^{99m}Tc 14F7 mAb as a non-invasive approach to assess the tumoral kinetic in an orthotopic ovarian tumor biomodel.

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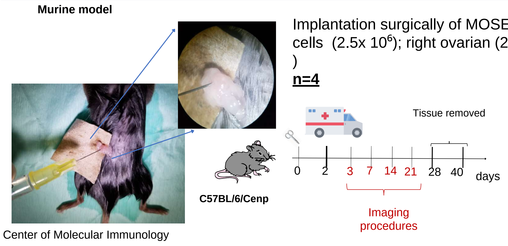
SUMMARY

Orthotopic tumor modeling is a valuable tool for pre-clinical ovarian cancer research, as it has multiple advantages over both subcutaneous and experimental mouse models. Unlike both models, orthotopic tumors contain more clinically accurate vasculature, tumor microenvironment, and responses to multiple therapies. Frequently, the standardization of a biomodels involves serial sacrifice of animals. However, the single-photon emission computed tomography and Computed tomography (SPET/CT) is a non-invasive method to evaluate the tumor progression, *in vivo*, using the same animals. This method is agreement with the 3Rs (reduction, refinement and replacement) recommended by the Guide for the Care and Use of Laboratory Animals. In addition, ^{99m}Tc-labelling of 14F7 antibody (^{99m}Tc-14F7 mAb) have been used to identify the NGcGM3 ganglioside in human breast tumors. The **objective** is to explore the imaging of ^{99m}Tc-14F7 mAb in monitoring ovarian orthotopic biomodels induced by MOSEC cells. **Methods:** Orthotopic mouse model of ovarian cancer were established by implantation surgically of MOSEC cells (2,5x 10^6) into the right ovarian (200µl); in five C57BL/6 mice. Weekly, the weight of animals was analyzed.

Six hours after the injection (intravenous rote through the mouse tail) of ^{99m}Tc-14F7 mAb, at days 7, 14 y 21 post-inoculation tumoral, SPET/CT imaging of mice bearing MOSEC cells, *in vivo*, were performed. All mice were maintained at the animal facility of Center of Molecular Immunology (CIM, Havana, Cuba). Animal care and experiments were done according to institutionally approved protocols of CIM and under supervision of Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of CIM. As the **results and discussion**, these mice developed orthotopic tumors that were non-invasively monitored *in vivo*, with the same number of animals that began the study. The intensity of radioimmunolabelling by ^{99m}Tc-14F7 mAb increased from day 7 to 21, at the inoculation site (right ovary). The histopathological studies corroborated the ovarian tumor and the spontaneous metastases in lungs, kidney and peritoneum, at day 28. Although further studies are needed to corroborate the expression of NGcGM3 in this tumoral tissues, the ^{99m}Tc-14F7 mAb may be a useful tool for the biology of NGcGM3-positive tumors and drug development research. Moreover, this study supports the concept of reduction of animals in agreement with the Reduction of animals in the preclinical studies.

Methods

1-Standardization of the murine orthotopic model of ovarian cancer induced with the MOSEC-cells line



Implantation surgically of MOSEC-cells (2.5x 10^6); right ovarian (200µl)

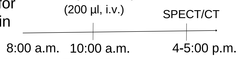
n=4

Non-clinical scenery: MOSEC-cells removed from intraperitoneal tumors are NGcGM3 positive. Immunofluorescence and Cytometric assays.

Clinical scenery: Breast cancer mama identified by ^{99m}Tc-14F7 mAb **(B.C.R., 2006)**

Binding of ^{99m}Tc-14F7 mAb

Radiochemical yield of >98% was obtained for ^{99m}Tc-14F7mAb within 2h.



National Institute of Oncology and Radiobiology (INOR)

Objective

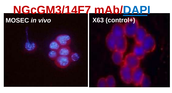
To explore the imaging of ^{99m}Tc-14F7 mAb in monitoring ovarian orthotopic biomodel induced by MOSEC cells, *in vivo*.

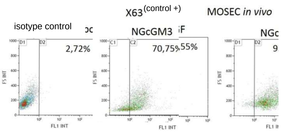
Human scenery

^{99m}Tc-14F7 mAb

Non clinical scenery

NGcGM3/14F7 mAb/DAPI

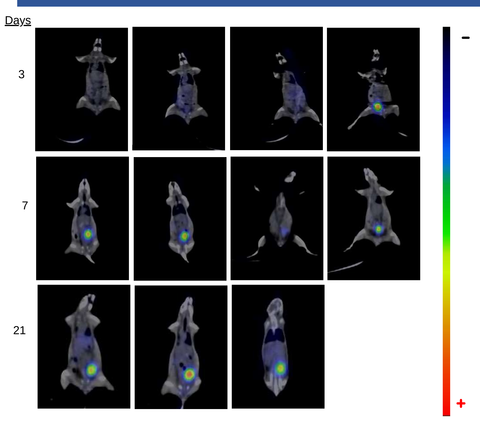




Institutional Animal Care and Use Committee of Center of Molecular Immunology (IACUC/CIM). Protocol approval number**: IACUC-2022-001-01**

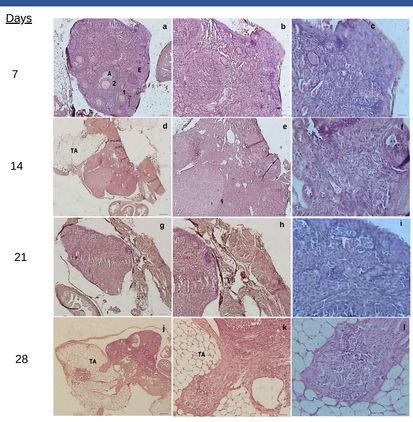
Results

2- Detection of orthotopic ovarian tumors by imaging of of ^{99m}Tc-14F7 mAb , *in vivo*.



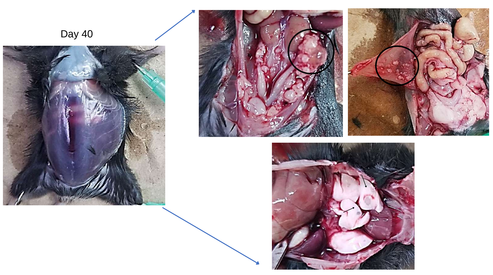
The intensity of radioimmunolabelling by ^{99m}Tc-14F7 mAb increased from day 7 to 21, at the inoculation site (right ovary)

4- Histopathological studies corroborated the ovarian ovarian tumors by MOSEC-cells.



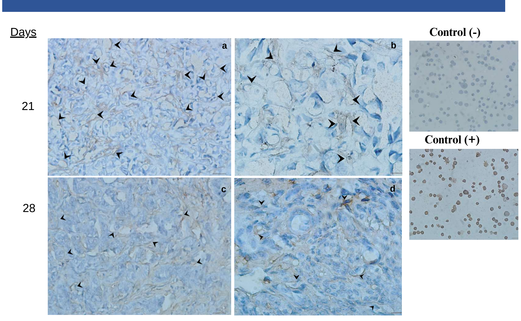
The microphotographs show the growth of primary tumor tissue in the ovary of one animal. C57BL/6 mice were inoculated on day 0 with MOSEC cells in the right ovary (orthotopic route). Animals were sacrificed on days 7 (a-c), 14 (d-f), 21 (g-i) and 28 (j-l). Hematoxylin and eosin staining. Stromata (E), Primordial follicles (1), Secondary growing follicles (2), Antrum (A), Adipose tissue (TA), Muscle tissue (TM). The figure is representative of the rest of the sample evaluated. 10X (a, d,g,j), 20X (b, e, h y k) y 40x (c, f, i, l).

3- Multiples peritoneal tumors with ascitic fluids at day 28 and 40 by microscopy study.



Biomodel of orthotopic ovarian tumor with the MOSEC cell line. Photographs show the growth of primary tumor tissue in the ovary of one animal. C57BL/6 mice were inoculated on day 0 with MOSEC cells in the right ovary (orthotopic route). Clinical monitoring of the mice (3) was performed twice daily starting on day 0. The photos show ballooning of the abdominal cavity due to the presence of ascitic fluid. Metastatic spread in the abdominal cavity is shown in the following figures. Photographs were taken with a steromicroscope at 12.5X.

5- NGcGM3 expression in orthotopic ovarian tumors



The expression of NGcGM3 ganglioside, was determined on ovarian tumors on days 21 and 28. NGcGM3 is localized at the cell membrane and/or cytoplasmatic cell (brown color) by immunohistochemistry staining (black arrowhead). Cell nuclear (blue color). Negative control (-): L1210 cells were use as negative control (-) and positive control (+). Tumor tissue from day 21 (a, b) and 28 (c, d); 40x (a, c), 100x (b, d).

Conclusions

- Spontaneous metastatic colonization of the abdominal and thoracic cavity by orthotopic inoculation of MOSEC cells, in C57BL/6 mice, generates a murine model similar to stage IV human ovarian cancer.
- Imaging techniques are an important tool to assess tumor progression, *in vivo*, in agreement with the reduction in animal research.
- *In vivo*, immune recognition of NGcGM3 ganglioside in ovarian tumor corroborate the value of this target for preclinical cancer immunotherapy.