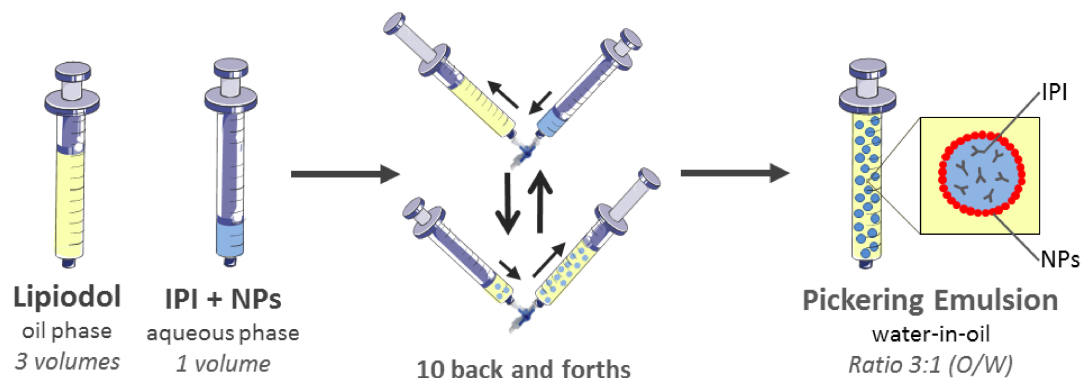
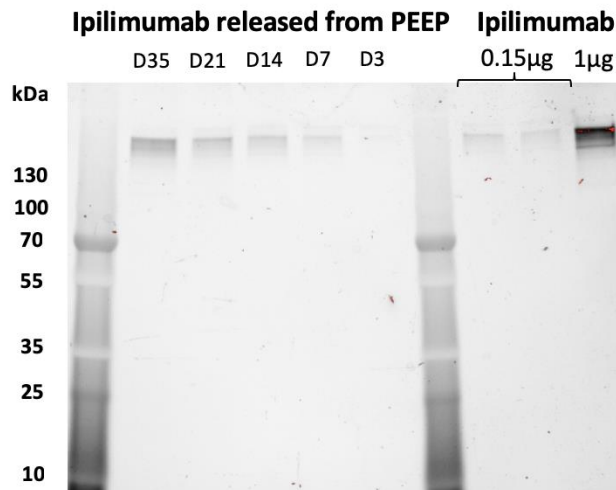


Supplemental data

Figure 1. Anti-CTLA4 pickering emulsions using ethiodized oil and PLGA nanoparticles formulation.



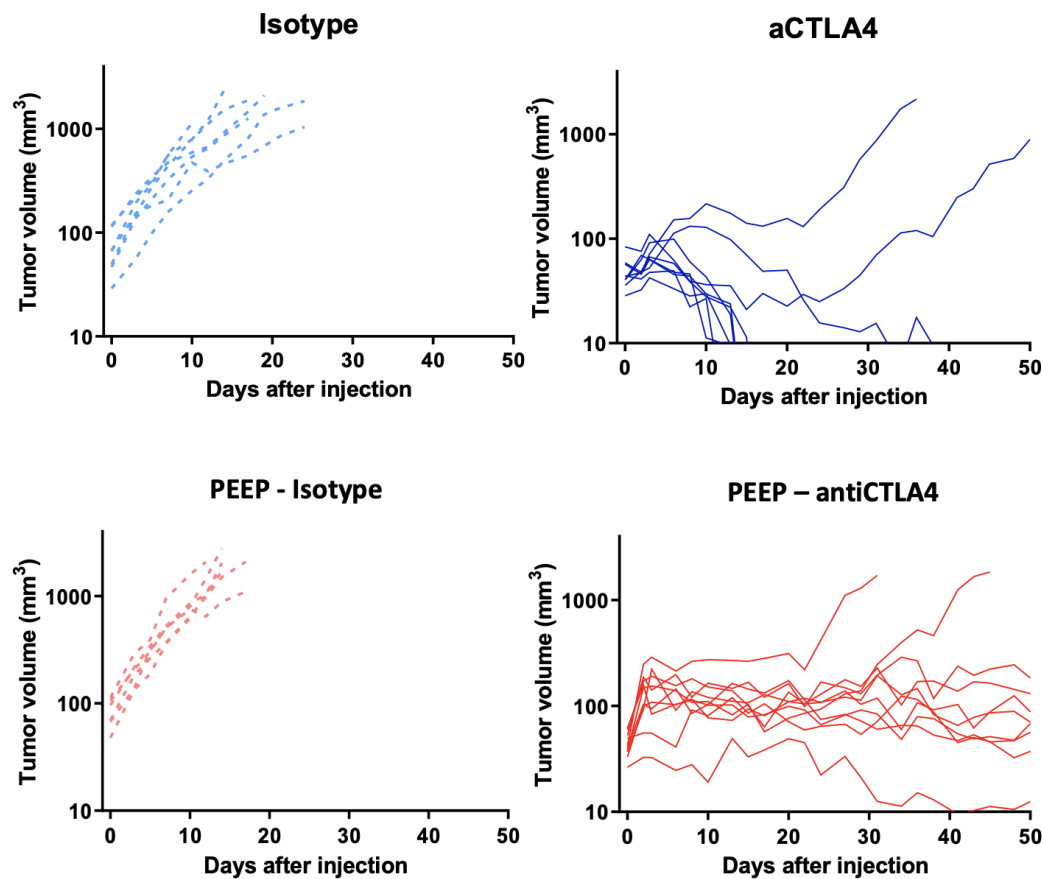
Formulation of a 3 (volumes of oil) to 1 (volume of aqueous phase) ratio Water-in-Oil Ipilimumab (Ipi) pickering emulsions using ethiodized oil and PLGA nanoparticles. First, 3 volumes of Lipiodol is withdrawn in a syringe and 1 volume of aqueous solution containing Ipilimumab and PLGA Nanoparticles (NPs) in another syringe. PLGA-NPs were added to the aqueous-phase during formulation of the Pickering-emulsion at a concentration of 15 mg/mL. Then, the two syringes are connected with a three-way stopcock and the aqueous phase is injected into the oily phase followed by repetitive pumping (20 times). The final emulsion is a water-in-oil emulsion with droplets of water containing Ipilimumab and PLGA nanoparticles at the oil-water interface. We replicated the experiments five times for both emulsions (with and without PLGA NPs).

Figure 2. Released antibody structural evaluation

Electrophoresis gel analysis showing similar migration profile (150 kDa) of Ipilimumab and Ipilimumab released from the aliquot of pickering emulsions using ethiodized oil and PLGA nanoparticles at various timepoints (D3=Day 3)

In order to preserve, as much as possible, the integrity of the antibodies, we chose to use partially non-denaturing conditions. No β -mercapto-ethanol was used and no pre-heating was done before migration, but sodium dodecyl sulfate (SDS) was used in the TRIS-Glycerol charge and migration buffers. Migration was run at a voltage of 80V during 30 minutes then the voltage was increased to 130 V during 90 minutes. Bands were observed under UV revelation.

Figure 3. *In vivo* antitumoral activity of intratumoral anti-CTLA4 and anti-CTLA4 released from pickering emulsions using ethiodized oil and PLGA nanoparticles.



Mice individual data per treated group showing objective response for both intratumoral anti-CTLA4 and PEEP – anti-CTLA4. Tumor growth within 3 weeks was observed in the isotype and PEEP – isotype groups.