Supplementary methods

Immunohistochemistry (IHC)

MC38 tumors were collected and snap frozen from the 5 surviving animals in each group on day 5 after oxaliplatin plus anti–programmed death-ligand-1 (OP) or trilaciclib plus oxaliplatin and anti–programmed death-ligand-1 (TOP) treatment. The frozen tissues were embedded in optimal cutting temperature compound and sectioned at ~5 μm for IHC staining to detect CD4, CD8 and FoxP3. After IHC staining, slides were visualized under light microscopy by the study pathologist, where strength and frequency of staining was assigned. Frequency of staining was estimated by counting the number of positive cells in 10 random 20x fields. Five random 20x fields were counted from the perimeter of the tumor and five random 20x fields were counted from the interior of the tumor using a 10 x 10 reticle micrometer (~1.1 mm^2 per field). The total cells counted in the 10 total fields were captured along with the average cells/field. Cell count information for all markers was captured in an Excel spreadsheet. These studies were conducted at Charles River Laboratories, Inc., Frederick, MD, USA.

Antibodies used for flow cytometry in animal studies

	Antibody target	**Clone**	**Fluorophore**	**Vendor**
Tumor analysis	CD45	30-F11	AlexaFluor 700	Biolegend
	CD3	17A2	PE	Biolegend
	CD4	RM4-5	FITC/PerCP-Cy5.5	Biolegend
	CD8	53-6.7	APC-H7/APC	BD Bioscience
	CD11b	M1/70	PE-Cy7/PE	BD Bioscience
	CD25	PC61	BV605	Biolegend
	Ly6G	1A8	BV421/PerCP-Cy5.5	Biolegend
	Ly6C	HK1.4	PerCP-	Biolegend

			Cy5.5/APC	
	FoxP3	FJK-16s	APC/PE	eBioscience
Spleen analysis	CD69	145-2C11	FITC	Biolegend
	CD4	RM4-5	PerCP-Cy5.5/FITC	Biolegend
	CD8	53-6.7	APC	Biolegend
	FoxP3	FJK-16s	PE	eBioscience
	CD25	PC61	PerCP-Cy5.5	Biolegend

Functional analysis of T-regulatory cells (Tregs) in vitro

Tregs were purified from spleens of C57BL/6 mice using the $CD4^+CD25^+$ Regulatory T cell Isolation Kit (Miltenyi Biotech; catalog number 130-091-041) and were cultured ex vivo with plate-bound anti-CD3ε (eBiosciences; catalog number 14-0031-86, clone 145-2C11) and soluble anti-CD28 (eBiosciences; catalog number 16-0281-85, clone 37.51) antibodies and 100 ng/mL interleukin (IL)-2 (Miltenyi Biotech; catalog number 130-094-054) in RPMI medium plus 10% weight per volume fetal bovine serum plus 2-mercaptoethanol for 48 hours with either 0, 250 or 1000 nM trilaciclib. Trilaciclib was removed from culture media, and equal numbers of trilaciclib-treated Tregs were then co-cultured for 3 days with carboxyfluorescein succinimidyl ester (CFSE)-labeled splenocytes to evaluate the ability of Tregs to suppress T-cell proliferation. $CD8^+$ T-cell proliferation was quantified by the level of CFSE dilution by flow cytometric analysis, normalized to controls (T cells stimulated in the absence of Tregs).

Figure S1 Tumor growth analysis of the (A) MC38 and (B) CT26 models treated with trilaciclib or trilaciclib in combination with αPD-L1 with the indicated schedule. Treatment was initiated (day 1) when tumor volume reached 80–120 mm^3. Data represent median tumor volume. Ten to 15 mice were analyzed in each group. (C) Tumor growth analysis of MC38 tumor-bearing mice treated with varying combinations of trilaciclib/oxaliplatin/αPD-L1 as indicated. Data represent median tumor volume. Ten to 15 mice were analyzed in each group. Treatment was initiated (day 1) when tumor volume reached 80–120 mm^3. (D) MC38 tumor growth of individual animals in Figure 1B treated with vehicle, OP or TOP (IM schedule). (E) MC38 tumor growth of individual animals in Figure 1C treated with vehicle, oxaliplatin + αPD-1, or trilaciclib + oxaliplatin + αPD-1 (top panel) or vehicle, 5-FU + αPD-L1, or trilaciclib + 5-FU + αPD-L1 (bottom panel). (F) CT26 tumor growth of individual animals in Figure 1F treated with vehicle, OP, or TOP. 5-FU, 5-fluorouracil; αPD-L1, anti–programmed death-ligand-1; IM, intramuscular; OP, oxaliplatin plus anti–programmed death-ligand-1; TOP, trilaciclib plus oxaliplatin and anti–programmed death-ligand-1.

Figure S2 Flow cytometry gating schemes for intra-tumor immune cell populations analyzed for cell proliferation status in figure 2. EdU, 5-ethynyl-2'-deoxyuridine; gMDSC, granulocytic myeloid-derived suppressor cell; mMDSC, monocytic myeloid-derived suppressor cell; NK, natural killer; Th, T-helper; Treg, T-regulatory cell.

Figure S3 (A) Proportion of EdU+ cells in each intra-tumor lymphoid and myeloid immune cell type at 6–24 hours (n=4 biological replicates) after trilaciclib treatment in Figure 2B and (B) days 2, 4 and 7 (n=4 or 5 biological replicates) after OP or TOP treatment in Figure 2C. Each biological replicate consists of a pool of three animals. Data represent mean ± standard deviation. *p<0.05; **p<0.01; ***p<0.001. EdU, 5-ethynyl-2'-deoxyuridine; gMDSC, granulocytic myeloid-derived suppressor cell; mMDSC, monocytic myeloid-derived suppressor cell; NK, natural killer; OP, oxaliplatin plus anti–programmed death-ligand-1; TOP, trilaciclib plus oxaliplatin and anti–programmed death-ligand-1; Treg, T-regulatory cell.

Figure S4 (A) Flow cytometry gating schemes for analysis of frequency of intra-tumor immune cell populations in figure 3. (B) Flow cytometry gating schemes for analysis of frequency of activated T cells in spleen. (C) Analysis of $CD8^+$ T cells, $CD4^+$ T cells and Treg ($FoxP3^+$) in tumor by IHC on days 5 and 9 (the box plots represent minimum and maximum of all data points for each treatment group), and the ratio of $CD8^+$ T cell to Treg count per field (left panel), and the ratio of $CD8^+$ T cells in the interior versus periphery of tumor. Five tumors were analyzed for each treatment group at each time point. *$p<0.05$ by Student's t-test. IHC, immunohistochemistry; OP, oxaliplatin plus anti–programmed death-ligand-1; TOP, trilaciclib plus oxaliplatin and anti–programmed death-ligand-1; Treg, T-regulatory cell.

Figure S5 Comparison of peripheral T-cell subsets in patients with SCLC by flow cytometry between the E/P and T/E/P treatment groups at the indicated time points. (A) Mean absolute count of $CD8^+$ T-cell subsets: naïve ($CD45RA^+CCR7^+$), effector ($CD45RA^+CCR7^-$), central memory ($CD45RA^-CCR7^+$) and effector memory ($CD45RA^-CCR7^-$) subsets. (B) Mean absolute count of $CD4^+$ T-cell subsets: naïve ($CD45RA^+CCR7^+$), effector ($CD45RA^+CCR7^-$), central memory ($CD45RA^-CCR7^+$) and effector memory ($CD45RA^-CCR7^-$) subsets. (C) Mean absolute count of activated $CD4^+$ Th1 cells ($CD3^+CD4^+CXCR3^+CCR6^-CD38^+HLA\text{-}DR^+$). (D) Mean proportion of $CD8^+$ T cells producing IFN-γ (IFN-γ^+IL-17^- cells within $CD3^+CD8^+$ population) after ex vivo stimulation. Error bars represent 95% confidence intervals. (E) Ratio of mean absolute count of activated $CD8^+$ T cells to Tregs, cell populations as defined in figure 1E–F. (F) PFS analysis of patients stratified into treatment groups with low (<median) or high (≥median) number of expanded T-cell clones at C3D1 (left panel) and C5D1 (right panel). The survival curves for patients with a high number of expanded clones in the E/P and T/E/P treatment groups were compared using the log-rank test. For C3D1, HR=0.66 for T/E/P relative to E/P (p=0.43). For C5D1, HR=0.42 for T/E/P relative to E/P (p=0.08). (G) Overall survival analysis of patients stratified into treatment groups with low (<median) or high (≥median) number of expanded T-cell clones at C3D1 (left panel) and C5D1 (right panel). The survival curves for patients with high number of expanded clones in E/P and T/E/P treatment groups were compared using the log-rank test. For C3D1, HR=0.67 for T/E/P relative to E/P (p=0.46). For C5D1, HR=0.76 for T/E/P relative to E/P (p=0.59). Comparison of peripheral T-cell subsets in patients with SCLC by flow cytometry between the E/P and T/E/P treatment groups. C1D1, cycle 1 day 1; C3D1, cycle 3 day 1; C5D1, cycle 5 day 1; E/P, etoposide and carboplatin; HR, hazard ratio; IFN,

interferon; PFS, progression-free survival; PTV, post-treatment visit; SCLC, small cell lung cancer; T/E/P, trilaciclib prior to etoposide and carboplatin; Th, T-helper; Treg, regulatory T cell.