

INCREASED SPLENIC BLOOD FLOW FOLLOWING MACROPHAGE ACTIVATION BY OLEIC ACID COMPLEXED WITH DE-GLYCOSYLATED VITAMIN D BINDING PROTEIN

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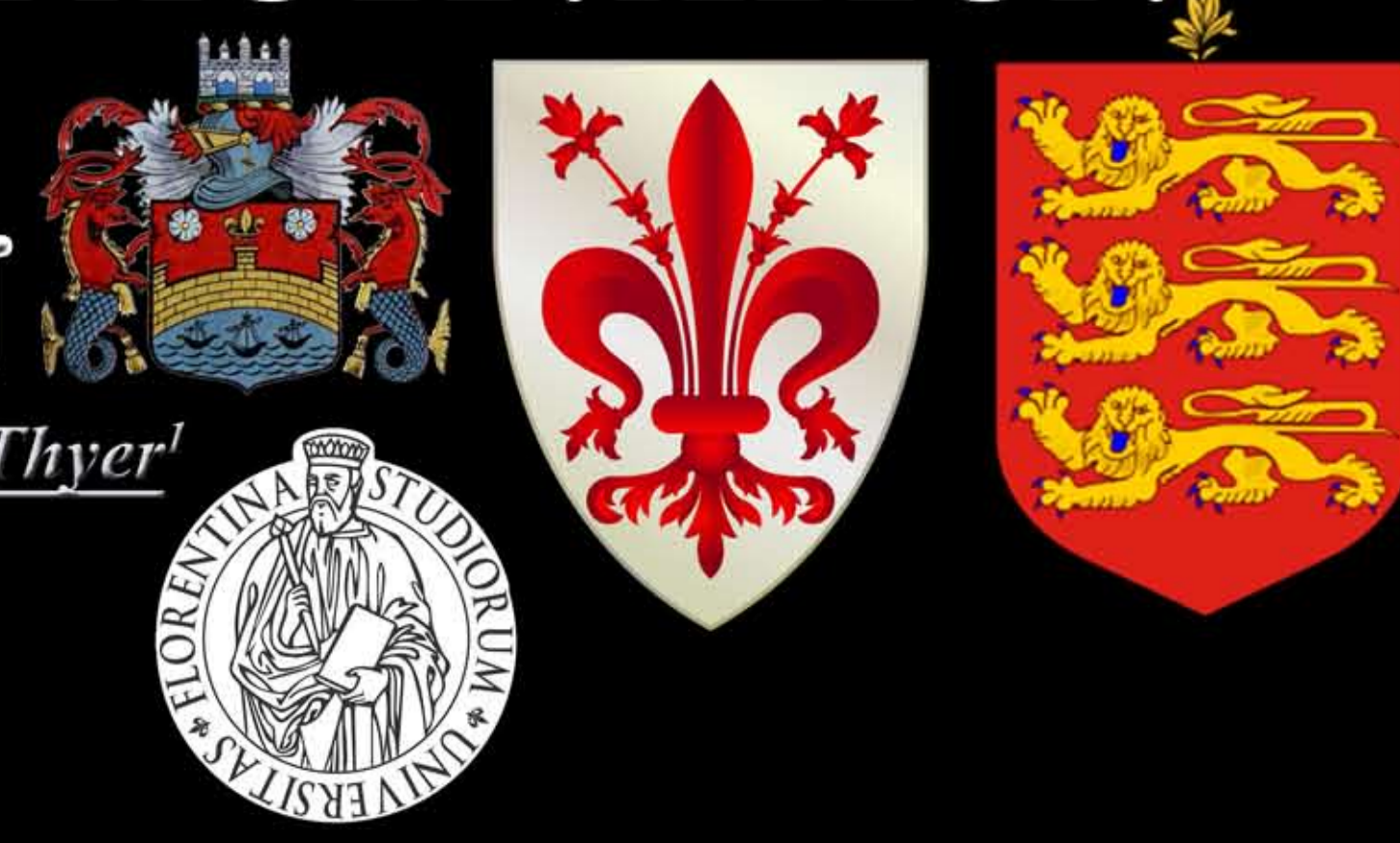
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INTRODUCTION

- The spleen is a site for storage and rapid deployment of monocytes; splenic monocytes have been identified as a resource that the body exploits to regulate inflammation and anticancer immuno-surveillance (*Science* 2009 325:612-6).
- We study the role of macrophages in human cancer since 1990, when we characterised the signalling transduction pathway of the human receptor for Macrophage-Colony-Stimulating Factor (c-fms) (*Proc. Natl. Acad. Sci. USA. Vol. 87, pp. 5613-5617, August 1990*).
- In the present study, a well characterised stimulator of macrophages deriving from vitamin D binding protein (DBP-MAF/GcMAF), was conjugated with oleic acid (OA), a known anti-cancer agent as well as a protein absorption enhancer.
- Here we demonstrate that the dramatic effects of OA-GcMAF in cancer patients (*Am J Immunol* 10(1): 23-32, 2014) are mediated by nitric oxide (NO) produced by activated macrophages.
- This identifies a triad of molecular elements, OA, GcMAF and NO, each one endowed with individual anti-cancer properties, that show synergistic effects and might open the way to a much improved cancer treatment strategy devoid of harmful side effects.

PATIENTS and METHODS

- At the Immuno Biotech Treatment Centre, patients with advanced cancer are currently being treated with OA-GcMAF-based integrative immunotherapy.
- OA-GcMAF (Goleic, *Am J Immunol* 10(1): 23-32, 2014) is used in combination with the following approaches:
 1. A very low carbohydrate, high protein, equicaloric diet that is known to slow tumour growth and prevent cancer initiation (*Am J Immunol* 10(1): 23-32, 2014).
 2. Fermented milk products containing naturally produced GcMAF (*Am J Immunol* 10(1): 23-32, 2014).
 3. High vitamin D3 supplementation (*Front Oncol* 3:250, 2013).
 4. Low-dose acetylsalicylic acid (*Nat Rev Clin Oncol* 9(5): 259-267, 2012).
 5. Omega-3 fatty acid supplementation (*BMJ* 346: f3706, 2013).
- These approaches, aimed at strengthening the immune system and reducing tumour growth, are considered complementary and not alternative to other anti-neoplastic therapeutic procedures.

RESULTS

- It is well assessed that OA-GcMAF stimulates macrophages (*Nutrients* 5(7): 2577-2589, 2013), and stimulated macrophages in turn release NO (*J Exp Med* 169(5): 1543-1555, 1989).
- Considering the impressive effects of NO in pre-clinical models of cancer where it slows tumour growth and enhances the efficacy of both chemotherapy and radiotherapy (*Cancer Chemother Pharmacol* 67(6): 1211-1224, 2011), as a first step we sought to determine whether OA-GcMAF stimulated the release of NO from macrophages *in vivo*.
- To this end, we chose to study the variation of the splenic blood flow, following the administration of OA-GcMAF.
- According to our hypothesis, OA-GcMAF would stimulate the production of NO by tissular macrophages at the site of injection, or by alveolar macrophages when administered by nebulisation.
- OA-GcMAF would then be absorbed into the bloodstream and it would eventually stimulate macrophages in other areas of the body.
- Considering the huge amount of macrophages residing in the spleen, we would expect a sustained release of NO by activated macrophages in this organ, with a concomitant increase of blood flow that we could measure by echo-colour-doppler (Esaote).

➤ Ultrasonographic appearance of the spleen of a 58 year old patient with pancreatic adenocarcinoma before OA-GcMAF administration. Splenic blood flow can be appreciated in correspondence of the hilum of the spleen. Little or no signal could be observed in the parenchyma of the organ.

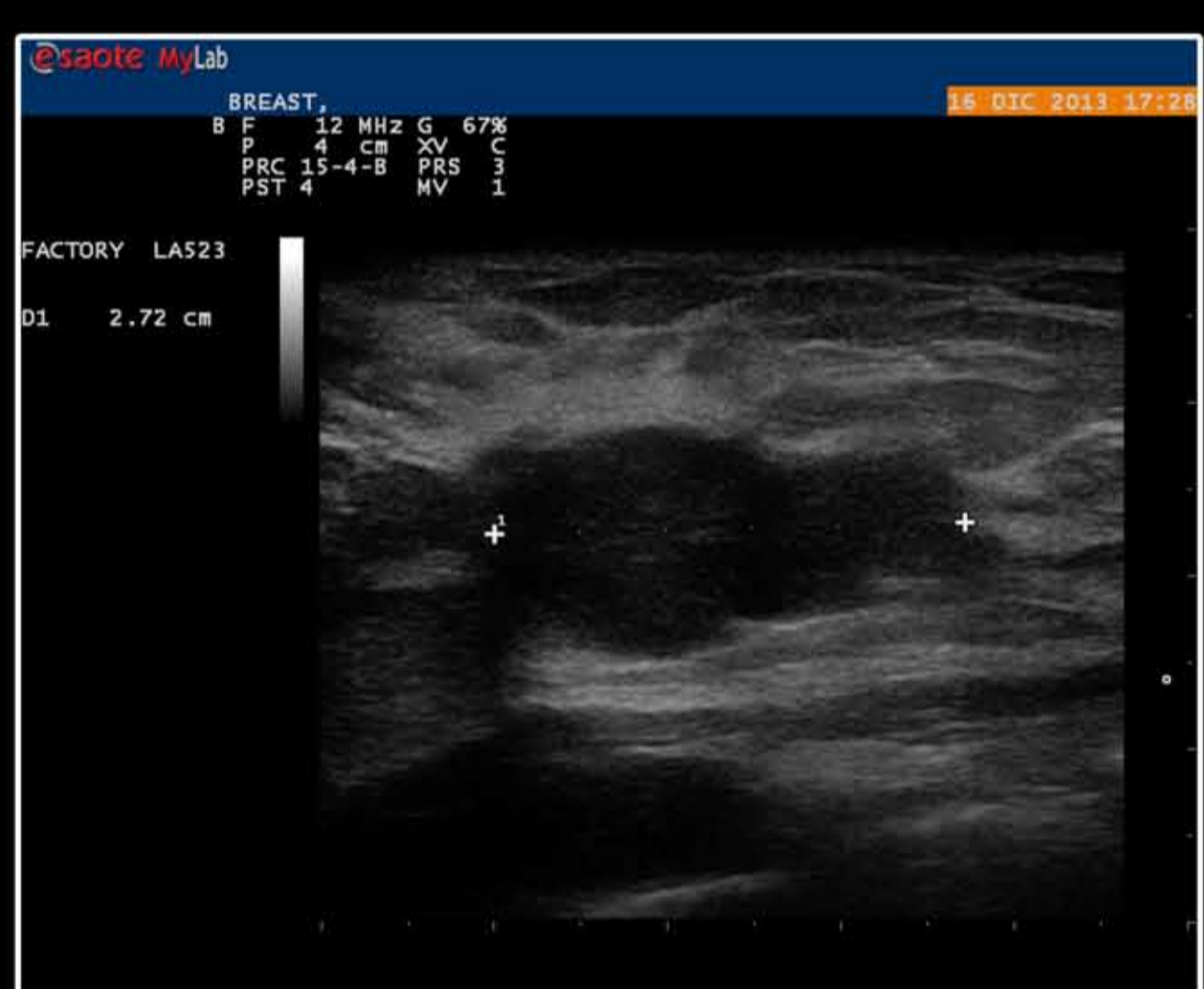


- Activation of tissular, alveolar and splenic macrophages by OA-GcMAF administration with sustained release of NO, was associated with significant decrease in tumour volume in all cases where the tumour, metastases or lymphnodes could be measured by ultrasonographic techniques.
- On average, we observed a decrease of tumour volume of about 25% in one week (*Am J Immunol* 10(1): 23-32, 2014).
- These results are consistent with the hypothesis that OA, GcMAF and NO, molecules endowed with individual anti-cancer properties targeting different aspects of neoplastic growth, indicate a synergistic effect when administered in such a way to exploit their physiological characteristics.
- Among the cases observed at the Immuno Biotech Treatment Centre, we present the cases of two metastasized breast cancer patients, for whom the integrative immunotherapy based on OA-GcMAF was remarkably effective.

➤ Sustained increase of splenic blood flow following administration of OA-GcMAF. OA-GcMAF was administered with a nebuliser containing 880 ng of the active principle dissolved in 7 ml of saline, with localised subcutaneous injections of 880 ng in the area of the inguinal nodes and with suppositories containing 200 ng of the active principle. Splenic blood flow was assessed by echo-colour-doppler ultrasonographic technique. The increase in parenchymal blood flow appears to be significant.



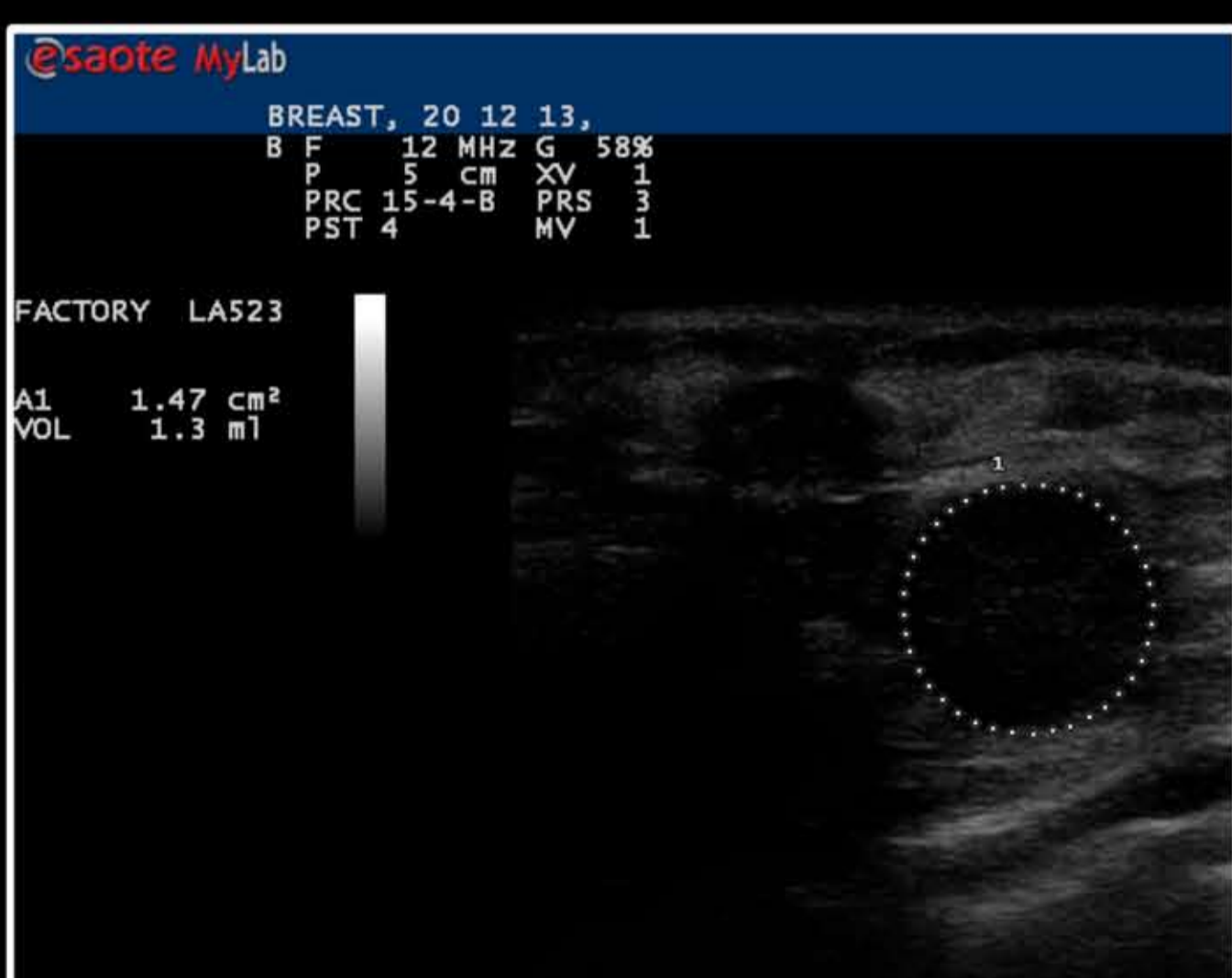
➤ 46 year old female. **Metastasised breast cancer.** Ultrasonography of the left breast was performed to assess modifications following immunotherapy. As previously reported, a mass of about 2.7 cm could be observed in the left breast in the peri-areolar area. The mass was inhomogeneously hypo-echoic and showed an elongated, ovoidal, morphology, with the major axis in the cranio-caudal direction. The margins were fairly regular and no spikes infiltrating the surrounding tissue could be observed.



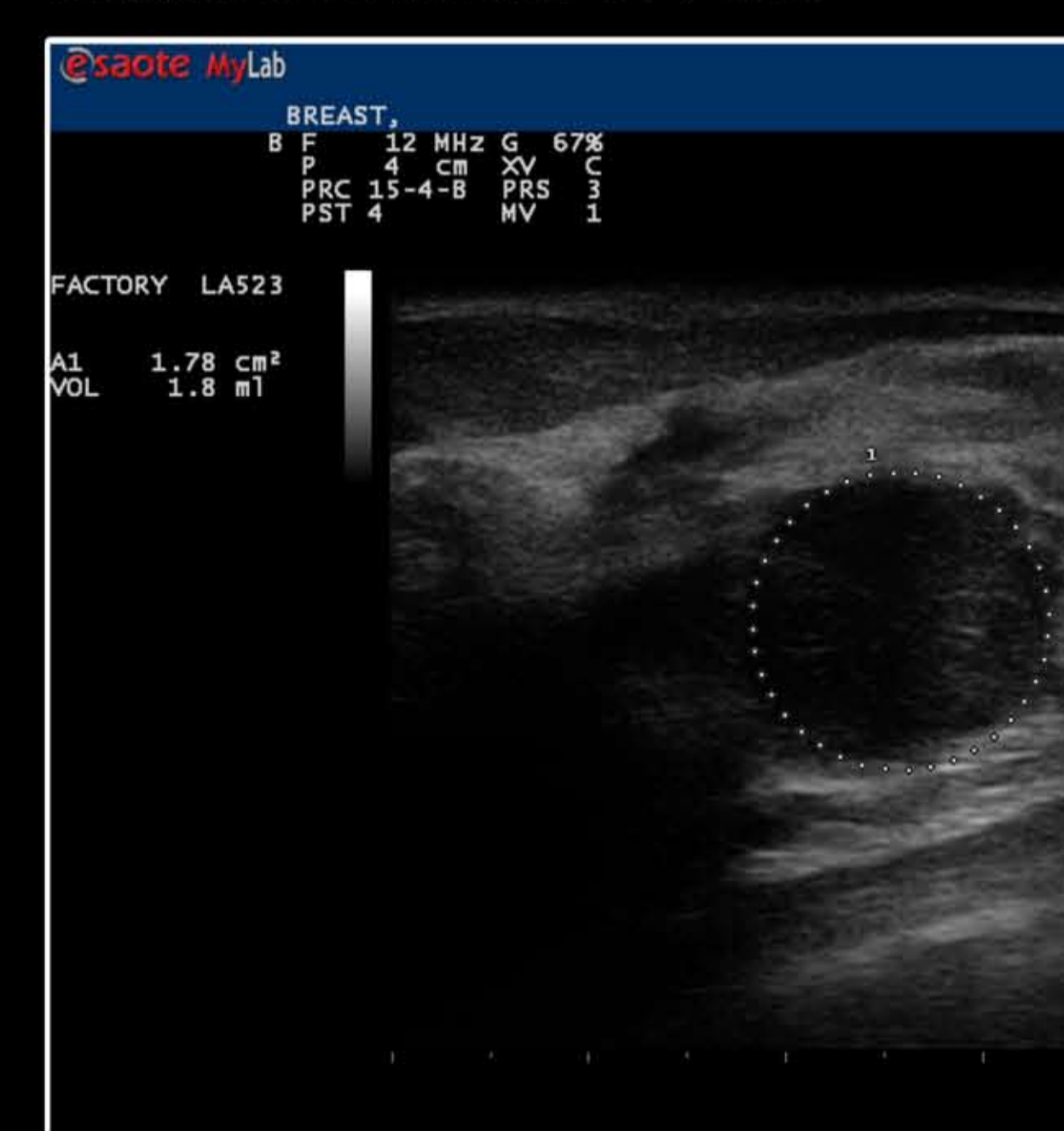
➤ 44 year old female. **Metastasised breast cancer.** Ultrasonography of the right breast was performed to assess modifications following immunotherapy. The lesion in the lower outer quadrant of the right breast, previously measuring 25 mm according to a MRI study of 06/12/2013, was taken as reference. Bearing in mind that measurements taken on ultrasonographic images may be affected by a number of variables, preliminary evidence appears to indicate a reduced diameter. The longest diameter of the inhomogeneous, mainly hypo-echoic, lesion in the lower outer quadrant of the right breast, presumably corresponding to that evidenced in the MRI, measured 17.5-18 mm.



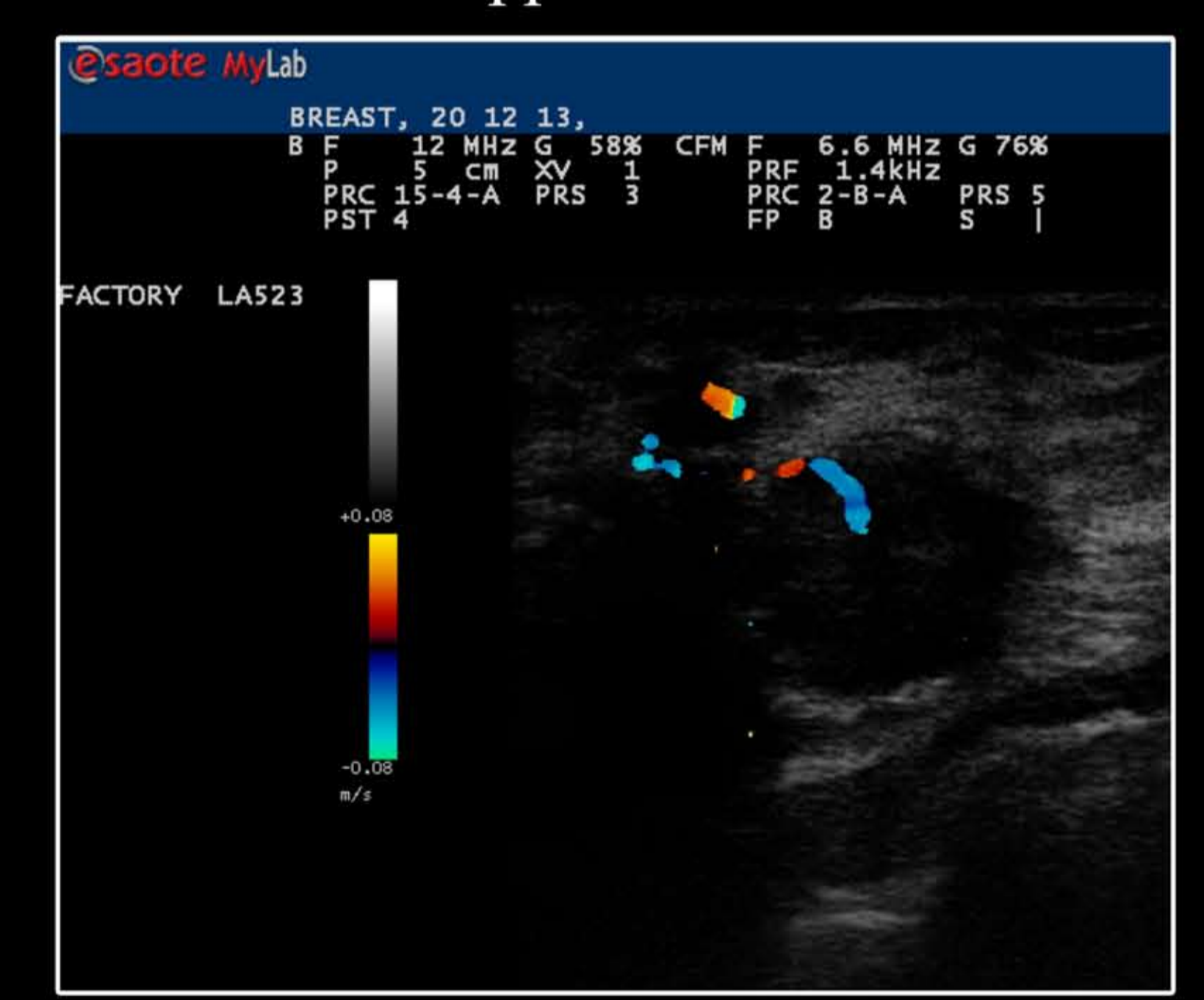
➤ 46 year old female. **Metastasised breast cancer.** Bearing in mind that measurements taken on ultrasonographic images may be affected by a number of variables, this major mass seems to "respond" to localised subcutaneous injections (880 ng/day) of OA-GcMAF, and consecutive measurements performed with the same ultrasound system and probe by the same operator, seem to indicate a reduction of the volume of the measured area within the lesion.



➤ 46 year old female. **Metastasised breast cancer.** In axial projection, the volume of the roundish area can be calculated in about 1.8 ml.



➤ 46 year old female. **Metastasised breast cancer.** Active vascularisation of the mass could be observed at the echo-colour-doppler.



CONCLUSIONS

- The observations presented in this study, together with the recent results of molecular modelling (*Nutrients* 5(7): 2577-2589, 2013), demonstrate that OA, GcMAF and NO, molecules endowed with individual anti-cancer properties, can be properly combined and specifically delivered to advanced cancer patients with significant effects on immune system stimulation and tumour volume reduction.
- These observations have the potential for revolutionising the field of cancer treatment since it appears that the proposed anti-cancer strategies are entirely devoid of any recorded harmful side effects.

ACKNOWLEDGEMENTS

➤ We wish to thank the Committees and the Patrons of the Conference for having accepted this study of ours. Quoting the words of the President of the SIICA 9th National Conference, we too "firmly believe that the SIICA 9th National Conference in Florence will become for all of you an unforgettable event."



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