Bioluminescent orthotopic PDX models of primary pancreatic cancer and residual/metastatic breast cancer to predict efficacy of standard of care and experimental treatments

Benjamin G. Cuiffo[1], Ingrid Rankin[1], Olivier Deas[2], Gregory D. Lyng[1], Stephano Cairo[2], Enora Le Ven[2], Jean-Gabriel Judde[2], Stephen T. Sonis[1].
[1]Biomodels, LLC, Watertown, MA, USA; [2]XenTech, Evry, France

Abstract

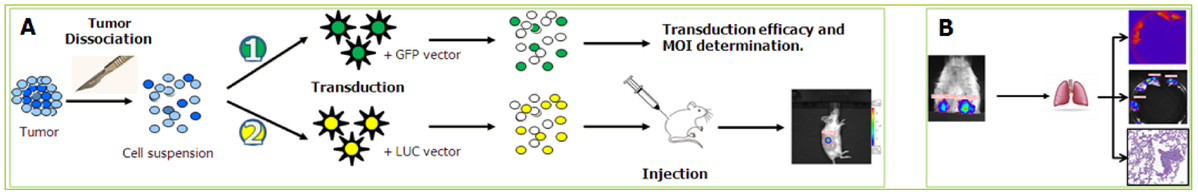
Background: Only ~5% of investigational anti-cancer agents are ultimately clinically successful. This low success rate may be partially attributed to the widespread historical use of imperfect ectopic xenograft models in preclinical studies of efficacy – utilizing established human cancer cell lines conventionally propagated in 2D monoculture that poorly recapitulate tumor heterogeneity. In order to model critical influences of tissue environment on tumor behavior and therapeutic response, heterogeneous patient-derived xenografts (PDX) can be ideally assayed following implantation at orthotopic sites in rodents. Challenges to this approach include inaccessibility of certain organs and inability to track residual or metastatic disease. To address these problems, we utilized pancreatic (PANx-005) or metastatic breast (HBCx-14) PDXs transduced with stable bioluminescent reporters in efficacy studies of standard of care (SOC) and experimental treatments in clinically recapitulative models.

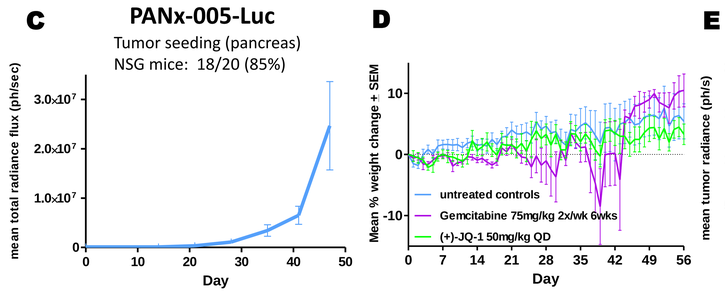
Methods: Freshly excised PDXs were transduced with lentiviral vectors stably expressing luciferase, and implanted orthotopically into NOD/SCID/γ (NSG) mice. For our primary pancreatic cancer model, the pancreas was surgically exposed, and ~2.5 x 10^6 PANx-005-Luc cells were inoculated directly. Post-op, tumor growth was monitored in-life (Xenogen IVIS® Lumina Series III instrument (IVIS)). Mice were randomized into treatment groups when mean tumor radiance (TR) reached 3.0 x 10^7 photons/sec. Control animals received no treatment; standard of care gemcitabine (75 mg/kg, 2QWx6-1wk rest, i.p.), or (+)-JQ-1 BET bromodomain inhibitor (50 mg/kg, QD, i.p.). For the residual/metastatic breast cancer model, ~1.5 x 10^6 HBCx-14-Luc were injected directly into the 4th inguinal mammary fat pad. Tumor growth and response were monitored throughout the study by IVIS. When individual TR reached 6.5 x 10^9 photons/sec, the primary tumor was resected and the animal enrolled into a treatment group to be treated for residual/metastatic disease. Control animals received no treatment; SOC groups received either radiation therapy of 12Gy (3x 4Gy fractions on Days 0, 4 and 8; capecitabine (540 mg/kg QDx5/ 1week rest, p.o.) or docetaxel (20 mg/kg, Q3Wx2, i.p.); while experimental animals received (+)-JQ-1 (50 mg/kg, QD, i.p.).

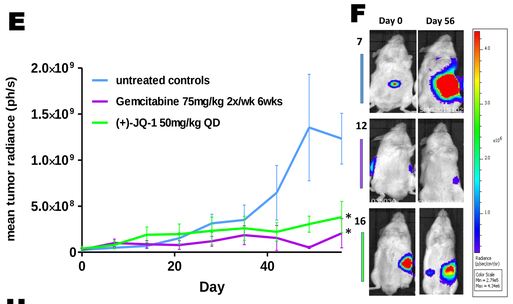
Results: Tumor seeding approached 100% in both models. Growth kinetics resembled clinical indications, including rapid growth and lung metastases for the HBCx-14 tumors, and slower growth of PANx-005 tumors. Efficacies of individual treatments recapitulated clinical responses: HBCx-14 tumors were high responders to capecitabine and (+)-JQ-1, and low-responders to docetaxel, while PANx-005 tumors displayed high response to (+)-JQ-1 and lower response to gemcitabine.

Conclusions: The translational predictivity of preclinical cancer models is enhanced by the use of human PDXs that preserve tumor heterogeneity, are assayed at orthotopic sites, and are utilized in models that recapitulate clinical situations (e.g. treatment of residual disease vs primary tumor). The use of stable bioluminescent reporters in such assays greatly enhances precision in monitoring tumor growth and treatment response. Currently available preclinical oncology models are more recapitulative, precise and predictive than ever before, and appear poised to engender improved translational success.

Studies were performed at Biomodels' facility in Watertown, MA, under Biomodels' IACUC Protocol 15-0622-1. Biomodels' Office of Laboratory Animal Welfare (OLAW) assurance number is A4591-01.
Characterization of PDX lines, as well as lentiviral luciferase transduction and model validation (Figure 1A and 1B) was performed by XenTech in conformity with National Veterinarian Regulations as defined by the French Ministry.







G

Treatment	#animals with metastases (%)	sites of metastases
untreated	2/7 (28.6%)	kidney, abdominal wall
gemcitabine	1/4 (25%)	abdominal wall
(+)JQ-1	3/5 (60%)	abdominal wall, spleen

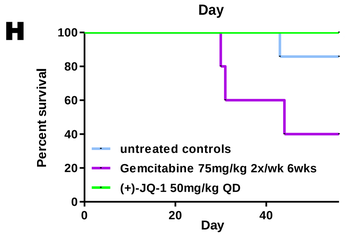
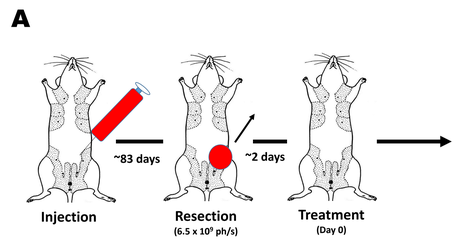
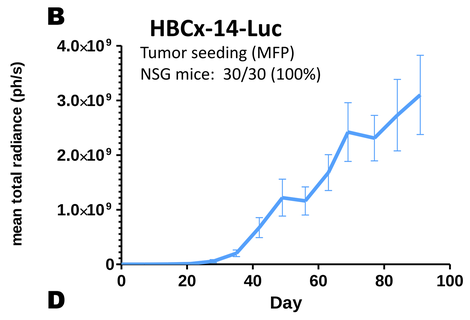
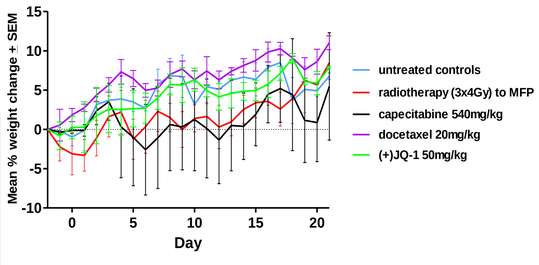
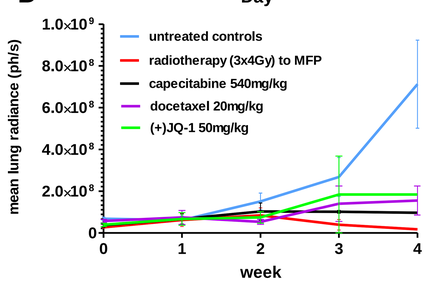


Figure 1: Bioluminescent Orthotopic PANx-005-Luc PDX Primary Pancreatic Cancer Model.
A. Workflow of generation of bioluminescent PDX. **B**. Schematic of validation of bioluminescent PDX. **C.** Tumor take rates and tumor growth kinetics as measured by IVIS radiance over time, pre-randomization. **D.** Mean percentage of weight change ± SEM following randomization over time. **E.** Tumor growth kinetics post-randomization as measured by IVIS radiance over time. Two-tailed unpaired t-test with Welch's correction was used to compare each treatment group to untreated controls on each Day. Statistical significance for each Group was achieved only at Day 56 (* = P < 0.05). **F.** Representative IVIS images from each Group at Day 0 and Day 56 and color scale. **G.** Table displaying number and percentage of animals with detectable metastases and location from each group. Zero statistically significant differences in number of animals with metastases were detected between Groups when tested by Fischer's Exact test. **H.** Kaplan-Meier curve indicating percentage of surviving animals over time. Animals treated with (+)-JQ-1 displayed a statistically significant enhancement of survival compared to animals treated with gemcitabine (* = P < 0.05), but not compared to untreated animals.









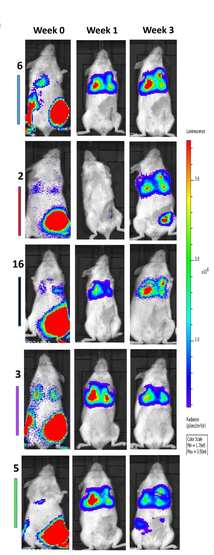


Figure 1: Bioluminescent Orthotopic HBCx-14-Luc PDX Residual/Metastatic Cancer Model.
A. Schematic of model. **B.** Tumor take rates and tumor growth kinetics as measured by IVIS radiance over time, pre-enrollment. **C.** Mean percentage of weight change ± SEM following randomization over time. **D.** Lung metastases kinetics post-resection of primary tumor as measured by IVIS radiance over time. **E.** Representative IVIS images from each Group at Day 0 and Day 56 and color scale.

Results

For the orthotopic bioluminescent human pancreatic cancer PDX model, twenty (20) male NSG mice (Jackson Labs) aged 5-7 weeks (~20g) were anesthetized with isoflurane and prepared for sterile surgery. Briefly, the pancreas of each animal was extruded for injection of bioluminescent PDX pancreatic tumor cells propagated in vivo, with viable cells counted by trypan blue exclusion. Eighteen out of twenty (18/20 (85%)) pancreatic inoculations of ~2.5 x 10^6 dissociated PANx-005-Luc cells resulted in primary tumors with detectable radiance by IVIS within 1 week. Forty-seven (47) days following tumor inoculation, seventeen (17) animals with a mean tumor radiance of 3.04 x 10^7 ± 1.07e7 ph/s were randomized by tumor radiance into treatment groups (Figure 1C). Day of randomization was considered Day 0 of the study. Animals were enrolled in the study for fifty-six (56) Days. Over the course of the study, animal weight was monitored daily. Untreated and (+)-JQ-1 treated animals displayed a similar slow steady weight gain over the course of the study, while some animals receiving gemcitabine treatment displayed periods of weight loss consistent with drug toxicity, especially on Days ~29 and ~35 (Figure 1D). Animal deaths in the gemcitabine treated group appear consistent with observed weight loss and drug toxicity, rather than tumor related morbidity (Figure 1H). Orthotopic tumor growth was tracked in vivo by IVIS. Untreated animals displayed a steady mean tumor growth through Day 49, while orthotopic tumor growth of gemcitabine or (+)-JQ-1 treated animals, displayed a slower growth rate (Figure 1E). Separation of mean tumor radiance between untreated and gemcitabine or (+)-JQ-1 treated groups was visible at Day 42 and differences became significant statistically (P=0.0323 for gemcitabine; P=0.0392 for (+)-JQ-1) at Day 56. Representative IVIS images from each Group at Day 0 and Day 56 are shown (Figure F). On Day 56, all animals were sacrificed and underwent necropsy to confirm sites of metastasis. Metastases were detected in abdominal wall (all Groups), kidney (untreated animal) or spleen ((+)-JQ-1) treated animals. Macrometastases were detected in two of seven (2/7 (28.6%)) untreated, one of four (1/4 (25%)) gemcitabine treated, and three of five (3/5 (60%)) of (+)-JQ-1 treated animals; however, these differences were not statistically significant when challenged with Fischer's Exact test.

For the orthotopic bioluminescent human metastatic breast cancer PDX model, thirty (30) female NSG mice (Jackson Labs) aged 5-7 weeks (~20g) were injected with ~1.5 x 10^6 dissociated HBCx-14-Luc PDX cells in the mammary fat pad. Thirty out of thirty (30/30 (100%)) injections produced primary tumors with detectable radiance by IVIS within 1 week. When individual tumor radiance reached 6.5 x 10^9 ph/s, the primary tumor was resected and the animal enrolled into consecutive treatment groups to be treated for residual/metastatic disease. Seventeen (17) animals have been enrolled to date, with the mean day of enrollment being 83 ± 1.94 days post-inoculation and mean tumor radiance at enrollment of 6.75 x 10^9 ± 6.28e8 ph/s (Figure 2B). Day of resection/enrollment was considered Day (-2) of the study. All enrolled animals survived to date. Animal weight was monitored daily. Animals receiving treatment with docetaxel gained the most weight, while animals receiving capecitabine or radiation treatment gained the least weight (Figure 2C). Area under the curve analysis followed by one-way ANOVA with Dunnett's Multiple Comparison test detected zero statistically significant differences in cumulative weight change between Groups. Progression of metastatic disease was tracked in vivo by IVIS of lungs. Untreated animal lungs displayed a steady increase in mean lung radiance through Week 4, while animals treated with docetaxel, capecitabine, (+)-JQ-1, or fractionated radiation displayed a slower increase in mean lung radiance over time (Figure 2E). Separation of mean lung radiance between untreated and treated groups was visible at week 4 following beginning of treatment, however these differences were not statistically significant at this time. Representative IVIS images from each Group prior to primary tumor resection and at week 3 are shown (Figure 2E). This study remains in progress.

Conclusions

Clinically recapitulative bioluminescent orthotopic PDX models of primary pancreatic cancer and residual/metastatic breast cancer following resection of primary tumor are can be employed to more accurately predict efficacy of standard of care and experimental anti-cancer therapeutics.