COMPARISON AND CONSEQUENCES OF DIFFERENT IMPLANTATION TECHNIQUES ON THE ORTHOTOPIC GROWTH OF SYNGENEIC HEPA1-6 LIVER CANCER CELLS

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Introduction

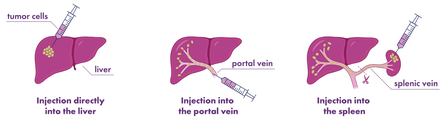
Checkpoint inhibitor treatment has become a common therapy of various cancer types. Still, there is a need for well-characterized preclinical mouse models, as clinical data indicates that patients only partially respond to immune-modulatory regiments.

When compared to the classic subcutaneous or subQperior™ (implantation into the mammary fat pad) syngeneic mouse models, orthotopic models are considered more predictive since the implantation of tumor cells into the organ of origin allows organotypic interaction between tumor cells and the surrounding stroma, including immune cells.

Orthotopic HEPA1-6 liver tumor growth can be initiated by different routes of tumor cell implantation:

- **Direct injection into the liver lobe** in Matrigel
- **Via the portal vein**
- **Via the spleen** with subsequent removal of the spleen

The growth of the luciferase-transduced Hepa1-6 cells can be monitored in vivo by bioluminescence imaging.



Results

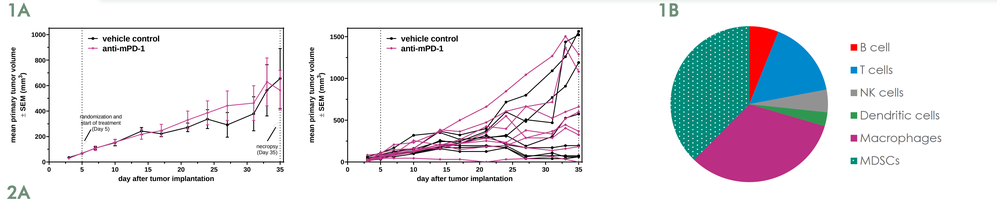


Fig. 1: HEPA1-6 cells were implanted into the mammary fat pad of C57BL6/N mice (subQperior™).
(A) Tumor growth curve of vehicle and anti-mPD-1 treated animals (left: mean values, right: single growth curves).
(B) Immune cell populations present at necropsy in vehicle-treated animals were determined by flow cytometry

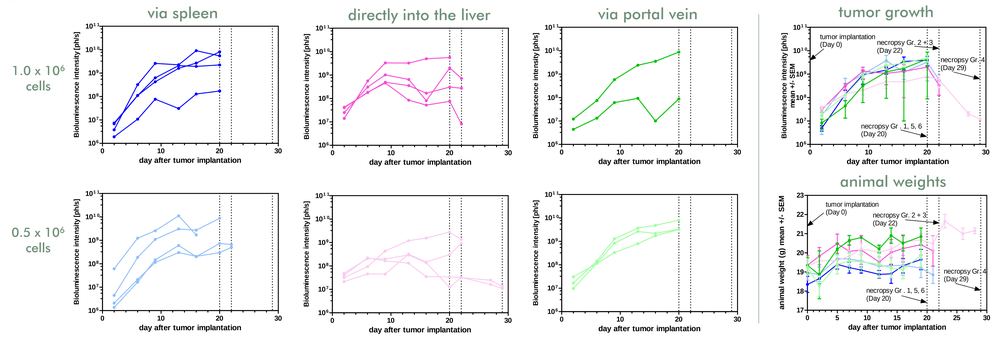
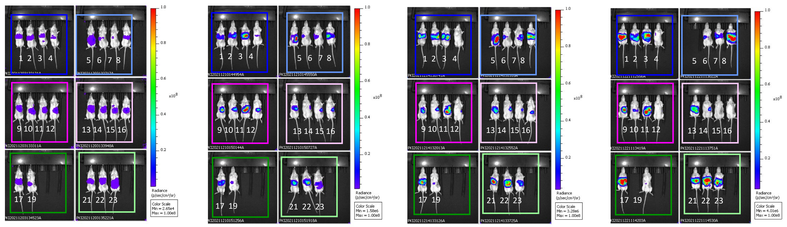
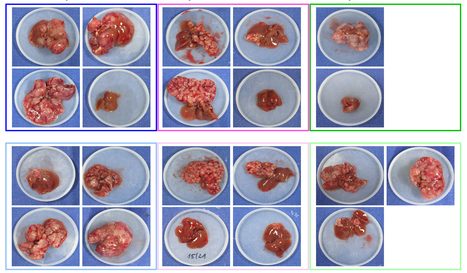


Fig. 2: HEPA1-6_Luc cells were engrafted by injecting cells via spleen, directly into the liver or via portal vein in C57BL6/N albino mice. Orthotopic liver tumor growth was followed by bio-luminescence imaging (BLI). Two different cell numbers were applied: 1.0 x 10^6 cells (solid color shades) and 0.5 x 10^6 cells (light color shades).
(A) Bioluminescence signal intensity is shown as single growth curves (blue: via spleen, pink: directly into the liver, green: via portal vein) and as mean values (right).
(B) BLI overlays are displayed.
(C) At necropsy liver/tumor was sampled and images taken.





Conclusion

- **C57BL/6 albino mice allow early detection of bioluminescence tumor signal**
- **Orthotopic tumor growth is more homogenous than ectopic (subQperior™) tumor growth**
- **Tumor cell inoculation via spleen or portal vein results in tumors distributed throughout the liver, whereas inoculation of tumor cells directly into the liver results in cauliflower-like tumors.**
- **Tumor growth after direct injection into the liver was most variable and showed slow growth for the low cell number**
- **Injection into the portal vein leads to loss of animals because of bleeding**

→ Standard application of liver cells via spleen is the method of choice if the removal of the spleen is acceptable

References

1. Rao et. al., PLoS One. 2016; 11(1): e0148263.
2. Kwong et. al., JHEP Rep. 2021 Apr; 3(2): 100224.

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