Supplemental Information

LTX-315 sequentially promotes lymphocyte-independent and lymphocyte-dependent antitumor effects

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Content

Two Supplemental Figures: Fig S1, Fig S2

Α

B16F10 Model

| B16F10 2x10⁵ i.d. ↓ -4 | LTX-315 i.t. (1 mg/50 µl Saline) ↓ ↓ ↓ 0 1 2 | Measure Tumor Progression | ► Time (d) |
|---------------------------------------------------|----------------------------------------------------------|------------------------------|------------|
| В | | | |
| Braf ^{v600E} /Pten ^{-/-} Model | | | |
| 4-Hydroxy- tamoxifen i.d. ↓ ~-28 | LTX-315 i.t. (1 mg/50 µl Saline) ↓ ↓ ↓ 0 1 2 | Measure Tumor Progression | ► Time (d) |
| C Kras ^{G12D} /p53 ^{fl/fl} N | | | |
| AdCre i.m. ↓ | LTX-315 i.t. (1 mg/50 µl Saline) ↓ ↓ ↓ | Measure Tumor Progression | ► Time (d) |
| ~-50 | 0 1 2 | | |

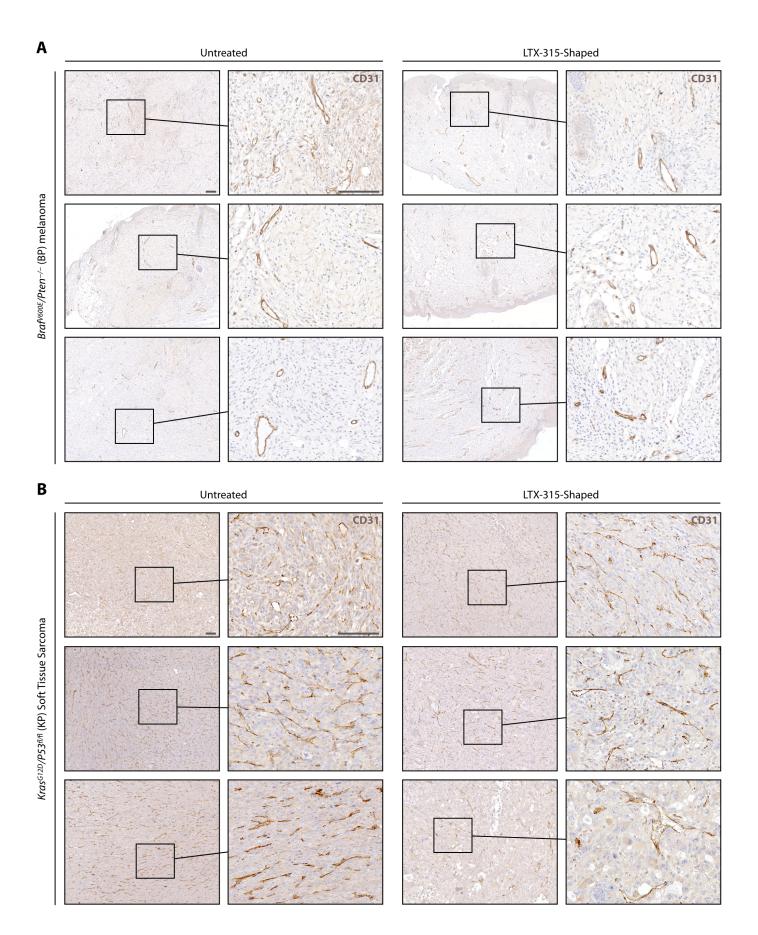


Figure S1. LTX-315 treatment strategy in syngeneic and conditional genetic mouse models.

- (A) Schematic of B16F10 melanoma experiments: C57BL/6 wild type mice or Rag2-/- mice bearing B16F10 melanoma tumor grafts were treated intratumorally (i.t.) with LTX-315 for three consecutive days. Tumor-bearing control mice were left untreated.
- (B) Schematic of BP melanoma experiments: *Braf^{V600E}/Pten^{-/-}* (BP) mice subjected to tamoxifen to produce tumors were treated intratumorally (i.t.) with LTX-315 for three consecutive days or left untreated.
- (C) Schematic of KP soft tissue sarcoma experiments: Kras^{G12D}/p53^{#/#} (KP) mice subjected to intramuscular leg injection with Adenovirus expressing Cre recombinase (AdCre) to produce tumors were treated intratumorally (i.t.) with LTX-315 for three consecutive days. Tumorbearing control mice were left untreated.

Figure S2. CD31 staining in untreated and LTX-315-shaped BP and KP tumors.

- (A) CD31 staining of tumor sections obtained from *Braf^{V600E}/Pten^{-/-}* (BP) mice that were either left untreated ("Untreated") or that regrew after LTX-315 treatment ("LTX-315-Shaped"). Both mouse cohorts had similar tumor burden (see also Figure 3A). Scale bar: 100 μm.
- (B) CD31 staining of tumor sections obtained from *Kras^{G12D}/p53^{#/#}* (KP) mice that were either left untreated ("Untreated") or that regrew after LTX-315 treatment ("LTX-315-Shaped"). Both mouse cohorts had similar tumor burden (see also Figure 3A). Scale bar: 100 μm.