Less Can Be More for Gene Dose and Drug Sensitivity
Susanne A. Gatz and Janet M. Shipley

CDK4 is preclinically validated as a therapeutic target in PAX3–FOXO1 fusion gene-positive rhabdomyosarcomas. Pharmacologic targeting showed sensitivity but, contrary to expectation, CDK4 genomic amplification and overexpression associated with 25% of cases that exhibited the lowest sensitivities. This emphasizes the importance of tumor-specific preclinical studies to define and understand drug sensitivity. Clin Cancer Res 21(21); 4750-2. ©2015 AACR. See related article by Olanich et al., p. 4947
trials with CDK4/6 inhibitors that speciﬁcally include cyclin D/CDK4 complexes which activate the kinase that releases the inhibitory effects of RB resulting in transcriptional activation of the E2F proteins. This leads to G1-S progression via upregulation of downstream targets such as CDC25A and CCNE2 that were assessed in RMS by Olanich and colleagues (1). Cyclin D/CDK4 is also reported to phosphorylate (p) the PAX3-FOXO1 fusion protein on Serine 430, contributing to its transcriptional activation of downstream targets (2) that will also drive cell-cycle progression and maintain an undifferentiated phenotype. p16 inhibits CDK4/6 catalytic activity through various conformational changes, and CDK4/6 inhibitors like LEE011 are ATP-competitive inhibitors, binding to the enzymes ATP binding site. Excess uncomplexed CDK4 may stoichiometrically compete with inhibitors and explain the decreased sensitivity of RMS cells with genomic ampliﬁcation and high levels of CDK4 (1).

In a previous study by the authors, 12q13-q14 ampliﬁcation has prognostic marker and is a potential indicator of pathway activation, the compelling results of Olanich and colleagues highlight the need for further studies to better understand the underlying molecular mechanisms and identify predictive biomarkers of response to CDK4/6 inhibitors in RMS and other tumor types.

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Figure 1.
Simplified schema for the role of CDK4 in PAX3–FOXO1 fusion gene-positive RMS with and without CDK4 gene ampliﬁcation. Mitogenic stimulation promotes cyclin D1/CDK4 complexes which activate the kinase that releases the inhibitory effects of RB resulting in transcriptional activation of the E2F proteins. This leads to G1–S progression via upregulation of downstream targets such as CDC25A and CCNE2 that were assessed in RMS by Olanich and colleagues (1). Cyclin D1/CDK4 is also reported to phosphorylate (p) the PAX3-FOXO1 fusion protein on Serine 430, contributing to its transcriptional activation of downstream targets (2) that will also drive cell-cycle progression and maintain an undifferentiated phenotype. p16 inhibits CDK4/6 catalytic activity through various conformational changes, and CDK4/6 inhibitors like LEE011 are ATP-competitive inhibitors, binding to the enzymes ATP binding site. Excess uncomplexed CDK4 may stoichiometrically compete with inhibitors and explain the decreased sensitivity of RMS cells with genomic ampliﬁcation and high levels of CDK4 (1).

Conﬁrmatory conditions that showed progressive disease on trial for “Patients With CDK4/6 Pathway Activated Tumors” (NCT02187783). Furthermore, the pediatric phase I study of LEE011 (NCT01747876) included a fusion-positive aRMS patient as suggested by the authors (1). This raises concerns for effective therapeutic targeting of highly expressed CDK4 in RMS. Evidence for other tumor types is mixed or lacking. Preclinical studies in glioblastoma indicate resistance to CDK4/6 inhibitor treatment in two cell lines with CDK4 ampliﬁcation (8). However, investigations of liposarcoma which harbor ampliﬁcation of CDK4 in > 90% of tumors indicate high sensitivity to pharmacologic inhibition in CDK4-ampliﬁed cell lines and encouraging results in a phase II trial of CDK4-ampliﬁed liposarcoma patients with RB pathway activity (6, 9). It will be interesting to see results of ongoing phase II trials with CDK4/6 inhibitors that speciﬁcally include CDK4 ampliﬁcation as one of the selection criteria [LUNG-MAP trial in squamous cell carcinoma of the lung (NCT02154490), SIGNATURE-trial for “Patients With CDK4/6 Pathway Activated Tumors” (NCT02187783)]. Furthermore, the pediatric phase I study of LEE011 (NCT01747876) included a fusion-positive aRMS patient with CDK4 ampliﬁcation that showed progressive disease on trial that may be in-keeping with reduced sensitivity (10).

In a previous study by the authors, 12q13-q14 ampliﬁcation that includes CDK4 was found to be associated with signiﬁcantly poorer survival in fusion gene-positive aRMS patients (3). Levels of CDK4 are also linked to poor prognosis in other cancer types (4). The reason behind these clinical correlations is unclear as functional relevance of increased levels of CDK4 in aRMS cells has not yet been seen. This could be due to the importance of another gene that is ampliﬁed at 12q13-q14 or some other cell context–speciﬁc association and functional effect. Although there is little evidence to date, CDK4 may also have a role beyond the cyclin D complex that is contributing to the phenotype of RMS.

The dependence on CDK4 in fusion gene-positive aRMS cell lines demonstrated by RNA interference is striking and in contrast to fusion gene-negative RMS (1). However, this is consistent with data from a cell line supporting CDK4-dependent phosphorylation of the PAX3–FOXO1 fusion protein and its increased transcriptional activity (11). Other kinases also phosphorylate the fusion protein, such as PLK1 that has been demonstrated to enhance its stability and activity (12). Therefore, targeting CDK4 may directly affect activity of the fusion protein unique to aRMS as well as inhibiting RB pathway activity (Fig. 1). Although CDK4 ampliﬁcation has prognostic marker and is a potential indicator of pathway activation, the compelling results of Olanich and colleagues highlight the need for further studies to better understand the underlying molecular mechanisms and identify predictive biomarkers of response to CDK4/6 inhibitors in RMS and other tumor types.
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No potential conflicts of interest were disclosed.

Authors' Contributions
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References
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