Rational drug combinations with CDK4/6 inhibitors in acute lymphoblastic leukemia

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Supplement

Methods

Patient derived xenograft models were generated as previously described. For drug treatment experiments, primary leukemia cells from peripheral blood or bone marrow were intravenously injected in to NSG mice. Splenocytes harvested from successfully engrafted xenografts were reinjected (10^6 cells/mouse) to create secondary and tertiary xenografts for treatment trials. After injection of leukemia blasts, mice were bled weekly. Engraftment was determined by flow cytometric analysis of peripheral blood using antibodies against human CD45. Once mice had > 1% peripheral blood blasts as determined by flow cytometry, the mice were randomized to receive/not receive drug.

Somatic gene testing:

DNA was extracted from leukemia blasts for 4 PDX samples, using a QIAamp DNA mini kit (Qiagen). CDKN2A/B status was assessed by next generation sequencing [31] by the Clinical Genomic Diagnostic Laboratory at the Children's Hospital of Philadelphia for samples that had not previously had sequencing performed and publicly available. If performed previously, references were cited in Table 1.

Figure S1: **Effect of ribociclib on G1 arrest in B-ALL cell lines *in vitro*.** B-ALL cell lines were treated for a minimum of 48-hours prior to harvesting for flow cytometry following treatment with palbociclib (A) versus ribociclib (B). Compared to palbociclib, higher concentrations of ribociclib are needed to induce G1 arrest. This correlates with human PK data.

Figure S2: **CDK inhibitors can successfully be combined with cytotoxic chemotherapy *in vivo*.** Original data presented in Figure 6B re-graphed using the same y axis across each PDX experiment including pre-treatment and concurrent treatment. Variations in disease burden occur

across experiments, however the trends relative to treatment approach indicate no differences in responsiveness to CDKi.

Figure S1: Effect of ribociclib on G1 arrest in B-ALL cell lines *in vitro*.

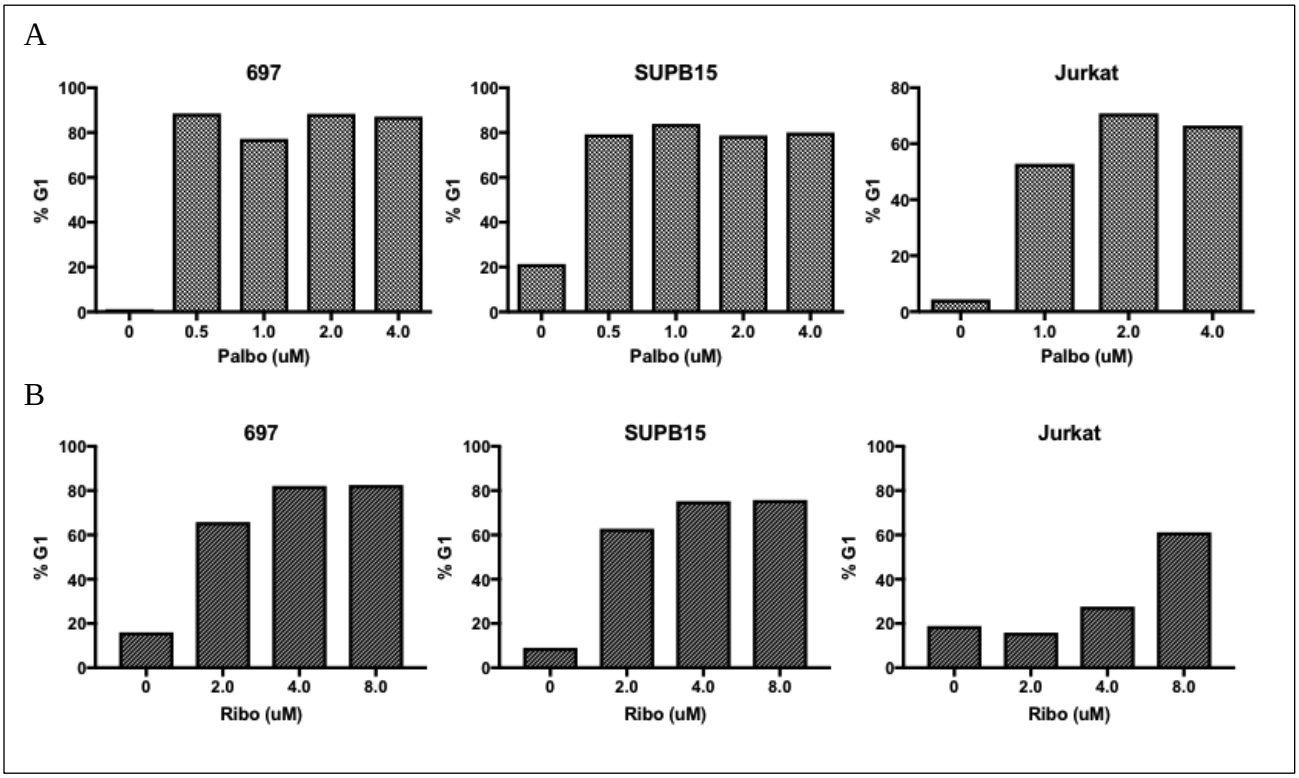
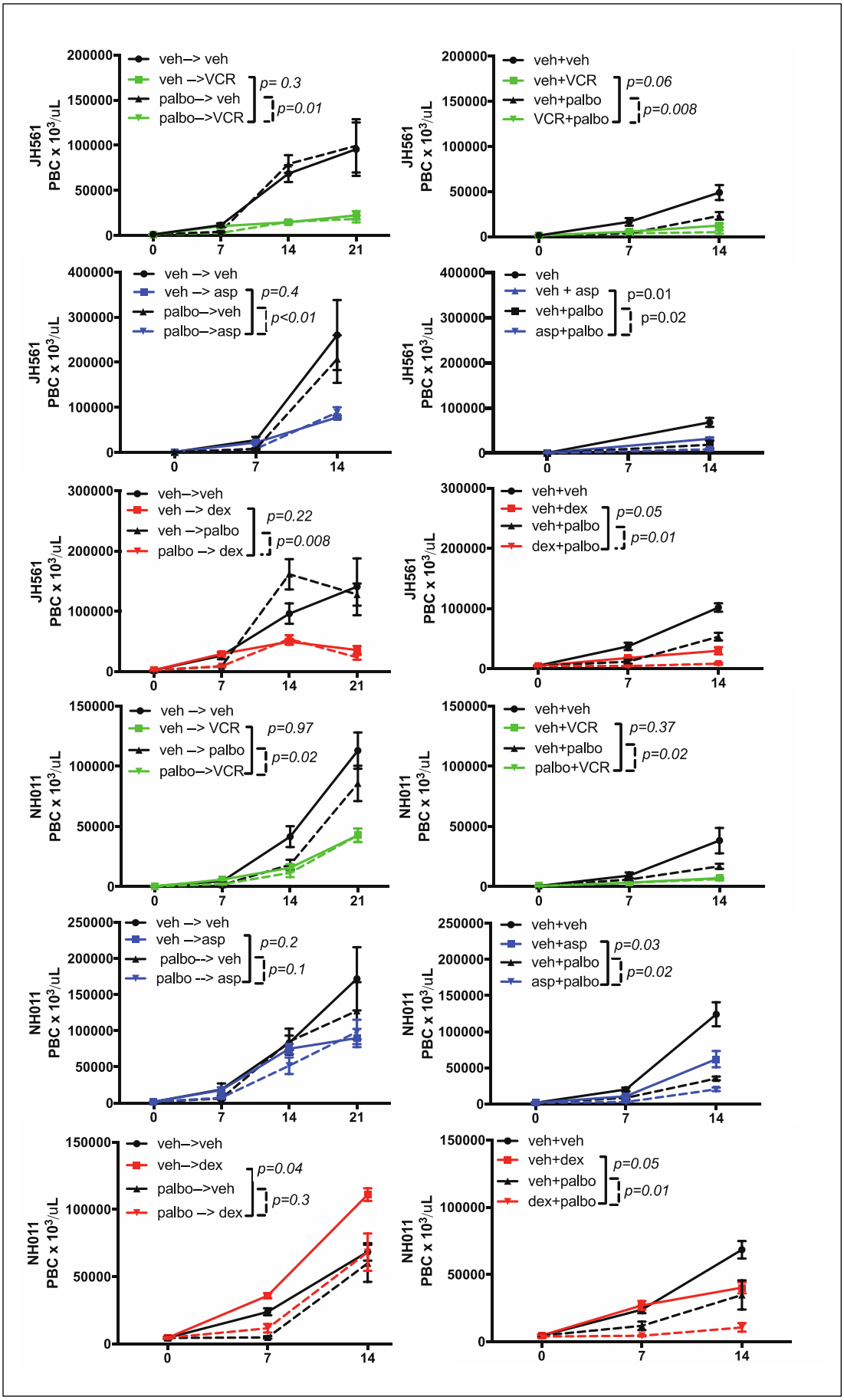


Figure S2: **CDK inhibitors can successfully be combined with cytotoxic chemotherapy *in vivo*.**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veh –>VCR
palbo–> veh
palbo–>VCR
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p=0.01
veh+veh
veh+VCR
veh+palbo
VCR+palbo
p=0.06
p=0.008
veh –> veh
veh –> asp
palbo–>veh
palbo–>asp
p=0.4
p<0.01
veh
veh + asp
veh+palbo
asp+palbo
p=0.01
p=0.02
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veh –> dex
veh –>palbo
palbo –> dex
p=0.22
p=0.008
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veh+dex
veh+palbo
dex+palbo
p=0.05
p=0.01
NH011 PBC x 10³/uL
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veh –> VCR
veh –> palbo
palbo–>VCR
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veh+VCR
veh+palbo
palbo+VCR
p=0.37
p=0.02
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veh –>asp
palbo--> veh
palbo --> asp
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p=0.1
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veh+asp
veh+palbo
asp+palbo
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p=0.02
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veh–>dex
palbo–>veh
palbo –> dex
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p=0.3
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veh+dex
veh+palbo
dex+palbo
p=0.05
p=0.01