization

Radioligand	Imaging Modality	Type of Study	Reference
^{99m}Tc-HYNIC-annexin A5	SPECT	Fas-mediated hepatocyte apoptosis in mice + Lymphoma-bearing mice receiving cyclophosphamide	[17]
		In vitro + Fas-mediated hepatocyte apoptosis in mice	[18]
		Biodistribution in rats + DEX induced thymic apoptosis in mice	[19]
		Hepatoma-bearing rats receiving cyclophosphamide	[21]
		Biodistribution + dosimetry in human volunteers	[22]
		Head and neck cancer patients	[23, 24]
		Intra-and inter-observer and day-to-day reproducibility in patients	[25]
		Influence of chemotherapy on biodistribution in patients	[26]
		Cancer patients receiving chemo- and/or radiotherapy	[27]
		Lung cancer patients receiving platinum-based chemotherapy	[28]
		Cancer patients receiving chemo- and/or radiotherapy	[29]
		Cancer patients receiving chemotherapy	[30]
		Lymphoma-and sarcoma-bearing mice receiving irradiation	[31]
		Lymphoma patients receiving irradiation	[32]
		Head and neck cancer patients receiving chemoradiation	[33]
^{99m}Tc-MAG$_3$-annexin A5	SPECT	Biodistribution in normal mice	[34]
^{99m}Tc-EC-annexin A5	SPECT	Breast tumour-bearing mice receiving paclitaxel	[35]
		Breast cancer patients receiving (chemo)therapy	[36]
^{99m}Tc-BTAP-annexin A5	SPECT	Biodistribution in healthy + patients with different malignacies	[37, 38]
		Lung, breast and lymphoma patients receiving chemotherapy	[39, 40]
^{99m}Tc-annexin-128	SPECT	Biodistribution in mice + cycloheximide induced liver apoptosis	[41]
^{99m}Tc-HYNIC-B1	SPECT	Biodistribution in mice + mice receiving DEX and anti-Fas antibody	[42]
^{99m}Tc-HYNIC-cys-annexin A5	SPECT	Biodistribution in mice + mice receiving anti-Fas antibody	[43]
		Cycloheximide induced liver apoptosis + castration in rats	[44]

^{123}I-annexin A5	SPECT	*In vitro*	[45]
		Biodistribution and dosimetry in mice and humans	[46]
		In vitro + colorectal tumour-bearing mice receiving farnesyltransferase inhibitor therapy	[47]
		DEX induced thymic apoptosis in rats	[48]
^{111}In DTPA-PEG-annexin A5	SPECT	Biodistribution + breast tumor-bearing mice receiving paclitaxel and/or C225	[49]
^{18}F - annexin A5	PET	*In vitro*	[50-52]
		Biodistribution + cycloheximide induced liver apoptosis in rats	[53]
^{64}Cu-DOTA-biotin-Sav annexin A5	PET	*In vitro* + breast tumour-bearing mice receiving PDT	[54]
[^{124}I]m-SIB + ^{125}I annexin A5	PET	*In vitro*	[55-56]
^{124}I annexin A5	PET	*In vitro* + mice receiving anti-Fas antibody	[57]
[^{125}I]SIB annexin A5 + ^{125}I annexin A5	PET	*In vitro* + fibrosarcoma-bearing mice treated with 5-FU	[58]
[^{124}I]4IB annexin A5	PET	*In vitro* + mice receiving anti-Fas antibody	[59]
^{125}I-MBP-annexin A5	PET	Mice receiving anti-Fas antibody	[60]

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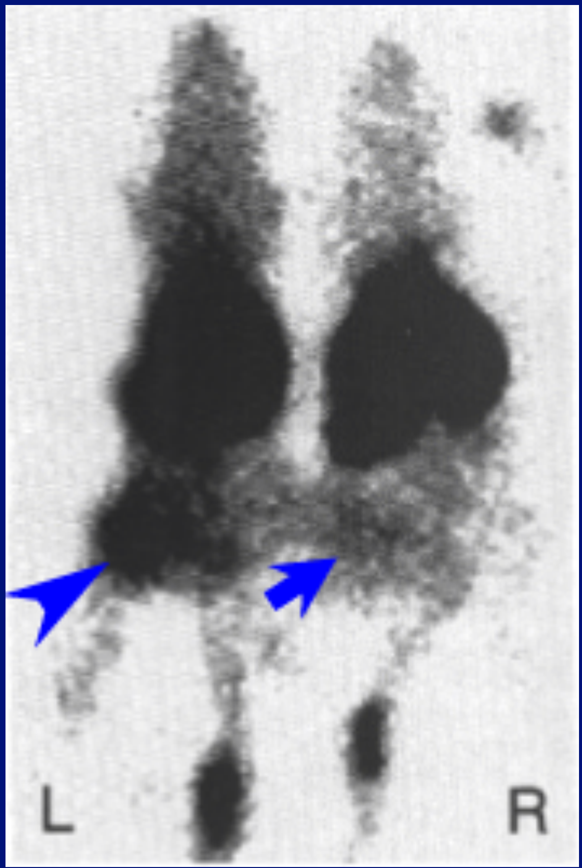
Preclinical data

Authors	Journal	Chelator	Setting	Results
Yang et al.	CBR 2001	EC	Breast Paclitaxel Delta T/N	T/N increase from 2 to 4 h.
Blankenberg et al.	PNAS 1998	HYNIC	Lymphoma Cyclo* Delta uptake	µ increase of 78% in uptake Related to apoptosis
Mochizuki et al.	JNM 2003	HYNIC	Hepatoma Cyclo* Delta uptake	µincrease of 50¨% in uptake r: 0.7 (vs TUNEL)

Annexin Imaging of Treated Murine Lymphoma



CH3.HeN mice implanted with 38C13 murine B cell lymphoma into left flank treated with cyclophosphamide (100mg/kg) 14 days post implantation, injected with Tc-99m annexin 20 hours following treatment, and imaged 1 hour later. Treated tumor (arrowhead) showed 363% increased uptake over control (arrow) by ROI analysis.

Blankenberg et. al., Proc. Natl. Acad. Sci. USA, 95:6349 (May 1998)

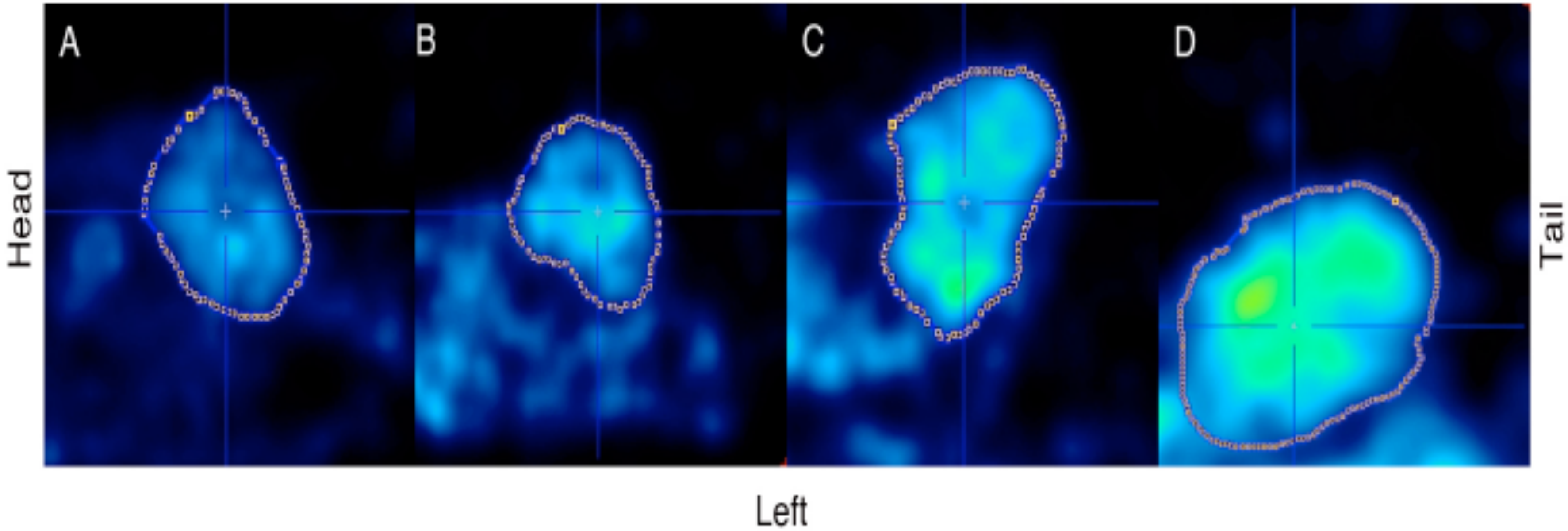


Fig. 3 MicroSPECT images of Colo205-tumors 3.5 hours after injection of 18.5 MBq ^{99m}Tc His-anxA5 in the tail vein. Mice received (A) 0.9% NaCl (=control) 4h before administration of the radiotracer, (B) 5-FU 4h before administration of the radiotracer, (C) oxaliplatin 12h before administration of the radiotracer and (D) irinotecan 24h before administration of the radiotracer. Representative sagittal slices demonstrates accumulation of ^{99m}Tc His-anxA5 in the tumors of untreated (=control) and treated mice.

Vangestel, JNM, 2011

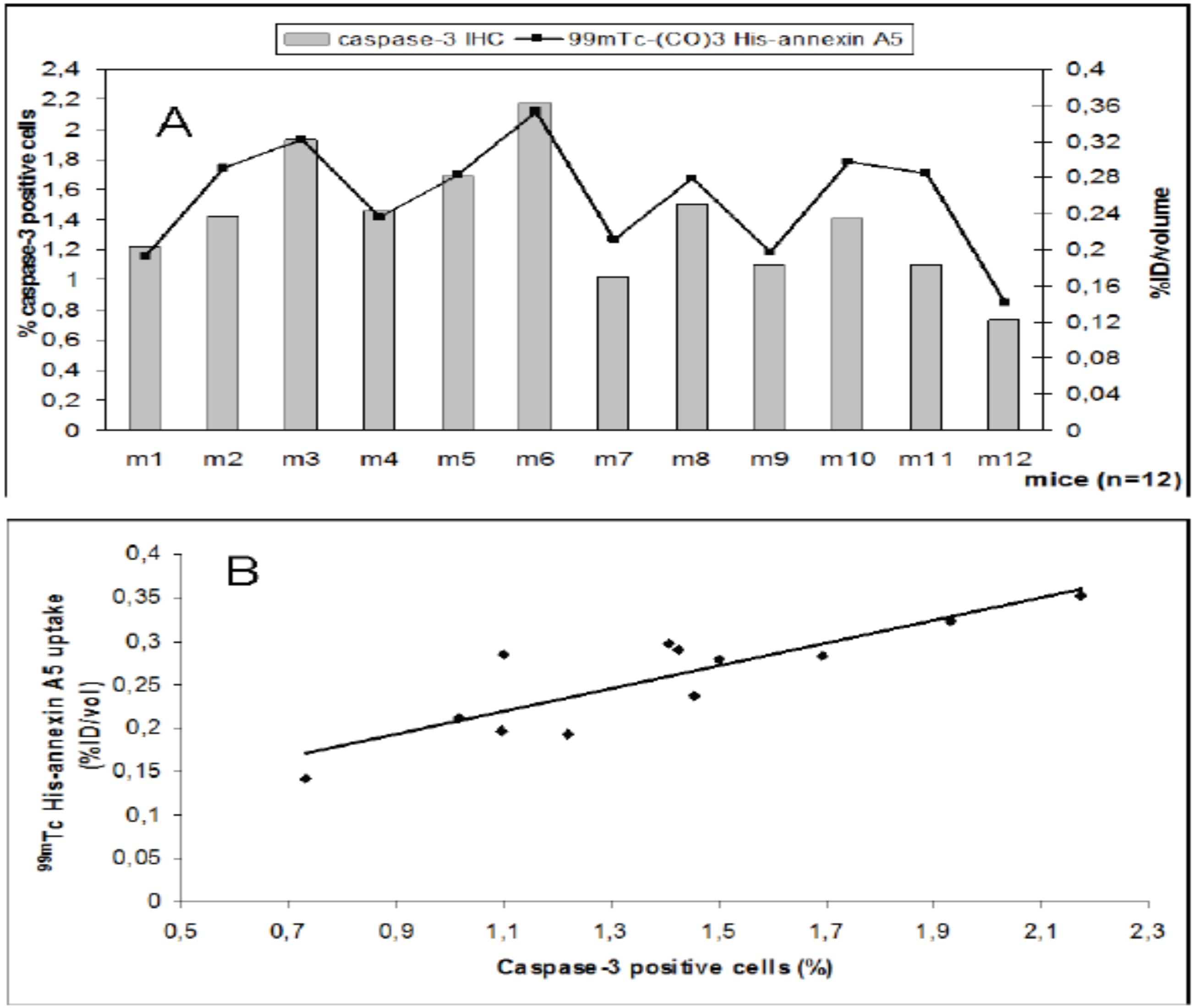


Fig. 7 A) [^{99m}Tc]-(CO)$_3$ His-annexinA5 tumor uptake in all 12 mice is demonstrated, together with percentage of caspase-3 positive cells. [^{99m}Tc]-(CO)$_3$ His-annexinA5 uptake is expressed as %ID/tumor volume.
B) Correlation of [^{99m}Tc]-(CO)$_3$ His-annexinA5 uptake and caspase-3 positive cells in Colo205 tumors. Tumor uptake of [^{99m}Tc]-(CO)$_3$ His-annexinA5 significantly correlated with percentage of caspase-3 positive cells in tumor ($R = 0.867$, $P < 0.01$).

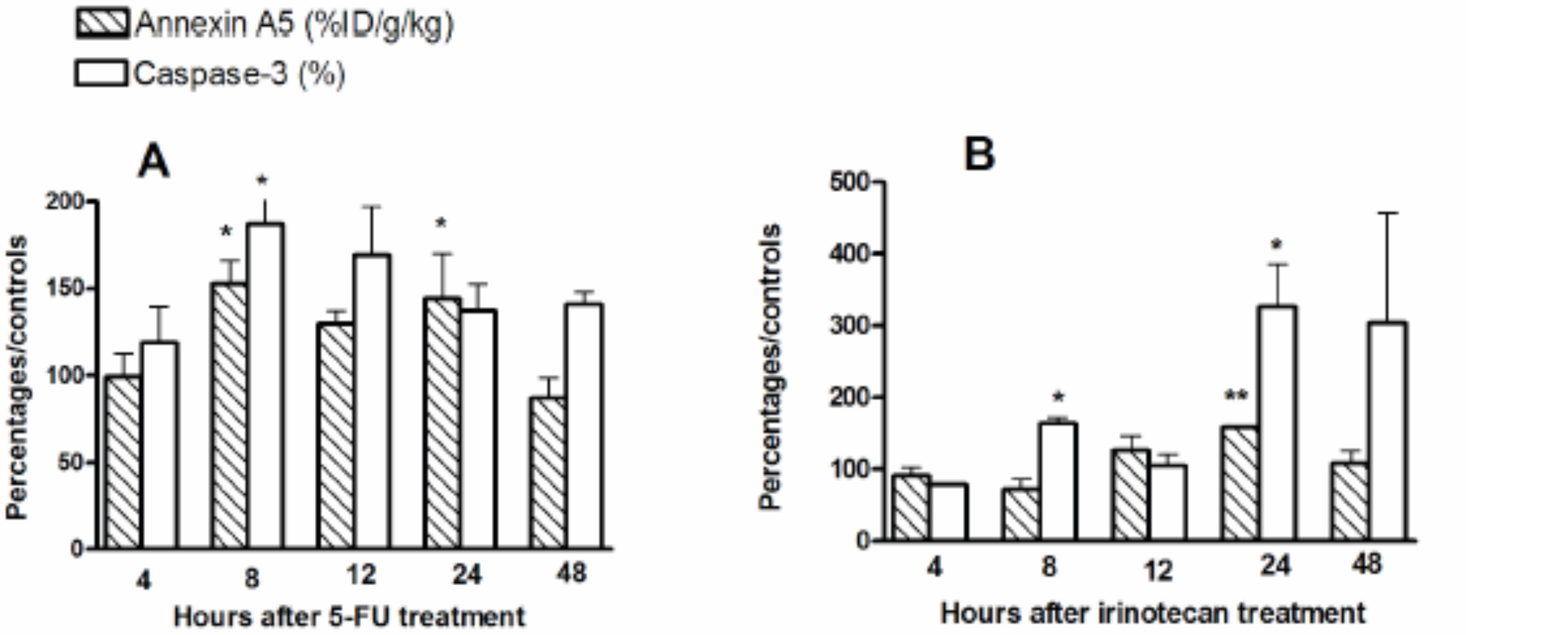
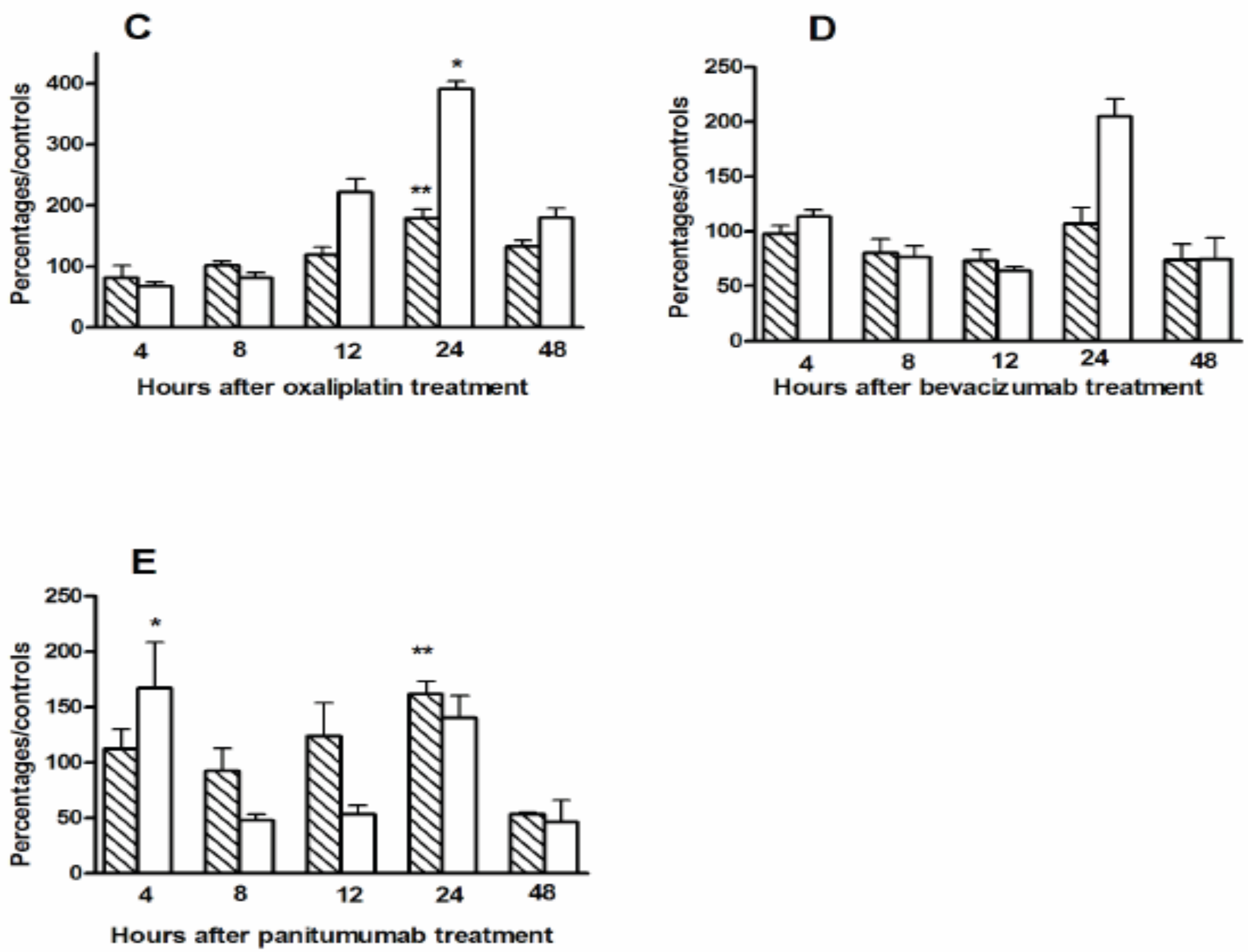


Fig. 2 Time course of ^{99m}Tc His-anxA5 uptake (%ID/g/kg) and caspase-3 positive cells (%), in tumors of Colo205-bearing mice, normalized to controls.
(A) Colo205-bearing mice received single-dose (ip) of 5-FU or, (B) irinotecan or (C) oxaliplatin or (D) bevacizumab or (E) panitumumab (n=3 in each time group)
* $p< 0.05$, ** $p < 0.01$; error bars represent SD

Vangestel, Molecular Imaging, 2012



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Vangestel, Molecular Imaging, 2012

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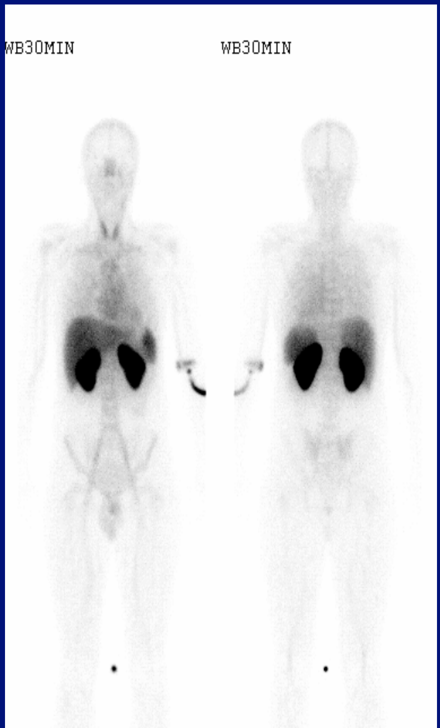
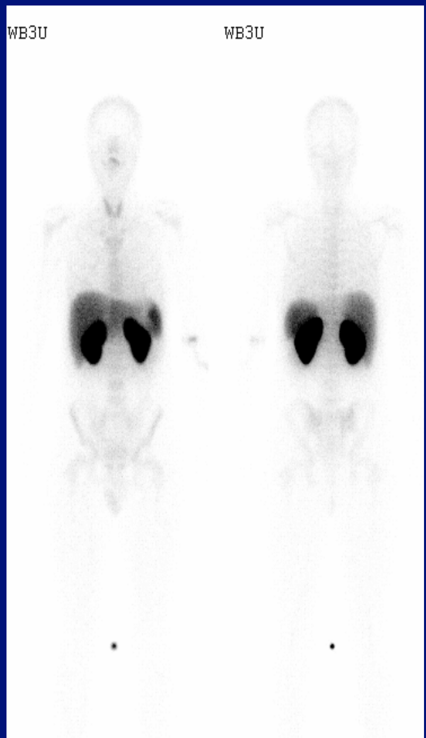
99mTc-Hynic-rh-Annexin V

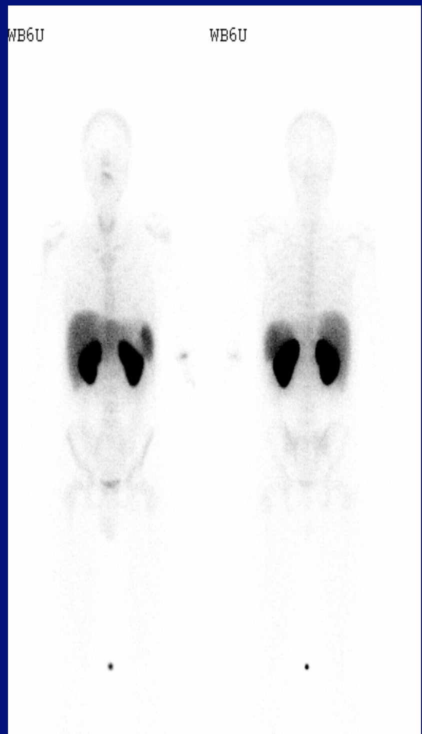
Healthy volunteer



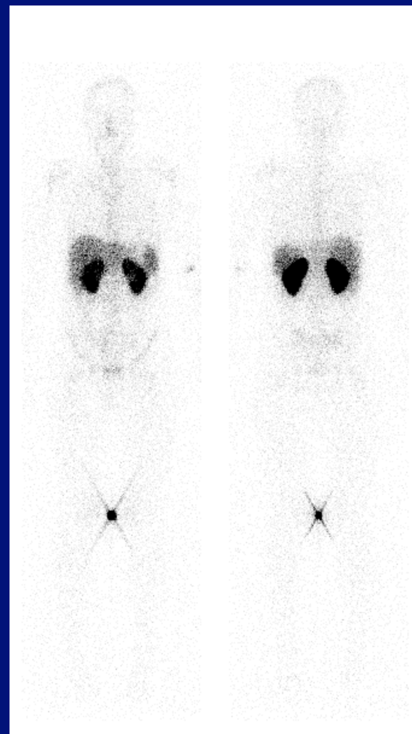
30 min



3 hrs

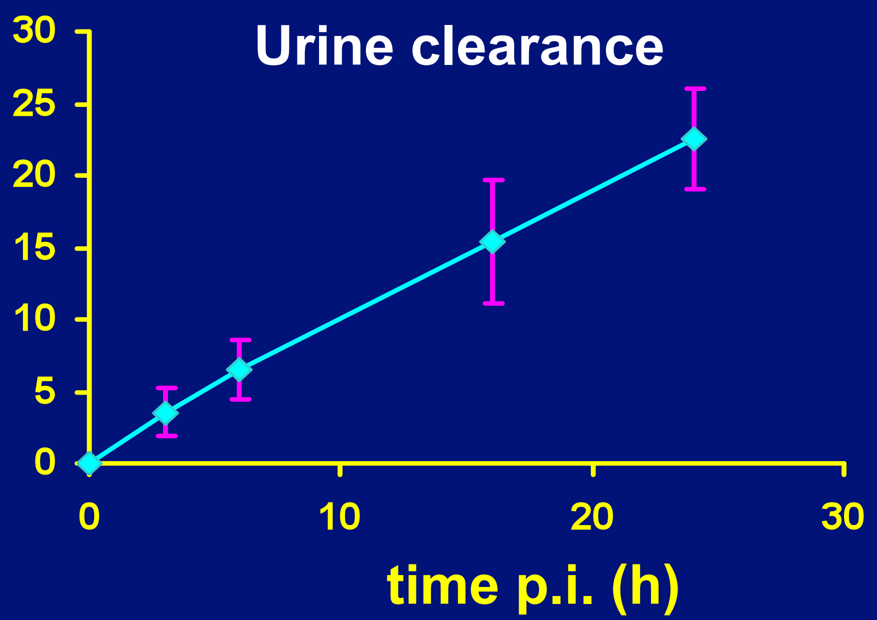


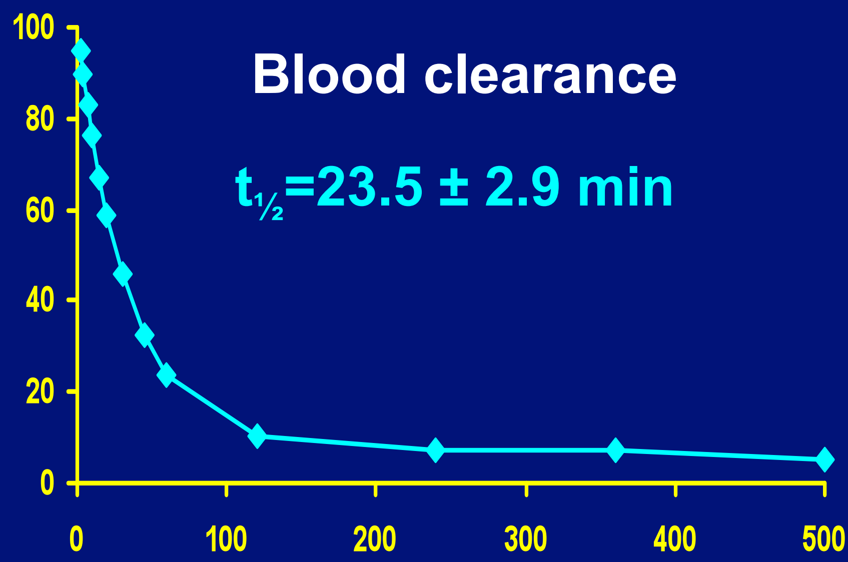
6 hrs



24 hrs

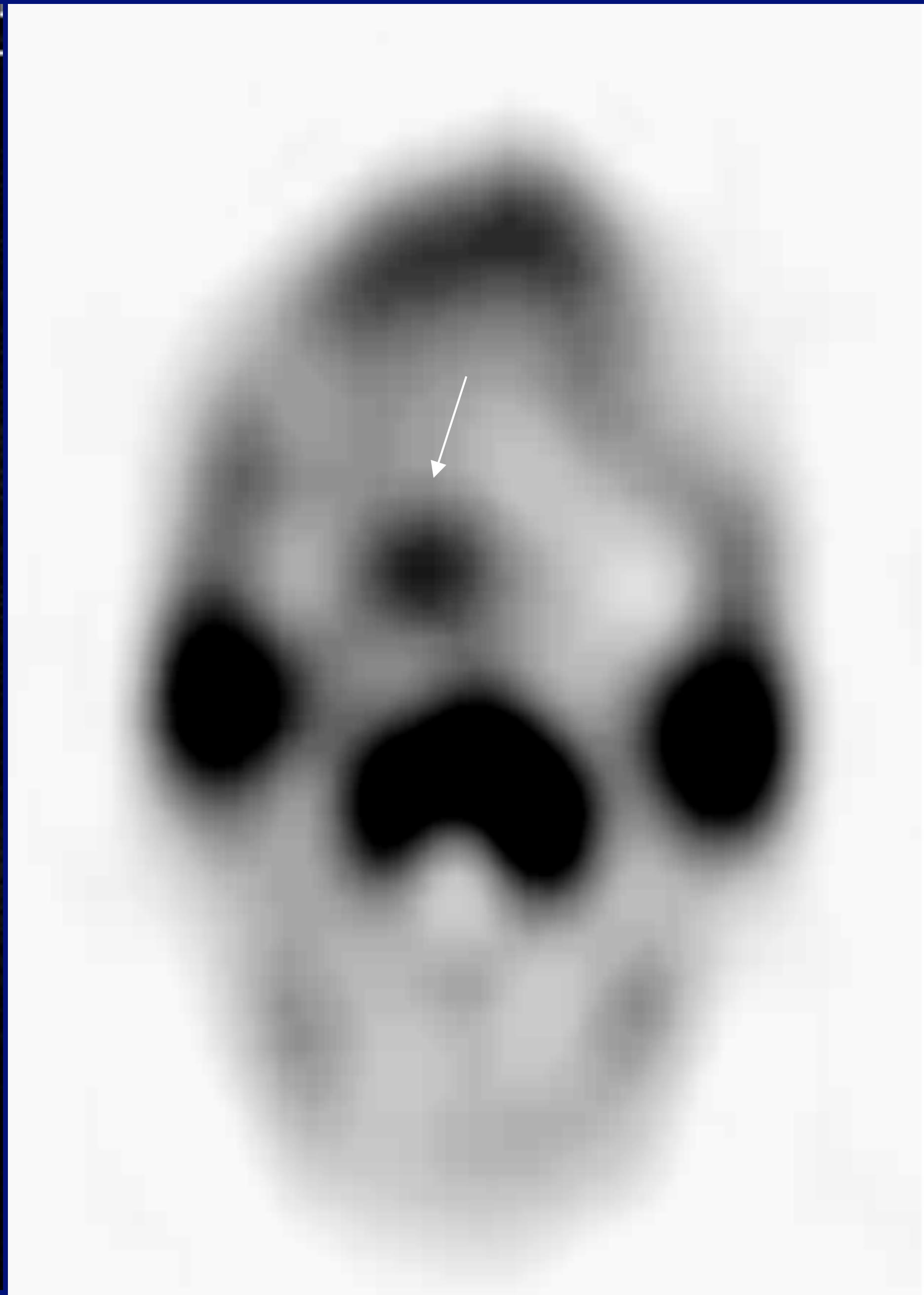
Body clearances in Human volunteers

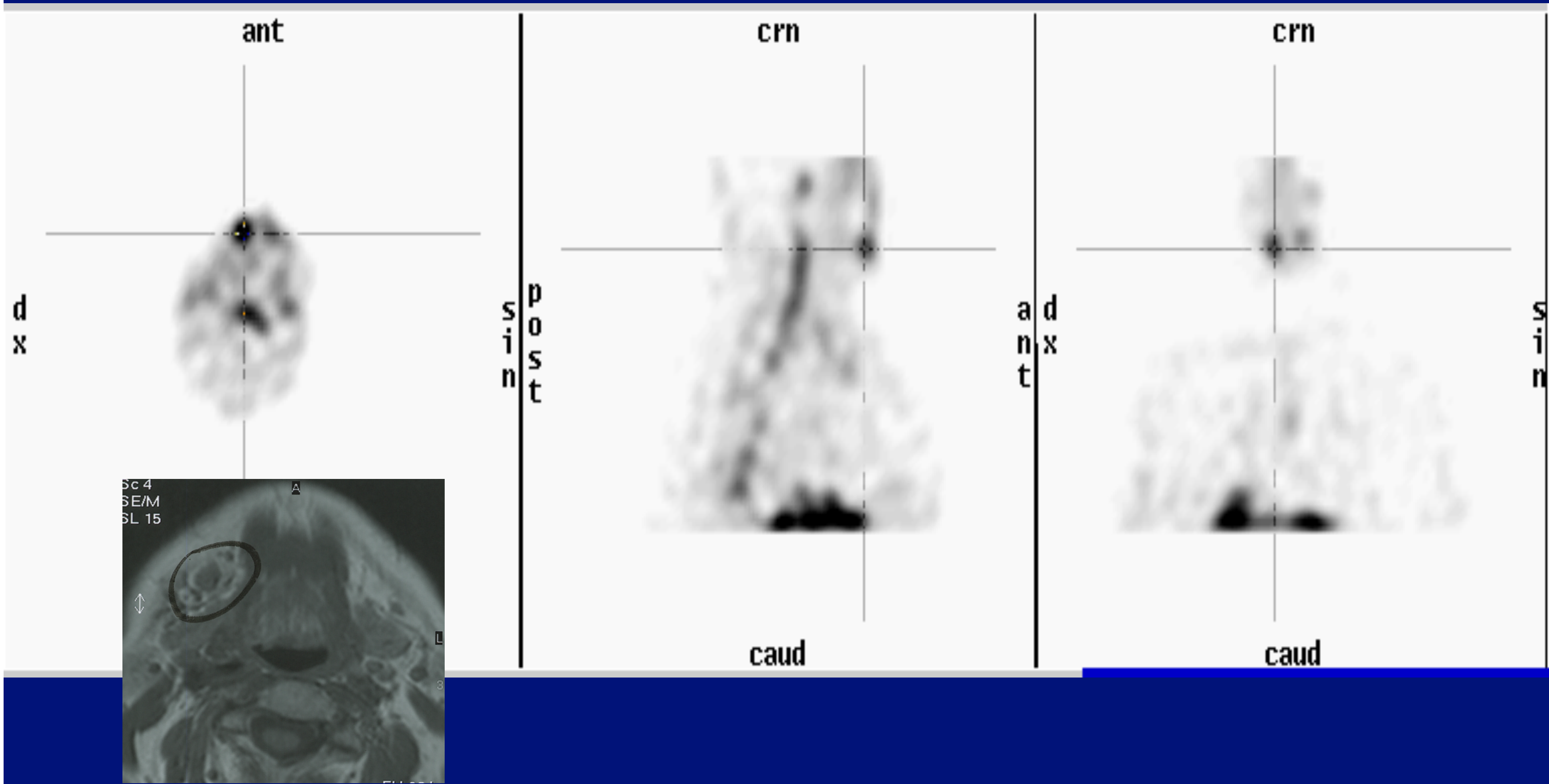




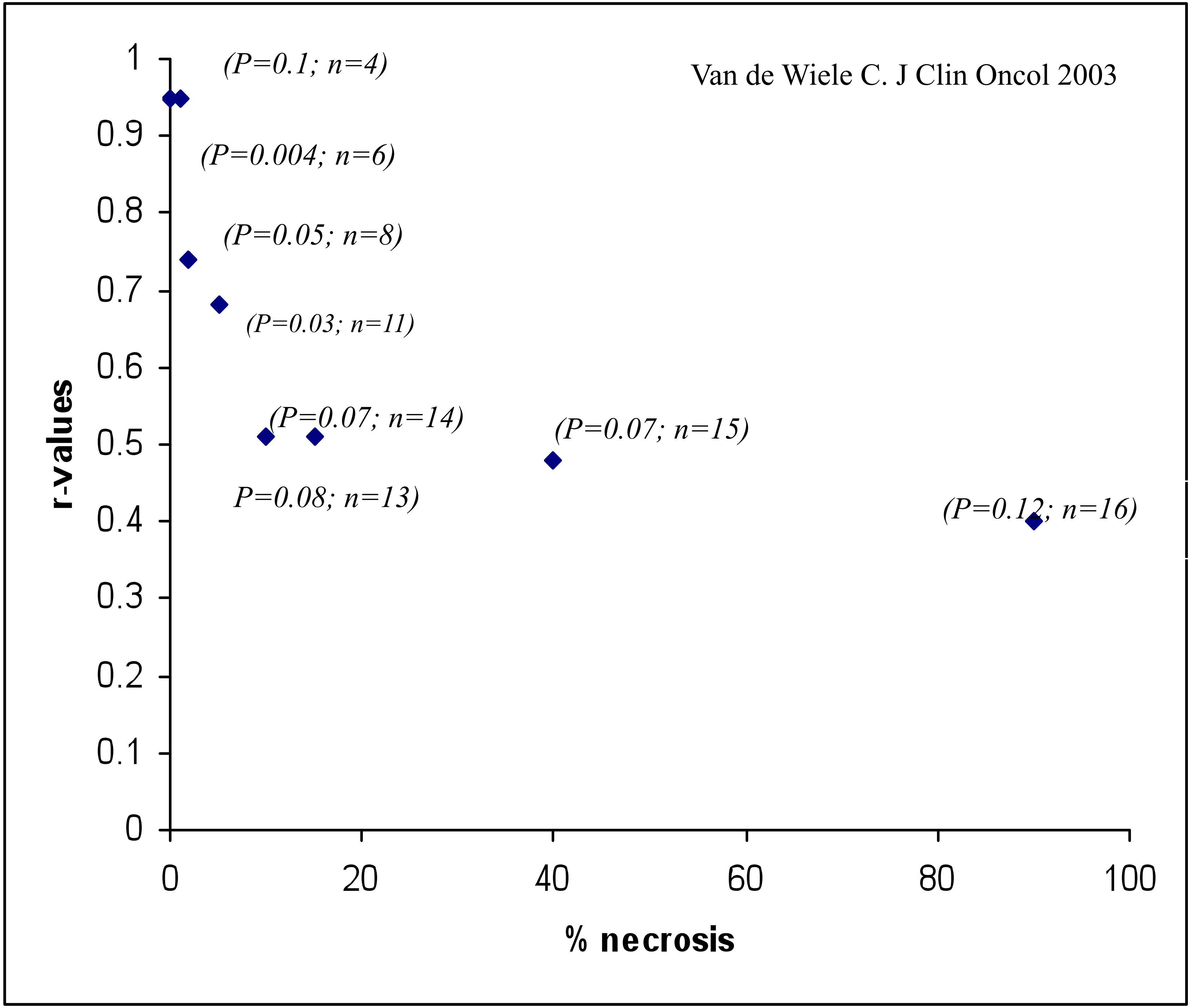
- cumulative urinary excretion after 24 h: 22.5 ± 3.5% of I.D.
- activity in stool after 24 h: 0.1 ± 0.2% of I.D.
- 0.8 rem/mCi to the kidneys


un-1952
ar-2002
:54.39
33
3.5
H-
W

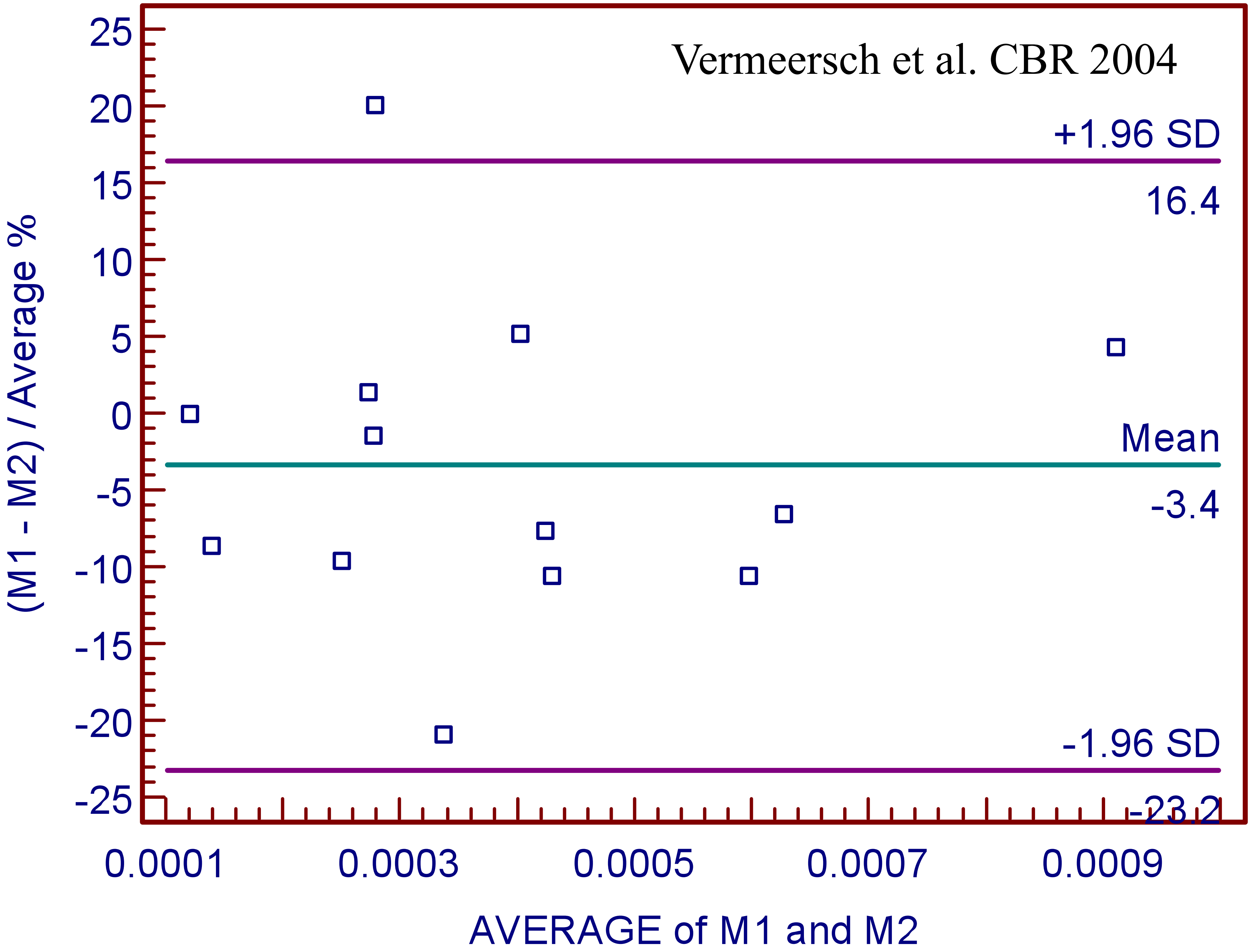


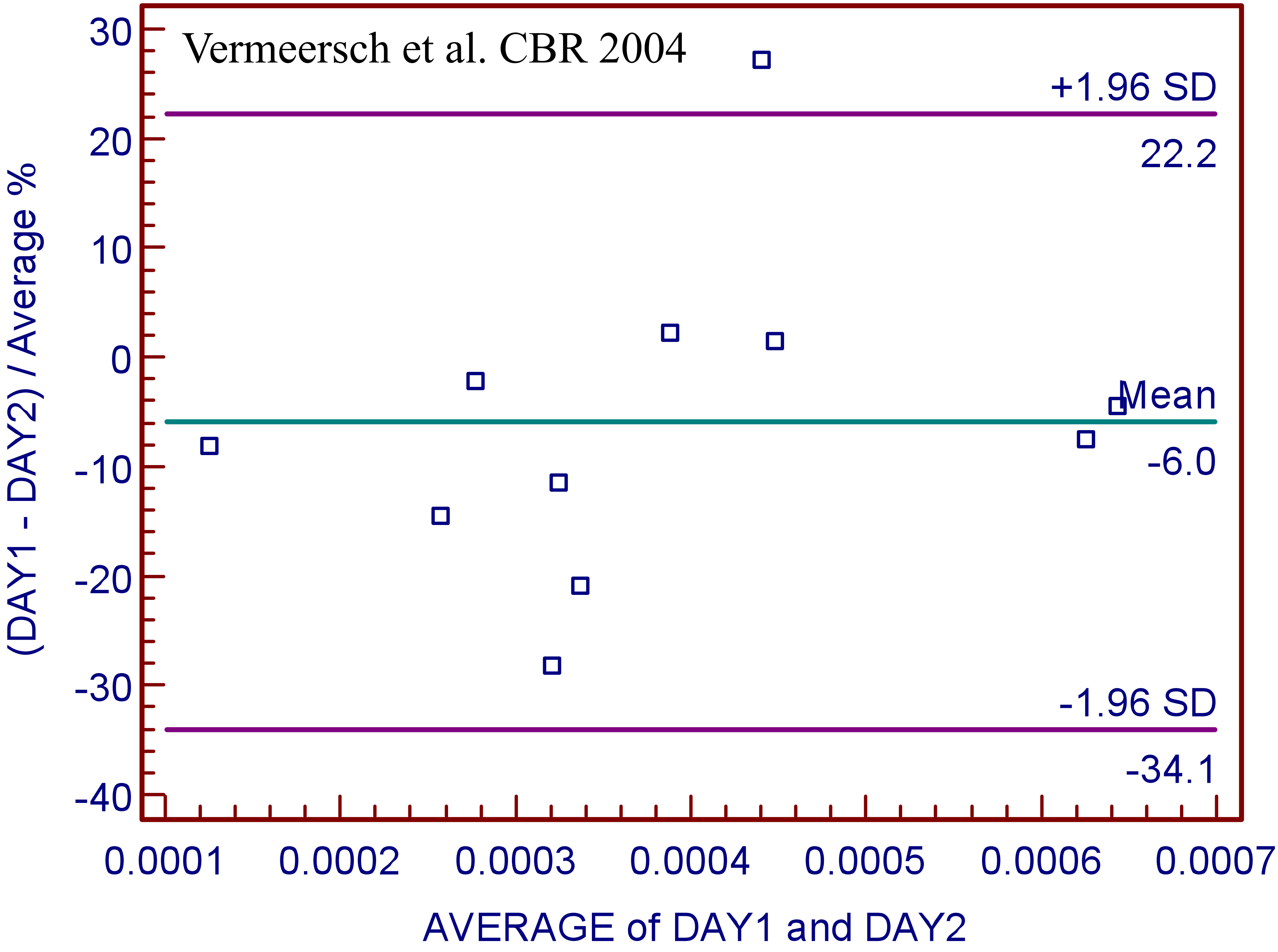


Van de Wiele C. J Clin Oncol 2003



Van de Wiele C. J Clin Oncol 2003
(P=0.1; n=4)
(P=0.004; n=6)
(P=0.05; n=8)
(P=0.03; n=11)
(P=0.07; n=14)
P=0.08; n=13)
(P=0.07; n=15)
(P=0.12; n=16)
r-values
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
% necrosis


Vermeersch et al. CBR 2004
+1.96 SD
16.4
Mean
-3.4
-1.96 SD
-23.2
(M1 - M2) / Average %
25
20
15
10
5
0
-5
-10
-15
-20
-25
0.0001
0.0003
0.0005
0.0007
0.0009
AVERAGE of M1 and M2


Vermeersch et al. CBR 2004
+1.96 SD
22.2
Mean
-6.0
-1.96 SD
-34.1
(DAY1 - DAY2) / Average %
AVERAGE of DAY1 and DAY2
30
20
10
0
-10
-20
-30
-40
0.0001
0.0002
0.0003
0.0004
0.0005
0.0006
0.0007

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Sequential ^{99m}Tc-Hydrazinonicotinamide-Annexin V Imaging for Predicting Response to Chemotherapy

Sylvie Rottey[1], Guido Slegers[2], Simon Van Belle[1], Ingeborg Goethals[3], and Christophe Van de Wiele[3]

[1]*Division of Medical Oncology, Department of Internal Medicine, University Hospital Ghent, Ghent, Belgium;* [2]*Department of Radiopharmacy, University of Ghent, Ghent, Belgium; and* [3]*Department of Nuclear Medicine, University Hospital Ghent, Ghent, Belgium*

J. NucL Med 2006

Patient	Sex	Age (y)	Tumor type	TNM classification	Localization of metastases	Largest diameter (cm) of most prominent lesion	Chemotherapy	Ratio of tumor activity to background activity			Treatment modalities	Response	
								Baseline	5–7 h	40–44 h		3 mo	6 mo
1	M	40	Melanoma	Stage IV	Liver	2.0, no necrosis	Dacarbazine	1	1	1	1	PD	
2	M	60	Bladder cancer	Stage IV	Bone, abdominal mass	3.5, no necrosis	Carboplatin and gemcitabine	1	1	1	1	PD	
3	F	48	Breast cancer	Stage IV	Liver	10.4, no necrosis	Carboplatin	1	1	1	6	PD	
4	M	65	Melanoma	Stage IV	Cutaneous	≤1, multiple lesions	Cisplatin and carmustine	1.41	1.53	1.32	2	PD	
5	F	50	Unknown primary cancer	Stage IV	Liver	4.5, no necrosis	CEF	1.88	1.91	2.57	1	PR	PR
6	F	52	Melanoma	Stage IV	Liver	1.0, no necrosis	Dacarbazine	1	1	1	1	CR	CR
7	F	75	Breast cancer	Stage IV	Liver	0.5, no necrosis	Vinorelbine	1	1	1	3	PD	
8	M	66	Esophageal cancer	Stage IV	Lungs	2.0, no necrosis	5-Fluorouracil and cisplatin	3.39	4.74	2.5	2	PR	PR
9	F	61	Breast cancer	T3 N1 M0		7.8, no necrosis	CEF neoadjuvant	1.77	5.35	2.98	1	PR	PR
10	M	50	Head and neck cancer	Stage IV	Mass in mediastinum	6.5, no necrosis	5-Fluorouracil and cisplatin	0.13	0.11	0.1	1	PD	
11	M	55	Hypernephroma	Stage IV	Bone	Multiple lesions found on bone scintigraphy	Bisphosphonate	1	1	1	1	SD	SD
12	F	52	Breast cancer	Stage IV	Supraclavicular lymph nodes	4.0, no necrosis	Vinorelbine	1	1	1	3	PD	
13	F	52	Small cell lung cancer	Limited disease*	Masses in left lung and mediastinum	8.2, necrosis	Carboplatin and etoposide	3.39	4.74	3.11	1	PR	CR
14	F	52	Breast cancer	Stage IV	Liver, mass in breast	10.3, necrosis	Doxorubicin	1	1	1	2	PD	
15	M	43	Head and neck cancer	Stage IV	Mass in tongue	2.0, no necrosis	5-Fluorouracil and cisplatin	1	1	1	1	PD	
16	M	22	Teratocarcinoma	NA	Mass in mediastinum	9.7, no necrosis	BEP	1.34	3.71	5.25	1	PR	CR
17	M	27	Yolk sac tumor	T2 NX M1a S1	Aortocaval lymph node	2.8, no necrosis	BEP	1	1.3	1	1	CR	CR

*Not TNM classification but 2-stage system for small cell lung cancer used by Veterans Affairs Lung Study Group.

PD = progressive disease; CEF = cyclophosphamide, epirubicin, and 5-fluorouracil; PR = partial remission; CR = complete remission; SD = stable disease; NA = not available; BEP = bleomycin, epirubicin, and cisplatin.

FIGURE 1. Significant increase in uptake of ^{99m}Tc-HYNIC-annexin V over time in primary breast tumor (arrows) in patient 9.

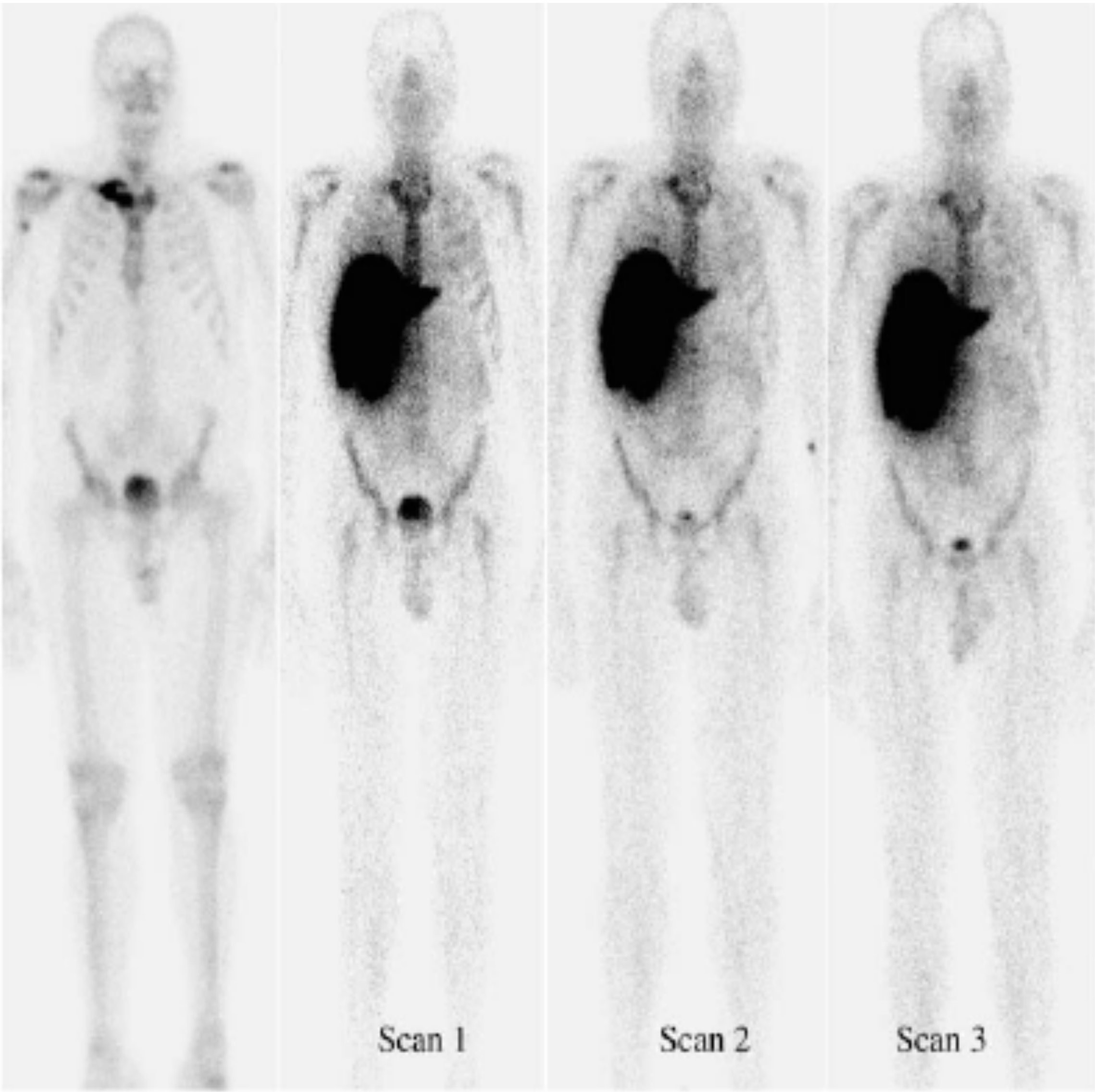


FIGURE 3. Absence of changes in uptake of ^{99m}Tc-HYNIC-annexin V over time in sternoclavicular bone metastasis relative to baseline bone scan (leftmost image) in patient 11.

Prognostic Significance of ^{99m}Tc Hynic-rh-Annexin V Scintigraphy During Platinum-Based Chemotherapy in Advanced Lung Cancer

Marina Kartachova, Nico van Zandwijk, Sjaak Burgers, Harm van Tinteren, Marcel Verheij, and Renato Alfredo Valdés Olmos

J Clin Oncol 2007

Table 1. Patient Characteristics, Changes in the Annexin V Tumor Uptake, and Therapy Outcome

Patient No.	Sex	Age (years)	Stadium	Histology	Localization of the Tumour As Determined by CT and Annexin V SPECT	ΔU%	ΔT% (cm)	RECIST
1	M	64	IIIB	Squamous cell carcinoma	Right upper lobe, mediastinal lymph nodes	15	−20	SD
2	M	72	IV	Adenocarcinoma	Left upper lobe, mediastinal lymph nodes, pleuritis carcinomatosa	0	0	SD
3	M	58	IV	Adenocarcinoma	Right upper lobe, pleuritis carcinomatosa	4	0	SD
4	F	56	IIIB	Large-cell carcinoma	Left upper lobe, mediastinal lymph nodes	16	−33	PR
5	M	52	IV	Adenocarcinoma	Right upper lobe, mediastinal lymph nodes, intrapulmonary metastases	19	−22	SD
6	M	55	IV	Adenocarcinoma	Right upper lobe, intrapulmonary metastases	23	−39	PR
7	M	49	IV	Squamous cell carcinoma	Right upper lobe and left upper lobe	Patient died before follow-up		
8	M	58	IIIB	Adenocarcinoma	Bronchogenic carcinoma (br. intermedius) with invasion of oesophagus	24	−21	SD
9	M	51	IIIB	Large-cell carcinoma	Right upper lobe	−30	3	PD
10	M	68	IIIB	Adenocarcinoma	Right upper lobe, mediastinal and supraclavicular lymph nodes left	−29	0	PD
11	M	52	IV	Large-cell carcinoma	Right upper lobe, mediastinal lymph nodes, intrapulmonary metastases	25	−51	PR
12	M	63	IV	Adenocarcinoma	Right upper lobe, mediastinal lymph nodes, solitary rib metastasis	22	−36	PR
13	F	66	IV	Squamous cell carcinoma	Left lower lobe, mediastinal and subclavicular metastases	Patient refused follow-up scan		
14	M	60	IIIB	Poorly differentiated large-cell carcinoma	Right upper lobe, mediastinal lymph nodes	123	−100	CR
15	F	73	IV	Large-cell carcinoma	Left upper lobe, pleuritis carcinomatosa links and contralateral intrapulmonary metastasis	−6	0	SD
16	M	59	IV	Squamous cell carcinoma	Left head bronchus with invasion of the mediastinum	−1	−6	SD

Abbreviations: CT, computed tomography; SPECT, single-photon emission computed tomography; ΔU, changes in the Annexin V tumor uptake as percentage to baseline value; ΔT, changes in the tumor size as percentage to base line value; RECIST, Response Evaluation Criteria in Solid Tumors Group; M, male; F, female; SD, stable disease; PR, partial response; PD, progressive disease; CR, complete response.

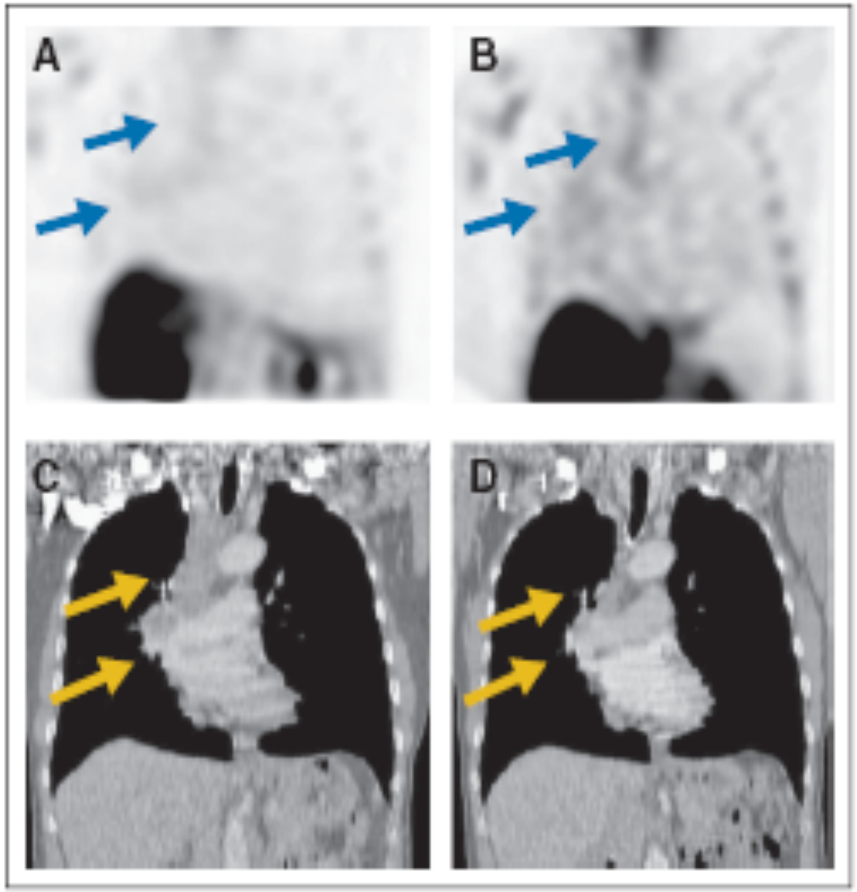


Fig 1. (A) Baseline and (B) follow-up ^{99m}Tc-Hynic-rh-annexin V single-photon emission tomography demonstrate chemotherapy-induced increase of tumor tracer uptake (arrows). (C) Baseline computed tomography demonstrates solid mass in the right upper lobe enlarged mediastinal lymph nodes (arrows). (D) Follow-up computed tomography scan 8 weeks after the start of chemotherapy shows complete response (arrows).

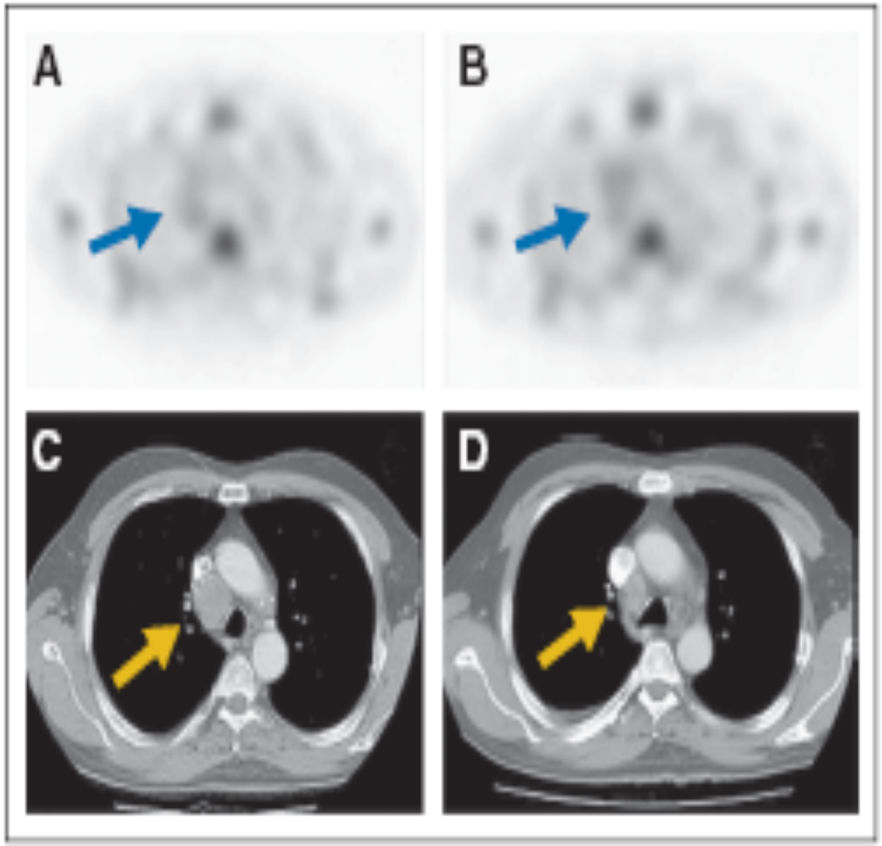


Fig 3. ^{99m}Tc-Hynic-rh-annexin V single-photon emission tomography (A) before and (B) 48 hours after the first administration of cisplatin demonstrate increase of the tumor tracer uptake in the mediastinal lymph nodes (arrows). (C) Baseline computed tomography scan demonstrates a large right paratracheal mass with partial response on (D) follow-up computed tomography scan, obtained 4 weeks later.

ANNEXIN-V IMAGING IN ONCOLOGY

- Basics
- Preclinical data
- **Clinical data**
  - Biodistribution and basic studies
  - Monitoring chemotherapy
  - **Monitoring radiotherapy**
- Future prospects

Changes in 99mTcHYNIC Annexin V tumor uptake versus respons to radiotherapy

- Kartachova et al. (Radiotherapy and Oncology 2004, 72: 333-339)
  - 29 evaluable patients
    - Lymphoma n= 26
    - Leukaemia n=1
    - NSCLC n=5
    - SCCHN n=1
  - Therapy
    - RT n=23
    - Chemo n=5
    - RT+chemo n=1
  - Annexin V scintigraphy before and within 72 hours following treatment instigation= results related to therapy outcome

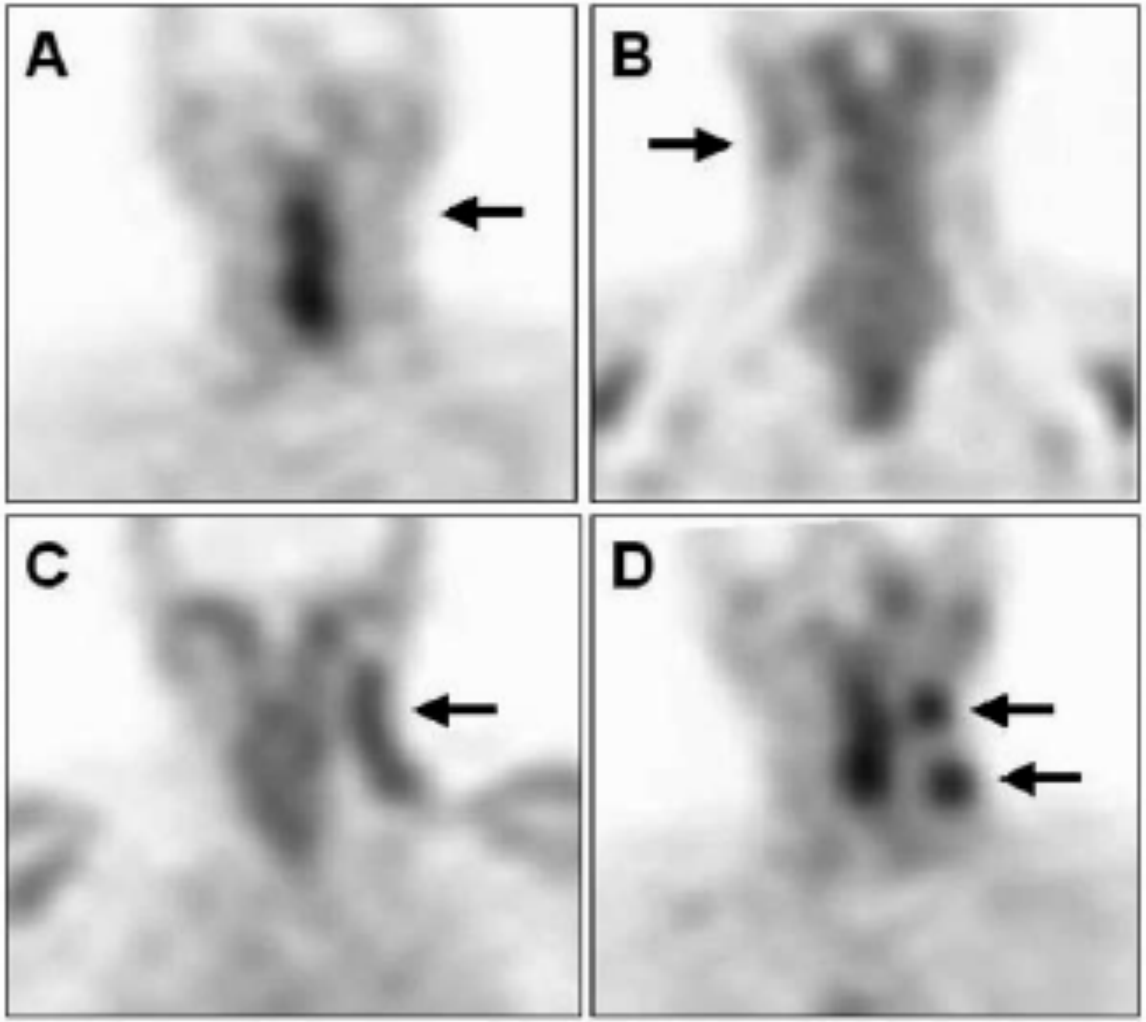
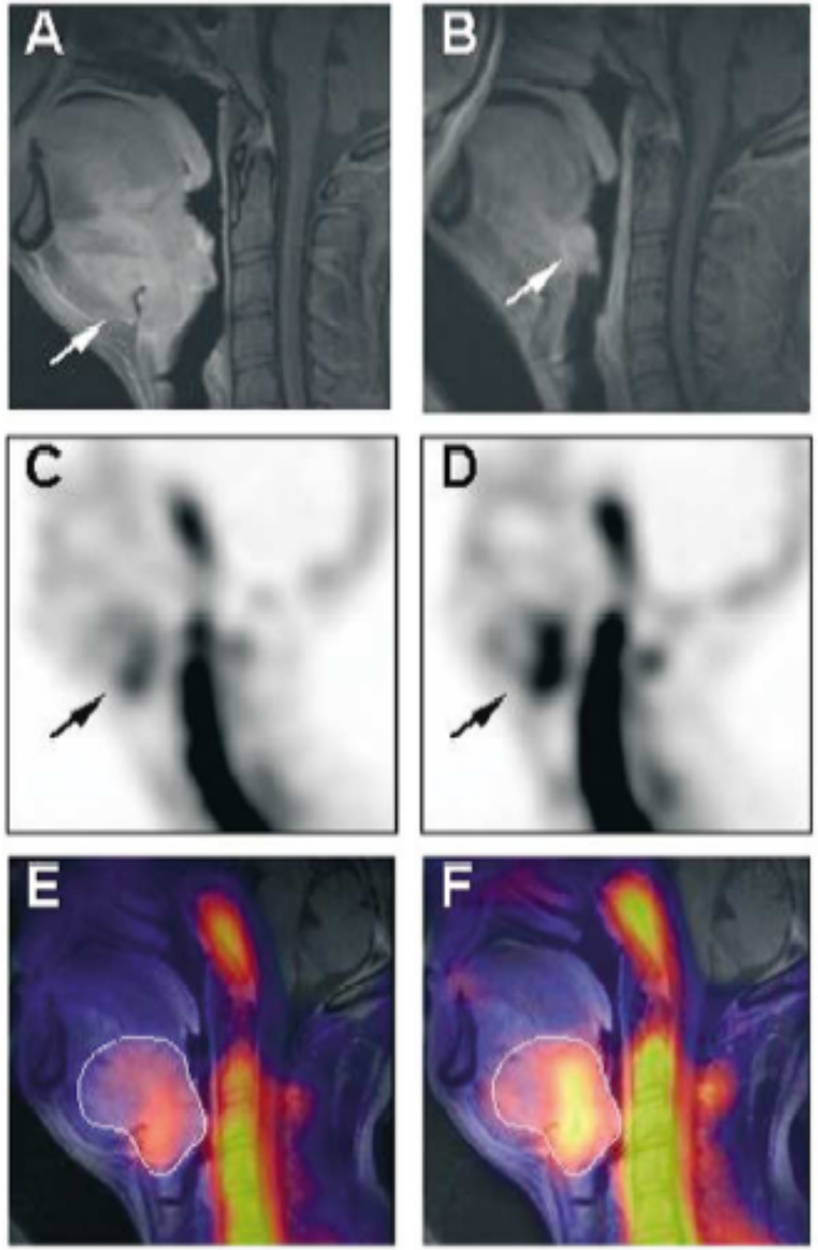
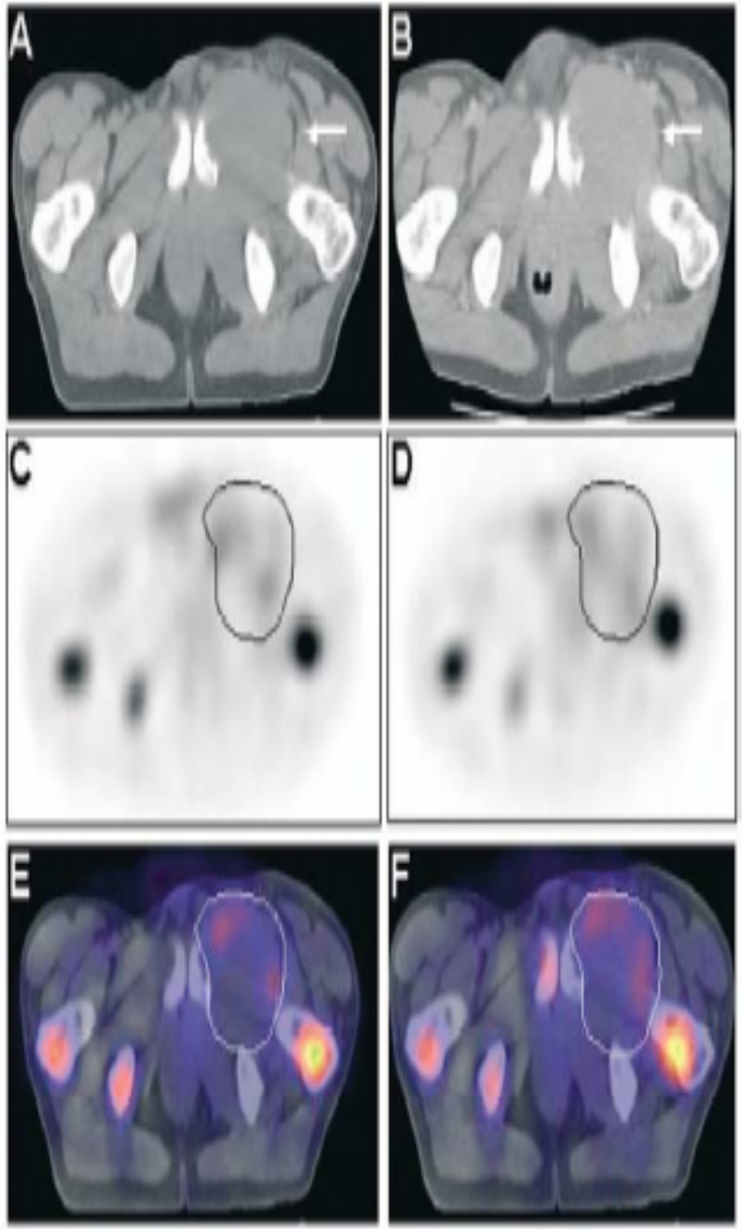


Fig. 1. Four-step grading scale of ^{99m}Tc-Annexin V tumour uptake on SPECT images of patients with malignancies localized in the neck (arrows): A, absent (grade 0); B, weak (grade 1); C, moderate (grade 2); D, intense (grade 3). Note: moderate to intense uptake is also observed in the cervical bone marrow (e.g. A and D).


A
B
C
D
E
F


A
B
C
D
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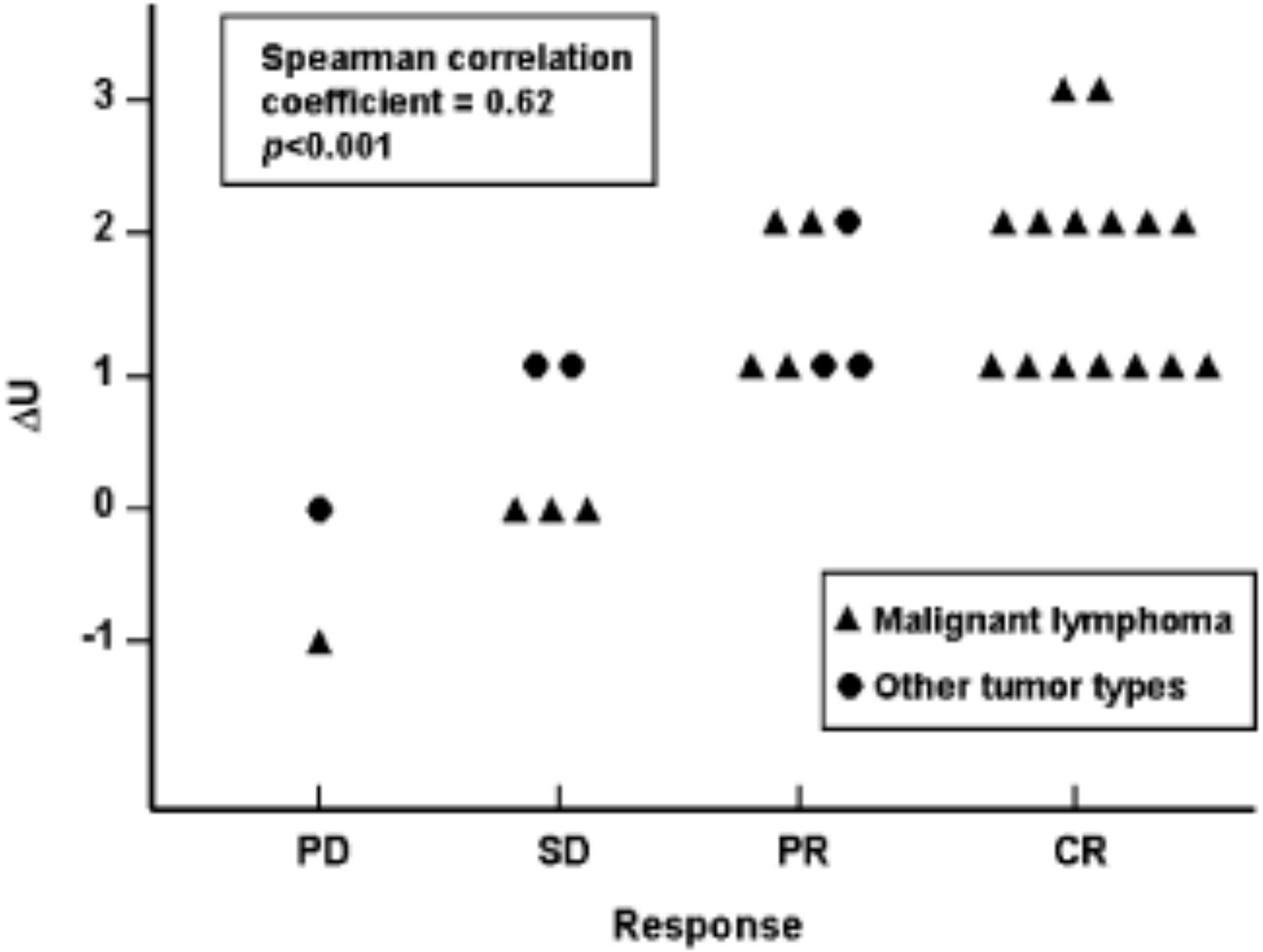

Fig. 5. Schematic correlation between changes in ^{99m}Tc-Annexin V tumour uptake (ΔU) and clinical response. PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

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From SPECT towards PET

- ^{18}F, ^{68}Ga

  - Better characterization apoptosis profile
  - Better resolution
  - Better quantification
  - CT map

CONCLUSIONS

- Limited available data on PET-labelled Annexin-V
- Revival foreseen for PET?