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$[^{123}\text{I}]\text{ICF01012}$ melanoma imaging and $[^{131}\text{I}]\text{ICF01012}$ dosimetry allow adapted internal targeted radiotherapy in preclinical melanoma models

**Running title: Melanoma Imaging and internal targeted radiotherapy**

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Abstract

Background: Melanin-targeting radiotracers are interesting tools for imaging and treatment of pigmented melanoma metastases. However variation of the pigment concentration may alter the efficiency of such targeting. Objectives: A clear assessment of both tumor melanin status and dosimetry are therefore prerequisites for internal radiotherapy of disseminated melanoma.

Materials & Methods: The melanin tracer ICF01012 was labelled with iodine-123 for melanoma imaging in pigmented murine B16F0 and human SK-Mel 3 melanomas. Results: In vivo imaging showed that the uptake of $[^{123}\text{I}]$ICF01012 to melanomas correlated significantly with melanin content. Schedule treatment of $3 \times 25\text{ MBq}^\text{[131}\text{I}]$ICF01012 significantly reduced SK-Mel 3 tumor growth and significantly increased the median survival in treated mice. For this protocol, the calculated delivered dose was 53.2 Gy. Conclusion: Radio-iodinated ICF01012 is a good candidate for both imaging and therapeutic purposes for patients with metastatic pigmented melanomas.

Keywords: Dosimetry; Imaging, Melanin, Melanoma, Radiotracer; Targeted Radionuclide Therapy
Melanins are complex biopolymers produced mainly in cutaneous and ocular melanocytes, choroid-retinal pigmented epithelium, iris and ciliary bodies [1]. While these pigments have a role in protection from UV-induced damage, their synthesis induces the formation of toxic intermediates, explaining why their production is restricted in subcellular organites, the melanosomes [2]. In addition, the melanins, i.e. eumelanin and phaeomelanin, share the protective property of linking xenobiotics to prevent DNA damage [3, 4]. Melanoma tumors result from the aberrant proliferation of melanocytes which specifically produce melanin pigments. However, as highly specialized components, melanins should have a positive role in melanoma imaging and therapy. Numerous studies have developed small organic molecules with a great affinity for melanins, such as iodoarylcarboxamides [5-7]. Detection of melanoma could then be performed after the radiolabeling of these molecules with γ or positron-emitting radionuclides for SPECT [8-10] or PET [11-14] imaging, respectively. These radiotracers targeting melanin-positive tumors could potentially improve staging and follow up as well as treatment of metastatic melanoma patients. Currently, disseminated melanoma can be treated by a combination of kinase inhibitors or by blocking melanoma-induced immunosuppression but resistance or serious adverse effects limit the efficacy of these novel therapies [15]. Therefore, alternative or complementary therapeutics are still of interest. Targeting the specific features of melanoma could include the above-mentioned melanins.

Besides a high specificity, melanins are not equally present in melanoma metastases. It has been previously reported that pigmentation is present in 25% of 70 analysed metastases [16]. Our group reported in a multicentre study aiming to compare the melanin radiotracer [123I]BZA2 with [18F]FDG performances for melanoma staging that such variations of melanin content in metastases as the technical performances of the “gold standard” [18F]FDG/PET radiotracer did not support the clinical use of [123I]BZA2 as a melanin radiotracer for melanoma imaging [9]. In this study, 45% of the metastatic lesions were pigmented. However, targeted radionuclide therapy approaches using melanin radiotracers labeled with iodine 131 gave favorable results in preclinical models [10, 17-19]. Recently, a first clinical study was conducted on 9 patients harboring melanoma metastases, preliminarily screened for inclusion by SPECT imaging with a melanin-targeting radioligand (i.e. [131I]BA-52), and treated with escalated doses ranging from
510 to 6600 MBq [20]. From this study, three out of five patients receiving a dose > 4.3 GBq had a survival of more than two years.

$^{[131I]}$ICF01012, an arycarboxamide, showed interesting properties for pigmented melanoma radiotherapy with a high efficiency on B16 models [10, 19], however, the decrease of tumor growth was less marked for human melanoma xenografts [17]. This observation could be associated to a lesser melanin content in xenografts. To compare these models in terms of melamins, imaging with $^{[123I]}$ICF01012 was performed, as this isotope could be used clinically. We also evaluated the $^{[131I]}$ICF01012 dosimetry and efficiency of $^{[131I]}$ICF01012 multiple injections on a human SK-Mel 3 tumor.

**Material and methods**

**Cell culture**

Murine B16F0 and human SK-Mel 3 melanoma cell lines were purchased from American Type Culture Collection (ATCC, Biovalley, Marnes La Vallée, France). B16F0 cells were maintained as monolayers in Dulbecco’s Modified Eagle’s Medium (DMEM-Glutamax, Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal calf serum (FCS, Biowest, Nuaillé, France) and 4 µg/µL gentamycin (Invitrogen). The SK-Mel 3 cell line was maintained in McCoy’s 5A medium (Invitrogen) supplemented with 15% FCS and 4µg/µL gentamycin. Cells were grown at 37 °C in a humidified incubator containing 5% CO₂.

**Animals**

Male C57BL/6J mice and male Swiss nu/nu mice (6 weeks old) were obtained from Charles River Laboratories (l’Arbresle, France). All animal studies were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” and approved by the local ethics committee (C2E2A) under numbers: CE03-11, CE18-09 and CE64-12. To establish the tumor, $3 \times 10^5$ B16F0 cells (mice C57BL/6J) and $5 \times 10^6$ SK-Mel 3 cells (nude mice) were suspended in 100 µL PBS buffer and injected subcutaneously into the right flank of each mouse.

**Radiochemistry**

ICF01012 was synthesised according to previously described methods [21]. $^{[123I]}$NaI (7.4 GBq/mL), produced using the $^{124}$Xe (p, 2n) reaction, was purchased from MDS Nordion SA (Ave
ICF01012 radiolabelling with $^{123}$I: To a solution of ICF01012 (1.2-2.5 mg) in citrate buffer pH = 4 (500 μL) were added, in a sealed vial equipped with a needle (25 G, 0.5 × 16 mm), an aqueous copper(II) sulfate solution (0.5 mg, 100 μL) and $[^{123}$I]NaI (165 MBq). The reaction mixture was heated at 150 °C for 1 hour. After cooling to room temperature, the residue was taken up in water (500 μL) and a 1.0 N aqueous sodium hydroxide solution (100 μL) was added. The vial cap and septum were removed. The resulting suspension was loaded on an extrelut® column and eluted after 10 minutes with dichloromethane (5 × 3 mL). The collected organic extracts were evaporated under reduced pressure, taken up with methanol (2 × 200 μL), and purified by semi-preparative RP-HPLC. Fractions containing the expected radiolabeled product were collected, evaporated to dryness, dissolved in dichloromethane (2 mL) and treated with a 2.0 N hydrochloric acid solution in anhydrous ether (5 mL). The resulting hydrochloride solution was evaporated under reduced pressure and the dry residue was suspended in anhydrous ether (5 mL). The solvent was then evaporated under vacuum for 30 minutes to give $[^{123}$I]ICF01012. The radiotracers were shown by analytical radio-RP-HPLC ($[^{123}$I]ICF01012: $R_f = 8.40$ min) to be identical to the authentic non-radioactive material and to be free of significant radiochemical impurities.

ICF01012 radiolabelling with $^{131}$I: $[^{131}$I]ICF01012 was synthesized by radio-iododestannylation as described previously [10, 17]. $[^{131}$I]ICF15002 was obtained in 150 minutes, with 81-88%
radiochemical yields, radiochemical purities in range of 92-99%, and specific activities in the range of 94-100 GBq/µmol.

In vivo planar scintigraphic imaging

B16F0 and SK-Mel 3 melanoma-bearing mice received an intravenous (i.v.) injection of 3.7 MBq $[^{123}\text{I}]$ICF01012 at day 16 and day 45 post cell inoculation, respectively. After anesthesia by intraperitoneal (i.p.) injection (200 µL/mice), with a mixture 4:1 of ketamine (Imalgène 500®; Rhône Mérieux, Lyon, France) and xylazine (Rompun®, 2%; Bayer, France), mice were submitted to serial scintigraphic acquisition at 3, 20 and 44 hours after radiotracer injection. Imaging was performed using a gamma-camera dedicated to small animal ($\gamma$-IMAGER®; Biospace Mesures, Paris, France). All the acquisitions were performed with a 15% window centered on the 160 KeV peak of $^{123}\text{I}$. Mice were placed in suitable position over the collimator of the $\gamma$-camera and images acquired for 10 min. Quantitative analyses of scintigraphic scans were performed using the $\gamma$Vision+ software (Biospace Mesures). Tumor uptake was determined from semi-quantitative analyses of scintigrams according to the method previously published [22]. Briefly, for each scintigram, regions of interest (ROIs) were drawn by the same investigator around melanomas and whole body mice with boundaries as closed as possible to their limits. The corresponding tumor surface was delineated in the muscle. The values in cpm/mm² were considered to calculate the ratios of tumor towards whole body and tumor to muscle activities.

At the end of image acquisition, mice were sacrificed; tumors were removed, weighed and immersed in liquid nitrogen and stored at -80 °C for melanin assay.

Therapy experiment on Nude mice bearing SK-Mel 3 melanoma

After 35 days of tumor growth, animals received $[^{131}\text{I}]$ICF01012 at 25MBq i.v. single dose (1 X 25 MBq) or once a week for 3 weeks (3 x 25 MBq). The length and width of tumors were measured three times per week using digital calipers. Tumor volume was determined according to the following formula: Volume (mm³) = (Length (mm) × Width² (mm))/2. Mice were weighed
to monitor potential toxicity. For ethical reasons, the animals were sacrificed when tumors reached 2000 mm$^3$. This endpoint used in survival analysis was considered as the death day.

*Biodistribution experiment in Nude mice bearing SK-Mel 3 tumors*

Thirty five days following xenograft, mice were i.v. injected with 0.37 MBq $[^{131}I]$ICF01012. Three mice per time (1, 3, 6, 10, 24, 48, 72, 120, 168 and 240 hours) were sacrificed, organs and tumors were removed and weighed. Their radioactivity was counted with a $\gamma$-counter (Wizard 1480, Perkin Elmer). The fraction of injected activity in tumors (\% IA/g) was determined by the ratio of counted activity per organ divided by the organ weight. Dosimetry was established using the MIRD methodology [23]. The absorbed dose is calculated by multiplying the cumulative activity (Bq.s/kg) in the organ with the so-called “S-value”, representing the energy deposited in the organ per decay (J/Bq/s). First, activity over time for each organ was fitted by a mono-exponential function. The cumulative activity was then estimated by the analytical integration of the mono-exponential over an infinite time. S-values were computed by a Monte Carlo approach allowing simulation of the physical interactions of the particles emitted by the $^{131}$I source in a CT scan-based mouse model [24].

*Melanin assay*

Tumor samples (20 mg) were crushed and dissolved in a 1 M aqueous potassium hydroxide solution (1 mL) and incubated at 60 °C for 30 minutes, until complete lysis [17]. The absorbance was measured at 405 nm and melanin concentration was determined using a standard curve generated with synthetic melanin (Sigma-Aldrich). Melanin content was expressed as µg per mg of tumor.

*Statistical analyses*

The statistical analyses were performed with the XLStat software (Addinsoft, VA) using ANOVA for tumor volumes and Log rank for median survival comparison respectively.
Results

Radiolabelling of ICF01012 with iodine 123 and in vivo uptake of $^{123}$IICF01012 in mouse and human melanoma tumors

ICF01012 (Fig.1) was prepared as previously described [21]. $^{123}$IICF01012 was obtained by radio-isotopic exchange in 180 min, with 55% uncorrected radiochemical yield. The radiochemical purity was >96.5%, the chemical purity >85% and the specific activity ranged between 16.8-35.6 MBq/µmol.

Different tumor uptakes were observed after $^{123}$IICF01012 radiotracer administrations in B16F0 and SK-Mel 3 models (Fig. 2A). However, in both models, 3 hours after radiotracer administration, a clear accumulation of $^{123}$IICF01012 occurred within the tumors and a rapid elimination of $^{123}$IICF01012 from non-specific organs was observed at 20 h post-injection. The accumulation of $^{123}$IICF01012 in pigmented eyes from the C57BL6 model attested its high specificity for melanin. The percentages of tumor $^{123}$IICF01012 uptake relative to whole body showed an increase in this ratio in a time dependent manner for highly pigmented B16F0, SK-Mel 3 being saturated at time 20h (Fig. 2B). The tumor to muscle ratio revealed a good selectivity of $^{123}$IICF01012 in B16F0 and SK-Mel 3, reaching 11.5 ± 1.7 and 4.8 ± 1.8 at 20h, respectively (Fig. 2B). The % of activity in melanomas at time 44h was related to the content of melanin 14.13 ± 4.65 vs 5.42 ± 0.39 µg/mg of B16F0 and SK-Mel 3 tumors, respectively (Fig. 2C). These results from two different in vivo pigmented models highlights the direct correlation between melanin content and $^{123}$IICF01012 uptake ($R^2 = 0.333$, $p = 0.019$). A $^{123}$IICF01012 deiodination was evidenced by thyroid uptake in both models. However, in the SK-Mel 3 model, this signal was more intense after 3h p.i., compared to B16F0 model. This could be linked to the lower $^{123}$IICF01012 tumor retention in SK-Mel 3. Indeed, we previously observed that $^{125}$IICF01012 was highly stable in the tumor, 84% of the unchanged form still being detected 8 days after injection, while it was quickly metabolized in blood [25]. A higher level of unbound $^{123}$IICF01012 will then generate a higher 123-iodine capture by the thyroid.

Evaluation of $^{131}$IICF01012 dosimetry in SK-Mel 3 tumors
Absorbed dose was evaluated on SK-Mel 3 tumor and non-target organs from biodistribution studies after i.v. injection of 37 MBq \[^{131}\text{I}]\text{ICF01012}\) and Monte Carlo S-values (Table 1). The biodistribution in the tumor showed a rapid and persistent strengthened accumulation of \[^{131}\text{I}]\text{ICF01012}\) (around 7% AI/gr) during 42 hours (Figure 4a). A significant dose was delivered with \[^{131}\text{I}]\text{ICF01012}\) on pigmented tumor (26.2 Gy) compared to the other organs. Low absorbed dose was deposited in excretion organs such as the kidney, liver, colon and stomach (3.2, 7.4, 9.2 and 7.4 Gy, respectively). A delivered absorbed dose of 30 Gy was classically used in melanoma radiotherapy protocols designed for regional control or palliative therapy [26]. The injected activity was then set up for efficient treatment in a schedule of 3 \(\times\) 25 MBq \[^{131}\text{I}]\text{ICF01012}\) (Table 1) with no expected side effects at the maximal delivered dose.

[^{131}\text{I}]\text{ICF01012}\) treatment inhibits the growth of SK-Mel 3 xenograft tumors and increases mice survival

The effects of \[^{131}\text{I}]\text{ICF01012}\) radiotherapy were observed on tumor growth and animal survival (Fig. 3A, B). After 20 days following the first 25 MBq \[^{131}\text{I}]\text{ICF01012}\) dose injection, the tumor growth (evaluated by the ratio of measured tumoral volume minus the initial volume to the initial one) of 3 dose-treated animals was significantly stopped compared to control (0.84 ± 0.34 vs 2.58 ± 1.24, at day 20) and this until 62 days (4.26 ± 1.76 vs 25.94 ± 5.68) (p<0.05; ANOVA test). During this time, this group harbored a tumor doubling time two-fold higher than the control group one (30.27 vs 14.50 days). In comparison, a single dose of \[^{131}\text{I}]\text{ICF01012}\) (25 MBq) slightly reduced tumor growth, although not significantly. The percent of surviving mice showed a significant increase of the median survival in 3 \(\times\) \[^{131}\text{I}]\text{ICF01012}\) treatment compared to the control group (81 vs 53 days) (p<0.05 ; Log-Rank test). After 60 days, the tumor escaped from treatment. For the 3 \(\times\) 25 MBq group, a significant slight weight loss compared to control and 1 \(\times\) 25 MBq was observed, which did not exceed the critical value of 10% of the initial values (Figure 4B).

Discussion
We show here for the first time the specific melanin binding property of $^{123}$IICF01012 for \textit{in vivo} imaging of mice bearing syngenic or human melanomas. The labeling of ICF01012 with iodine-123 was performed with excellent radiochemical yield and purity. $^{123}$IICF01012 imaging assessed in melanoma-bearing mouse models gave good results in terms of contrast, elimination and uptake in pigmented tissues (\textit{Figure} 2). \textit{In vivo}, tumor $^{123}$IICF01012 activity was correlated with melanin concentration. This is in total accordance with previous observations using ICF01012 labeled with iodine 125 for imaging mice bearing different human melanomas. Indeed, in a non-pigmented A375 model, $^{125}$IICF01012 concentration was near background level. $^{131}$IICF01012 therapy was without effect on an M3Dau amelanotic xenograft [17]. Labeling of ICF01012 with iodine 123 was developed for a more convenient clinical use. Despite a lower melanin concentration in SK-Mel 3 vs B16F0, $^{123}$IICF01012 imaging was sensitive enough to detect these tumors. Another melanin radiotracer, $^{123}$IBZA2, was compared to $^{18}$F]FDG in a phase 3 clinical trial and showed a significantly higher sensitivity in the detection of pigmented melanoma metastases, despite the difference in terms of sensitivity between PET ($^{18}$F]FDG ) and SPECT imaging ($^{123}$IBZA2) [9]. The elimination rate of $^{123}$IBZA2 from target organs in preclinical models was faster than for $^{123}$IICF01012 (data not shown). Therefore $^{123}$IICF01012 seems more suitable than $^{123}$IBZA2 for both melanin-positive patient stratification and $^{131}$IICF01012 treatment follow-up.

For therapy, B16Bl6 tumors were more sensitive and one injection was sufficient to induce an antitumoral effect, the threshold to get an objective radiotherapeutic response being from 14.8-22.2 MBq [19]. However, as a direct consequence of the relationship between melanin content and ICF01012 tumor uptake, the absorbed dose was 108 Gy in highly pigmented B16Bl6 [19] \textit{vs} 26.2 Gy in SK-Mel 3 tumors, for 37 MBq $^{131}$IICF01012 injected. Three doses of 25 MBq $^{131}$IICF01012 injections were associated with a strong reduction of SK-Mel 3 growth and an increase in mouse survival without significant impact on weight. Dosimetry calculations of 188-Re-Labeled melanin-binding antibody (7.4 GBq injected) showed 28.80-19.90 Gy deposits for a wide range of melanin concentration [27]. The major challenge in antibody therapies is their inability to penetrate solid tumors efficiently [28]. In contrast, the high uptake of ICF01012 is due to its small size and the abundancy of the target. $^{125}$IICF01012 was homogeneously distributed in the tumor (data not show), allowing a similar effect on the overall tissue.
The absorbed dose for melanoma external beam radiation therapy usually ranges from 30 Gy to 60 Gy with a fractionated regimen (for a review see [26]). An external radiation dose of 37.5 Gy given in 15 fractions (2.5 Gy/fraction) was suggested for effective palliation in patients with metastatic melanomas. Interestingly, in a phase I clinical study of targeted radionuclide therapy with a melanin-targeting radiotracer, $^{[131]}$I$^{[131]}$BA-52, the absorbed dose to melanoma metastases was in a range of 1.1 to 12 Gy per GBq, leading to an objective response in 3 out of 5 patients (receiving a dose superior to 4.3 GBq) surviving more than 2 years [20]. Even if extrapolation of treatment efficacy from preclinical models to clinical trials is difficult, the resulting data indicate a promising potential for $^{[131]}$I$^{[131]}$ICF01012 radiotherapy of pigmented metastases. However, we are aware that the pigmented status of melanoma metastases remains the main limitation of our therapeutic strategy, as it is well established that all melanoma metastases are not pigmented [16, 29]. However, a recent clinical analysis showed a significant association between the presence of pigmented metastases and a decrease in life survival [30]. Therefore specifically killing pigmented cells will be of great interest in reducing melanoma recurrence. Furthermore, the use of radiosensitizers could overcome the radio-resistance of human melanomas. For example, small DNA molecules (DBait), which disorganize the repair system, enhanced radio-induced DNA damages in different preclinical models [31]. Interestingly, a B-RAF kinase inhibitor (i.e. PLX-4032) was shown to radiosensitize melanoma cells [32]. Combined treatment involving both $^{[131]}$I$^{[131]}$ICF01012 and PLX-4032 could allow the targeting of both achromic and melanized disseminated melanomas in patients harboring the B-RAF$^{V600E}$ mutation.

**Conclusions** Melanin is an attractive target for melanoma treatment [10, 17-20, 33]. We demonstrated herein that radio-iodinated melanin-targeting ligands, $^{[123]}$ICF01012 and $^{[131]}$ICF01012, are useful tools for imaging and therapy of pigmented melanomas respectively, in preclinical models. All these results support the clinical use of $^{[131]}$ICF01012 for the selection of melanin positive sub-populations, and as targeted radionuclide therapy of metastatic melanoma patients.

**Competing interests**
The authors declare that they have no conflicts of interest.

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References


Table 1: Organ absorbed dose in Gy for $^{[131]I}$ICF01012 single injection (37 MBq) or for $^{[131]I}$ICF01012 three injections (3 x 25 MBq), estimated using the MIRD formalism in nude mice bearing SK-Mel 3 melanoma

<table>
<thead>
<tr>
<th>Organs/Tissues</th>
<th>Calculated dose (Gy) for 37 MBq</th>
<th>Calculated dose (Gy) for 3x25 MBq</th>
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<tr>
<td>Bladder</td>
<td>2.5 ± 0.6</td>
<td>5.1</td>
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<tr>
<td>Blood</td>
<td>0.8 ± 0.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Brain</td>
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</tr>
<tr>
<td>Colon</td>
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<td>18.7</td>
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<tr>
<td>Eyes</td>
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<tr>
<td>Heart</td>
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<tr>
<td>Kidneys</td>
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</tr>
<tr>
<td>Liver</td>
<td>7.4 ± 0.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Lungs</td>
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</tr>
<tr>
<td>Muscle</td>
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<tr>
<td>Ovaries</td>
<td>1.0 ± 0.1</td>
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</tr>
<tr>
<td>Pancreas</td>
<td>1.9 ± 0.1</td>
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<tr>
<td>Skin</td>
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<tr>
<td>Stomach</td>
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<tr>
<td>Tumor SK-Mel 3</td>
<td>26.2 ± 2.4</td>
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</tr>
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Legends of Figures

**Figure 1:**
Structure of ICF01012

**Figure 2:**
$[^{123}\text{I}]\text{ICF01012}$ imaging and correlation with cell melanin content in both B16F0 and SK-Mel 3 melanoma models. (A) Representative serial imaging normalized with the same color scaling (set at 75% of the maximum pixel value) of *in vivo* $[^{123}\text{I}]\text{ICF01012}$ biodistribution on C57BL/6J mice bearing B16F0 or nude mice bearing SK-Mel 3 tumors. Tumors were indicated by white arrows, thyroid and eyes by orange and red arrows, respectively. (B) % of $[^{123}\text{I}]\text{ICF01012}$ activity in tumor relative to the whole body and tumor-to-muscle ratios. (C) The melanin content was determined in B16F0 (n = 5) and SK-Mel 3 (n = 4) tumors. A significant correlation between the percentage of injected activity (AI) present in the tumor at 44 h and the melanin concentration was observed (Pearson’s test, p=0.019)

**Figure 3:** Efficiency of $[^{131}\text{I}]\text{ICF01012}$ on SK-Mel 3 melanoma growth and mice survival. Nude mice received a single (25 MBq) or three (3 × 25 MBq) i.v. injections of $[^{131}\text{I}]\text{ICF01012}$. The results were obtained with 8 mice per group. (A) Tumor growth was expressed as % of the baseline volume and doubling time (DT) was determined. A significant effect of the three injection protocol was observed compared to the other groups (ANOVA, Tuckey post-test, p<0.001). (B) Animal survival curve was calculated as the number of animals that remained in each group at indicated times, a median survival was calculated (MS). A significant increase in percentage survival of animals receiving the three-injection treatment was observed compared to the control and the one-injection groups (Log rank test, p<0.05).
(A) Time course accumulation of $[^{131}\text{I}]\text{ICF01012}$ in SK-Mel 3 xenografts (B) Animals were weighed steadily and variations to the basal values were calculated, a transient significant decrease between the control and the three-injection groups (ANOVA, Tuckey post-test, p<0.05) was observed from days 18 to 48 but stayed below the 10% variation compared to initial weight.
Figure 1

ICF01012
Figure 2

A. [123I]ICF01012

B16F0

SK-Mel 3

B.

C. % activity in tumor/whole body

Tumor-to-muscle ratio

Melanin content (µg/mg of tumor)

% IA in tumor 44h after [123I]ICF01012 injection
Figure 3

A. Tumor growth ((FV-IV)/IV) vs. Days post the first $^{131}$IICF01012 injection

- Control
  - DT: 14.50 days
- 1x $^{131}$IICF01012
  - DT: 15.76 days
- 3x $^{131}$IICF01012
  - DT: 30.27 days

B. Percent survival vs. Days post the first $^{131}$IICF01012 injection

- Control
  - MS: 53 days
- 1x $^{131}$IICF01012
  - MS: 62 days
- 3x $^{131}$IICF01012
  - MS: 81 days
**Figure 4 (not supplementary figure)**

**Supplementary Figure 1**

A. Biodistribution of $[^{131}\text{I}]\text{ICF01012}$ in SK-Mel 3 tumor (% AI/\text{gr})

B. Weight variation (%) days post the first $[^{131}\text{I}]\text{ICF01012}$ injection

- **Control**
- 1x $[^{131}\text{I}]\text{ICF01012}$
- 3x $[^{131}\text{I}]\text{ICF01012}$

Days post the first $[^{131}\text{I}]\text{ICF01012}$ injection