In necrotic areas of tumors from Group 4 (mouse n°7971 R and L) and Group 5 (mouse n°7975 R and L), blue-stained iron granular debris were found in more amounts and appeared as a dark blue, granular, inter-cellular framework. In such areas, some necrotic cells presented a light blue staining of their cytoplasm, giving them an appearance of necrotic "ghost cells". Similar bluish necrotic "ghost cells" were also observed in very low numbers in some tumors of Group 2 (mouse n°5227 R) and Group 3 (mouse n°7967 R).

CONCLUSIONS

Five mice were subcutaneously grafted with human tumors (MDA-MB-231, breast cancer cell line; 2 xenografts per mice) and included in this histopathological and histochemical study.
At the end of the study, all tumors were collected and fixed in 4% formalin. Representative samples of fixed material were embedded in paraffin, and then transferred to BiodOxis for processing and microscopic evaluation.
One of the two xenografts from each mouse was processed for evaluating histopathological features of the tumors (HES staining). Both xenografts samples from each mouse were processed for evaluating tumor cells iron accumulation (Perls special stain). Microscopic analysis was performed by light microscopy using an Olympus BH2 microscope.

In this study, the ten evaluated MDA-MB-231 xenografts presented comparable histological features, and appeared as undifferentiated carcinomas.
Necrotic areas were seen in the central parts of every tumor. They varied from 60% to 80% of the tumor surface in analyzed tumors. They were slightly more extensive in tumors of Group 2 (mouse n°5227 R) and Group 3 (mouse n°7967 R), in comparison to the other groups.

Iron deposits were only observed in the cytoplasm of inflammatory cells with macrophage morphology or as granular debris, located in the peri-tumoral and intra-tumoral fibrous stroma and in necrotic areas. They appeared as brown granular deposits on HES stained slides and as dark blue granular deposits in Perls stained slides.
No iron deposits were identified in tumor cells, in any tumor of any group.
Iron deposits were found in moderate to important amounts in peri-tumoral areas. Those data should be considered with caution, as they are related to the amount of peri-tumoral tissue simultaneously sampled with the tumor.
In intra-tumoral stroma, iron deposits were absent or found in low amounts in tumors of Group 3 (mouse n°7967 R and L). They were slightly more abundant (low to moderate amounts) in the tumors of the other groups (Group 4 ≈ Group 5 ≤ Group 2 ≤ Group 1), without patent differences.
In necrotic areas, iron deposits were mostly observed in low amounts, except in tumors of Group 4 (mouse n°7971 R and L) and Group 5 (mouse n°7975 R and L) where they appeared in more amounts, with some to many reactive necrotic "ghost cells".

Study limitations.
Nothing to report