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(71) Applicant (for all designated States except US): **CENTRE LEON BERARD** [FR/FR]; 28, rue Laënnec, F-69008 Lyon (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **DUTOIR, Aurélie** [FR/FR]; 239 cours Emile Zola, F-69100 Villeurbanne (FR). **ROUSSEAU, Raphaël** [FR/FR]; 2 Impasse Jean Cotton, F-69300 Caluire (FR).

(74) Agent: **TETAZ, Franck**; Cabinet Regimbeau, 139 rue Vendôme, F-69477 Lyon Cedex 06 (FR).

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(54) Title: INTRAOPERATIVE DIAGNOSIS OF PRIMARY TUMORS AND SECONDARY TUMORS OR METASTASES

(57) Abstract: The invention relates to the improvement of the detection of the surgical margins of primary tumors as well as to the improvement of the resection of secondary tumors (metastases), most notably pulmonary metastases. The invention notably relates to the use of a RAFT scaffold on which have been grafted both a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins.



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Intraoperative diagnosis of primary tumors and secondary tumors or
metastases

5 The invention relates to improving the detection of the surgical margins of primary tumors as well as improving the resection of secondary tumors (metastases), notably pulmonary metastases.

10 The goal of tumor resection is the complete excision of the tumor with adequate safety margins of healthy tissue. Among patients bearing a primary tumor, the quality of tumor control is correlated with survival. To date, only preoperative evaluation by means of classic imaging techniques (MRI, scanner) enables the surgeon to determine the extent of the safety margins to anticipate. Thus, it is sometimes difficult for the surgeon to
15 distinguish peritumoral edema or inflammation from the tumor itself. This uncertainty generally leads the surgeon to wide resection margins of healthy tissue, making functional recovery problematic (notably in the case of limb resection). To date, no intraoperative method is available or reliable enough to help the
20 surgeon to delimit *in situ* (i.e., during the procedure) with sufficient precision the extension of the tumor in healthy tissue.

Among patients in metastatic relapse, the surgical control of metastatic localizations, notably pulmonary, is also correlated with survival. Currently, the surgical indication is guided by the
25 results of conventional imaging (MRI, CT scanner) whose sensitivity is limited by the sometimes subcentimeter size of the metastatic lesions. With regard to pulmonary metastases, no reliable technique for intraoperative detection exists beyond manual palpation of the pulmonary tissue.

30 In the field of mammary adenocarcinomas, the sentinel node technique makes it possible to detect the propagation of the breast cancer toward the axillary lymph nodes. However, the purpose of the sentinel node technique is not tumor resection. The purpose of this technique is to determine if the breast cancer has propagated toward the axillary lymph nodes. In this technique, 450
35 microcuries of technetium are injected around the tumor followed by 5 ml of isosulfan blue (1%, Lymphazurin dye) 5-10 minutes before axillary dissection. As the sentinel lymph node becomes

mildly radioactive, the surgeon uses a Geiger counter to precisely locate the sentinel lymph node. This enables the surgeon to make a very small incision above the sentinel node. Thus, this is not an intraoperative diagnostic method.

5 These methods have the disadvantage of using radioactive markers and do not have as a goal a true visualization of the tumor but rather aim to provide an indication of the localization of the tumor. Moreover, tumor binding of markers or tracers is generally not uniform and it is not suitable for precisely
10 delimiting surgical margins.

 Conventional methods for determining tumor margins by preoperative imaging and anatomopathological analyses of the excised tumor are thus quite insufficient. To date, genuine intraoperative diagnostic methods for guiding the surgeon during
15 tumor excision have not been described.

 In contrast to the intraoperative field, a number of techniques have been described for the preoperative diagnosis of primary or secondary tumors as well as for the specific targeting of tumors with imaging markers. Others specific ligands of tumor
20 cells have been described for carrying and targeting therapeutic molecules toward tumors.

 Thus, RAFT (regioselectively addressable functionalized template) is a cyclodecapeptide possessing functionalizable amino acid side chains. RAFT is a multifunctional molecule that combines
25 the ability to target specifically and to carry a diagnostic or therapeutic molecule. The RAFT molecule has two functional domains. On the lower face, two motifs are for grafting diagnostic or therapeutic molecules. The upper face or "targeting domain" of the RAFT enables the grafting of four target-specific ligands.
30 RAFT maintains spatial separation between the "targeting" and "therapeutic" functional domains, thus avoiding the situation in which one of the functions disturbs the other (WO 2004/026894).

 In particular, a RAFT scaffold has been described bearing on one of these faces four cyclic RGD motifs for targeting $\alpha_v\beta_3$
35 integrins and on the other face the cyanine 5 (Cy5) fluorophore: RAFT-c(RGD)₄-Cy5 (Garanger *et al.*).

Due to its conformation, RAFT enables the combination targeting/drug/imaging. A single molecule specifically carries the drug and is used to image the tumor or, subsequently, to quantify the real activity of the carried drug by using "intelligent probes." The multivalent presentation of cRGD ligands by the RAFT cyclodecapeptide (RAFT-cRGD) enables specific targeting of tumor neovascularization and tumor cells overexpressing $\alpha_v\beta_3$ integrins. Binding of the cRGD motif to $\alpha_v\beta_3$ integrin is followed by internalization of the RAFT by endocytosis, thus enabling delivery of the therapeutic or diagnostic agent within the target cell. In several animal models, it has thus been shown that it is possible not only to target tumors and their vascularization but also to deliver in a specific and above all targeted manner a diagnostic marker or a molecule of therapeutic interest (Jin et al.).

Thus, RAFT has been envisaged essentially as a system for carrying and targeting molecules of therapeutic interest toward target tumor cells. The use of RAFT or RAFT-cRGD for intraoperative diagnosis has neither been described nor envisaged.

Labeled ligands bound to $\alpha_v\beta_3$ integrins can be used for detection of tumors and for molecular imaging to detect specific targeting of therapeutic molecules to tumors in clinical trials.

A great number of markers, labels and ligands may be used for the detection and molecular imaging of tumors. However, for these various markers it remains to be determined to what extent such molecules enable satisfactory detection of tumor margins in per operative conditions. As discussed above, intra-operative or per-operative diagnostic methods for guiding the surgeon during tumor excision have not been described.

The essential difficulty is to delimit *in situ* with sufficient precision the extension of the tumor in healthy tissue.

Another problem arising is that expression of some receptors/molecules on tumor cells may vary especially after chemotherapy. This has been reported for $\alpha_v\beta_3$ integrins for which chemotherapeutic treatments induce a significant decrease in integrin expression. Labeled ligands of the integrins were therefore not envisioned as particularly useful in per operative detection of tumor margins as patients are often treated by chemotherapy before performing surgery.

Surprisingly, it has now been shown that a RAFT on which is grafted both a ligand of the $\alpha_v\beta_3$ integrins (the RGD motif) and a marker can be used for the intra-operative or per-operative diagnosis of primary and secondary tumors.

5 Even more surprisingly, treatment by chemotherapy does not alter the detection of tumor margins in per-operative conditions.

The techniques of the present invention make it possible to delimit with much better precision the margins of primary and secondary tumors, to facilitate the surgical procedure and,
10 finally, to limit extension of the resection in healthy tissue all while maintaining tumor control.

Moreover, the inventive techniques make it possible to detect intraoperatively the existence of pulmonary micrometastases invisible to the naked eye or not palpable by the surgeon.

15

SUMMARY OF THE INVENTION

The present invention is related to a composition comprising a tracer for use in surgery wherein the tracer comprises a
20 molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.

In a first embodiment, the invention is related to a composition comprising a tracer for use in surgery for the
25 intraoperative detection of the surgical margins of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said intraoperative
30 detection of the surgical margins of a tumor in an individual comprises a) administration to the individual of said tracer, b) circulation of the tracer in the individual and c) detection of the tumor margins in near infrared light.

In a second embodiment, the invention is related to a
35 composition comprising a tracer for use in surgery for the resection of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces

and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection of a tumor in an individual comprises a) administration to the individual of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins
5 in the near infrared and d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control.

In a third embodiment, the invention relates to a composition comprising a tracer for use in surgery for the resection of a
10 tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection of a tumor in an individual comprises a) administration
15 to the individual of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins in near infrared light, d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control, and e) verification of the precision of the resection of
20 the tumor in near infrared light.

Advantageously, said resection of a tumor in an individual comprises a repetition of steps c) to e) for the resection of the same tumor and / or for the resection of several distinct tumors.

In some embodiments, the tumor is an osteosarcoma, an
25 uncategorized sarcoma, a myxoid liposarcoma, a nondifferentiated liposarcoma, a chondrosarcoma, a Ewing's sarcoma, a rhabdomyosarcoma, a hepatic carcinoma, a colorectal carcinoma, a gastrointestinal stromal tumor (GIST), a mammary adenocarcinoma, an ovarian carcinoma or a neuroblastoma.

30 In other embodiments, the tumor is a secondary tumor or a metastasis.

In another embodiment, the tumor is a pulmonary metastasis.

Preferably, the molecular scaffold is a cyclopeptide with two faces, the marker detectable in the near infrared being grafted on
35 one of the faces and the ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.

Preferably, the molecular scaffold is the cyclic decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-] or the cyclic decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Ala-Lys-Pro-Gly-].

Advantageously, the marker detectable in the near infrared is
5 a fluorophore.

More preferred, the marker detectable in the near infrared is Indocyanine Green (ICG).

Advantageously, the ligand of the $\alpha_v\beta_3$ integrins is selected among the peptide Arg-Gly-Asp (RGD), the motif cyclo[RGDfK] and
10 the motif cyclo[RGDyK].

Preferably, the molecular scaffold is the cyclic decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-] having two faces, four cyclo[RGDfK] motifs being grafted on one face and a Indocyanine Green fluorophore being grafted on the other face.

Even more preferred, the molecular scaffold is the cyclic
15 decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-] having two faces, four cyclo[RGDfK] motifs being grafted on one face on the lysine in positions 1, 3, 6, 8 and a Indocyanine Green fluorophore being grafted on the other face on the lysine(s) in
20 position 2 and/or 7.

The invention relates to a method for the intraoperative detection of the surgical margins of a tumor in an individual comprising the following steps:

- 25 a) administration in the individual of a sufficient quantity of a tracer comprising a molecular scaffold on which is grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,
b) circulation of the tracer in the individual,
30 c) detection of the tumor margins in near infrared light.

Another object of the invention is a method for the resection of a tumor in an individual comprising the following steps:

- a) administration in the individual of a sufficient quantity of a tracer comprising a molecular scaffold on which is
35 grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,
b) circulation of the tracer in the individual,
c) detection of the tumor margins in the near infrared, and

- d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control.

Another object of the invention is a method for the resection
5 of a tumor in an individual comprising the following steps:

- a) administration in the individual of a sufficient quantity of a tracer comprising a scaffold on which is grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,
- 10 b) circulation of the tracer in the individual,
- c) detection of the tumor margins in near infrared light,
- d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control, and
- 15 e) verification of the precision of the resection of the tumor in the near infrared.

In a particular embodiment of the invention, the inventive method comprises a repetition of steps c) to e) for the resection of the same tumor and / or for the resection of several distinct
20 tumors.

In an advantageous embodiment, the tumor is an osteosarcoma, an uncategorized sarcoma, a myxoid liposarcoma or a nondifferentiated liposarcoma. In other embodiments, the tumor is a chondrosarcoma, a Ewing's sarcoma, a rhabdomyosarcoma, a hepatic
25 carcinoma, a colorectal carcinoma, a gastrointestinal stromal tumor (GIST), a mammary adenocarcinoma, an ovarian carcinoma or a neuroblastoma.

In another embodiment, the tumor is a secondary tumor or a metastasis.

30 Advantageously, the tumor is a pulmonary metastasis.

Advantageously, the molecular scaffold is a cyclopeptide with two faces, the marker detectable in the near infrared being grafted on one of the faces and the ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.

35 Preferentially, the scaffold is the cyclic decapeptide c[-Lys(Boc)-Lys(Alloc)-Lys-Lys(Boc)-Pro-Gly-Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-].

Advantageously, the marker detectable in the near infrared is a fluorophore.

In a preferred embodiment, the ligand of the $\alpha_v\beta_3$ integrins is selected among the peptide Arg-Gly-Asp (RGD), the motif
5 cyclo[RGDfK] and the motif cyclo[RGDyK].

Still more advantageously, the scaffold carries four cyclo[RGDfK] motifs on one face and an ICG' marker on the other face.

10 DESCRIPTION OF THE INVENTION

The invention relates both to compositions comprising a tracer for use in surgery and to intraoperative methods or surgical methods combining a molecular scaffold, a ligand of the
15 $\alpha_v\beta_3$ integrins, a marker detectable in the near infrared and a near infrared camera. These compositions and methods make it possible to precisely define tumor margins diagnostically and intra-operatively or per operatively (i.e. during surgery).

The present invention is related to compositions comprising a
20 tracer for use in surgery wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face. The compositions comprising a tracer according to the invention are for use in per-
25 operative or intraoperative diagnostic methods for detection of the surgical margins of a tumor during surgery.

The compositions and methods of the present invention provide for improvement of tumor resection thanks to a precise detection of the margins of a tumor during surgery.

30 In a first embodiment, the invention is related to a composition comprising a tracer for use in surgery for the intraoperative detection of the surgical margins of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being
35 grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said intraoperative detection of the surgical margins of a tumor in an individual comprises a) administration to the individual of said tracer, b)

circulation of the tracer in the individual and c) detection of the tumor margins in near infrared light.

In a second embodiment, the invention is related to a composition comprising a tracer for use in surgery for the resection of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection of a tumor in an individual comprises

5 a) administration to the individual of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins in the near infrared and d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control.

In a third embodiment, the invention relates to a composition comprising a tracer for use in surgery for the resection of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection of a tumor in an individual comprises a) administration to the individual of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins in near infrared light, d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control, and e) verification of the precision of the resection of the tumor in near infrared light.

Advantageously, said resection of a tumor in an individual comprises a repetition of steps c) to e) for the resection of the same tumor and / or for the resection of several distinct tumors.

The invention relates also to methods for the intraoperative detection of the surgical margins of a tumor in an individual comprising the following steps:

- a) administration in the individual of a sufficient quantity of a tracer comprising a molecular scaffold on which is grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,
- b) circulation of the tracer in the individual,

c) detection of the tumor margins in near infrared light.

The term "tracer" means the combination marker-scaffold-ligand of the $\alpha_v\beta_3$ integrins. Surprisingly, it has been shown in the present invention that this tracer circulates and is distributed in the organism and tumor tissues in such a way that tumor margins can be detected with precision without observing background noise.

The tracer can be administered in the individual by all commonly used routes of administration. Preferably, the administration is by intravenous route.

A sufficient or efficient amount/quantity of tracer is administered to the individual. This amount is determined according to standard techniques.

Preferably, the quantity of tracer administered to the individual is between 50-1000nmol/kg, preferably between 75-500nmol/kg and even more preferably between 75-150 nmol/kg.

In order to enable distribution or circulation of the tracer in the individual, the tracer is administered for a sufficient length of time before the intraoperative diagnosis of the tumor. This distribution time is typically between 6 and 24 hours, more preferentially between 12 and 24 hours and still more preferentially the distribution time is 18 hours. In preferred embodiments, administration of the tracer is performed at least 6, 9, 12, 18 or 24 hours before surgery to allow for adequate distribution of the tracer in the individual.

The marker is a marker detectable in the near infrared and consequently the detection of tumor margins takes place in near infrared light with a near infra red camera. Advantageously, this detection is carried out using a near infrared camera connected to a computer and a display for visualizing the surgical margins. Such a system can be implemented by placing the camera on an articulated arm that the surgeon can reposition during the operation.

The invention also relates to a method for the resection of a tumor in an individual comprising the following steps:

a) administration in the individual of a sufficient quantity of a tracer comprising a molecular scaffold on which is

grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,

- b) circulation of the tracer in the individual,
- c) detection of the tumor margins in the near infrared, and
- 5 d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control.

The precise definition of the tumor margins obtained using the tracer and detection in the near infrared provides a
10 significant improvement in surgical methods for the resection of primary and secondary tumors such as pulmonary metastases.

The invention also relates to a method for the resection of a tumor in an individual comprising the following steps:

- a) administration in the individual of a sufficient quantity
15 of a tracer comprising an RGD scaffold on which is grafted a marker detectable in the near infrared and a ligand of the $\alpha_v\beta_3$ integrins,
- b) circulation of the tracer in the individual,
- c) detection of the tumor margins in near infrared light,
- 20 d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control, and
- e) verification of the precision of the resection of the tumor in near infrared light.

25 The intraoperative detection of tumor margins enables the surgeon to move back and forth between the resection and the detection of the tumor in order to be sure of the complete resection of the tumor while limiting the resection of healthy tissue.

30 Thus, steps c) to d) can be repeated for the resection of the same tumor and / or for the resection of several distinct tumors.

In the above described methods, detection of the tumor margins and precise resection of the tumor may further be controlled by histopathology analysis.

35 The inventive compositions for use in surgery and the intraoperative diagnostic methods prove particularly effective when the tumor is an osteosarcoma, an uncategorized sarcoma, a myxoid liposarcoma, a nondifferentiated liposarcoma, a

chondrosarcoma, a Ewing's sarcoma, a rhabdomyosarcoma, a hepatic carcinoma, a colorectal carcinoma, a gastrointestinal stromal tumor (GIST), a mammary adenocarcinoma, an ovarian carcinoma or a neuroblastoma.

5 The inventive compositions for use in surgery and the intraoperative diagnostic methods have also been shown effective for secondary tumors or metastases, notably pulmonary metastases.

 The tracer used in the inventive methods consists of a molecular scaffold comprising two faces. Persons skilled in the
10 art are familiar with these molecular scaffolds and will understand that the scaffold can take various forms. The function of the scaffold is to avoid interference between the marker detectable in the near infrared and the ligand or ligands of the $\alpha_v\beta_3$ integrins. In addition, the scaffold improves the presentation
15 of the ligands of the $\alpha_v\beta_3$ integrins and their binding with the tumor cells or tumors. Typically, the scaffold has two faces, a marker detectable in the near infrared being grafted on one face and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.

 In a preferred embodiment, the molecular scaffold is a
20 cyclopeptide with two faces, the marker detectable in the near infrared being grafted on one of the faces and the ligand(s) of the $\alpha_v\beta_3$ integrins being grafted on the other face.

 Preferably, the scaffold is a RAFT scaffold (RAFT cyclic
decapaptide) as described in WO 2004/026894 or by Garanger *et al.*

25 RAFT (regioselectively addressable functionalized template) is a cyclodecapeptide possessing functionalizable amino acid side chains. RAFT is a multifunctional molecule that combines the ability to target specifically and to carry a diagnostic or therapeutic molecule.

30 The RAFT molecule has two functional domains. On the lower face, one or two motifs are for grafting diagnostic or therapeutic molecules. The upper face or "targeting domain" of the RAFT enables the grafting of four target-specific ligands. RAFT maintains spatial separation between the "targeting" and
35 "therapeutic" functional domains, thus avoiding the situation in which one of the functions disturbs the other.

 In preferred embodiments the scaffold is a RAFT cyclic
decapaptide. Advantageously, the scaffold is the cyclic

decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-].
Particularly advantageously, the scaffold is the cyclic
decapeptide c[-Lys(Boc)-Lys(Alloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-
Lys(Alloc)-Lys(Boc)-Pro-Gly-] wherein protection of the lysine in
5 positions 1, 3, 6 or 8 and with a protective group such as Boc and
protection of the lysine in positions 2 and 7 with a protective
group such as Alloc results in RAFT molecules having two
orthogonally addressable domains pointing on either side of the
cyclopeptide backbone. Typically, four ligands of the $\alpha_v\beta_3$
10 integrins (such as an RGD motif or a cyclic RGD motif) are
grafted on the lysines in positions 1, 3, 6 and 8 for recognition
and binding to the integrin whereas a marker detectable in near
infrared is grafted on the lysine in position(s) 2 and/or 7.

In other embodiments, the scaffold is c[-Lys-Lys-Lys-Pro-Gly-
15 Lys-Ala-Lys-Pro-Gly-]. Typically, four ligands of the $\alpha_v\beta_3$
integrins (such as an RGD motif or a cyclic RGD motif) are grafted
on the lysine in positions 1, 3, 6 and 8 for recognition and
binding to the integrin where as a marker detectable in near
infrared is grafted on the lysine in position 2.

20 Other scaffolds or vectors can be envisaged for the tracer
used in the inventive compositions and methods. In particular,
liposome vectors comprising in their membrane specific antibodies
to tumor epitopes can be envisaged.

The tracer also comprises a marker detectable in the near
25 infrared (NIR). This is typically a near infrared emitting
fluorophore such as Cy5 or ICG (Indocyanine Green). Indocyanine
Green has excitation/emission wavelengths of 780-830 nm.
Advantageously, this fluorophore has received regulatory approval.

The $\alpha_v\beta_3$ integrins have been the subject of a number of
30 studies due to their role in tumor angiogenesis and in the
formation of metastases. Ligands of the $\alpha_v\beta_3$ integrins have thus
been studied and implemented for the preoperative detection of
tumors, for therapy or for carrying and targeting therapeutic
molecules toward tumors.

35 In the present invention the extraordinary effectiveness of
ligands of the $\alpha_v\beta_3$ integrins for marking and defining tumor
margins is shown in an astonishing manner.

Numerous ligands of the $\alpha_v\beta_3$ integrins have been described in the literature and are well known to persons skilled in the art. In a preferred embodiment of the invention, the ligand of the $\alpha_v\beta_3$ integrins is selected among the peptide Arg-Gly-Asp (RGD) or among
5 cyclic RGD motifs such as cyclo[RGDfK] and cyclo[RGDyK]. The motif cyclo[RGDfK] or cyclo(-Arg-Gly-Asp-D-Phe-Lys) has been described by Van Hagen *et al.* Int. J. Cancer 90, 186 (2000) and Hu *et al.* Biochem. 39, 2284 (2000). The motif cyclo[RGDyK] or cyclo(-Arg-Gly-Asp-D-Tyr-Lys) has been described by Chen *et al.* Bioconjug. Chem
10 15, 41 (2004). Particularly advantageously the ligand is the RAFT cyclopeptide cyclo[RGDfK].

Preferably, the tracer implemented in the inventive compositions and methods has several copies of the cyclic RGD (cRGD) motifs, which have a greater affinity for the integrins
15 than the non-cyclic RGD motif.

Advantageously, the tracer is ICG-RAFT-c(RGD)₄ or ICG-RAFT-c(-RGDfK)₄; RAFT-c(-RGDfK)₄ notably has been described by Garanger *et al.* and in WO 2004/026894. Four cyclo[RGDfK] motifs are exposed on one of the faces of a RAFT scaffold to which they
20 are bound by an oxime bond (-O=N=C-). The other face of the RAFT carries a marker detectable in the near infrared such as Indocyanine Green (ICG or ICG').

In preferred embodiments of the invention, the tracer comprises a scaffold which is a RAFT cyclic decapeptide having the
25 sequence c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-] or c[-Lys-Lys-Lys-Pro-Gly-Lys-Ala-Lys-Pro-Gly-]. The tracer further comprises four cyclic RGD (cRGD) motifs grafted on the lysine in positions 1, 3, 6 and 8. Preferably, the four cyclic RGD motifs are c(RGDfK) motifs. A marker detectable in near infrared (NIR) is
30 grafted on the other surface of the cyclic decapaptide on the lysine(s) in positions 2 and/or 7.

The multivalent presentation of cRGD ligands by the RAFT cyclodecapeptide (RAFT-cRGD) makes it possible to specifically
35 target tumor neovascularization and tumor cells overexpressing the $\alpha_v\beta_3$ integrins. Binding of the cRGD motif to the $\alpha_v\beta_3$ integrin is followed by internalization of the RAFT by endocytosis, thus enabling the delivery of the diagnostic agent within the target cell.

The invention is also related to a tracer or to a composition comprising a tracer as described herein above.

FIGURES

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Figure 1: Specific tumor localization of RAFT-cRGD-ICG' administered by IV route. Exposure of the tumor zone under the NIR camera makes it possible to distinguish transcutaneously the tumor/muscle limit (B) invisible under white light (A). Tumor localization specificity is confirmed by detection of the light signal in the tumor only whereas healthy muscle in contact (M) has no interfering signal *in situ* (C). Image analysis (D) shows the accumulation of RAFT-cRGD-ICG' in the tumor and its absence in healthy tissues in direct contact.

15

Figure 2: The combination of RAFT-cRGD-ICG' with the NIR probe makes it possible to significantly decrease ($p < 0.01$) the size of the tumor fragments resected compared to the size of the fragments determined by preoperative imaging.

20

Figure 3: Detection of pulmonary metastases using the near infrared probe. After thoracotomy, exposure of the lungs under near infrared light makes it possible to distinguish millimeter-size metastases invisible under white light (arrow). Comparison of number of metastases (M Pulm) detected according to the conventional technique or with the near infrared probe (RAFTs + NIR) for 9 rats is also shown in table I.

25

Figure 4: Tumor resection under near infrared lighting after intravenous injection of RAFT-cRGD-ICG'. (A) The 4-5 mm primary tumor, invisible under white light, is visible directly beneath the skin when the zone of interest is exposed to near infrared light. (B) The tumor margins appear clearly under near infrared lighting; no signal is detected in the muscles surrounding the tumor. This distinction makes it possible to perform an optimal resection of the primary tumor (C). The specificity and sensitivity of RAFT-cRGD-ICG' are demonstrated by the detection of

35

millimeter-size pulmonary metastases under near infrared light whereas no signal is detectable in the surrounding tissue (D).

Figure 5: Quantification of the fluorescence signal in tumor and healthy tissues. No significant difference in the quantity of intra-tumor signal is observed between the control group (mean tumor signal: 302+/- 25 RLU/pix/ms) and the group having received the chemotherapy treatment (mean tumor signal: 273 RLU/pix/ms).

Figure 6: Relative expression of genes of αv integrin/reference gene (HPRT) and genes of $\beta 3$ integrin/reference gene (HPRT) in control groups (A) and those treated with ifosfamide (B).

EXAMPLES

15

Example 1

All elements of the procedure will be carried out by experimenters experienced in the handling of animals, according to good laboratory practices, under suitable anesthesia and analgesics, and after agreement of the ethics committee of the Lyon veterinary school.

Objective 1. Improve the detection of the surgical margins of the primary tumor by means of diagnostic nanoparticles.

We will use an animal model that accurately reproduces the clinical operational context. *Animal model.* Our model of metastatic osteosarcoma in the rat has been described previously. In brief, the primary tumor is obtained by the transplantation of a tumor graft (0.5 mm³) in the paratibial position on 3-week-old immunocompetent rats. This model has the advantage of perfectly mimicking the human osteosarcoma due to its rapid growth, its hypervascularized and chemoresistant character and its high metastatic potential. Four weeks after implantation of the tumor graft pulmonary metastases are observed in 90% of the animals. We have recently shown that non-invasive medical imaging techniques make it possible to follow the response of the tumor to a therapy. *Determination of doses and administration times of RAFT-cRGD-ICG'*

RAFT-cRGD-ICG' molecules will be injected by intravenous route according to a dose escalation protocol (three levels:

0.25 nmol/g, 0.5 nmol/g and 0.75 nmol/g, 3 rats per level). We will measure by *in vivo* spectrometry the intra-tumor accumulation kinetics of RAFT-cRGD-ICG' in order to determine the optimal intra-tumor signal/noise ratio.

5 *Detection of tumor margins*

We will compare the quality and effectiveness of the surgical resection using *in vivo* intraoperative imaging with conventional techniques (*i.e.*, quality of resection margins by fluorescent marking on anatomopathological slices after excision visually).

10 The primary tumor will be resected 10 days after implantation of the tumor graft. The animals of the treated group will receive four hours before the procedure an IV injection of RAFT-cRGD-ICG' at the established concentration. The surgical procedure will be carried out using the near infrared probe. The comparison with the
15 control group (*i.e.*, RAFT-cRGD without ICG') will involve the anatomopathological analysis of the tumor margins (comparison of the fluorescence due to the presence of ICG' nanoparticles and classic H&E counterstaining) and the comparison of resected tumor volumes.

20 ***Expected results***

At the end of these experiments, we expect to have determined: i) the improvement of the tumor margins and of the resection of the primary tumor (*e.g.*, less resection of healthy tissue, better tumor control) in the rats when the tumor margins
25 are visualized using RAFT-cRGD-ICG' molecules detected by the near infrared probe compared to conventional methods for determining tumor margins (*i.e.*, preoperative imaging and anatomopathological analyses of the excised tumor).

30 ***Objective 2. Improve the resection of pulmonary metastases by means of diagnostic nanoparticles.***

On the basis of the RAFT-cRGD-ICG' doses and injection times established above, we will compare the sensitivity of the near infrared probe with the standard surgical technique (*i.e.*, detection visually and by palpation) for detecting pulmonary
35 metastases in our model of metastatic osteosarcoma in the rat. The animals of the control group will receive RAFT-cRGD without ICG'. The animals of the experimental group will receive RAFT-cRGD-ICG'. Four hours after administration of RAFT, the animals will be

5 euthanized, the lungs will be removed and the number of pulmonary metastases will be determined according to the conventional method (counting of detectable metastases visually and by palpation) or by means of the near infrared probe. Next, the resection of pulmonary metastases will be performed on the anesthetized animals. The surgical procedure will be carried out using the near infrared probe for the animals of the treated group (having received an intravenous injection of RAFT-cRGD-ICG') or according to the conventional technique for the control group. The quality of the intraoperative resection by means of the near infrared probe will be evaluated by comparing the number of persistent metastases/not detected *in situ*. This comparison will involve anatomopathological and fluorescence analyses. To us it does not appear possible to evaluate the benefit to the survival of animals in which pulmonary metastases have been resected by one or the other of the approaches as the rat model does not lend itself to keeping alive animals having undergone a thoracic surgical approach.

Expected results

20 At the end of these experiments, we expect to have determined the improvement in the detection of pulmonary metastases by means of RAFT-cRGD-ICG' compared to simple visualization and to intraoperative palpation. Due to the limitations of the animal model, a benefit in terms of survival could be determined only during a future phase I test in humans.

RESULTS OBTAINED

Objective 1. Improve the detection of the surgical margins of the primary tumor by means of diagnostic nanoparticles.

30 We confirmed the specific tumor localization of RAFT-cRGD-ICG' molecules injected by intravenous route (**Figure 1**). Exposure of the tumor zone under NIR light makes it possible to visualize transcutaneously the tumor/muscle limit (**Figure 1B**), invisible under white light (**Figure 1A**). Tumor localization specificity of RAFT-cRGD-ICG' is confirmed by the detection of the fluorescent signal only in the tumor (**Figure 1C-D**), whereas no signal is present in the healthy muscles in contact with the tumor (**Figure 1C-D**).

Detection of tumor margins.

We resected the primary tumor of 9 rats bearing osteosarcoma using intraoperative imaging. Anatomopathological analyses (localization of fluorescence due to the presence of ICG' nanoparticles and classic H&E counterstaining) confirmed the quality of the resection. The comparison of resected tumor volumes using intraoperative imaging compared to those determined by conventional preoperative imaging (MRI) shows a significant benefit (*i.e.*, a significant decrease in the size of the resected fragment, $p < 0.01$) in favor of the resection carried out under intraoperative guidance (**Figure 2**).

Objective 2. Improve the resection of pulmonary metastases by means of diagnostic nanoparticles.

We were able to show that the near infrared probe makes it possible to visualize *in situ* millimeter-size metastases invisible under white light (**Figure 3**). We established that the number of metastases visualized by the near infrared probe is significantly ($p < 0.01$) greater than that detected by the standard technique (*i.e.*, detection visually and by palpation). This is shown by the table below.

Rat ID	M Pulm	M Pulm (RAFTs + NIR)
A-1	3	6
A-2	0	0
A-3	9	12
A-4	2	4
A-5	5	9
A-6	5	9
A-7	0	0
A-8	0	4

Table I

Example 2: Results obtained with RAFT-cRGD₄

In several animal models, we show that it is possible not only to target tumors (lung, osteosarcoma, prostate) and their vascularization but also to deliver in a specific and above all targeted way a diagnostic marker. The figure shows the specificity of RAFT-cRGD for the $\alpha_v\beta_3$ integrins and the sensitivity of RAFT-

cRGD-Cy5 in the *in situ* detection of a peritoneal carcinosis of an ovarian tumor in a mouse model.

Preliminary experiments using ICG' (indocyanine green), a fluorophore similar to Cy5 and approved for clinical use, made it possible to show a specific tumor targeting of RAFT-cRGD-ICG' in the rat model of grafted osteosarcoma.

Tumors 4-5 mm in diameter, undetectable in visible light, are detected under exposure to near infrared light after administration of RAFT-cRGD-ICG' (fig. 4A). Dissection of the region of interest under near infrared light makes it possible to clearly discriminate the limit between muscles and tumor (fig. 4B). The total resection of the tumor with minimal healthy margins is obtained under near infrared light (fig. 4C). Moreover, use of the near infrared camera enables the detection of millimeter-size pulmonary metastases, invisible under white light, after intravenous administration of RAFT-cRGD-ICG'.

Example 3: Procedure

As currently applied for the resection of the primary tumor in the rat:

Eighteen hours before the procedure, the animals receive an injection of RAFT-cRGD by intravenous route.

During the operation the animals are maintained under anesthesia by isoflurane inhalation.

The tumor region is placed under the field of the near infrared camera. The tumor is examined/visualized by NIR on the display in order to begin to visualize the extension of the tumor in surrounding tissues (tumor visible transcutaneously). The resection of the tumor is carried out by NIR guidance. The best approach to the tumor is given by virtue of the near infrared image displayed on the screen. After incision of the skin (generally in the posterior muscle cavity), the tumor is freed from the muscles, the successive layers of muscles located around the tumor (which appear non-fluorescent by near infrared imaging) are removed according to the limits of the fluorescent signal: the last muscle freed (not resected, left *in situ*) corresponds to the last non-fluorescent muscle nearest the tumor (muscle in contact). Next, the resection margins are determined on the bones: the limit

of resection is set by the detection of fluorescence: the bones are cut at the limits of the fluorescence visualized on the display. The tumor, freed of all muscles, is removed and the non-resected region around the tumor (i.e., the healthy region) is
5 observed under near infrared light to verify the quality of the resection (verification of the absence of the fluorescence indicative of total tumor resection).

Example 4: Clinical protocol

10 This phase I/II clinical protocol (toxicity/feasibility study) is comprised of three parts:

1) Study of the toxicity of the diagnostic nanoparticles. In a dose escalation protocol (3 doses; 4 patients per dose level), we will evaluate the toxicity (determine the dose
15 limit) of clinical grade RAFT-cRGD₄-ICG' administered by IV route in patients with a refractory or relapsing osteosarcoma.

2) In this same cohort of patients we will compare the sensitivity of detection of tumor margins of RAFT-cRGD₄-ICG' to the sensitivity of detection of the conventional technique (anatomopathological analyses). Clinical grade RAFT-cRGD₄-ICG' will be administered by IV route to
20 patients 18 hours before the surgical procedure. The resection of the tumor will be carried out according to the conventional technique (preoperative imaging for the determination of margins). Tumor margins will be determined by near infrared imaging after the resection (exposure of the resected tissue under the near infrared camera) and according to the conventional method
25 (anatomopathology). We think that the use of RAFT-cRGD₄-ICG' will make it possible to determine tumor margins with a sensitivity equal to (or better than) the conventional technique.

3) Determination of the sensitivity of intraoperative
35 determination of tumor margins of RAFT-cRGD₄-ICG' and of the near infrared camera. In a second cohort of patients, we will verify that the clinical grade RAFT-cRGD₄-ICG' administered by IV route before the surgery makes it

possible to correctly determine the margins of the tumor (or to determine exactly the extension of the tumor in healthy tissues) when the tumor region is exposed under near infrared light. Resection of the tumor will be guided by intraoperative imaging. Conventional anatomopathological and fluorescent analyses of the resected tissues will make it possible to determine the quality of the resection of the tumors and to verify the margin detection sensitivity of RAFT-cRGD₄-ICG'. We think that the use of RAFT-cRGD₄-ICG' and the intraoperative detection probe will improve the resection of the tumor (will make it possible to ensure local control of the tumor while avoiding the sacrifice of too much healthy surrounding tissue).

4) Typically, the computer and display are placed on a cart and the camera on an articulated arm that the surgeon is able to reposition.

Example 5: Extrapolation to other tumors overexpressing $\alpha\beta 3$ integrins

The results obtained in the rat osteosarcoma model have made it possible to: i) establish the sensitivity and specificity of tumor targeting by RAFT-c(RGD)4-Fluo and ii) determine the feasibility of tumor surgery assisted *in situ* by use of the RAFT-c(RGD)4-Fluo/detection probe combination peroperatively.

In a model of orthotopic and metastatic rat osteosarcoma, we have shown that it is possible to significantly improve tumor resection quality by virtue of assistance by near infrared imaging after intravenous injection of RAFT-c(RGD)4-Fluo. Comparison of resected tumor volumes shows a significant increase (i.e., a significant decrease in resected fragment size; $p < 0.01$) in favor of resection performed under peroperative guidance. We have also shown that the RAFT-c(RGD)4-Fluo/near infrared probe combination makes it possible *in situ* to visualize metastases that are invisible under white light.

In view of the promising results obtained in the rat graftable osteosarcoma model, we defined the field of application of the peroperative RAFT-c(RGD)-Fluo/detection probe.

More particularly concerning sarcomas, we defined for which types of sarcomas surgery under peroperative guidance will be applicable.

We believe that the peroperative RAFT-c(RGD)-Fluo/detection probe can be used to aid the resection of all tumors that overexpress $\alpha\beta 3$ integrins.

To determine precisely which tumors overexpress these integrins, we evaluate the expression of $\alpha\beta 3$ integrins, targets of RAFT-c(RGD)4-Fluo, by immunohistochemistry on samples of human sarcomas having or not having received neoadjuvant chemotherapy and sampled at the time of surgery.

Results obtained

Up to now, it has been possible for us to evaluate by immunohistochemistry the expression of $\alpha\beta 3$ integrins on 12 human sarcoma samples including liposarcomas and uncategorized sarcomas (**Table II**). These initial analyses reveal that all sarcomas do not express $\alpha\beta 3$ integrins in an identical manner and that within a given histological subtype (liposarcoma) differences can be observed. Dedifferentiated or even differentiated liposarcomas do not express this molecule whereas nondifferentiated or mixed liposarcomas express it weakly and its expression is clearly detected in uncategorized sarcomas.

<i>Sample ID</i>	<i>Histological Type</i>	<i>Integrin labeling</i>
Sarc1	Uncategorized sarcoma	++
Sarc2	Differentiated liposarcoma	0
Sarc3	Myxoid liposarcoma	++
Sarc4	Differentiated liposarcoma	0
Sarc5	Uncategorized sarcoma	+
Sarc6	Differentiated liposarcoma	0

liposarcoma			
Sarc7	Myxoid liposarcoma	++	
Sarc8	Liposarcoma	++	5
Sarc9	De- differentiated liposarcoma	0	
Sarc10	Liposarcoma	+	
Sarc11	Uncategorized sarcoma	++	10
Sarc12	Liposarcoma	+	

15

Table II: Semi-quantitative analysis of the expression of $\alpha v \beta 3$ integrins by immunohistochemistry. It arises from this analysis that all sarcomas do not express integrins with the same intensity. 0: no expression detected, +: weak expression of integrins, ++: average expression; +++: strong expression.

In view of these results it appears to us that the peroperative RAFT-c(RGD4)-Fluo/detection probe can be used at least for uncategorized sarcomas, myxoid liposarcomas and nondifferentiated liposarcomas. This approach can then be extended to any tumor overexpressing $\alpha v \beta 3$ integrins. For example, it could be applied to certain neuroendocrine tumors and to ovarian carcinomas. Indeed, it has been shown by qRT-PCR in neuroendocrine tumors that the level of expression of integrins - especially $\beta 3$ integrin - is extremely variable and weaker in these tumors than in hepatic metastases of colorectal cancer. (Oxboel J. et al. Oncol rep. 2009; 21 (769-775). In the case of ovarian carcinomas, a team has demonstrated that in all cases the αv and $\beta 5$ integrin subunits ($\alpha v \beta 5$ being the other dimmer target of the cRGD motif with $\alpha v \beta 3$) are expressed in these carcinomas. Expression of the $\beta 3$ subunit is more variable (Maubant et al. J Mol Histol. 200 (36): 119-129). These results led us to think that RAFT-c(RGD4)-Fluorophore would target these tumors. The literature and our

results confirm the possibility of expanding use of the peroperative RAFT-c(RGD)-Fluorophore/detection probe combination to other sarcomas (rhabdomyosarcoma, Ewing sarcoma, leiomyosarcomas, etc.) and tumors expressing the target pre- and
5 post-chemotherapy treatment.

Example 6: Neoadjuvant chemotherapies do not affect the binding of RAFT-cRGD4-ICG' to tumors

In the rat osteosarcoma model, we tested the sensitivity of
10 RAFT-c(RGD)-Fluo injected by IV route to enable the detection *in situ* of primary tumors in animals having received chemotherapy treatments (ifosfamide).

The treatment was as follows: 21 days after tumor implantation, the rats with a progressive tumor receive an IP
15 injection of ifosfamide (increasing doses from 5 mg/kg to 10 mg/kg). One week after treatment, the primary tumor is resected using the peroperative RAFTc(RGD)4-Fluo/detection probe combination.

We have demonstrated the specific tumor localization of RAFT-
20 c(RGD)4-Fluo injected by intravenous route on animals having received chemotherapy. Exposure of the tumor region under NIR light makes it possible to visualize transcutaneously the tumor/muscle limit which is invisible under white light. The specificity of tumor localization is confirmed by detection of the
25 light signal only in the tumor whereas healthy muscle in contact has no parasitic signal *in situ*.

The specific tumor localization of RAFT-(cRGD)4-Fluo is confirmed by detection of the fluorescent signal only in the tumor (**Figure 5**), whereas no signal is present in the healthy muscles in
30 contact with the tumor (**Figure 5**). The intensity of the fluorescence signal detected in tumors in control animals (i.e., having received no treatment) and that detected in tumors of animals having received ifosfamide displays no significant difference (**Figure 5**). These results indicate that chemotherapy
35 does not modify the specific tumor targeting of RAFT(cRGD)4-Fluo.

In parallel to these tests, and to verify the expression of post-chemotherapy integrins, we analyze by qRT-PCR the expression

of $\alpha\beta 3$ integrins in the tumors of animals from each group (control and treated with ifosfamide).

The results obtained to date and presented in **Figure 6** indicate that chemotherapy does not diminish the expression of these molecules. An increase in their expression is observed after treatment with ifosfamide (**Figure 6 B**).

This result is surprising. Indeed, it has been reported in ovarian carcinomas that, although the expression of $\beta 3$ integrins does not vary as a function of the response to chemotherapy, that of αv integrin does vary according to response to chemotherapy. (Guo HY et al. Zhonghua Fu Chan Ke Za Zhi. 2004;39:750-753). Moreover, in the case of osteosarcoma, a team analyzed by immunohistochemistry the expression of integrins and described that polychemotherapeutic treatments (adriamycine /cisplatine/ ifosfamide) induce a significant decrease in αv integrin expression.

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PATENT REFERENCES

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CLAIMS

1. A composition comprising a tracer for use in surgery wherein the tracer comprises a molecular scaffold
5 having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.
- 10 2. A composition comprising a tracer for use in surgery according to claim 1 for the intraoperative detection of the surgical margins of a tumor in an individual, wherein the tracer comprises a molecular scaffold
15 having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said intraoperative detection of the surgical margins of a tumor in an individual comprises
20 a) administration to the individual of said tracer, b) circulation of the tracer in the individual and c) detection of the tumor margins in near infrared light.
- 25 3. A composition comprising a tracer for use in surgery according to claim 1 for the resection of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection
30 of a tumor in an individual comprises a) administration to the individual of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins in the near infrared and d) resection of the tumor while limiting the

extension of the resection in healthy tissue all while maintaining tumor control.

4. A composition comprising a tracer for use in surgery according to claim 1 for the resection of a tumor in an individual, wherein the tracer comprises a molecular scaffold having two faces, a marker detectable in the near infrared being grafted on one of the faces and a ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face, and wherein said resection of a tumor in an individual comprises a) administration to the individual of a sufficient quantity of said tracer, b) circulation of the tracer in the individual, c) detection of the tumor margins in near infrared light, d) resection of the tumor while limiting the extension of the resection in healthy tissue all while maintaining tumor control, and e) verification of the precision of the resection of the tumor in near infrared light.
5. A composition comprising a tracer for use in surgery according to claim 4 wherein said resection of a tumor in an individual comprises a repetition of steps c) to e) for the resection of the same tumor and / or for the resection of several distinct tumors.
6. A composition comprising a tracer for use in surgery according to anyone of claims 1-5 wherein the tumor is an osteosarcoma, an uncategorized sarcoma, a myxoid liposarcoma, a nondifferentiated liposarcoma, a chondrosarcoma, a Ewing's sarcoma, a rhabdomyosarcoma, a hepatic carcinoma, a colorectal carcinoma, a gastrointestinal stromal tumor (GIST), a mammary adenocarcinoma, an ovarian carcinoma or a neuroblastoma.

7. A composition comprising a tracer for use in surgery according to anyone of claims 1-5 wherein the tumor is a secondary tumor or a metastasis.
- 5
8. A composition comprising a tracer for use in surgery according to claim 7, wherein the tumor is a pulmonary metastasis.
- 10
9. A composition comprising a tracer for use in surgery according to anyone of the preceding claims wherein the molecular scaffold is a cyclopeptide with two faces, the marker detectable in the near infrared being grafted on one of the faces and the ligand of the $\alpha_v\beta_3$ integrins being grafted on the other face.
- 15
10. A composition comprising a tracer for use in surgery according to claim 9 wherein the molecular scaffold is the cyclic decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-Gly-] or the cyclic decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Ala-Lys-Pro-Gly-].
- 20
11. A composition comprising a tracer for use in surgery according to anyone of the preceding claims wherein the marker detectable in the near infrared is a fluorophore.
- 25
12. A composition comprising a tracer for use in surgery according to claim 11 wherein the marker detectable in the near infrared is Indocyanine Green (ICG).
- 30
13. A composition comprising a tracer for use in surgery according to anyone of the preceding claims wherein the ligand of the $\alpha_v\beta_3$ integrins is selected
- 35

among the peptide Arg-Gly-Asp (RGD), the motif cyclo[RGDfK] and the motif cyclo[RGDyK].

14. A composition comprising a tracer for use in
5 surgery according to anyone of the preceding claims
wherein the molecular scaffold is the cyclic
decapeptide c[-Lys-Lys-Lys-Pro-Gly-Lys-Lys-Lys-Pro-
Gly-] having two faces, four cyclo[RGDfK] motifs
being grafted on one face and a Indocyanine Green
10 flurophore being grafted on the other face.

15. A composition comprising a tracer for use in
surgery according to claim 14 wherein the molecular
scaffold is the cyclic decapeptide c[-Lys-Lys-Lys-Pro-
15 Gly-Lys-Lys-Lys-Pro-Gly-] having two faces, four
cyclo[RGDfK] motifs being grafted on one face on the
lysine in positions 1, 3, 6, 8 and a Indocyanine Green
flurophore being grafted on the other face on the
lysine(s) in position 2 and/or 7.

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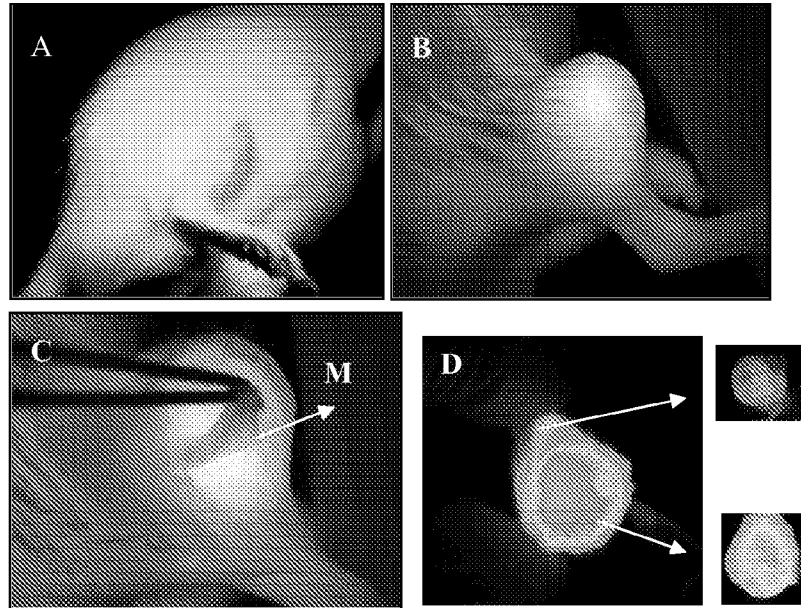


Fig. 1

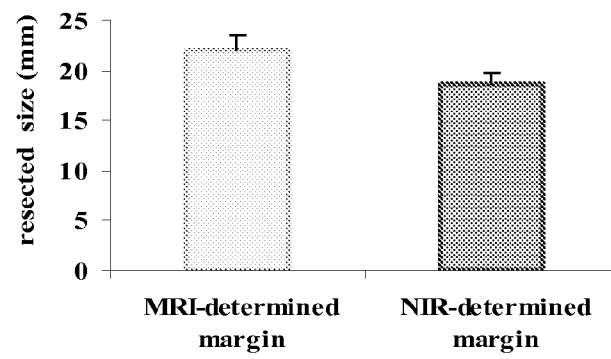


Fig. 2

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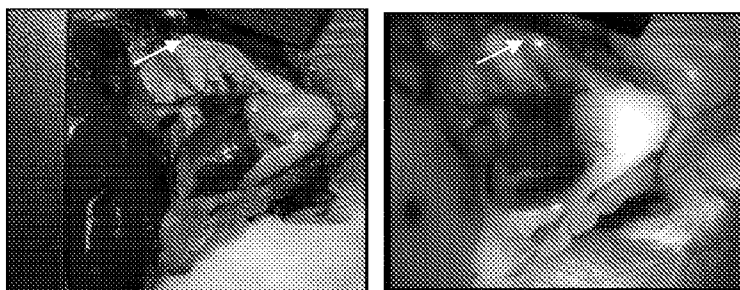


Fig. 3

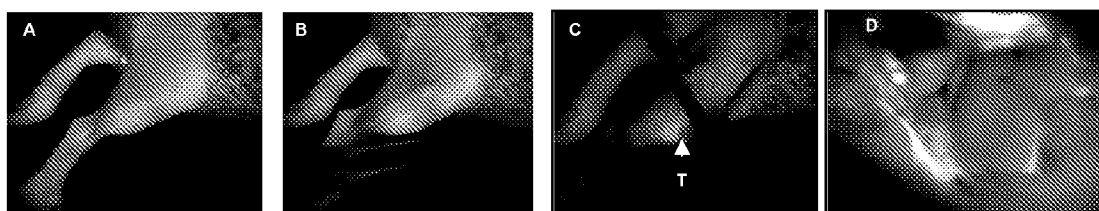


Fig. 4

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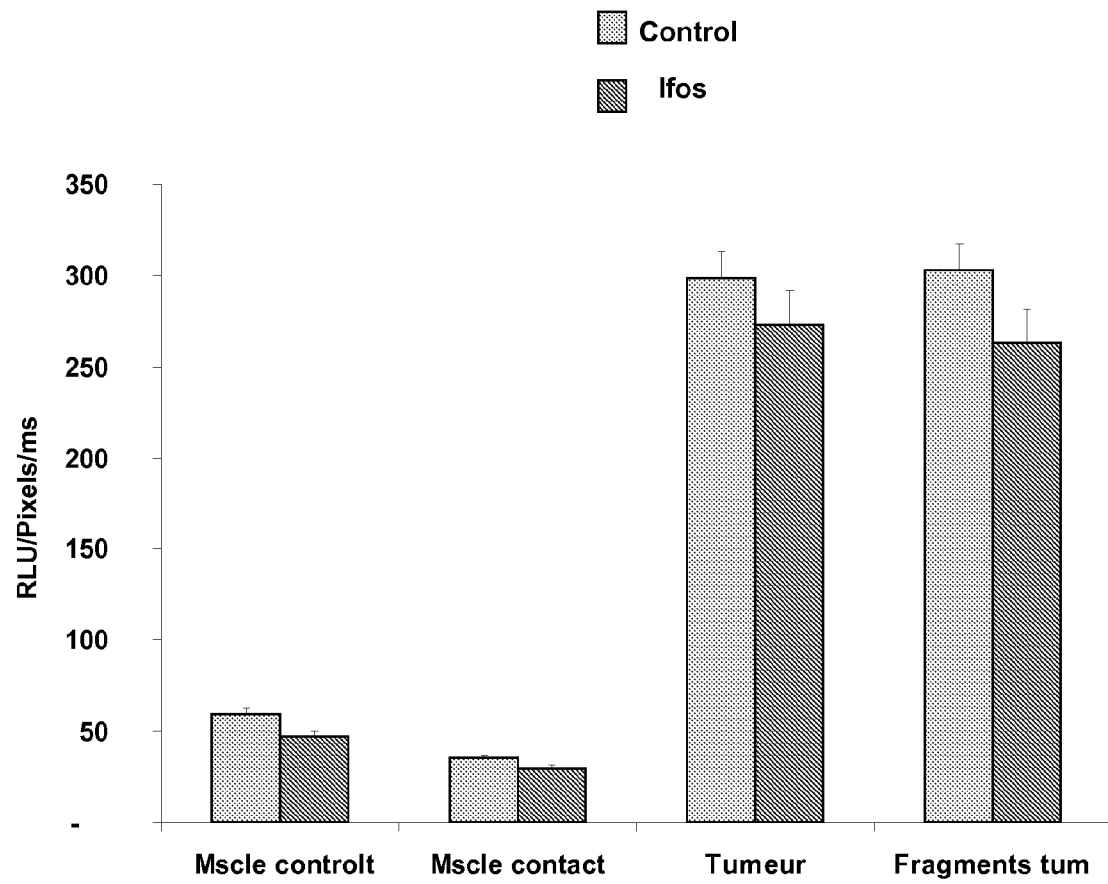


Fig. 5

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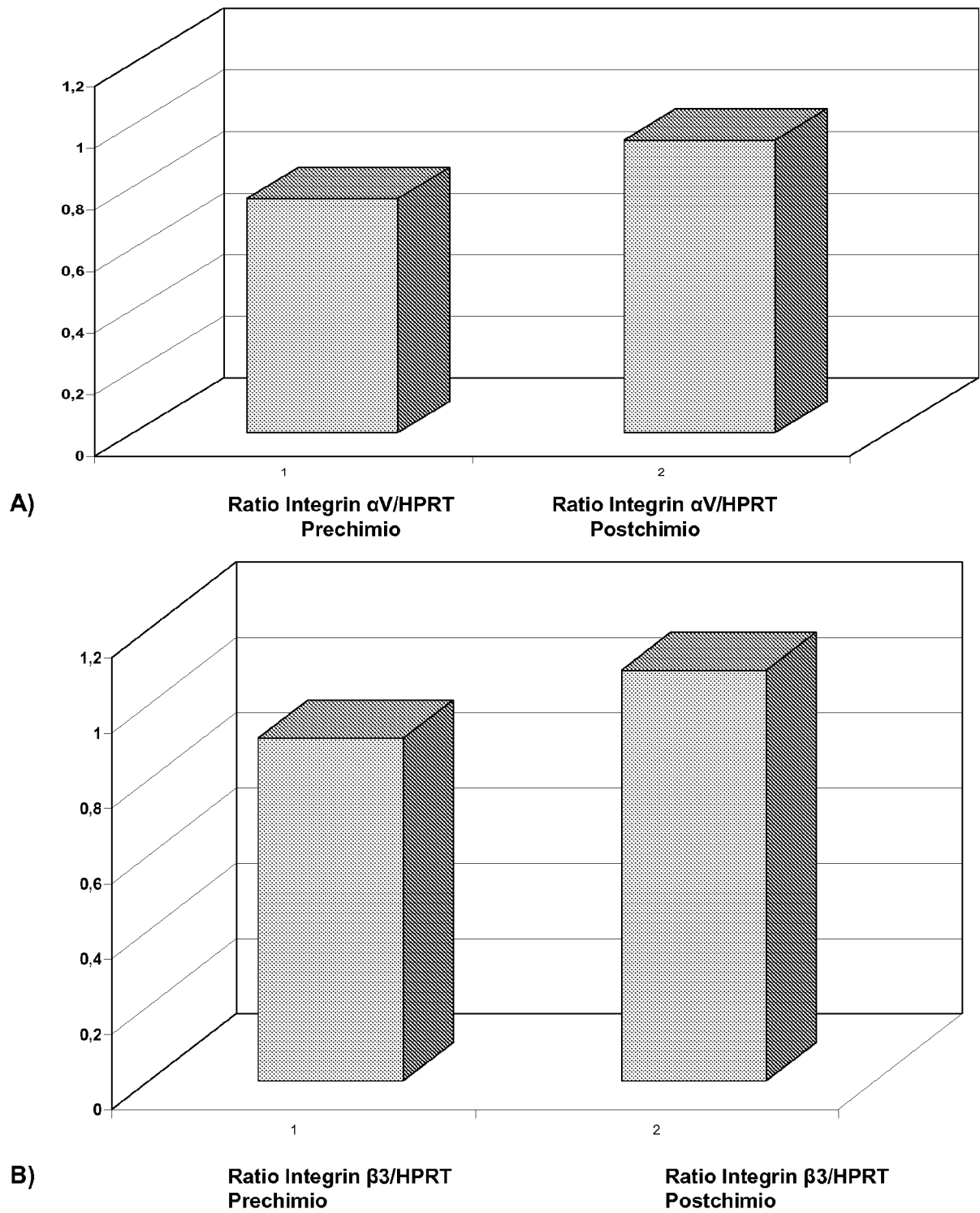


Fig. 6