Supplementary figure 1
Supplementary figure 1. These data correspond to figure 1. **A. metastasis in tumour-bearing mice at necropsy.** P815, 4T1, and CT26 tumours were induced as described in figure 1 and treated with 50µl PBS (blue) or CFA (red). At the time of euthanasia (due to tumour size or illness) mice were analysed for the prevalence of metastasis to the lungs or liver. (Note that in the P815 and 4T1 tumour models, CFA treated mice live longer, on average than PBS-treated mice, so would have had more time, on average, to develop metastases). A generalized linear regression model was applied to binomial data for statistical analysis. Error bars represent the binomial uncertainty of the data. P-values are displayed above the bar plots. A trend of reduced lung metastases with CFA treatment was observed in the 4T1 model (p=0.056). P815 (exp. N=5); PBS (n=29), CFA (n=32). 4T1 (exp. N=3); PBS (n=16), CFA (n=19). CT26 (exp. N=3); PBS (n=25), CFA (n=38). **B. Tumour growth of regressing mice in the P815 model treated with CFA.** These data correspond to Figure 1A. Six complete tumour regressions were observed in CFA-treated mice and one mouse initially regressed but relapsed approximately 50 days post-treatment. Some mice received FNAs at 6-7 day intervals (up to three analyses) or 2-3 day intervals (up to seven analyses), and some mice did not receive FNAs. The mean tumour growth of PBS treated mice in the corresponding experiments (n=18) is shown for comparison. **C. IFA (incomplete Freund’s adjuvant) is not an effective treatment for P815 mastocytoma.** P815 mastocytoma murine tumours were induced as previously described and treated intratumourally with 50µl PBS (blue), incomplete Freund’s adjuvant (IFA ,black), or CFA (red). Data were analysed using Tukey adjustments for multiple comparisons. CFA-treated mice had significantly increased survival compared to IFA-treated mice (coxph p=0.0203, HR: CFA:IFA=0.3101, 95%CI[0.115, 0.834]). There was no significant survival difference between PBS-control and IFA-treated mice (coxph p=0.6314, HR: PBS:IFA=0.8145, 95%CI[0.352, 1.883]). The significant survival difference between CFA-treated and PBS-control mice was maintained (coxph p=0.0492, HR: CFA:PBS=0.3656, 95%CI[0.134, 0.996]).
Supplementary figure 2. Histopathology post-CFA of grey horse melanoma. Panels A and B show intratumoral lymphocytic infiltrates in clusters (A: #003; 11 weeks after treatment) or more evenly dispersed (B: #008; 8 weeks after treatment) in horses which showed tumour regressions post treatment. Animals which showed no tumour regression typically had no (C: #009; 6 weeks after treatment) or sparse lymphocytic infiltrates (D: #001; 18 weeks after treatment). Paraffin-embedded formalin-fixed tissue stained with HE after melanin bleaching procedure; magnification 200x. # refers to numbering of horses in supplementary table 1.
Top: Extensive immune infiltrate; patients 3,5,6.

Middle: Prominent necrosis patients 3,5,6

Bottom: Less immune infiltrate, minimal necrosis patients 1,2 and 7

Supplementary fig 3
Supplementary figure 3. Histology of patients after CFA injection. Biopsies were taken from patients on day 5 (compulsory) and day 28 (optional) after CFA injection (day 1). Biopsies were stained with H&E. All images x400. The top row shows biopsies with extensive immune infiltrate at day 5, the middle row shows biopsies from the same patients, all with prominent necrosis (patients 3, 5, and 6 shown left, middle and right, respectively in both the top and middle rows). The bottom row biopsies (left = patient 1 on day 5; middle two panels = patient 2 on days 5 and 28; right = patient 7 on day 5) have less immune infiltrate and minimal necrosis (all x400). Patient samples are numbered according to supplementary table 2, which contains details of each patient, and the type of cancer from which they are suffering. Histology from patient #4 is presented in figure 4, so is not included here. * indicates viable tumour; # indicates necrotic tumour; circles show focal inflammation.
**Supplementary figure 4.** CT scans of chest of patient #4, 3 months before CFA treatment. These images correspond to figures 6 C and D, and show that the lung metastatic deposit that reduced in size after CFA treatment, had also decreased after previous treatment with everolimus, the last dose of which was administered 6 weeks before the first CFA injection. However as the half-life of everolimus is only 30 hours, it was considered very unlikely that the shrinkage shown in Fig 6C could be attributed to this previous treatment. Tumour measurements: 39mm (left, green arrow), and 5mm (right, dark blue arrow).