DEVELOPMENT OF PRECLINICAL ADVANCED MODELS OF HUMAN SOLID TUMORS FOR THE IDENTIFICATION OF EFFECTIVE THERAPEUTIC STRATEGIES

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Background. Despite many antineoplastic compounds showed favorable tumor responses in preclinical studies, more than 95% of these therapeutics failed to confirm efficacy in clinical trials and many factors are responsible for this high failure rate, including the lack of predictive cancer models. For decades, established tumor cell lines and xenografts have represented the standard for preclinical cancer research; notwithstanding these models maintain utility for a first antitumor efficacy assessment, their behavior diverged from original tumors, in terms of tumor heterogeneity and patterns of gene expression. Moreover, xenografts are implanted in ectopic sites which do not represent the microenvironment of origin of tumors and very rarely produce metastatic dissemination, thus limiting the investigation on a key point of cancer progression and death.

Aim. In this context, the use of advanced preclinical models such as tumor organoids, patient derived xenografts (PDX), patient-derived organoids xenografts (PDOX) and orthotopic implant in mice, could increase the robustness of drug discovery studies.

Advanced preclinical models of colorectal cancer

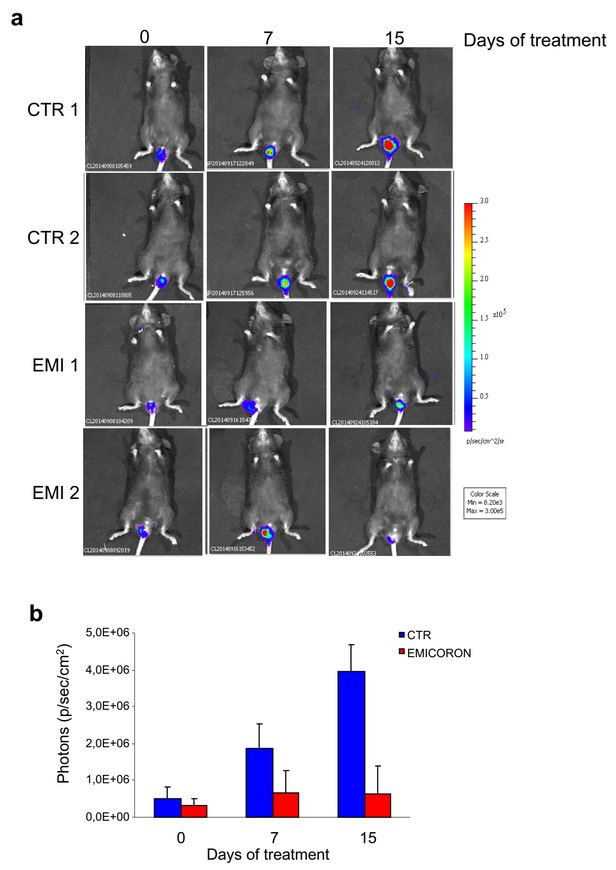

Figure 1. EMICORON is efficacy against orthotopic colorectal model.
A90-LUC GEMMs colorectal murine cells were injected in the rectal mucosal surface of syngenic C57 BL6 mice. Mice were treated with EMICORON given orally at 20 mg/Kg for 15 consecutive days. **a**) Real time tumor growth of A90- LUC cells was monitored by optical imaging. Imaging was performed at baseline (day 0) before the administration of compound and at day 7 and 15 of treatment and representative images are shown. Data were acquired and analyzed using the Living Image Software version 3.0. **b**) Histogram reports bioluminescence in tumors from untreated and treated groups at day 0, 7 and 15. Errors bars indicate ±SD.

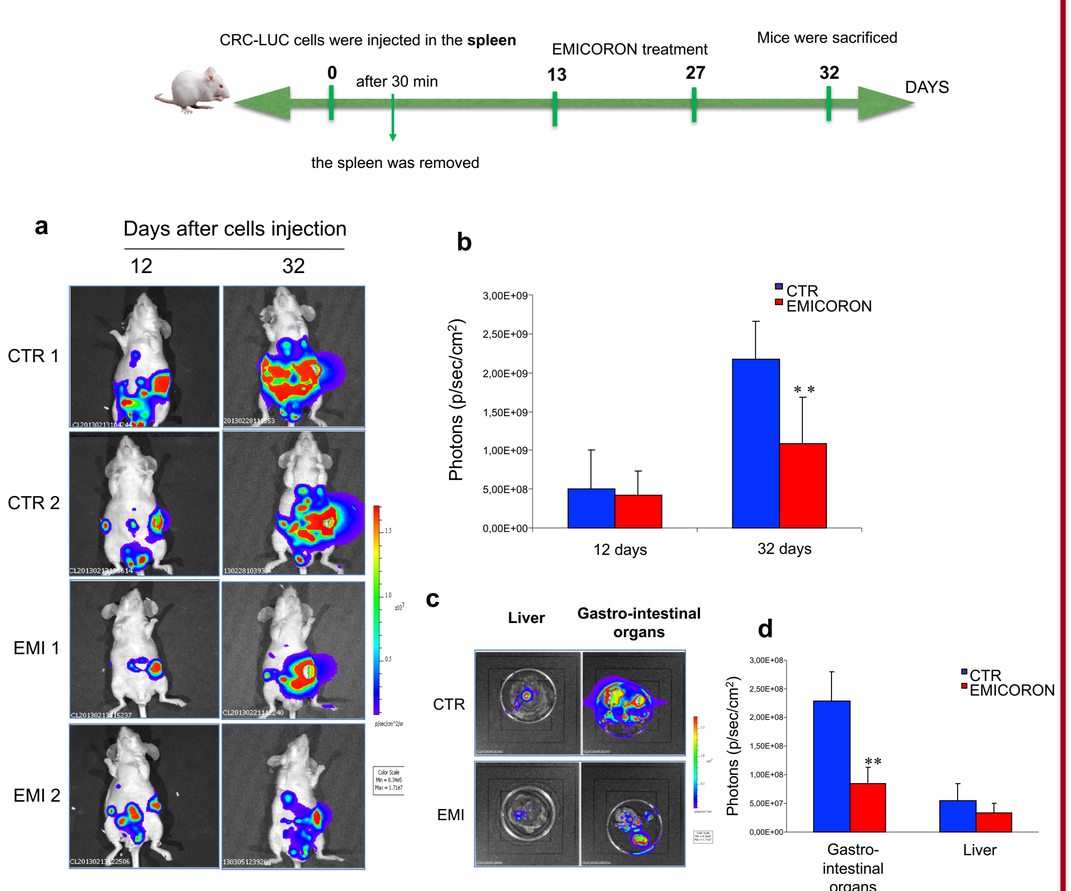

Figure 2. ***EMICORON has antitumor efficacy in a tumor dissemination model.***
a) Human HCT116LUC2 colon cancer cells were stably transfected with firefly luciferase gene (luc2) and were injected in the spleen of immunosuppressed mice (n=12). After 30 min, the spleen was removed by spleenectomia and the mice were sutured using 1mm surgical sutures. Real time tumor dissemination was monitored using the IVIS imaging system 200 series (Caliper Life Sciences, Hopkinton, MA, USA). Twelve days post- injection 6 mice were treated *o.s.* with Emicoron at 20 mg/Kg for 15 consecutive days. **b**) Histogram reports bioluminescence in mice from untreated (blue) or treated with EMICORON (red) at day 12 and at day 32 after tumor cells injection. Error bars indicate mean values ±SD. **c**) Thirty-two days post- injection, the mice were sacrificed, organs were harvested and acquired using the IVIS imaging system 200 series (Caliper Life Sciences, Hopkinton, MA, USA). **d**) Histogram reports bioluminescence intensity in liver and gastrointestinal organs. Error bars indicate mean values ±SD. * * $p < 0.01$.

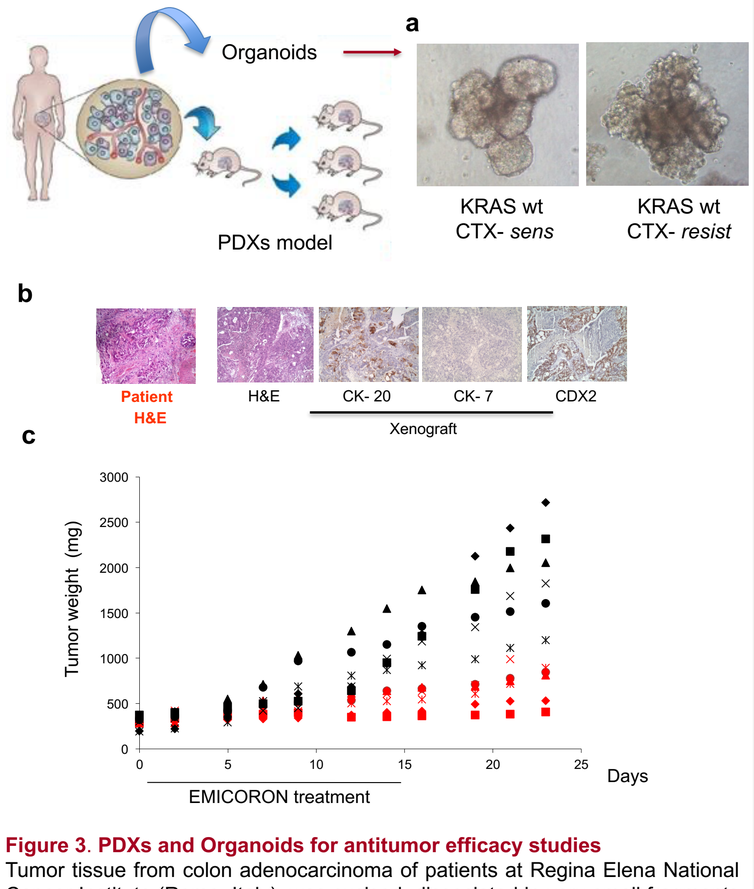

Figure 3. PDXs and Organoids for antitumor efficacy studies
Tumor tissue from colon adenocarcinoma of patients at Regina Elena National Cancer Institute (Rome, Italy), was excised, dissociated in very small fragments and resuspended in a drop of BME with organoids medium. **a**) Tumor organoids will appear 1-2 weeks after plating. We obtained organoids KRAS wild type sensitive and resistant to the treatment with Cetuximab (CTX). **b**) Small fragments of a metastatic CRC KRAS mut were coated in Matrigel and implanted in NOD-SCID mice in a subcutaneous pocket. After mass formation in mice, H&E staining was performed to determine the biological stability of the xenograft compared with the patient tumor tissues. In addition, immunoistochemistry of markers of the epithelium, anty-cytokeratin 7 (CK-7), anti-cytokeratyn 20 (CK- 20) and anti-CDX2 was performed. **c**) Following transplantation, tumors were allowed to grow to about 200 mg before initiation the treatment with EMICORON. Each point represents mice untreated (black) or treated with EMICORON (red). Six mice for each group were evaluated.

Advanced preclinical models of BRCA1/2 mutated breast and ovarian tumors

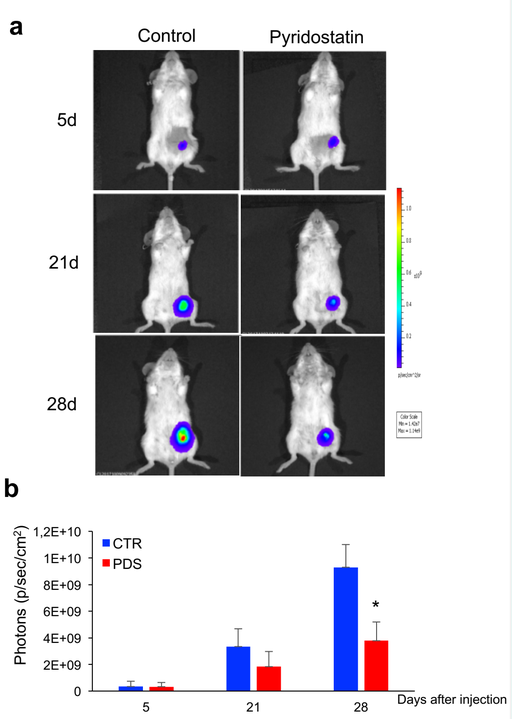

Figure 4. Pyridostatin has a marked antitumor efficacy in orthotopic model of breast cancer
a) MDA-MB-436 LUC cells (BRCA1 defective) were implanted into the mammary fat pad of NSG female mice. When the tumor was palpable the mice were treated with vehicle (control) and with Pyridostatin (PDS 7.5 mg/Kg) *iv* at days 8-12;15-19. Real time tumor growth was monitored using the IVIS imaging system 200 series (Caliper Life Sciences, Hopkinton, MA, USA). **b**) Histogram reports bioluminescence intensity in mice untreated (blue) or treated with Pyridostatin (red) at day 5, 21 and 28 after tumor cells injection. Error bars indicate mean values ±SD. * $p < 0.5$.

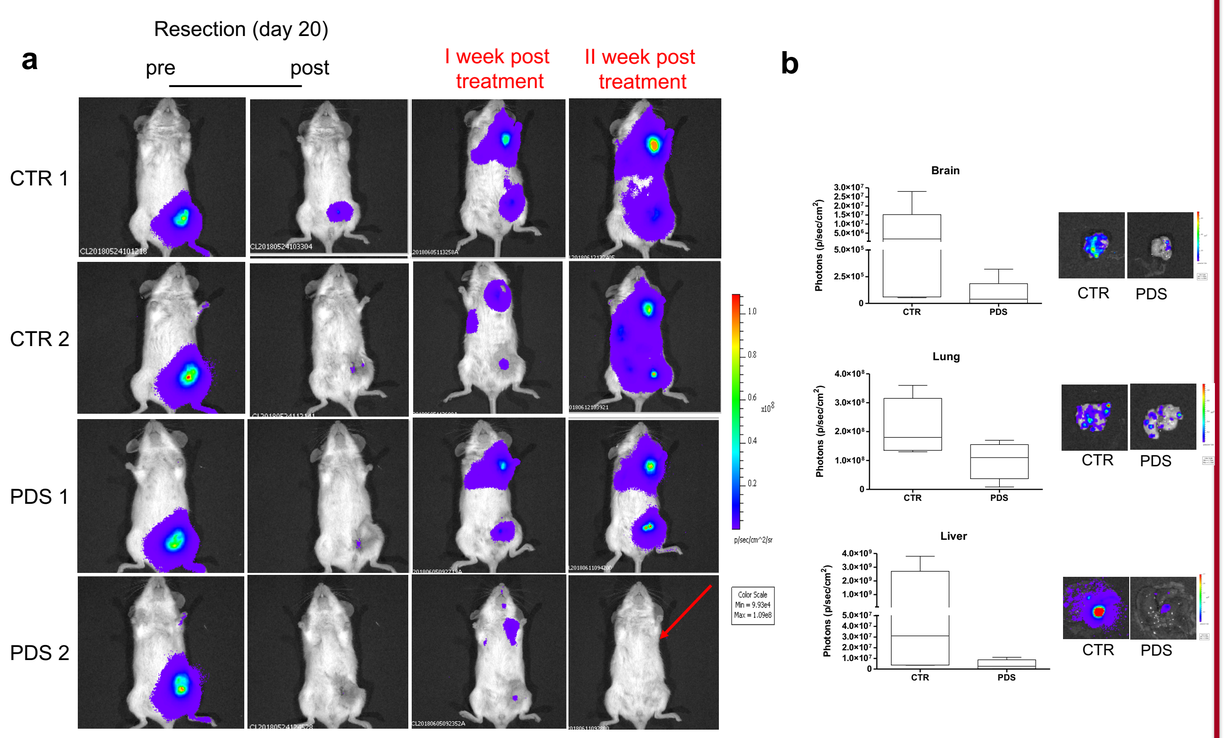

Figure 5. ***Pyridostatin reduces the growth of spontaneous breast cancer metastasis.***
a) MDA-MB-436 LUC cells were implanted into the mammary fat pad of NSG female mice. Twenty days after cells injection, primary tumor was resected and mice were treated with vehicle (control) or Pyridostatin *iv* (7.5 mg/kg daily) for 2 weeks. Real time tumor growth was monitored at the end of the first and second weeks of treatment using the IVIS imaging system 200 series (Caliper Life Sciences, Hopkinton, MA, USA).
b) At the end of treatment mice were sacrificed and multiple organs including brain, lung and liver were dissected and analyzed for tumor metastasis by IVIS imaging. Box plots report bioluminescence signal of ex- vivo organs. Each group included five mice.

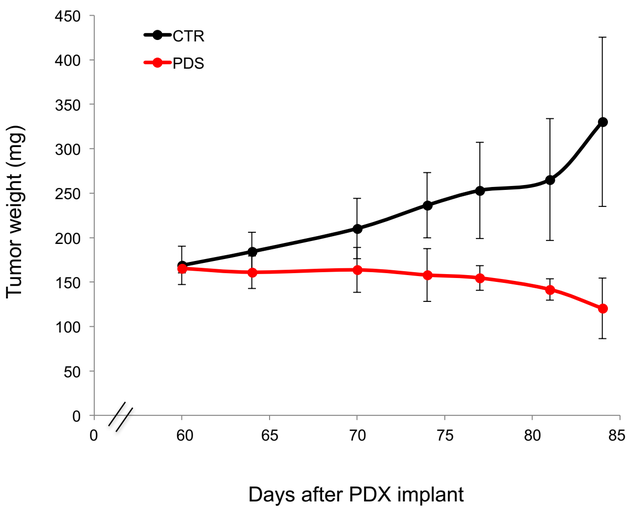

Figure 6. ***Pyridostatin has antitumor efficacy in a PDX model of BRCA2$^{-/-}$Ovarian Cancer.***
At passage 2 in mice, a PDX of ovarian cancer BRCA2$^{-/-}$ was explanted and diced into 15-20 mm^3 pieces, coated in Matrigel and implanted in NSG female mice by a small incision in a subcutaneous pocket. After 60 days the tumors reached 150 mg and the mice were treated with vehicle (CTR) or Pyridostatin at 7,5 mg/Kg i*v* for 15 days. Tumor size were measured three times a week in two dimensions by a caliper and tumor weight was calculated using the formula $axb^2/2$, where a and b are the long and short sizes of the tumor, respectively. Each group included five mice. Error bars indicate mean values ±SD.