Background
Trilaciclib, an intravenously administered transient cyclin-dependent kinase (CDK)4/6 inhibitor, is indicated to decrease the incidence of chemotherapy-induced myelosuppression in adult patients with extensive-stage small cell lung cancer. In an open-label phase 2 trial in patients with metastatic triple-negative breast cancer, adding trilaciclib prior to gemcitabine plus carboplatin improved overall survival, potentially through protection and direct activation of immune function.1 Based on its broad effects on the cancer-immunity cycle, including enhanced antigen presentation and effector CD8 T-cell function, and sensitivity of Tregs to transient CDK4/6 inhibition, we hypothesized that trilaciclib may enhance the antitumor efficacy of inhibitory receptor immunotherapy (IRI).

Methods
Syngeneic murine models of breast cancer (MMTV-PyMT) and colorectal cancer (CT26) were utilized to evaluate the synergy between trilaciclib and IRI. Trilaciclib 100 mg/kg was administered weekly, and α-PD-1 (5 mg/kg; clone RMP1-14), α-CD73 (5 mg/kg; clone TY/23), α-TIGIT (5 mg/kg; clone TY/23), α-TIM3 (5 mg/kg; clone RMT3-23), and α-LAG3 (10 mg/kg; clone C9B7W) administered biweekly. Treatment was administered intraperitoneally and continued until animals reached humane or study endpoint. Tumor volume and weight were measured 2–3 times a week.

Results
In both MMTV-PyMT and CT26 models, adding trilaciclib to IRI delayed tumor growth and improved survival compared with treatment with IRI alone. In the CT26 model, the combination of trilaciclib plus α-PD-1 delayed tumor growth ($P=0.004$) and improved survival ($P=0.02$) versus α-PD-1 monotherapy. Additional benefit was observed when trilaciclib was added to α-TIGIT therapy compared with α-TIGIT alone, with delayed tumor growth ($P=0.007$) and improved survival ($P=0.002; P=0.04$) in the MMTV-PyMT and CT26 models, respectively. Trilaciclib plus α-LAG3 delayed tumor growth ($P=0.03$) compared with α-LAG3 alone in the CT26 model. When evaluating the combination of trilaciclib with multiple IRIs, an increase in survival was observed when trilaciclib was added to α-PD-1 plus α-CD73, α-TIGIT, or α-TIM3. Survival was significantly increased with trilaciclib plus α-PD-1 and α-LAG3 ($P=0.006$). Compared with α-TIGIT alone, trilaciclib plus α-PD-1 demonstrated efficacy irrespective of tumor model or starting day of treatment.

Conclusions
Adding trilaciclib to IRI combinations heightened antitumor benefits. The combination of trilaciclib plus α-PD-1 was consistently effective, irrespective of when treatment was initiated, or the tumor model used. The data suggest that trilaciclib provides complementary immune modulatory benefits that support the mechanism of IRI and provide a rationale for combining trilaciclib with IRI to enhance clinical efficacy, including in populations resistant to checkpoint blockade or who have received prior IRI treatment.

REFERENCES

Ethics Approval
This study was performed in compliance with federal laws and institutional guidelines as approved by the Animal Ethics Experimentation Committee of the Peter MacCallum Cancer Centre.