APPENDIX
A. IMMUNOHISTOCHEMICAL STAINING PROTOCOL

Tissue sections were baked for 30 minutes (50°C), followed by dewaxing with Histoclear (Electron Microscopy Sciences, Hatfield, Pennsylvania) and rehydration in graded ethanol series. Subsequently, samples were subjected to heat-induced antigen retrieval with either citrate (pH = 6.0), ethylenediaminetetraacetic acid (pH = 8.0), or tris-ethylenediaminetetraacetic acid (pH = 9.0) buffers at 95°C–100°C. Upon cooling, slides were washed (1×phosphate-buffered saline, 5% bovine serum albumin, and 0.1% Tween-20) and blocked with 200 µL of Dako Dual Endogenous Protein Block (Agilent Technologies, Santa Clara, California) for 45 minutes at room temperature (RT). After an additional washing step, the sections were incubated for 1 hour with 250 µL 2.5% normal goat serum and incubated with the primary antibody targeting CD3, HLA-DR, CTLA-4, PD-1, or PD-L1 for either 90 minutes (RT) or alternatively overnight (4°C). The optimal titer was objectively defined as the one with the highest dynamic range of specific staining. For detection, slides were washed, incubated with an horseradish peroxidase-conjugated goat anti-mouse/rat secondary antibody (Vector Laboratories, Burlingame, California) for 1 hour (RT), and developed for 1–3 minutes with horseradish peroxidase ImmPACT DAB Substrate Peroxidase (Vector Laboratories). Finally, the sections were counterstained with hematoxylin, followed by dehydration with an increasing ethanol series and Histoclear. The slides were mounted with VectaMount Permanent Mounting Medium (Vector Laboratories) for light microscopy analysis.

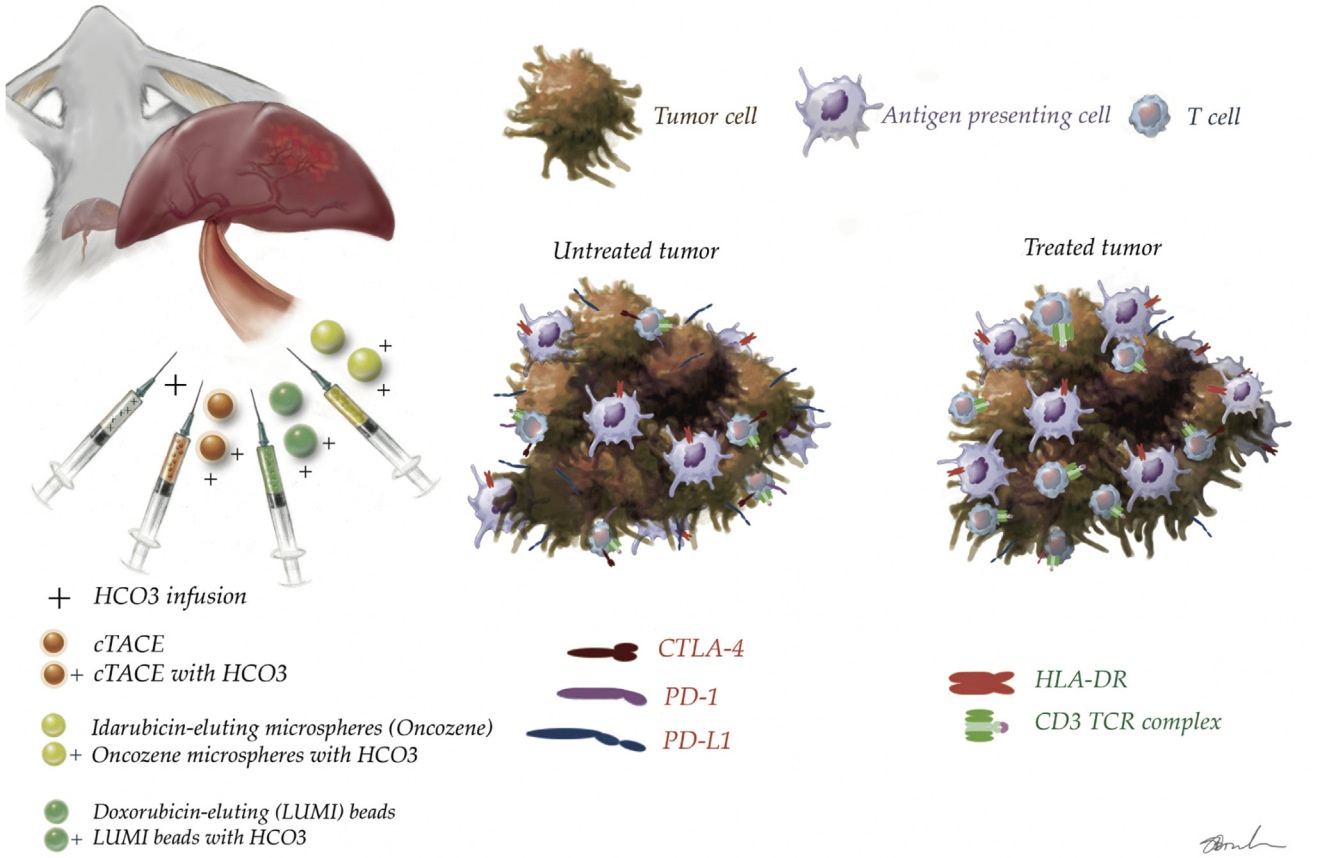

Figure E1. Illustratration of the experimental design to assess the impact of intra-arterial therapy with different embolic agents on the immune response. VX2 liver tumor-bearing New Zealand white rabbits were assigned to groups underdoing locoregional treatment as follows: bicarbonate infusion, conventional transcatheter arterial chemoembolization (cTACE) with Lipiodol, drug-eluting embolic-TACE with either idarubicin-eluting Oncozene microspheres or doxorubicin-eluting LUMI beads. Each transcatheter arterial chemoembolization treatment arm was performed either with or without additional bicarbonate infusion. Intra-arterial therapy resulted in substantial increase of the tumor-infiltrating $CD3^+$ T-lymphocytes and $HLA\text{-}DR^+$ antigen-presenting cells and lower expression of the cytotoxic T-lymphocyte–associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1)/PD-1 ligand (PD-L1) pathway axis. CD = cluster of differentiation; HLA-DR = human leukocyte antigen DR type; TCR = T-cell receptor.

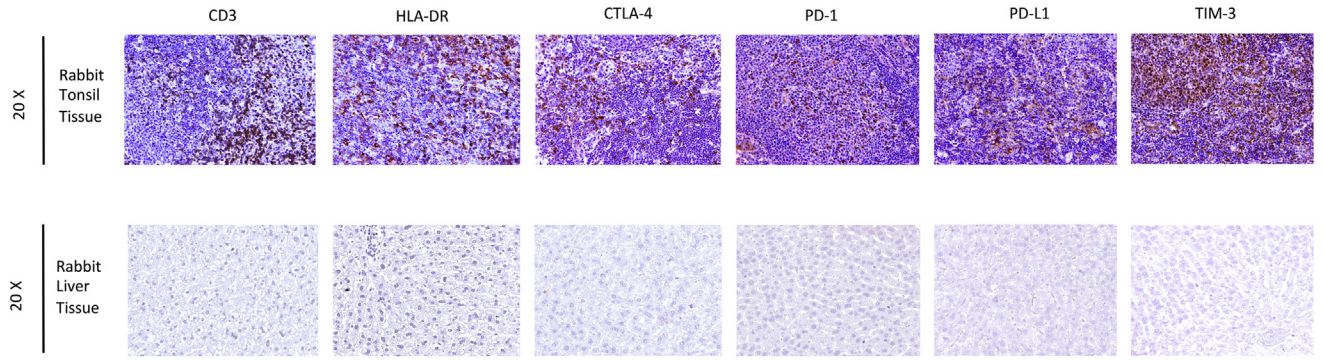

Figure E2. Positive and negative control stainings (20× magnification). Staining specificity of the surface markers in rabbit tonsil tissue (positive control) and rabbit liver tissue (negative control) was verified by a board-certified pathologist (X.Z.). CD = cluster of differentiation; CTLA-4 = cytotoxic T-lymphocyte–associated protein-4; HLA-DR = human leukocyte antigen DR type; PD-1 = programmed cell death protein-1; PD-L1 = programmed cell death protein-1 ligand; TIM-3 = T cell immunoglobulin and mucin protein 3.

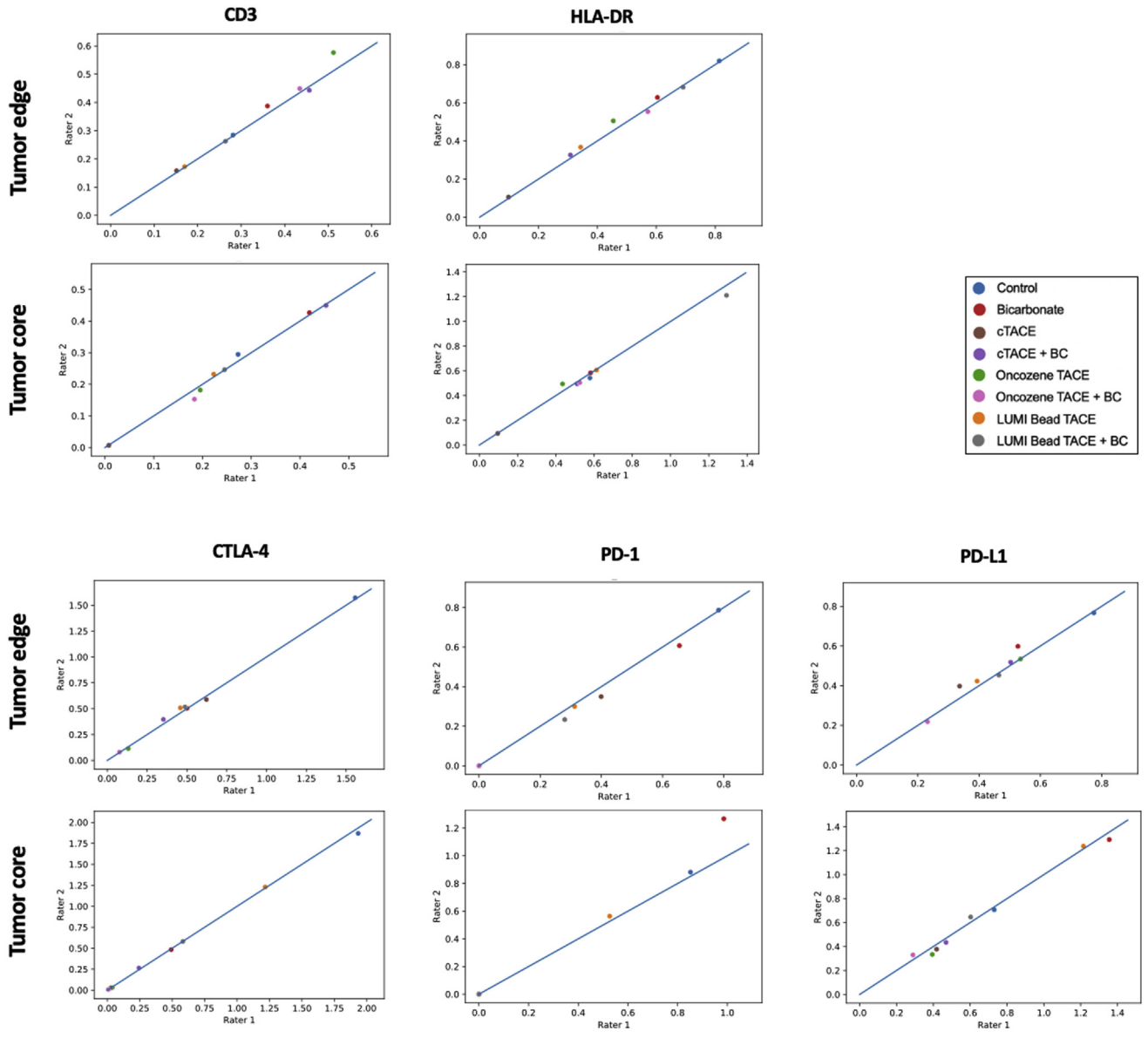

Figure E3. Scatter plots of the 2 percentage cell positivity measurements. Interreader agreement between the 2 raters (rater 1: X.X., rater 2: X.Y.) on a subset of the data (1 tumor/group for each marker) demonstrates the reproducibility of the results. BC = bicarbonate; CD = cluster of differentiation; cTACE = conventional transcatheter arterial chemoembolization; CTLA-4, cytotoxic T-lymphocyte–associated protein-4; HLA-DR = human leukocyte antigen DR type; PD-1 = programmed cell death protein-1; PD-L1 = programmed cell death protein-1 ligand; TACE = transcatheter arterial chemoembolization.

Table E1. Intraclass Correlations Coefficients (ICC3) across the Markers.

Region	Marker	ICC3	95% CI	*P* value
Tumor edge	CD3	0.985390258	[0.93, 1.0]	≤.0001
	HLA-DR	0.995537171	[0.98, 1.0]	≤.0001
	CTLA-4	0.998019535	[0.99, 1.0]	≤.0001
	PD-1	0.996652009	[0.98, 1.0]	≤.0001
	PD-L1	0.978543438	[0.9, 1.0]	≤.0001
Tumor core	CD3	0.993890486	[0.97, 1.0]	≤.0001
	HLA-DR	0.992324757	[0.96, 1.0]	≤.0001
	CTLA-4	0.999269454	[1.,1.0]	≤.0001
	PD-1	0.978223446	[0.9, 1.0]	≤.0001
	PD-L1	0.993910123	[0.97, 1.0]	≤.0001

Note–A *P* value <.05 shows significant agreement between the 2 measurements.

CD = cluster of differentiation; CI = confidence interval; CTLA-4 = cytotoxic T-lymphocyte–associated protein-4; HLA-DR = human leukocyte antigen DR type; PD-1 = programmed cell death protein-1; PD-L1 = programmed cell death protein-1 ligand.