Evaluation of pharmacological response

Virtual THERACAT meeting

Sep 2020

Tel Aviv University

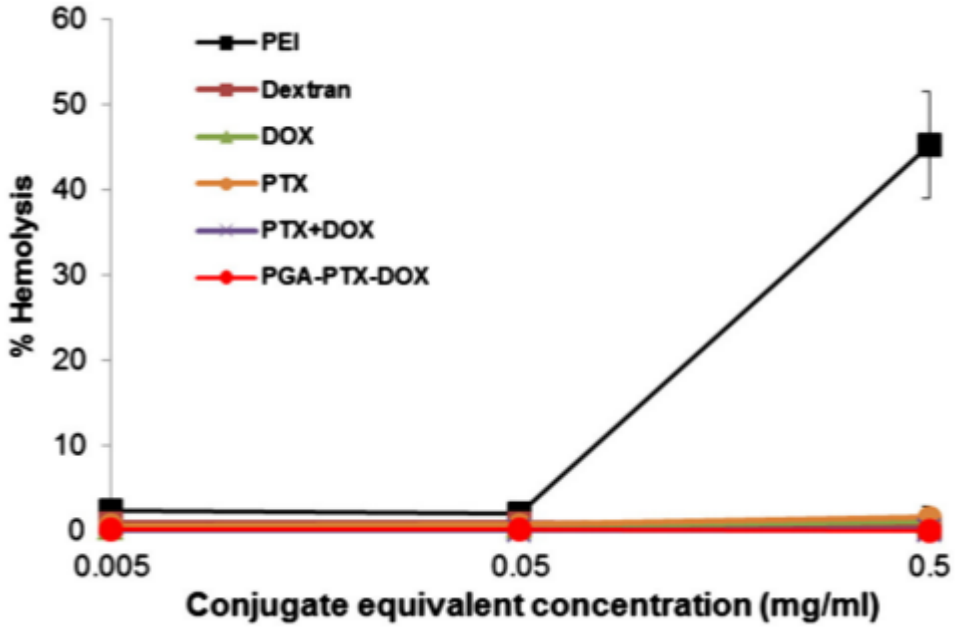
Prof. Ronit Satchi-Fainaro, Ph.D.
Head, Cancer Research and Nanomedicine Laboratory
Kurt and Herman Lion Chair in Nanosciences and Nanotechnologies
Director, Kahn 3D-BioPrinting Initiative; Director, Cancer Biology Research Center
Sackler Faculty of Medicine, Tel Aviv University, Israel

Evaluation of biocompatibility

Biocompatibility: Red Blood Cell (RBC) lysis

- Hemolysis Biocompatibility of the conjugate can also be assessed by measuring red blood cell (RBC) lysis. The concentrations used should be the ones relevant to the in vivo concentrations used, adjusted to dilution in the mouse blood volume (1.5 ml).

- For example- Hemolysis assay Rat red blood cells (RBC) solution (2% wt/wt) was incubated with serial dilutions of PGA–PTX–DOX, PTX, DOX and the combination of free drugs, for 1 hour at 37 °C. Highest concentration of the treatments was the one used in the in vivo experiment, adjusted to dilution in mouse blood volume (0.5 mg/ml conjugate and equivalent concentrations of free drugs). Dextran (Mw 70 kDa, Sigma) was used as negative control and polyethyleneimine (Mw 25 kDa, Sigma) was used as positive control. Following centrifugation, the supernatants were transferred to a new plate and absorbance measured at 550 nm using a SpectraMax M5e plate reader (Molecular Devices). The results were expressed as percentage of hemoglobin released by 1% wt/vol solution of Triton X100 (100% lysis).at the attached graph, the results clearly show that at these concentrations PGA–PTX–DOX conjugate and the free drugs did not cause hemolysis ex vivo and are therefore suitable for i.v. administration. Polyethyleneimine (PEI), a cationic polymer, was used as a positive control and dextran was used as a negative control.



Markovsky E*, Baabur-Cohen H*, Satchi-Fainaro R. J Control Release 2014, 187: 145-157.

Biocompatibility: Body weight change

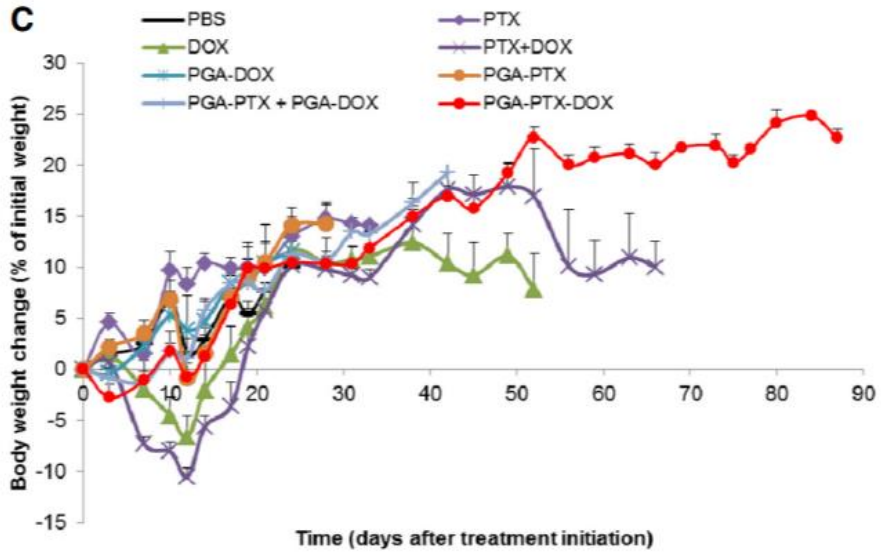
Measurement of cachexia at an extent of more than 20% body weight loss compared to initial weight represents toxicity

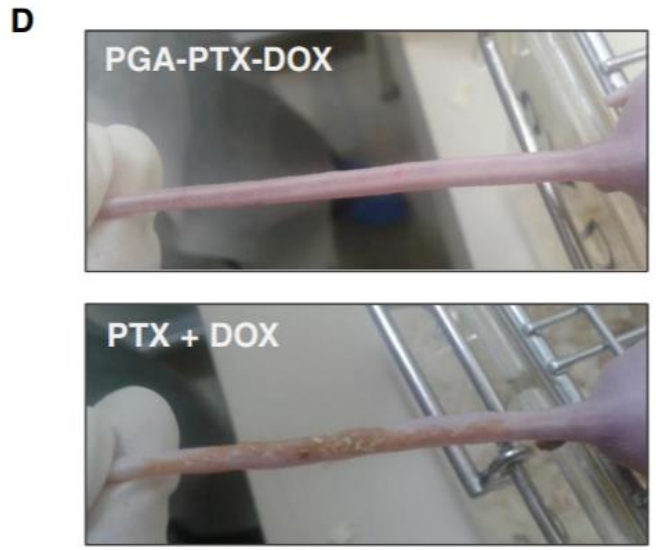
- Weigh the mice 3 times a week

For example: Body weight and tumor size were monitored every other day. In all the experiments, humanitarian end point was set at 20% body weight loss, or 1000 mm^3 tumor size.

Injection site-

- Tail vein collapse/wounded site



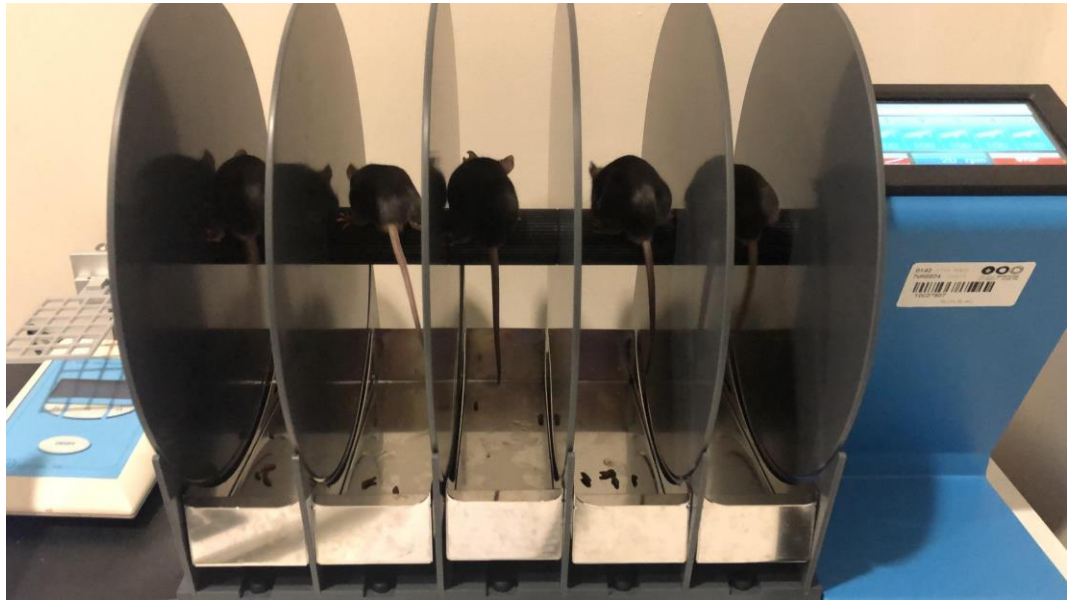


Markovsky E*, Baabur-Cohen H*, Satchi-Fainaro R. J Control Release 2014, 187: 145-157.

Biocompatibility: Evaluation of behavioral changes: neurotoxicity

Rotarod assay

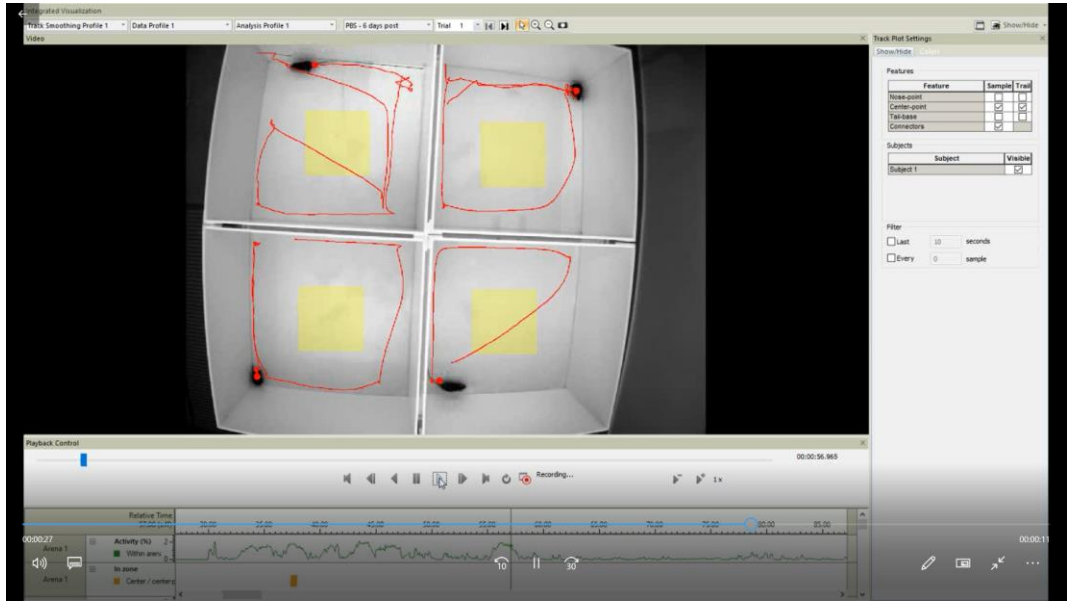
- Rotating rod at increasing velocity to measure motor coordination and imbalance



OpenField Maze

- Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice
- Animal models have proven to be invaluable to researchers trying to answer questions regarding the mechanisms of behavior. The Open Field Maze is one of the most commonly used platforms to measure behaviors in animal models. It is a fast and relatively easy test that provides a variety of behavioral information ranging from general ambulatory ability to data regarding the emotionality of the subject animal.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4354627/



Biocompatibility: Evaluation of cardiotoxicity by ultrasound and histology

Ultrasound

- Left ventricle systolic volume (υl)
- Ejection fraction %

For example: Echocardiography examination. Mice treated every other day with 5 mg/kg (cumulative dose 15 mg/kg) of Dox or Dox-equivalent dose of (FA-PEG)-Pull-(Cyst-Dox), (NH2-PEG)-Pull-(Cyst-Dox), PLD, PLD-FA, were anesthetized with 2% isoflurane and echocardiograms were performed with a commercially available mouse 2Dechocardiography system (Vevo 2100, VisualSonics, Toronto, Canada) equipped with 35 MHz phased array transducer. Transthoracic echocardiography was performed, 10 days after the last treatment, as previously described [56]. An experienced technician, blinded to the treatment groups, performed all measurements that were averaged for 3 consecutive cardiac cycles.

Histological evaluation

- Histological staining with Masson's Trichrome of hearts of mice

Histological analysis. After functional evaluation, animals were euthanizedwith an overdose of pentobarbital and heartswere perfused with 4% formaldehyde (15 mmHg) for 10 min. Hearts were harvested, sectioned and adjacent blocks were embedded in paraffin, sectioned into 5-µmslices. To evaluate pathological changes in cardiac morphology and structure, heart sections were stained withMasson's Trichrome.

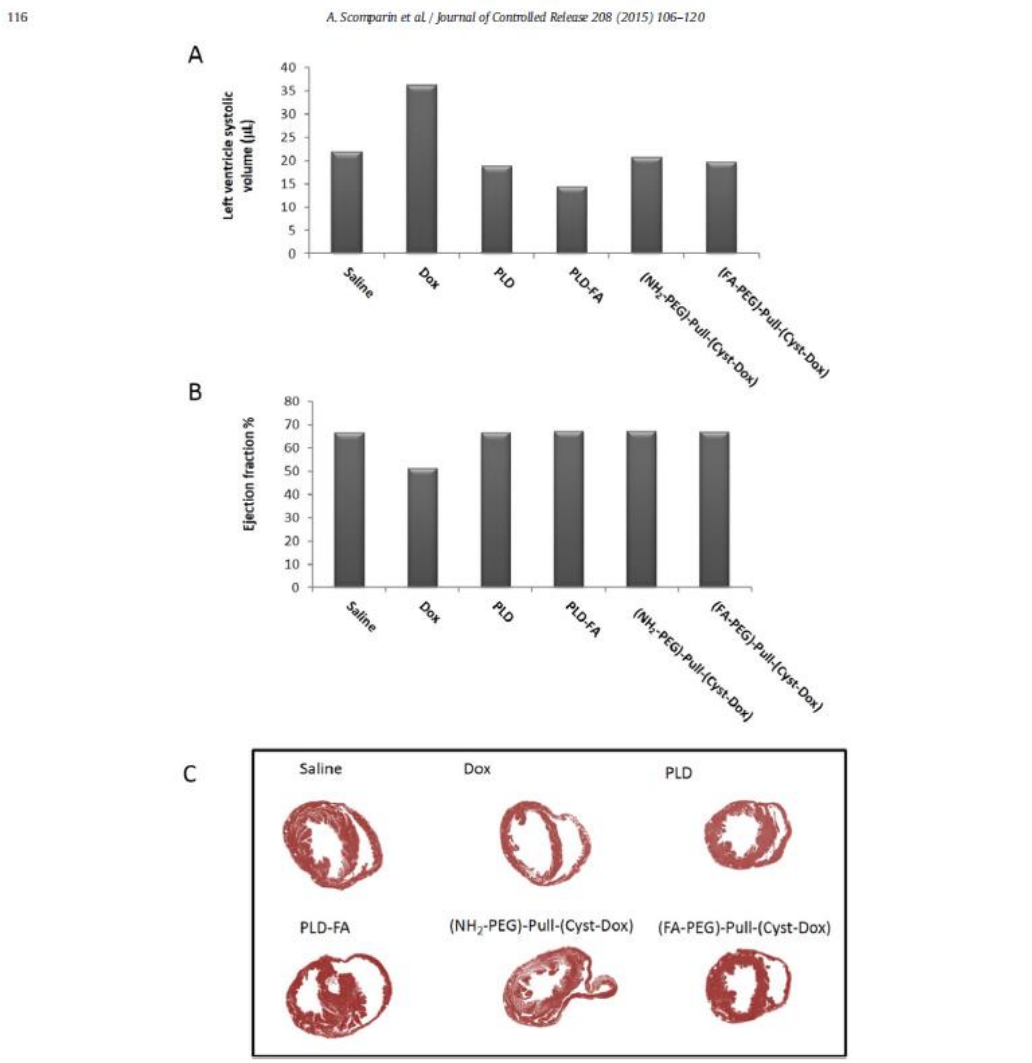
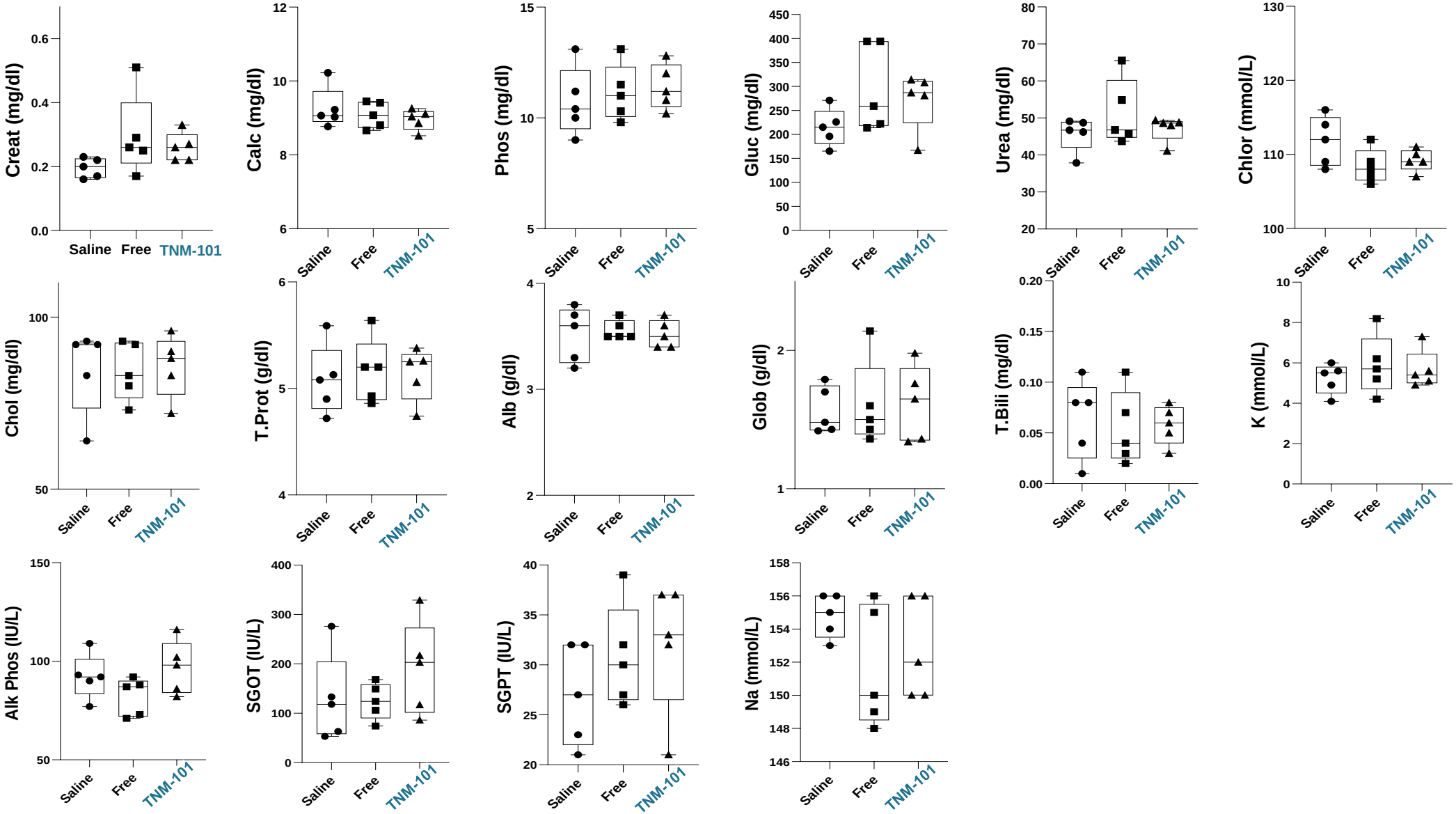


Fig. 7. FR-targeted and non-folated polymeric and liposomal formulations reduce Dox-induced cardiotoxicity in mice. Mice treated with Dox showed impaired functionality displaying (A) greatest left ventricle systolic volume and (B) lowest LV ejection fraction ten days following treatment discontinuation. (C) Histological staining with Masson's Trichrome of hearts of mice treated three times in 6 days with saline or 5 mg/kg of Dox or Dox-equivalent dose of PLD-FA, PLD, (FA-PEG)-Pull-(Cyst-Dox), or (NH_2-PEG)-Pull-(Cyst-Dox).

Scomparin A, et al., (Satchi-Fainaro R). *J Control Release* 2015, 208 : 106–120.

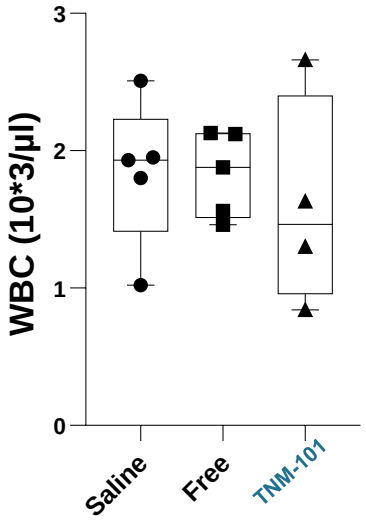
Biocompatibility: Kidney and liver functions and blood chemistry

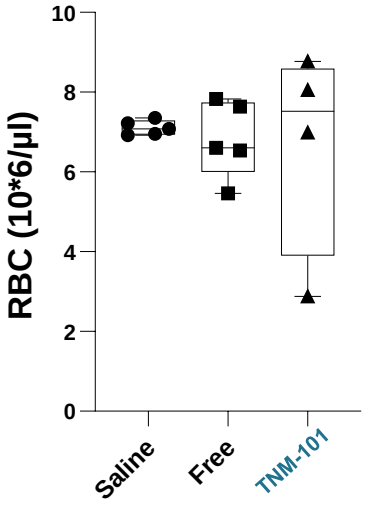
CHANGES IN BLOOD CHEMISTRY

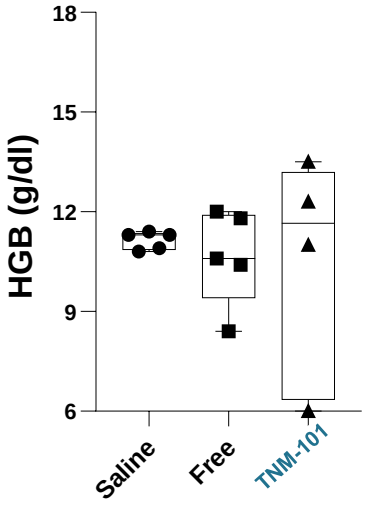


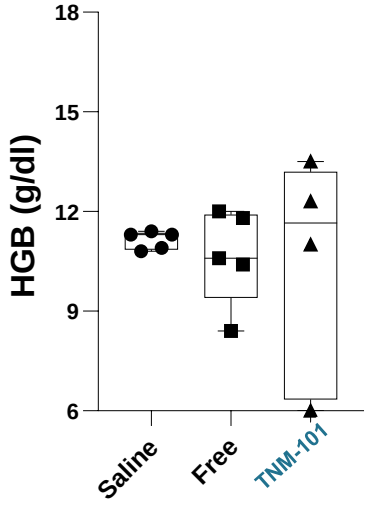
Biocompatibility: Blood count

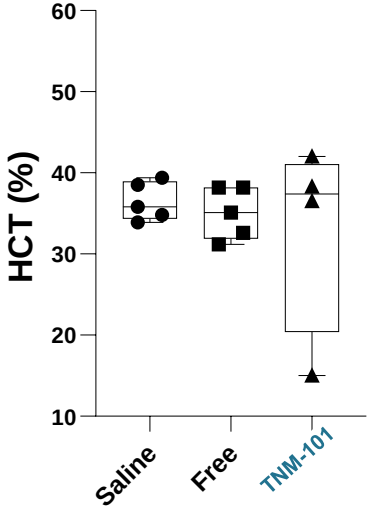
CHANGES IN BLOOD COUNT

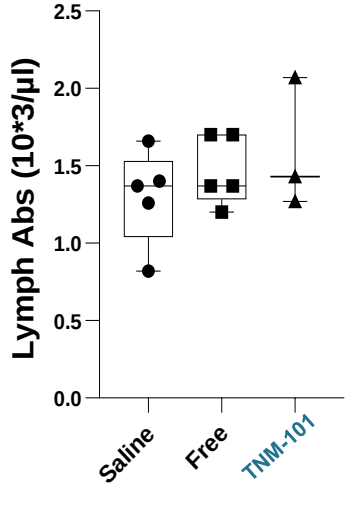


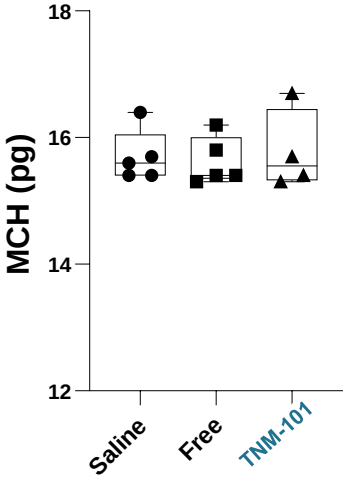


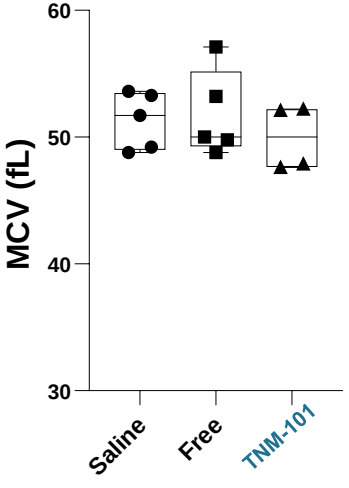


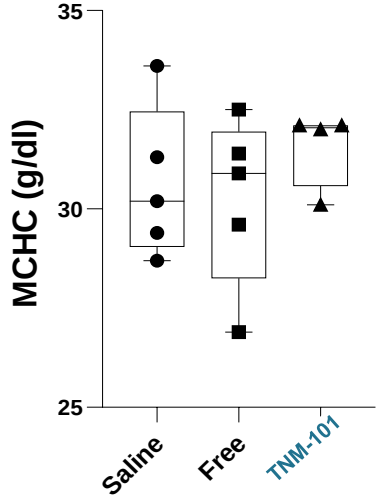


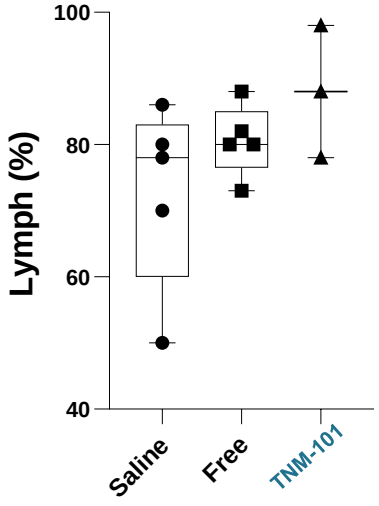


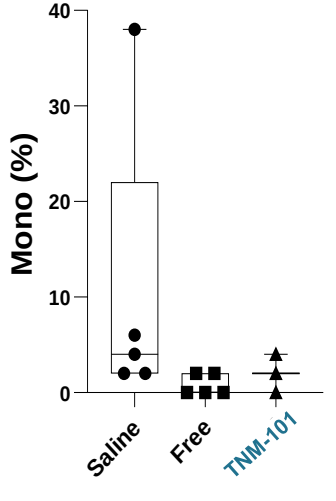


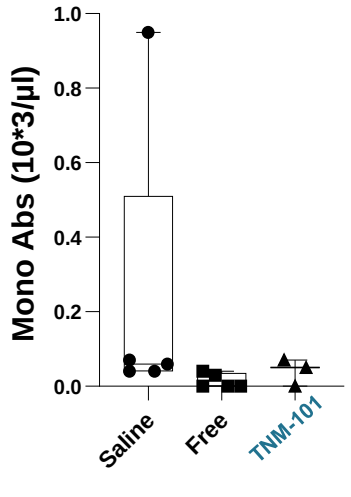












Evaluation of immune-reactivity

Evaluation of immune-reactivity

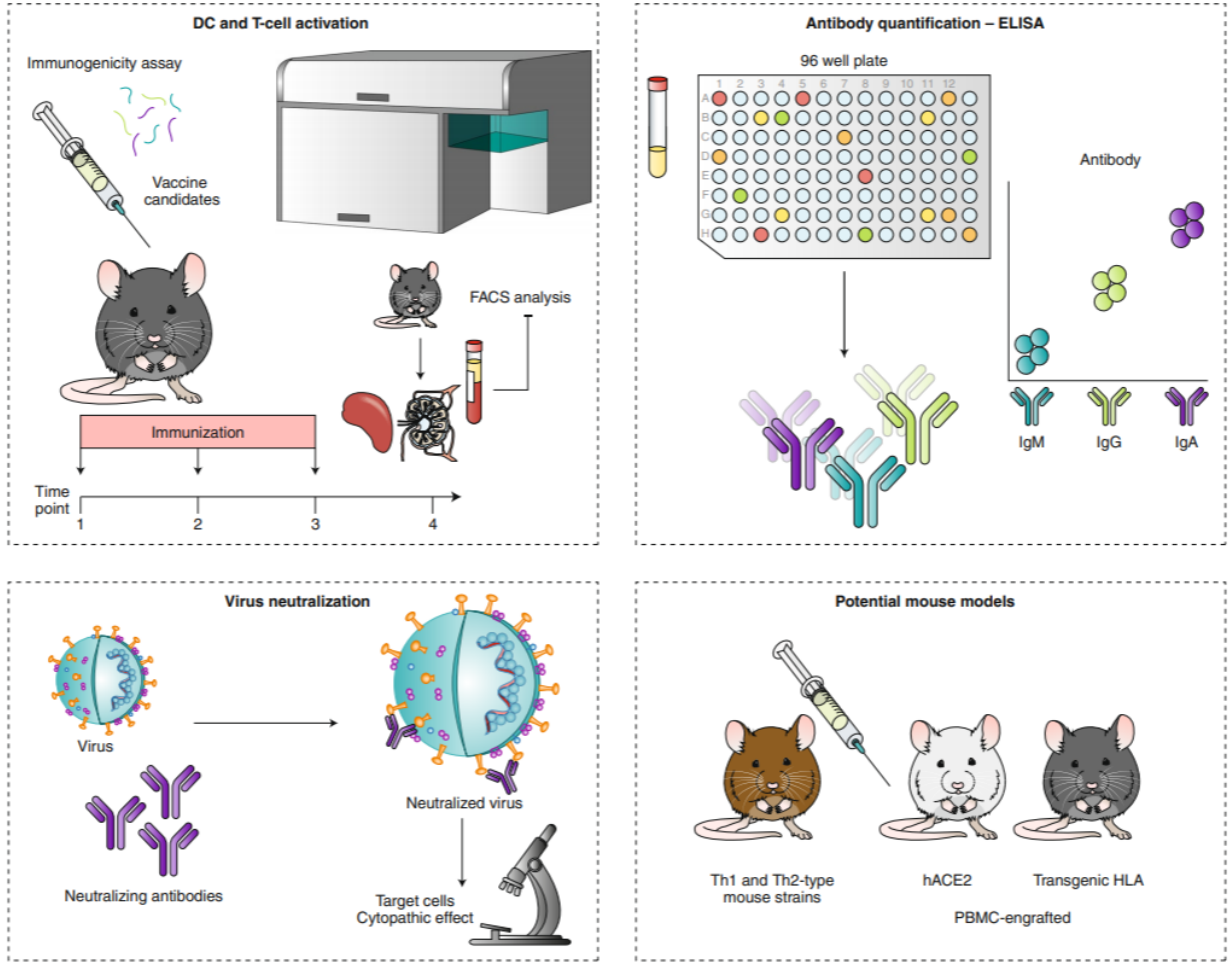
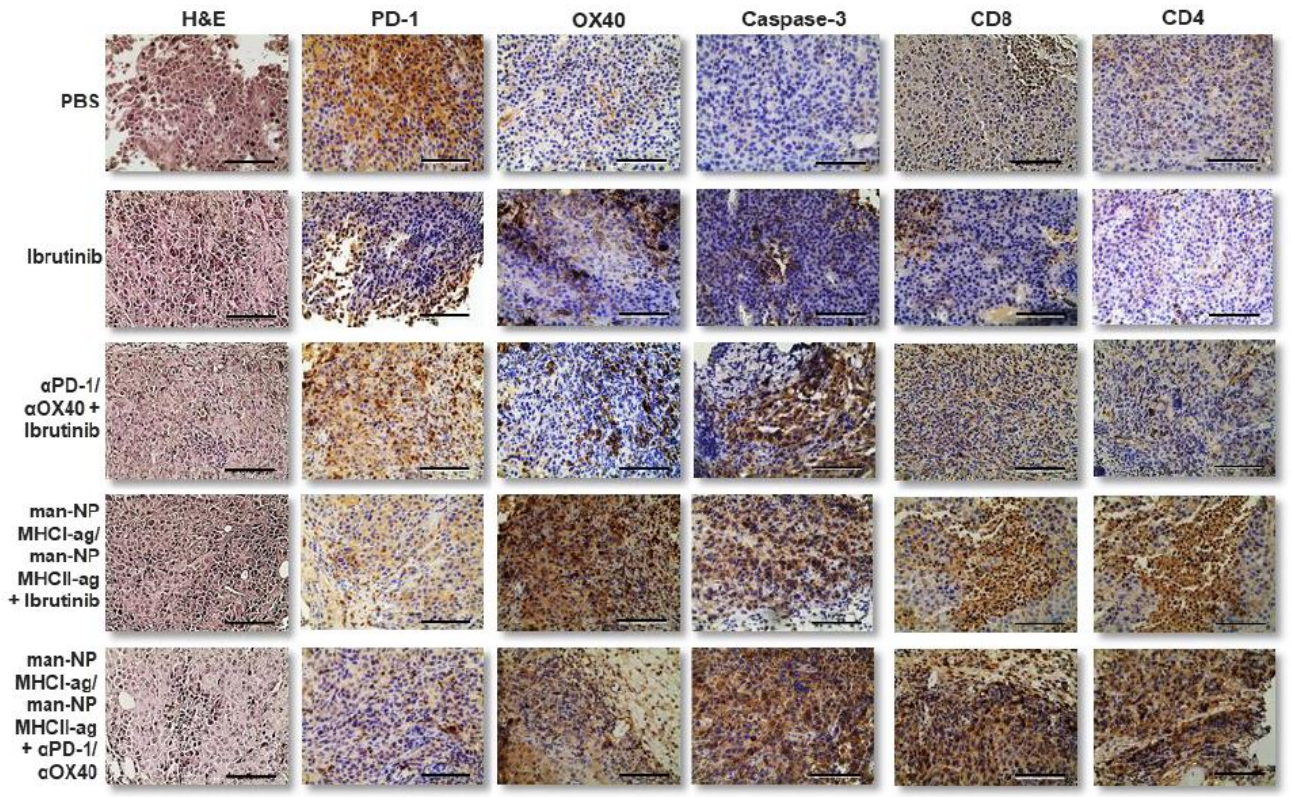


Fig. 6 | Schematic representation of key methodologies to characterize the immune response and related anti-SARS-CoV-2 effect induced by vaccine candidates. These assays include the evaluation of dendritic cell (DC) and T cell function upon immunization by flow cytometry and quantification of levels of antigen-specific binding and neutralizing antibodies at different time-points. These studies are still limited by the mouse models of SARS disease currently available, but different options are emerging as potentially useful for the study of SARS-CoV-2 infection mechanisms and COVID-19 vaccine development. DC, dendritic cell; FACS, fluorescence-activated cell sortin; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulins (Ig); hACE2, human angiotensin-converting enzyme 2; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cell.

Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini Y, Tiram G, Liubomirski Y, Satchi-Fainaro R. *Nature Nanotechnology*, 2020 Jul 13:1-16

Histological analysis of response



Conniot J, Scomparin A, Peres C, Yeini E, Pozzi S, Matos AI, Kleiner R, Moura LIF, Zupančič E, Viana AS, Doron H, Gois PMP, Erez N, Jung S, Satchi-Fainaro R*, Florindo HF*. *Nature Nanotechnol.* 2019 Sep;14(9):891-901

Evaluation of body distribution

Human cell line-derived xenograft (CDX) melanoma models

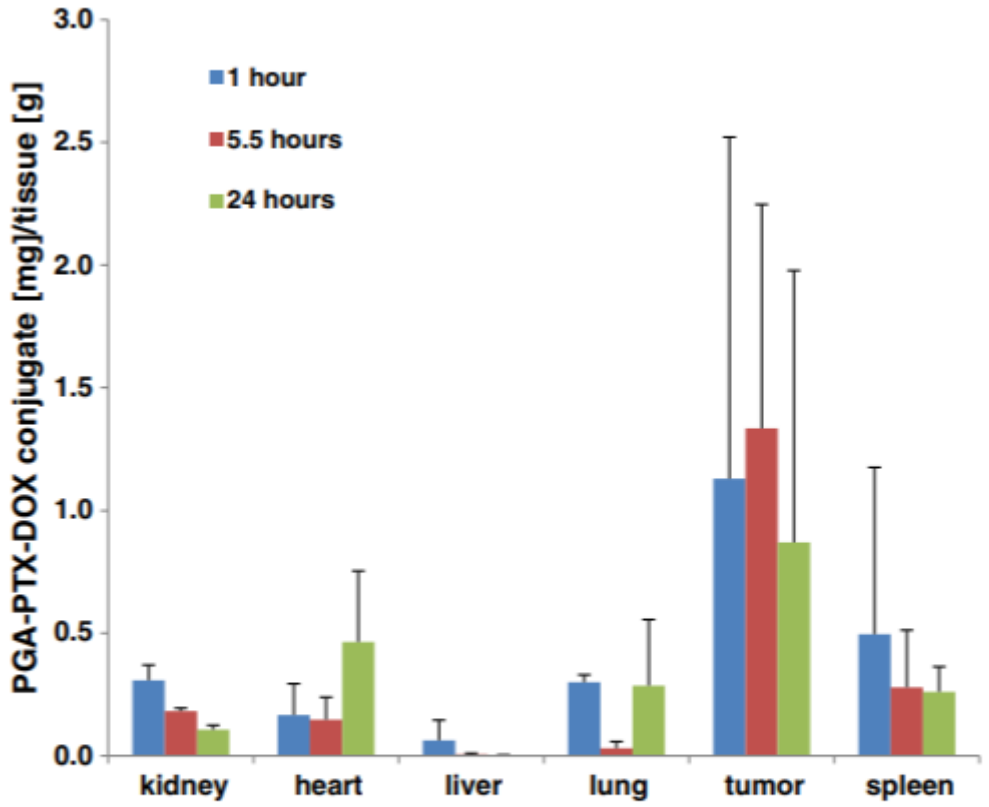


Fig. 8. Biodistribution of PGA–PTX–DOX in mammary tumor bearing mice. Biodistribution of the conjugate at 1.5, 5.5 and 24 hours following i.v. injection. The conjugate accumulated mainly in the tumor at all time points.

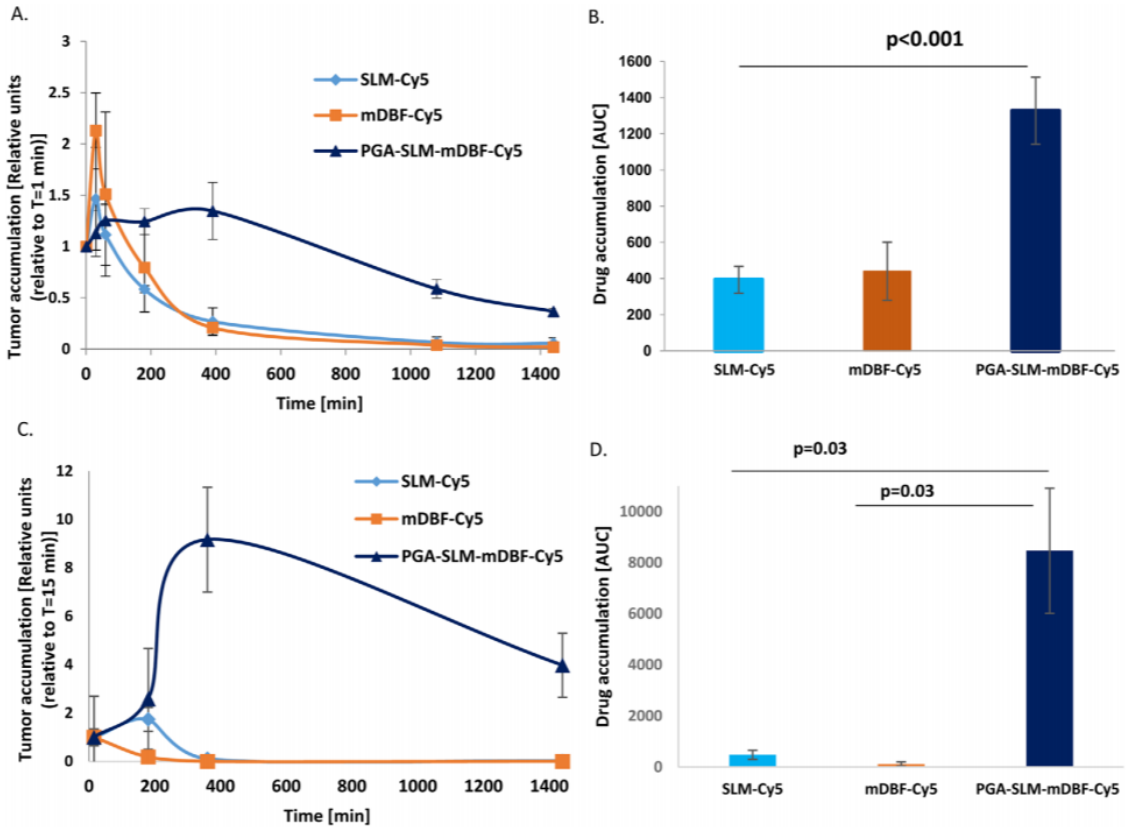
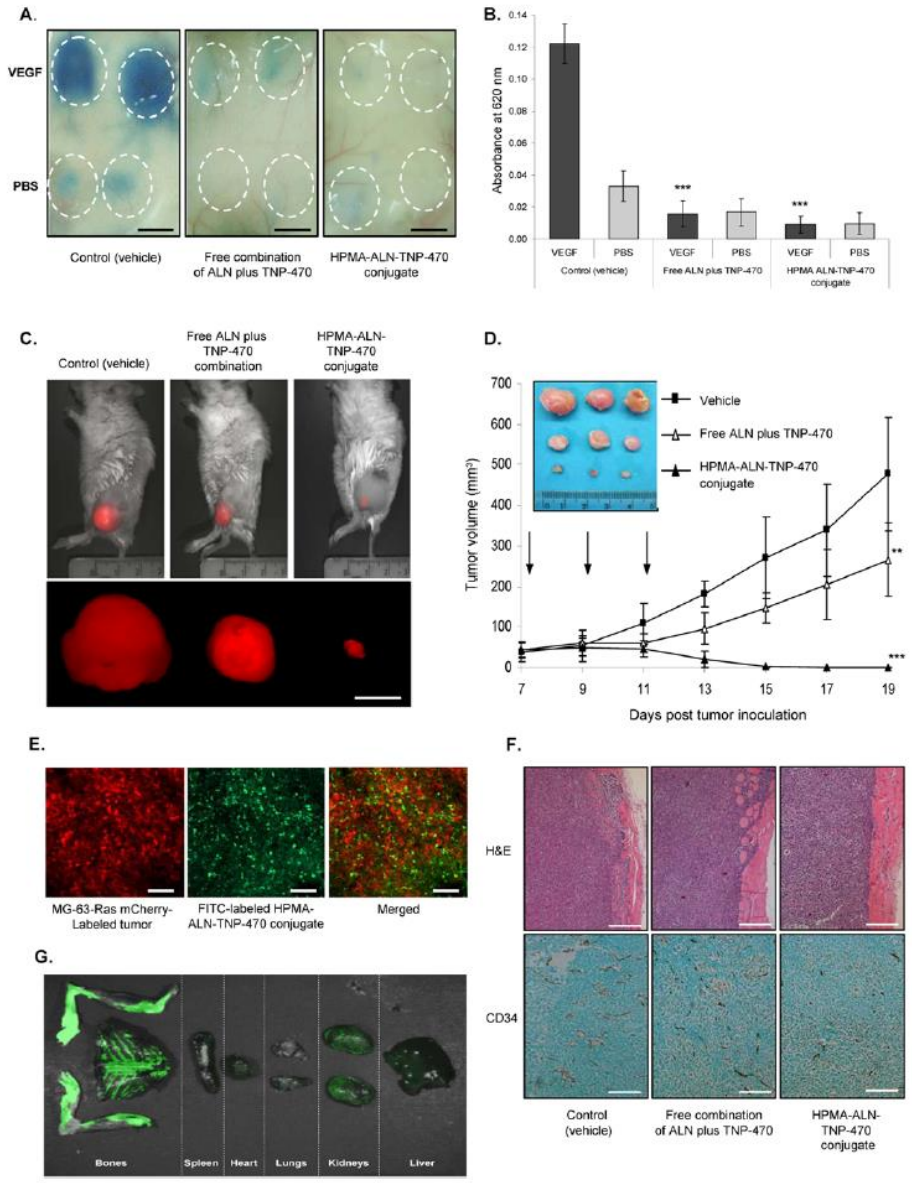


Figure 7. PGA–SLM–mDBF–Cy5 conjugate accumulates at the tumor site. D4M.3A tumor bearing mice were treated with SLM-Cy5, mDBF-Cy5 or PGA–SLM–mDBF–Cy5 i.v. (A,B) or i.p. (C,D) at mDBF equivalent dose. CRI Maestro noninvasive intravital fluorescence imaging system was used to follow tumor accumulation for 24 h. A,C) Accumulation of the different treatments at the tumor site at different time points. B,D) Total tumor accumulation within 24 h (AUC-area under the curve) of the different treatments. $N = 5$ or 3 mice per group for i.v. and i.p., respectively, and one-way ANOVA was used for statistical analysis.

Evaluation of vascular hyperpermeability (Miles assay), tumor volume, tumor accumulation and histology

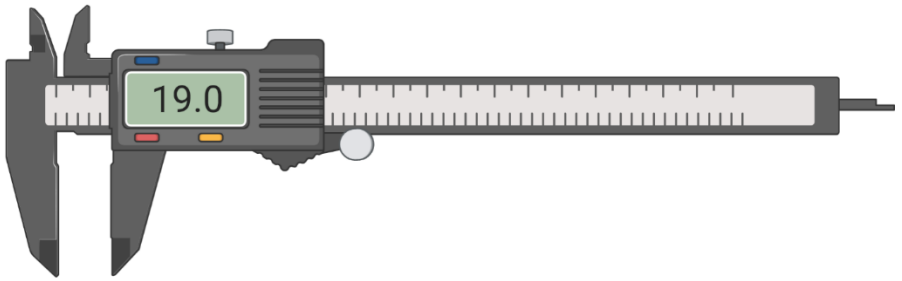


Evaluation of tumor volume

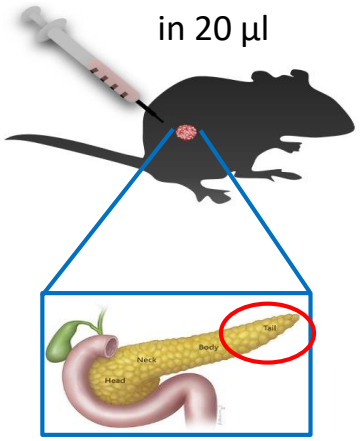
Measuring physically tumor volume by caliper

Human cell lines commonly used for melanoma xynogeneic models:

. Tumors are usually measured by a digital caliper and tumor volume is calculated as: $width^2 \times length \times 0.52$.



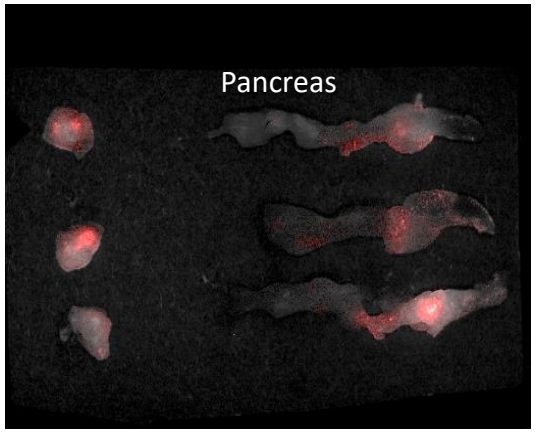
Pancreatic tumor imaging for tumor growth monitoring

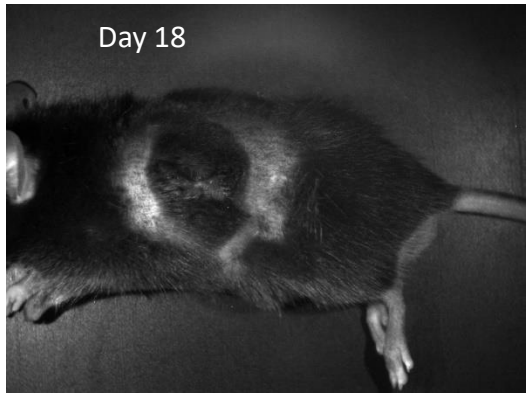


10^6 KPC cells in 20 µl

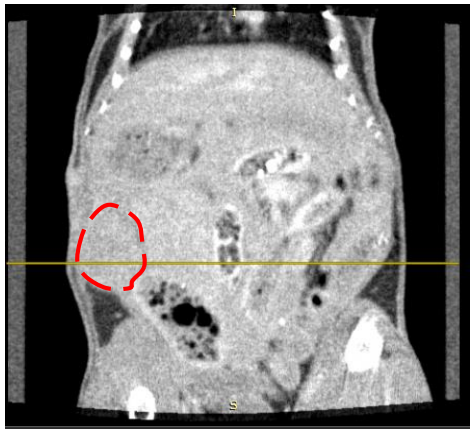
With 5% Trypan-Blue for easier detection of the injection site

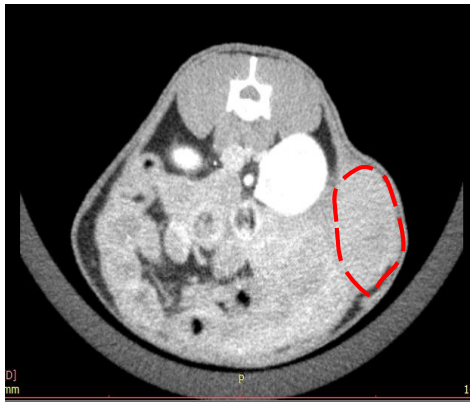
SCID mice / C57BL/6J mice



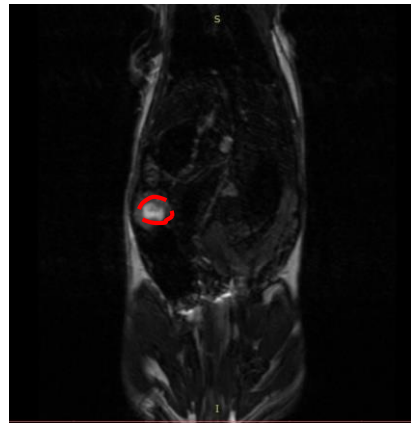


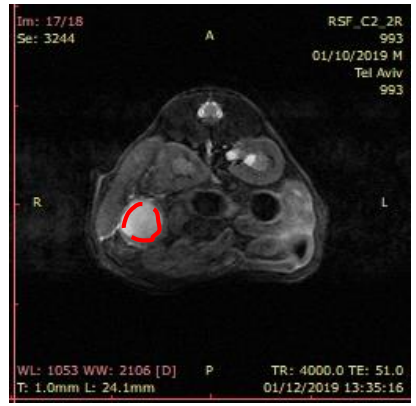
Fluorescent imaging (**Maestro CRi™**)





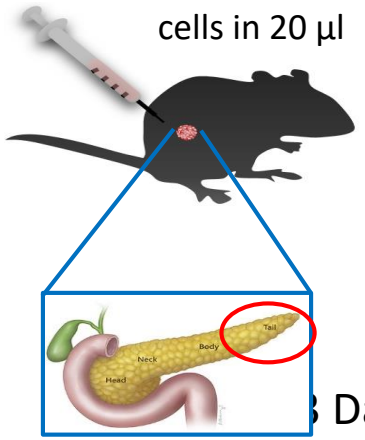
CT





MRI

Pancreatic tumor imaging for tumor growth monitoring
T2 tumor volume evaluation



$2x10^6$ KPC cells in 20 µl

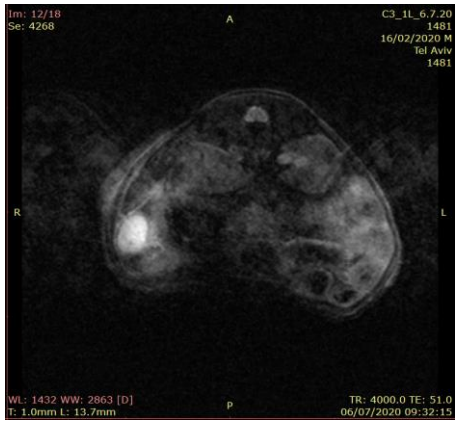
With 5% Trypan-Blue for easier detection of the injection site

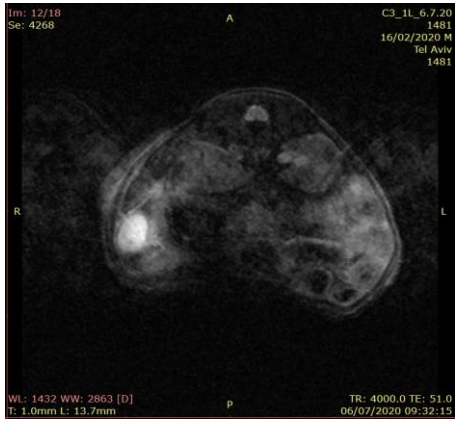
7 Days

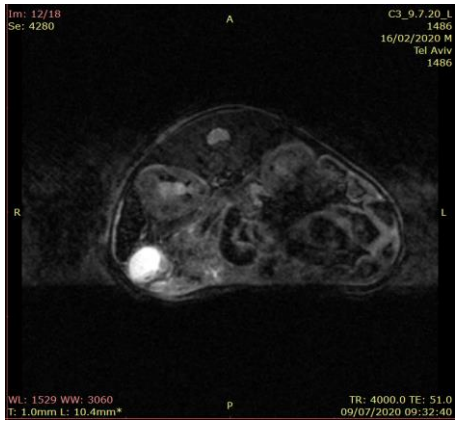
13 Days

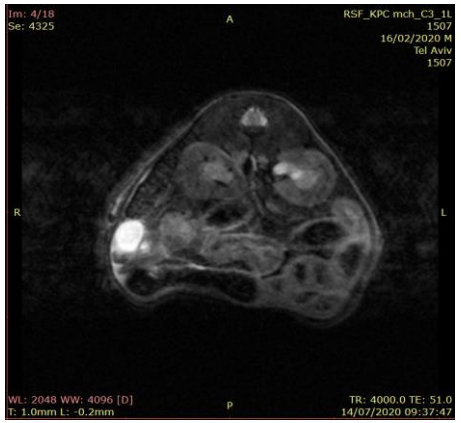
16 Days

21 Days



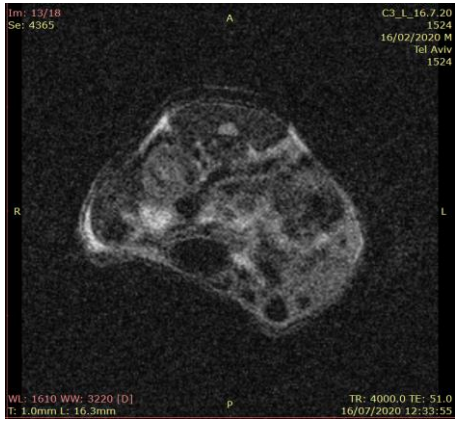


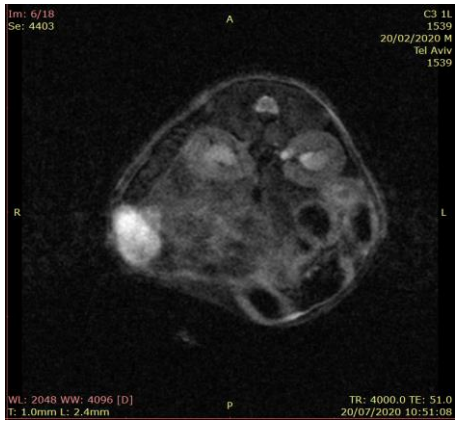


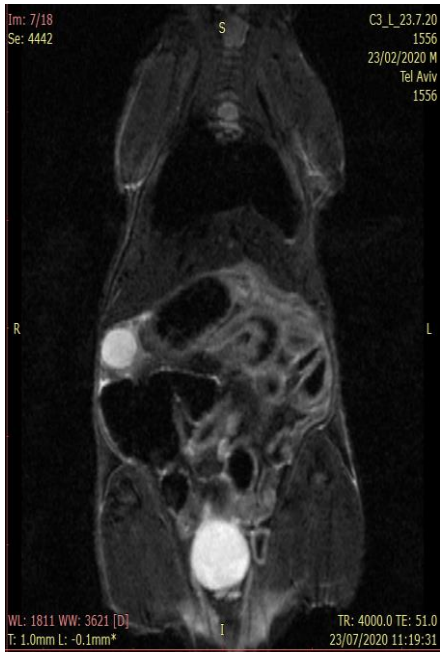


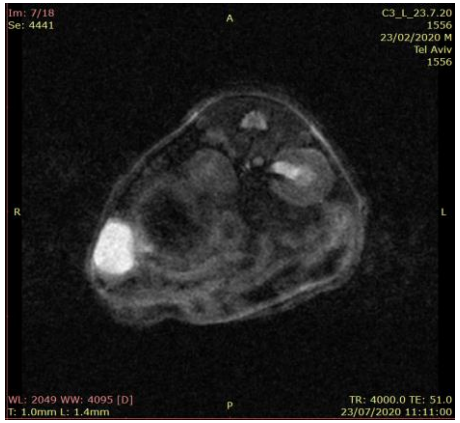
27 Days

30 Days

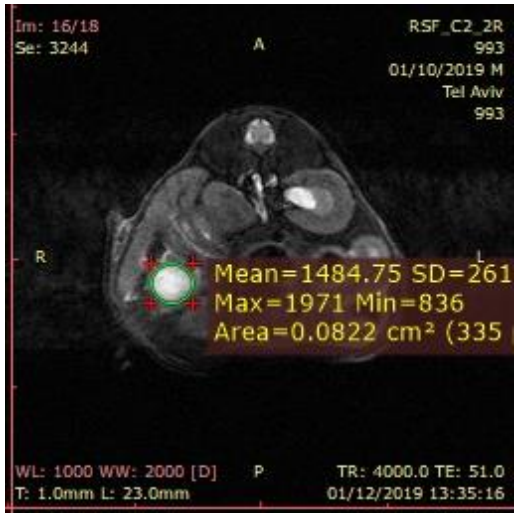


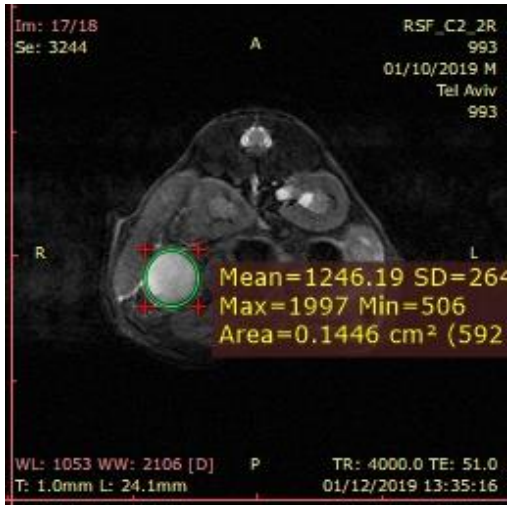


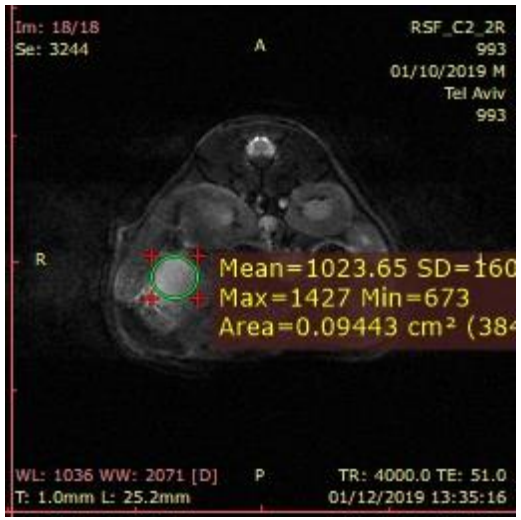




Pancreatic tumor imaging for tumor growth monitoring
T2 tumor volume evaluation







$$\text{Tumor area sum} = 8.22\ \text{mm}^2 + 14.46\ \text{mm}^2 + 9.443\ \text{mm}^2 = 32.123\ \text{mm}^2$$

$$\text{Tumor volume} = \text{slice width (1 mm)} \times \text{tumor area sum} = 32.123\ \text{mm}^3$$

- Excellent soft tissue contrast resolution
- Reliable tumor size estimation
- Good anatomical localization
- Suitable for soft tissue imaging

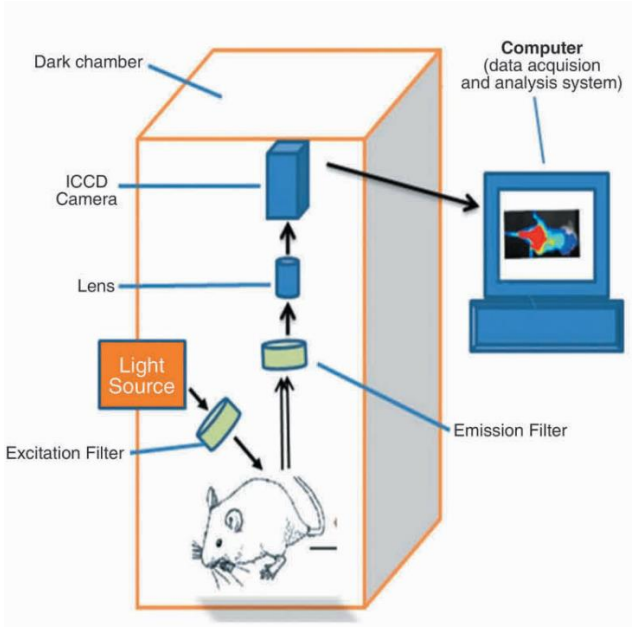
Pros

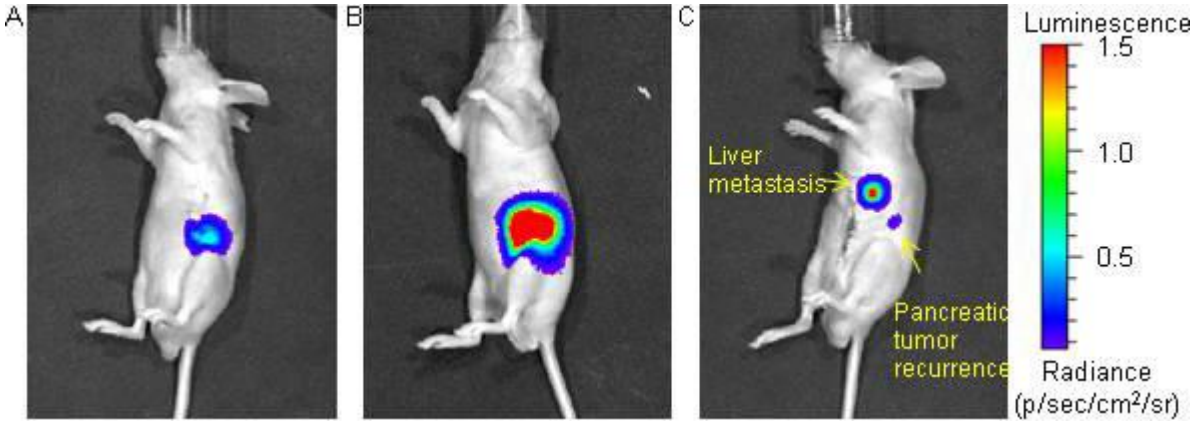
Cons

- long scanning time
- Complicated to use

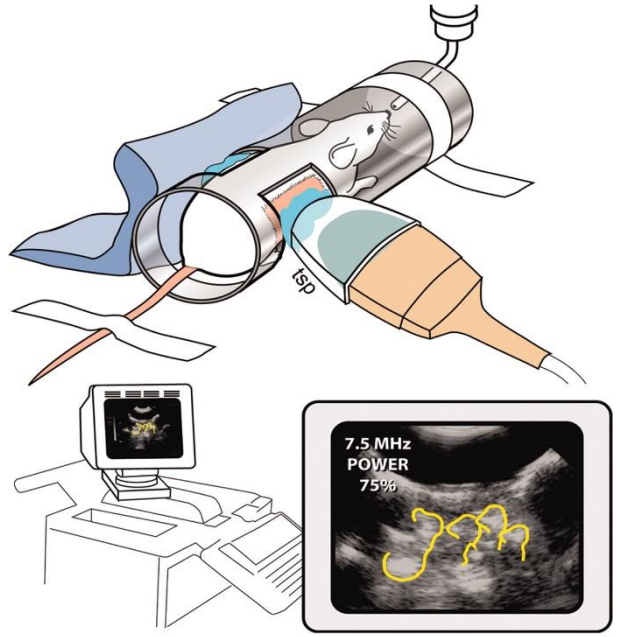
Alternative imaging methods

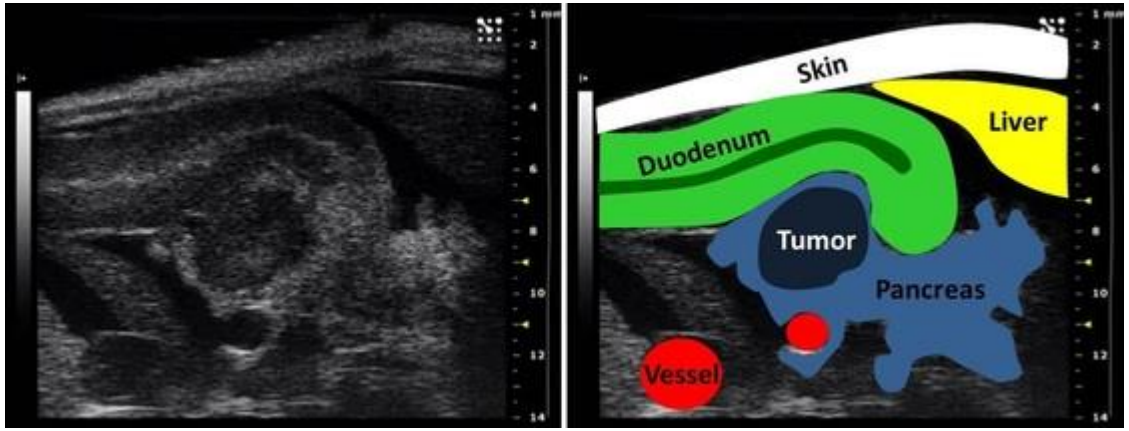
Biospace / IVIS





Ultrasound



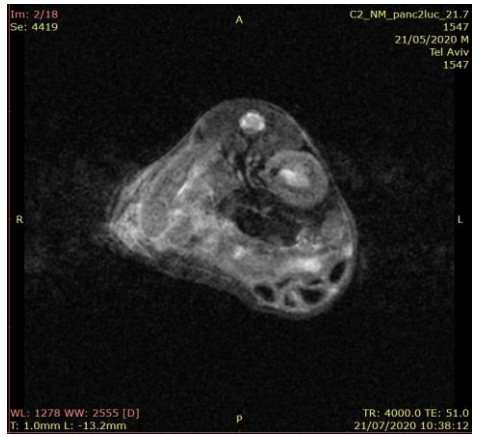


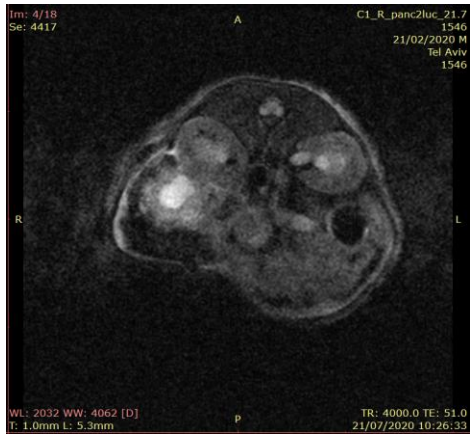
IVIS

NM

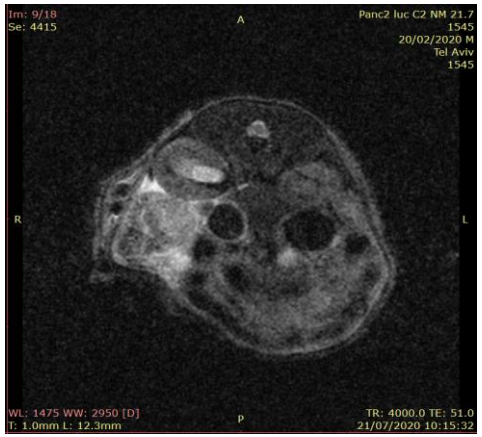
1R

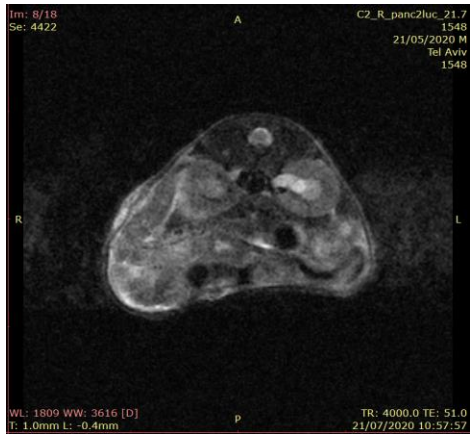
Cage 1

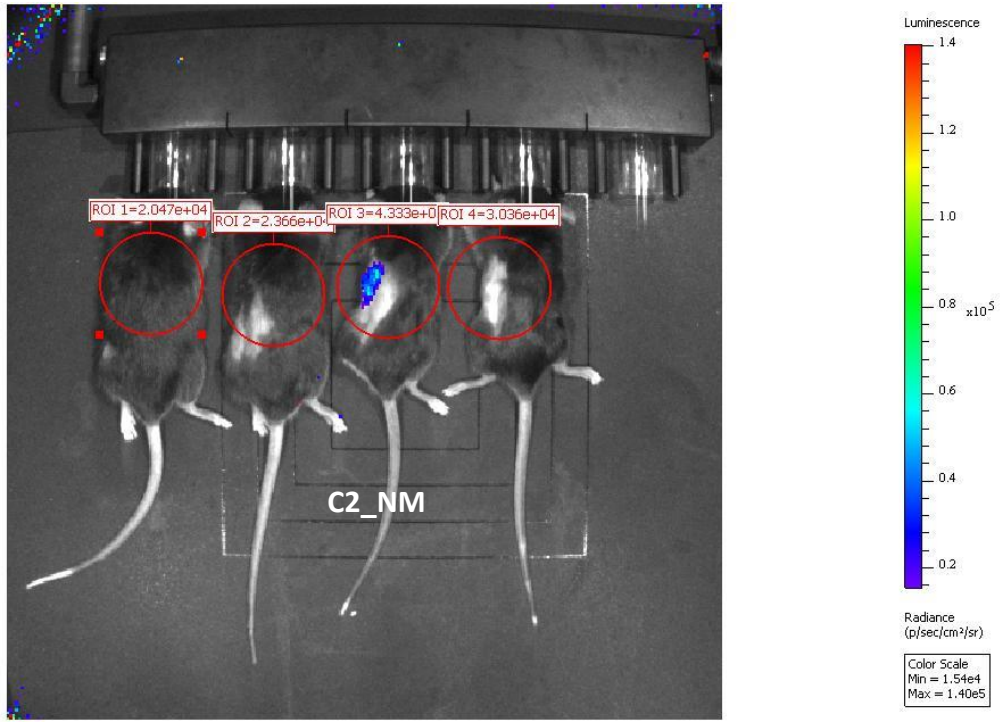




Cage 2







Survival: Kaplan Meier curve

The Kaplan-Meier plot needs survival data as input and it can also handle censor events.
The Kaplan Meier survival curves shows and compares how survival in subject groups changes with different conditions (e.g treatments etc).
Additional statistical information can be added to the plot, see below:

- *Median*: The median survival can be displayed for each sample group in the sample group legend.
- *Log-Rank*: In the case of two sample groups, a Log-Rank p-value can be displayed in a separate legend.