CJ215, A NEW ULTRASENSITIVE NEAR-INFRARED FLUORESCENT PROBE FOR ENHANCED TUMOR DETECTION IN VIVO: A COMPARATIVE STUDY WITH ICG IN A PRECLINICAL MODEL.

Valérie Rouffiac[1], Corinne Laplace-Builhé[1], Michel Urbain[2], Mélanie Polrot[3], Francois Scherninski[2]

[1]*Gustave Roussy, Université Paris-Saclay, Plate-forme d'imagerie et Cytométrie , UMS AMMICa INSERM* US23-CNRS 3655. *ViLLEJUIF, F-94805, France*

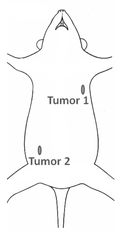
[2] *Proimaging, rue Dupont-de-l'Eure, PARIS, F-75020, France*

[3]*Gustave Roussy, Université Paris-Saclay, Plate-forme d'Evaluation Préclinique, UMS AMMICa INSERM* US23-CNRS 3655. *ViLLEJUIF, F-94805, France*

Introduction

The optimization of detection of subclinical lesions and intraoperative margins delineation is an important challenge in oncology to decrease the risk of local recurrence and preserve healthy tissue. For many years, clinical trials using near-infrared (NIR) optical imaging combined with ICG as contrast agent, try to address these issues. ICG is already approved for clinical use, but as a blood pool agent, it is not specific to tumor tissue. ICG shows a passive targeting based on enhanced permeability and retention effect, and thus is not ideally suited for image-guided surgery. In the present work, we assessed a new NIR fluorescent probe, the CJ215, in comparison with ICG, for tumor detection in a preclinical model of orthotopic mammary cancer.

Materials & Methods



Tumor model

30 nude mice were injected at day 0 (D0) into two contralateral mammary glands with 20µL/gland containing 50 000 4T1-Dendra2 cells suspended in PBS. At D9, tumors reached a mean volume of 74,8 and 44 mm^3 for tumor 1 and tumor 2 respectively.

Fluorescent probes

In vivo fluorescent specificity and sensitivity of a new carbocyanine, CJ215, (ProImaging®) was compared with ICG, the only Near Infra Red (NIR) fluorophore already approved in human. Preliminary experiments (data not shown) revealed a maximal fluorescent enhancement for a 2 mg/kg intravenous injection dose of CJ215. Dilutions were adapted according to the molar masses of ICG and CJ215.

In vivo imaging

Fluorescent images were acquired before and after intravenous injection of CJ215 or ICG onto an IVIS Spectrum CT®(Perkin Elmer) using both 745 nm (+/-15 nm) and 820 nm (+/-10 nm) filters for excitation and emission peaks respectively. Images were acquired at D9 post-injection of tumor cells, before NIR probes injection and 2h, 24h, 48h, 4 and 6 days post-probes injection.

For all acquisitions, optimal settings were automatically fixed. Fluorescent radiant efficiency (normalized unit) was quantified inside tumor 1 (T1), tumor 2 (T2) and abdominal region (T3), (fig 1). Normalized tumor fluorescent uptakes (nTFUs) were then computed as the ratio of T1/T3 and T2/T3.

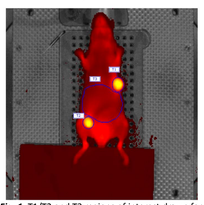


Fig. 1: T1/T2 and T3 regions of interest drawn for fluorescent intensity quantification.

Ex vivo confocal imaging

At D15, 6 days post-injection of NIR probes, mice were sacrificed. Both tumors and livers were removed and fixed in 4% paraformaldehyde. Each sample was incubated with Hoechst 33258 (1µg/ml final) for nuclei staining before being examined onto a confocal microscope SP8® (Leica) for the evaluation of CJ215 or ICG distribution in tissue. Confocal acquisitions were performed with identical settings (laser power, resolution, offset and gain) and :

- A 405 nm laser excitation for nuclei tracking
- A 488 nm laser excitation for tumoral cells detection (green form of the Dendra2 protein)
- A 638 nm laser excitation with a long-pass filter (>670nm) for CJ215 and ICG signals.

Results & Discussion

In vivo imaging

Qualitative analyses of CJ215 and ICG biodistributions show that *(fig.2)* :

- ✓ **ICG signal** is located in the abdominal region at 2h post-injection and is not longer detectable at 24 hours. Tumors are not detectable at any time under the acquisition setting used for CJ215 imaging.
- ✓ **CJ215 signal** is distributed over the whole body with a specific contrast enhancement at tumor location at 4 and 6 days post-injection

Quantitative analyses reveal higher nTFUs for CJ215 than ICG for all times considered *(fig.3)* , indicating that tumors are better and earlier detected with CJ215 than with ICG.

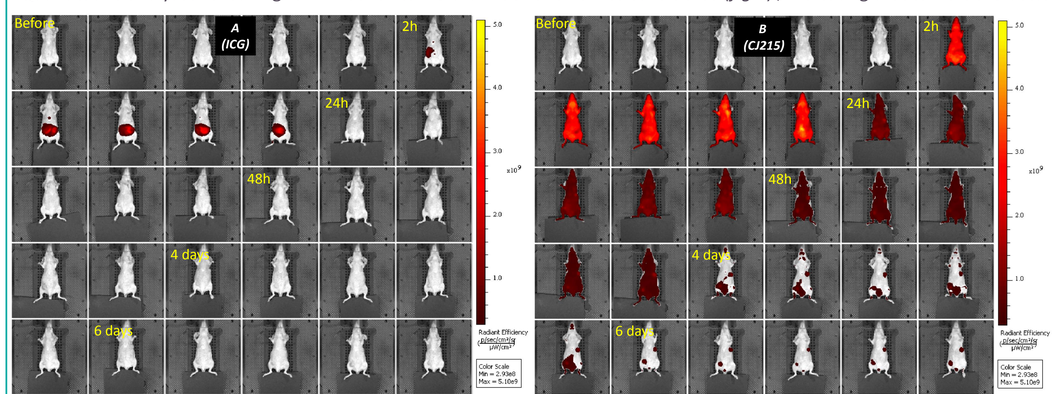


Fig. 2: Example of 5 mice per group followed at each time point : before, 2h, 24h, 48h, 4 and 6 days after intravenous injection of ICG (A) or CJ215 (B) at 2mg/kg. For direct quantitative comparison, all images are displayed with a same color scale (same min and max values for radiant efficiency).

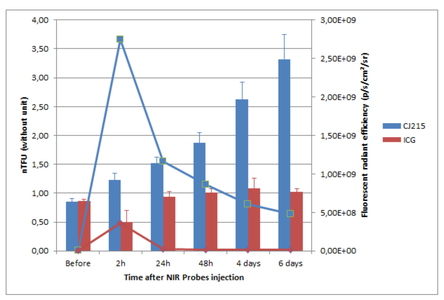


Fig. 3: Fluorescent intensities normalized with surrounding tissues (nTFUs) are represented in histograms. Non normalized fluorescent intensities are represented in dot lines. For both representations, T1 and T2 values were mixed. Differences between nTFUs of CJ215 and ICG were statistical significant for all times considered (Wilcoxon test).

Ex vivo confocal imaging

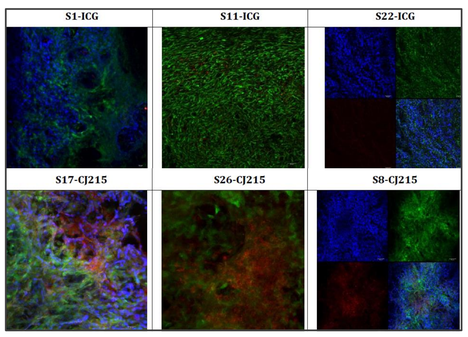


Fig. 4: Biodistribution of CJ215 and ICG at 6 days post-injection. Tumor cells appeared in green, nuclei in blue and NIR Probes in red. Images were acquired under standardized conditions allowing direct comparison of fluorescence intensities.

Tissue imaging in confocal microscopy confirmed data obtained with whole-body imaging :

- ➢ a lack of signal in the tumor tissue with ICG, at 6 days post injection (same imaging setting as CJ215) *(fig. 4).*
- ➢ a strong detectable signal of CJ215 inside tumors, 6 days post injection while no signal is detectable outside tumor volumes *(fig 4 and 5).*
- ➢ An absence of CJ215 or ICG fluorescence in livers at 6 days post injection *(fig 4 and 5).*

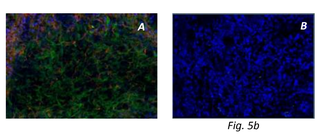


Fig. 5b

Fig. 5: Biodistribution of CJ215 inside (A) and outside (B) tumors area at 6 days post-injection. Tumor cells appeared in green, nuclei in blue and NIR-Probes in red.

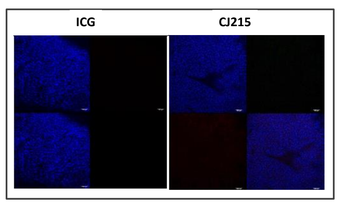


Fig. 6: Biodistribution of ICG and CJ215 in livers at 6 days post-injection. No NIR fluorescence can be seen at that time meaning a good clearance of both NIR-probes.

Conclusion

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Proimaging

Based on whole-body and confocal microscopy imaging, we have demonstrated :

- ➢ A higher fluorescence intensity of CJ215 in tumors compared to ICG whatever the time post-injection
- ➢ A greater signal to noise ratio for CJ215 compared to ICG allowing a significant improvement of tumor specific detection
- ➢ A highly persistent CJ215 fluorescence signal in tumors until 6 days post-injection, whereas a good clearance in blood and liver is seen at the same time.

CJ215 may have a greater value than ICG for intraoperative assessment of tumor margins thanks to a significant improvement of tumor specific fluorescence.

In addition, the significant persistence of CJ215 fluorescence in the tumor volume may provide greater flexibility and reproducibility in clinical protocols.