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## **A new FCM based method to quantify surface monoclonal antibody as targeting agent with nanoparticles: application on a docetaxel-trastuzumab immunoliposome**

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HAL Authorization

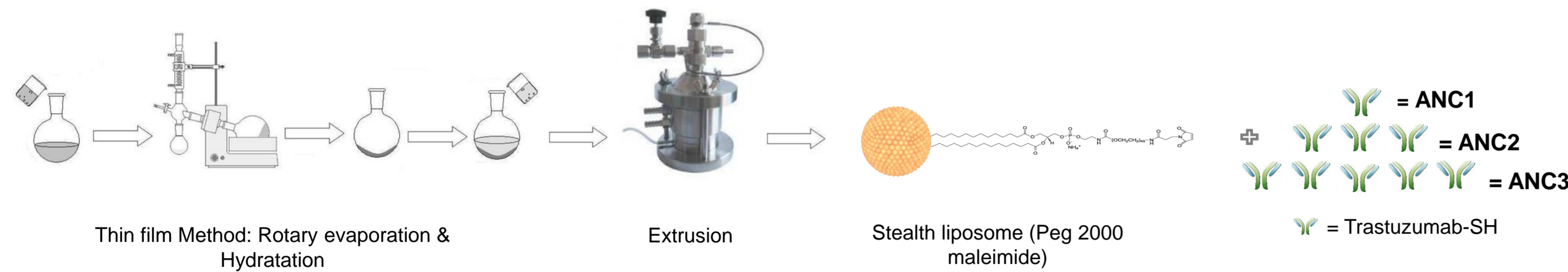
**BACKGROUND**

Improving drug delivery in oncology, developing targeted nanoparticles loaded with cytotoxics is a rising strategy in oncology. Antibodies are the most commonly used targeting agents. However, determination of their optimal density on nanoparticles surface remains an open question, mainly due to the difficulties in measuring accurately the actual level of coating. We have developed a stealth immunoliposome encapsulating docetaxel and harboring anti-Her2 trastuzumab. To better prototyping the nanoparticles, we have developed a method for the absolute quantitation of trastuzumab, so as to compare the *in vitro* performance of the immunoliposomes in terms of efficacy in breast cancer depending on the final number of antibodies.

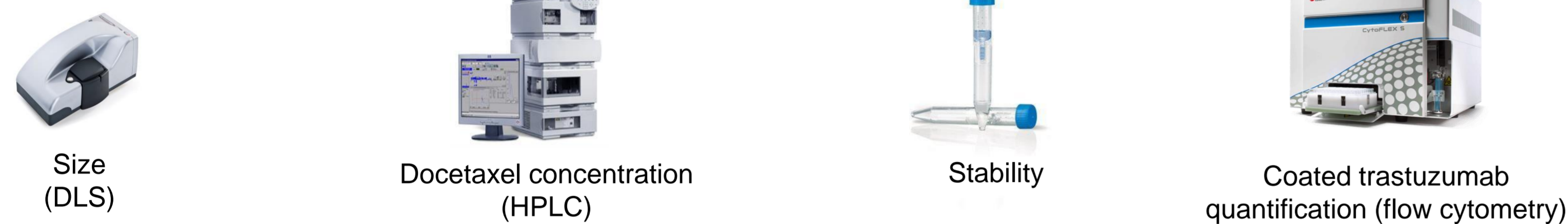
**MATERIAL & METHODS**

**Biopharmaceutical development:**

Three synthesis steps

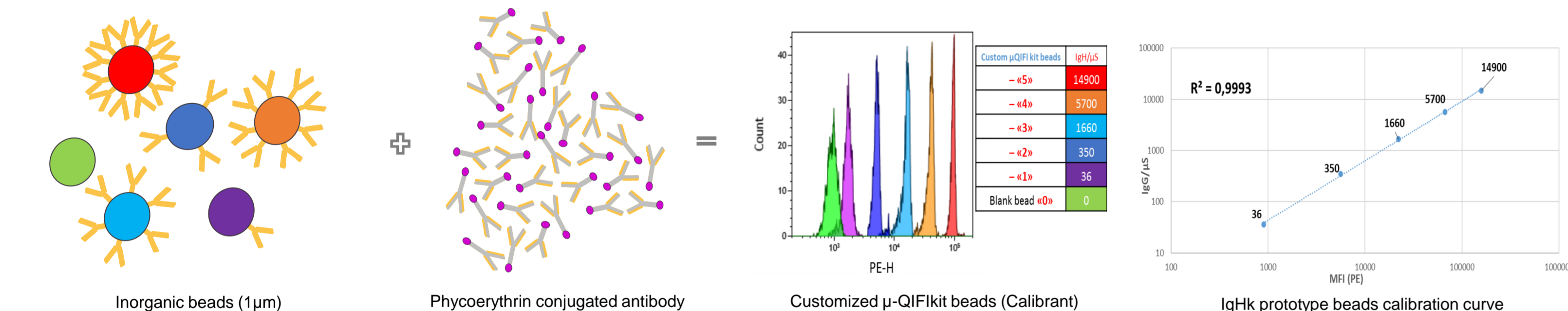


**Characterization studies**



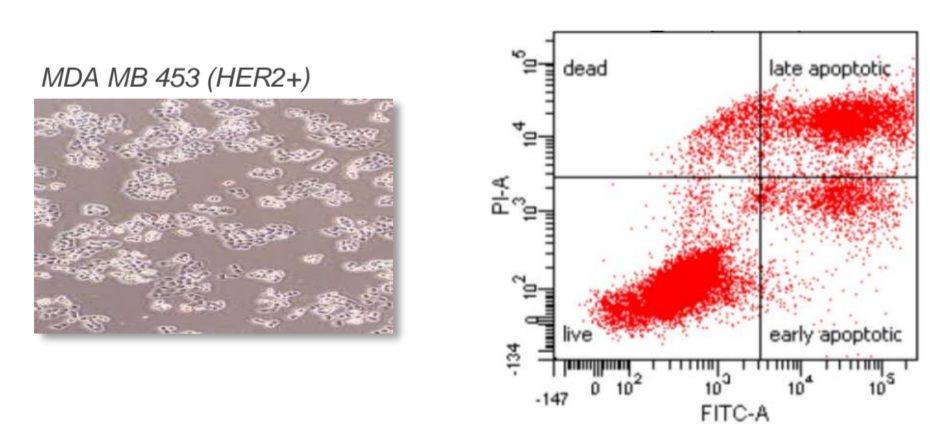
**Focus on coated trastuzumab quantification kit (Cytoflex®)**

Quantitative Immuno-Fluorescence Indirect assay. Adapted from QIFikit® (Beckman-Coulter) on μsphere size and IgG measuring range.

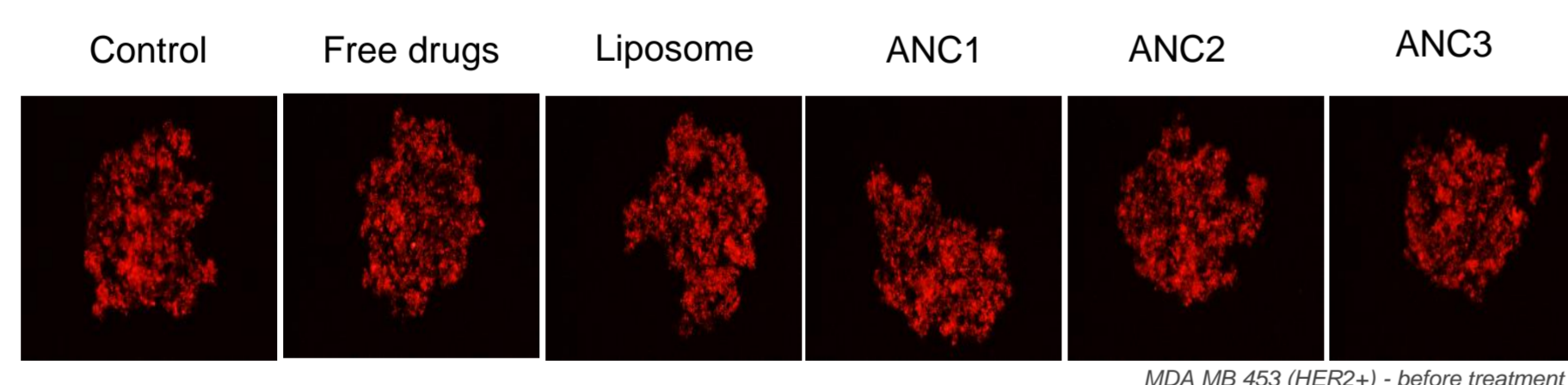


**In vitro efficacy studies (MDA MB 453/HER2+)**

**2D model: Apoptosis Assay (Annexin V-FITC/PI)**



**3D model: Bioluminescent spheroids (CellTiterGlo®)**

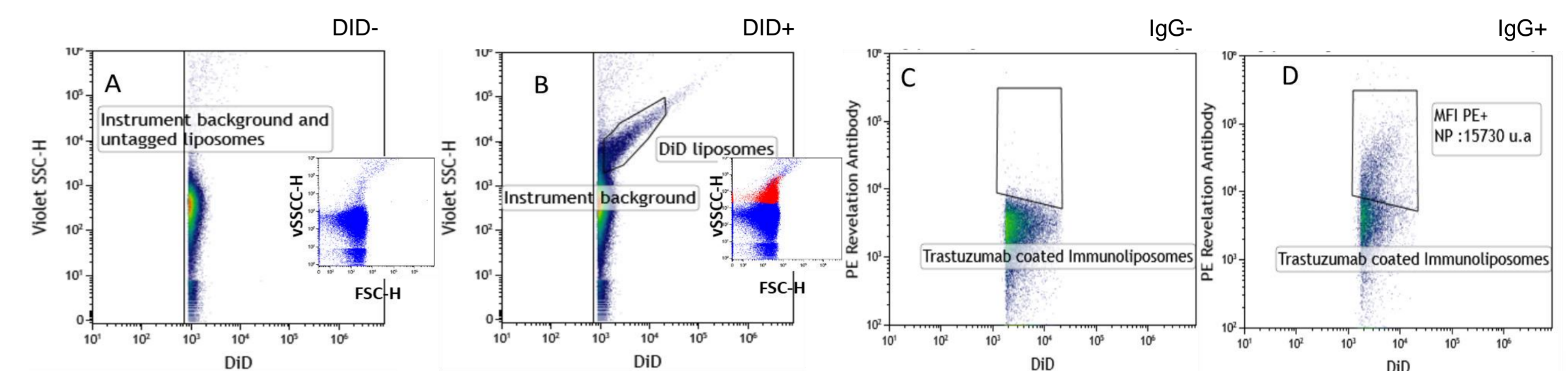


**Biopharmaceutical development:**

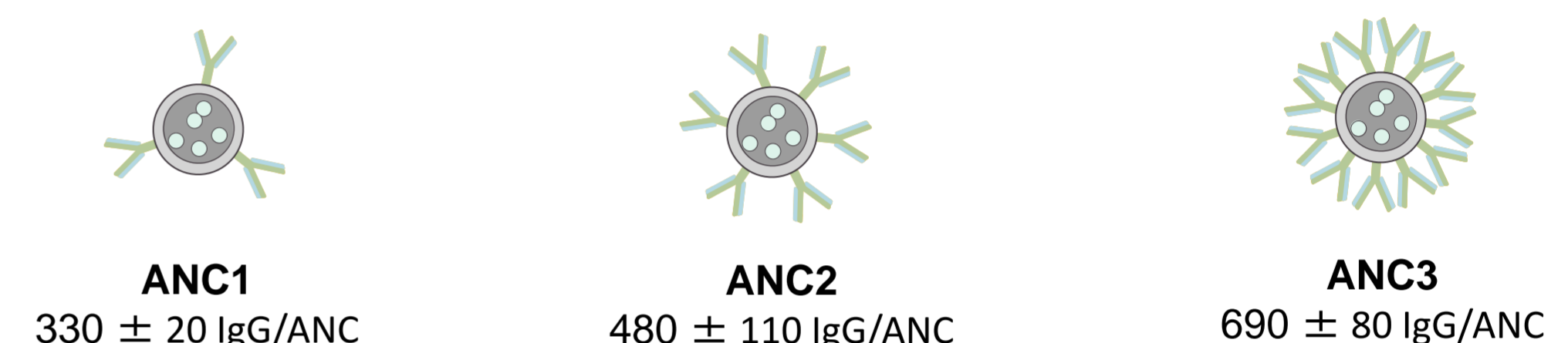
Size (nm)*	Docetaxel encapsulation rate (%)*	Stability*
140 ± 3	> 90	Size: ok; Docetaxel leakage: 18 % weekly

\*Similar data were found for all formulations (n>3)

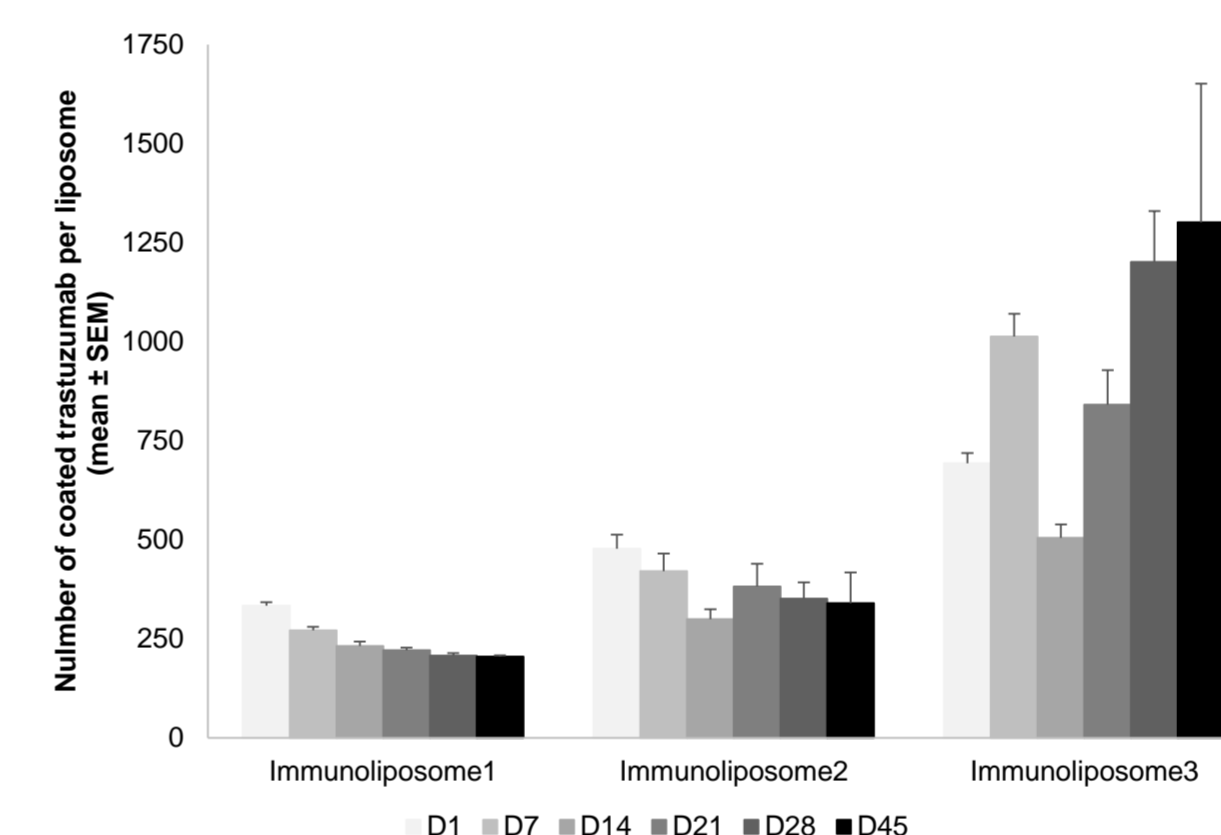
**Focus on coated trastuzumab quantification kit (n=3)**



ANCs and trastuzumab were detected; 65 % of the population was analyzed (Qnano®) ANC1, ANC2 and ANC3 trastuzumab engraftments was statistically different (p<0,004, One-way ANOVA)



**Stability studies on trastuzumab engraftment (n=3)**

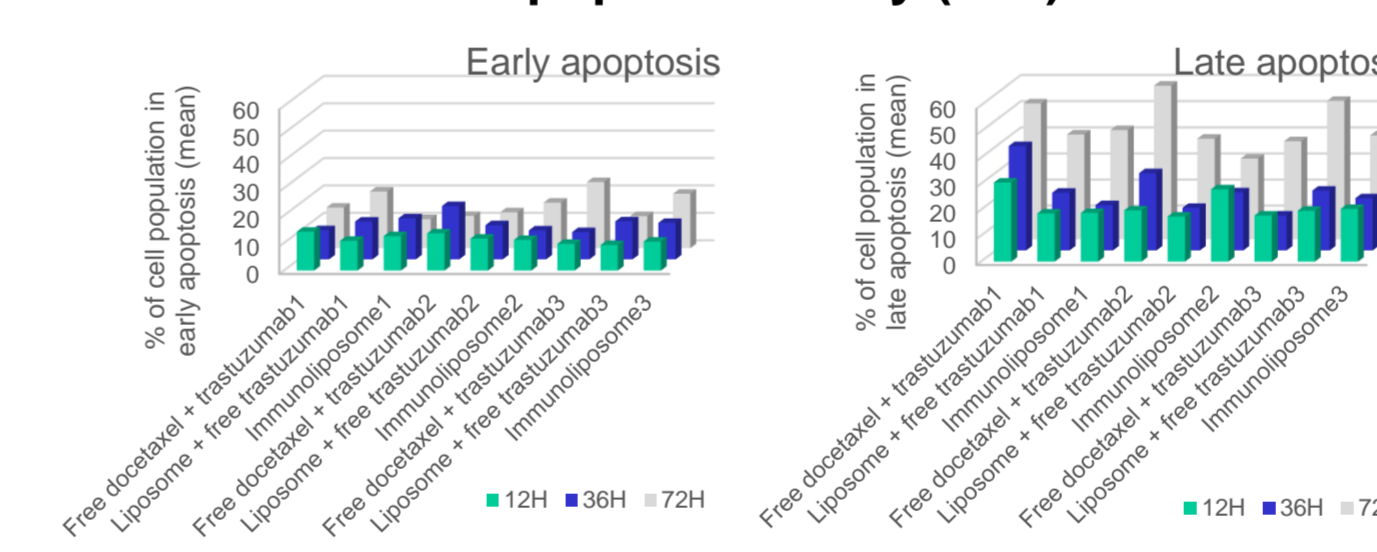


ANC1 and ANC2: Initial max loss of 20% (i.e., 19 % and 12 %, p>0.05, t-student) then stable for 45 days (p<0.05, t-student)?

ANC3: strong variations with an apparent increase of 87 % at day 45 (p=0.011, One-way ANOVA).

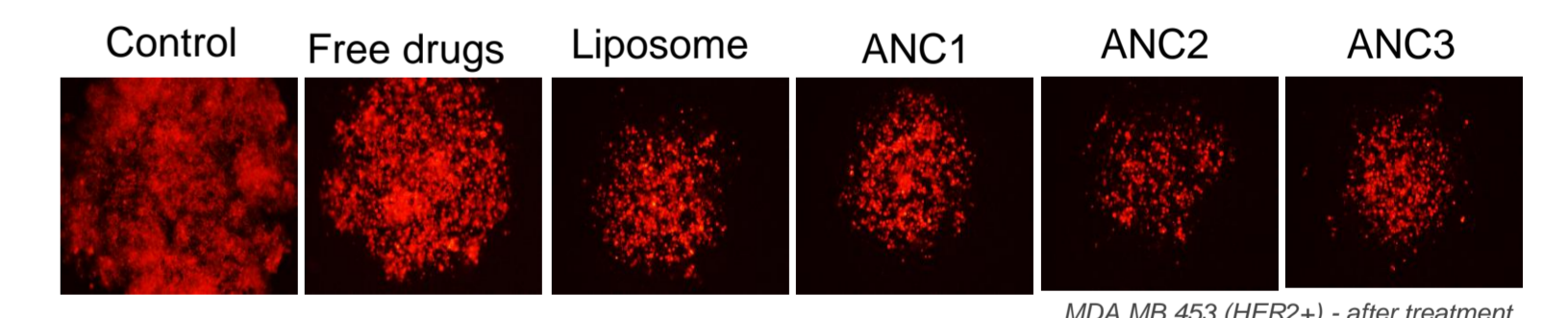
**In vitro efficacy studies (MDA MB 453/HER2+)**

**2D model: Apoptosis Assay (n=3)**



Empty liposomes and trastuzumab did not show cytotoxic effect. Apoptosis increased over time for all conditions. No significant difference in efficacy was observed between treatments.

**3D model: Bioluminescent spheroids (n=3)**



Empty liposomes and trastuzumab did not show cytotoxic effect. Liposomal formulations performed better than free drugs. No significant difference in efficacy was observed between treatments.

**DISCUSSION**

We developed a **new fully calibrated assay based on flow cytometry** of submicron particles that can accurately quantify the number of coated antibodies on single nanoparticles. This single particle analysis quantitative approach was used for **quality control** of different immunoliposomes batches targeting HER2+ breast cancer cells, thus allowing to determine precisely the optimal engraftment rate when prototyping candidates.

While comparative *in vitro* 2D studies were not conclusive, 3D models demonstrated higher efficacy of our immunoliposomes when compared to free trastuzumab docetaxel used in combo. Moreover, **higher density of targeting agent on nanoparticles was not a prerequisite** for maximal anti-tumor effect.

**PERSPECTIVES**

This original method should help us to further **determine optimal characteristics** of immunoliposomes so as to rationalize early development steps with 3d generation nanoparticles.

**Extensive pharmacometrics** (i.e., PB-PK modeling) will be used to analyze experimental data so as to **define the optimal trastuzumab density engraftment depending on tumor model** and Her2 expression.

**Lyophilization** studies will be performed to improve the stability of the nanoparticle so as to facilitate shifting towards in vivo studies.

**REFERENCES**

Rodallec, A *et al.* Int. J. Nanomedicine, 2018  
 Poncelet, P. *et al.* J. Immunol. Method, 1985