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MEK Inhibitor Resistance in Acute Myeloid Leukemia

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Mutations of the *RAS* family of genes are frequent events in AML, occurring in 10% of children and 10-20% of adults. *NRAS*mutations promote proliferation through activation of the Ras/Raf/MEK/ERK signalling pathway. Several MEK inhibitors have shown promising pre-clinical activity in AML, with a number of compounds currently in adult phase I/II clinical trials, including AZD6244, GSK1120212 and AS703026. Given not all patients respond to MEK inhibitor treatment we undertook a comprehensive preclinical evaluation of MEK inhibitors in AML and developed a clinically relevant model of resistance.

The *in vitro*efficacy of 7 MEK inhibitors was determined using a diverse panel of 6 paediatric and 5 adult AML cell lines (Table 1). All AML cell lines were sensitive to at least one MEK inhibitor with the exception of the Down syndrome associated AML line, CMK, and the adult erythroblastic line, HEL, that showed overt resistance (IC50 >20μM) to MEK inhibition (Table 1). In sensitive cell lines, the reduced proliferation was associated with apoptosis, as assessed by Annexin V+ staining. To confirm mechanism of action, inhibition of MEK phosphorylation as well as the downstream kinase, pERK, were assessed by immunoblotting. The level of basal MEK activation was variable across the cell line panel and pMEK was increased upon exposure to active MEK inhibitors. In contrast, levels of pERK were reduced suggesting that MEK inhibitors may disrupt the interaction of MEK with its downstream transducers rather than a direct inhibition of MEK phosphorylation.

Molecular and clinical resistance to kinase inhibitors is well described for targets such as FLT3 and BCR-ABL1. Since clinical responses to MEK inhibitors have been variable, we investigated the potential mechanisms of resistance to MEK inhibitors *in vitro*. Long-term culture of THP-1 cells (*MLL*-rearranged, *NRAS*mutated) with AZD6244 and AS703026, resulted in high-level resistance. Importantly, cells displayed cross-resistance not only to these two compounds but also a third MEK inhibitor, GSK1120212 (Table 2). Resistance was associated with reduced basal pMEK expression. In order to establish the mechanism of resistance we performed comprehensive mutation and gene expression analyses utilising whole-exome sequencing and RNAseq respectively. These data revealed a spectrum of acquired molecular aberrations common to both resistant cell lines compared to the parental THP1 cells. Together, these data indicate that whilst MEK inhibition is a promising strategy to treat AML, resistance to one MEK inhibitor may lead to cross-resistance to other compounds targeting MEK.

Table 1. *In vitro* sensitivity of AML cell lines to MEK inhibitors.

	IC50[#]						
Cell Line	**PD0325901**	**AZD6244**	**MEK162**	**GSK1120212**	**CI1040**	**TAK733**	**AS703026**
MV411	0.17	1.4	0.88	0.0035	6.9	0.098	0.41
THP1	1.1	0.66	1.2	1.8	6.3	0.33	0.22
CMK	>20	>20	>20	>20	>20	>20	>20

AML193	0.24	2.9	2.1	0.014	4.8	0.25	0.42
Kasumi1	0.20	1.7	1.1	0.0039	6.5	0.051*	0.31
Mo7e	0.11	1.1	0.34	0.0061	4.8	0.038	0.078
ME1	0.0037	0.023	0.065	0.00065	0.53	0.0047	0.075
HL60	0.00092	0.12	0.019	0.00081	0.74	0.00075	0.0060
ML2	0.0043	0.066	0.047	0.0014	0.68	0.0099	0.022
HEL	>20	>20	>20	>20	>20	>20	>20
OCIAML3	0.0078	0.072	0.040	9.2e-005	1.1	0.0043	0.023

#IC50 is the concentration (µmol/L) of drug required to reduce cell viability by 50% at 96h and was calculated using non-linear regression of transformed normalised data.

Table 2. Cross-resistance to MEK inhibitors

	IC50#		
Cell Line	**AZD6244**	**AS703026**	**GSK1120212**
THP1 Parental	0.66	0.22	1.8
THP1 AZD6244 Resistant	>20	>20	>20
THP1 AS703026 Resistant	>20	>20	8.6

#IC50 is the concentration (µmol/L) of drug required to reduce cell viability by 50% at 96h and was calculated using non-linear regression of transformed normalised data.

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