# MEK inhibition and resistance in acute myeloid leukaemia

*Amanda Smith[1], Achal Neupane[2], Jonathan Ellis[3], Sadia Afrin[1], Rachel Burow[1], Caedyn Stinson[1], Paul Leo[2,3], Alex Cristino[4], Andrew Moore[1,5,6]*

[1]Childhood Leukaemia Research Laboratory, The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane; [2]UQ Centre for Clinical Genomics, The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane; [3]Institute of Health and Biomedical Innovation, Queensland University of Technology , Translational Research Institute, Brisbane; [4]Systems Biology Group, The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane; [5]Children's Health Queensland Hospital & Health Service, Brisbane; [6]UQ Child Health Research Centre, The University of Queensland, Brisbane.

## 1.1 Background

- *RAS* mutations occur in 10% paediatric and up to 20% of adult AML
- Mutations confer constitutive activation of Ras/Raf/MEK/ERK pathway→ hyperproliferation
- MEK inhibitors currently under evaluation in clinical trials

**- AZD6244 -GSK1120212 -AS703026**

- Potential resistance mechanisms poorly described

## 2.1 Methods

- Panel of 6 paediatric and 5 adult AML cell lines including

**-THP1 (NRAS G12D) -AML193 (NRAS G13V) -HL-60 (NRAS Q61L) -ML-2 (KRAS A146T)**

- Assessed 7 commercially available MEK inhibitors

**-Proliferation -Cell cycle -Apoptosis -MEK expression**

- Potential mechanisms of resistance to MEK inhibitors

**-THP1 cells with resistance to AZD6244 or AS703026 (denoted AZD6244_R or AS703026_R respectively) were established by long term co-culture with drug.**

## 3.1 AML exhibit variable sensitivity to MEK inhibitors

**Table 1. MEK inhibition reduces the proliferation of AML cells**

| | | $IC_{50}$[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cell line** | **PD0325901** | **AZD6244** | **MEK162** | **GSK1120212** | **CI1040** | **TAK733** | **AS703026** | **Ara-C** |
| Paediatric | MV411 | 0.17** | 0.41* | 0.33** | 0.003*** | 6.9 | 0.098** | 0.41** | 1.6 |
| | **THP1** | 0.19*** | 0.3** | 0.43** | 0.08*** | 2.2* | 0.23*** | 0.23*** | 6.7 |
| | CMK | >20 | >20 | >20 | >20 | >20 | >20 | >20 | 0.48 |
| | **AML193** | 0.24* | 2.9 | 2.1 | 0.014* | 4.8 | 0.25* | 0.42* | 2.6 |
| | Kasumi1 | 0.2 | 1.7 | 1.1 | 0.004 | 6.5 | 0.051 | 0.31 | 0.15 |
| | Mo7e | 0.11 | 1.1 | 0.34 | 0.006 | 4.8 | 0.038 | 0.08 | 0.033 |
| Adult | ME1 | 0.003 | 0.022 | 0.037 | 0.00004 | 0.55 | 0.005 | 0.08 | 0.41 |
| | **HL60** | 0.001 | 0.12 | 0.02 | 0.001 | 0.73 | 0.001 | 0.007 | 0.64 |
| | **ML2** | 0.004 | 0.066 | 0.047 | 0.001 | 0.68 | 0.01 | 0.02 | 0.069 |
| | HEL | >20 | >20 | >20 | >20 | >20 | >20 | >20 | 0.085 |
| | OCI-AML3 | 0.008 | 0.07 | 0.04 | 9.20E-05 | 1.1 | 0.004 | 0.02 | >20 |
| | Median all[2] | 0.11 | 0.3 | 0.33 | 0.003 | 2.2 | 0.038 | 0.08 | 0.445 |

[1]$IC_{50}$; is defined as the concentration of drug that reduces cell viability by 50%, calculated by non-linear regression.[2] Median calculated excluding those values >20uM where an exact IC50 could not be established. Bolded cell lines highlights those with known *Ras* mutations. P-value determined by students t-test; *p<0.05, **<0.01, **p<0.005, compared to Ara-C. Abbrev: Ara-C; cytarabine arabinoside.

## 3.2 MEK inhibition induces apoptosis and pMEK

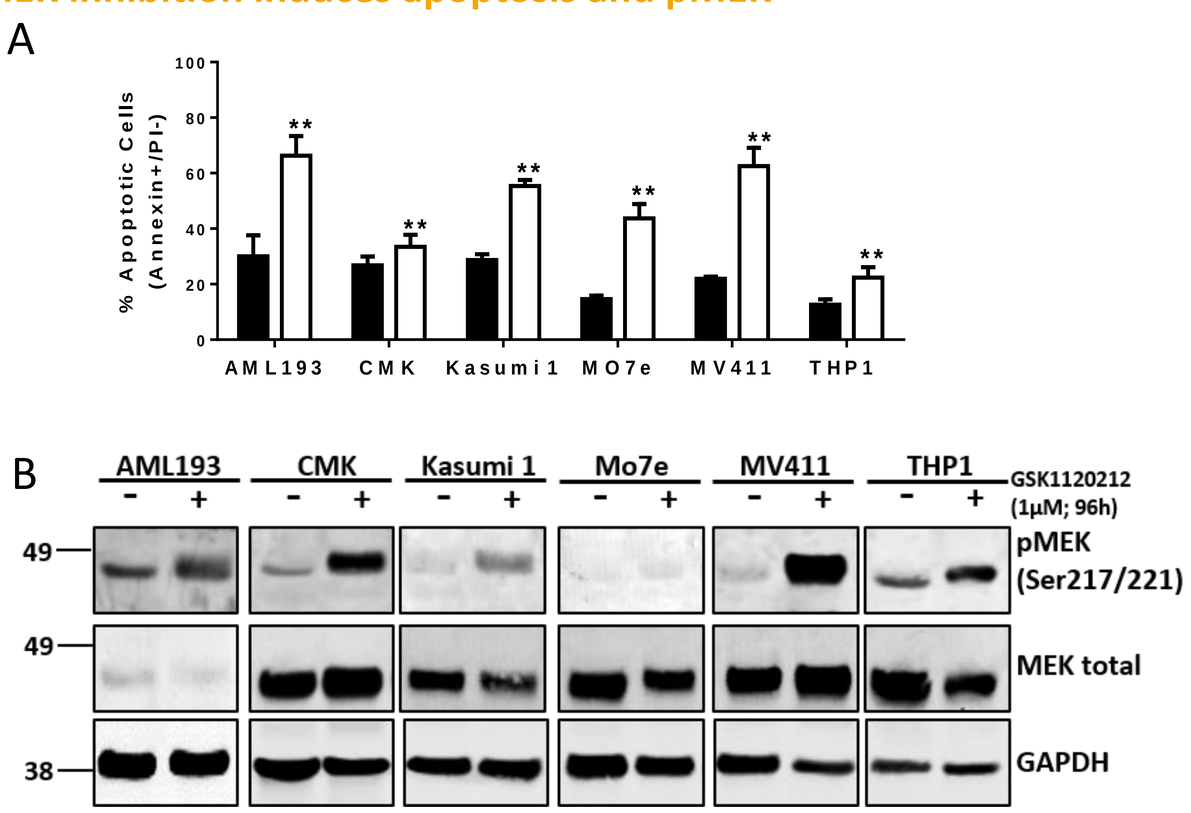


**Figure 1. MEK inhibitors induce apoptosis and MEK phosphorylation.** AML cell lines were treated with 1μM GSK1120212 for 96h and assessed for **A)** Apoptosis with Annexin V staining, and **B)** MEK phosphorylation by immunoblotting. GAPDH was used as a loading control. *Column*, mean (n ≥ 3); *Bars*, SEM. **p<0.01. Student's t-test relative to DMSO control.

## 3.3 Resistance does not affect MEK pathway response to inhibitors

**Table 2. MEK inhibitor resistance in THP1 AML cells**

| | $IC_{50}$ | | | | | |
|---|---|---|---|---|---|---|
| **Cell Line** | **AZD6244** | **MEK162** | **GSK1120212** | **AS703026** | **Ara-C** | **Daunorubicin** |
| **Parental** | 0.3 | 0.43 | 0.08 | 0.23 | 6.7 | 0.12 |
| **AZD6244_R** | >20 | >20 | >20 | >20 | 4.6 | 0.03 |
| **AS703026_R** | >20 | 7.4 | >20 | 12.5 | 5.43 | 0.02 |

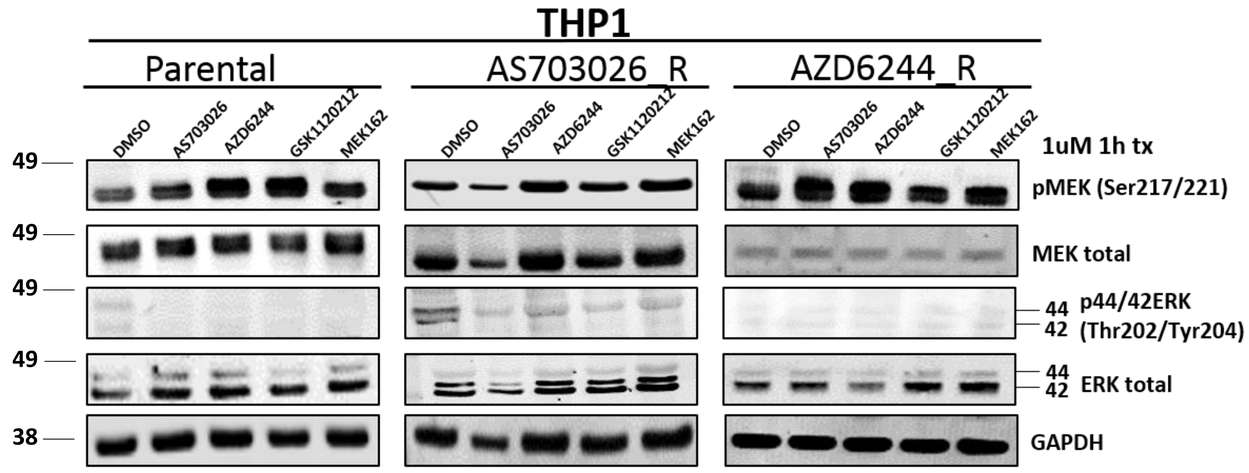


**Figure 2. Resistant cells' response to MEK inhibitors.** THP1 parental, AS703026_R and AZD6244_R were treated with indicated MEK inhibitors (1μM; 1h) and subject to immunoblotting for pMEK (Ser217/Ser221), MEK total, p44/42ERK (Thr202/Tyr204), 44/42ERK total, and GAPDH (loading control).

## 3.4 Genomic analysis of resistance

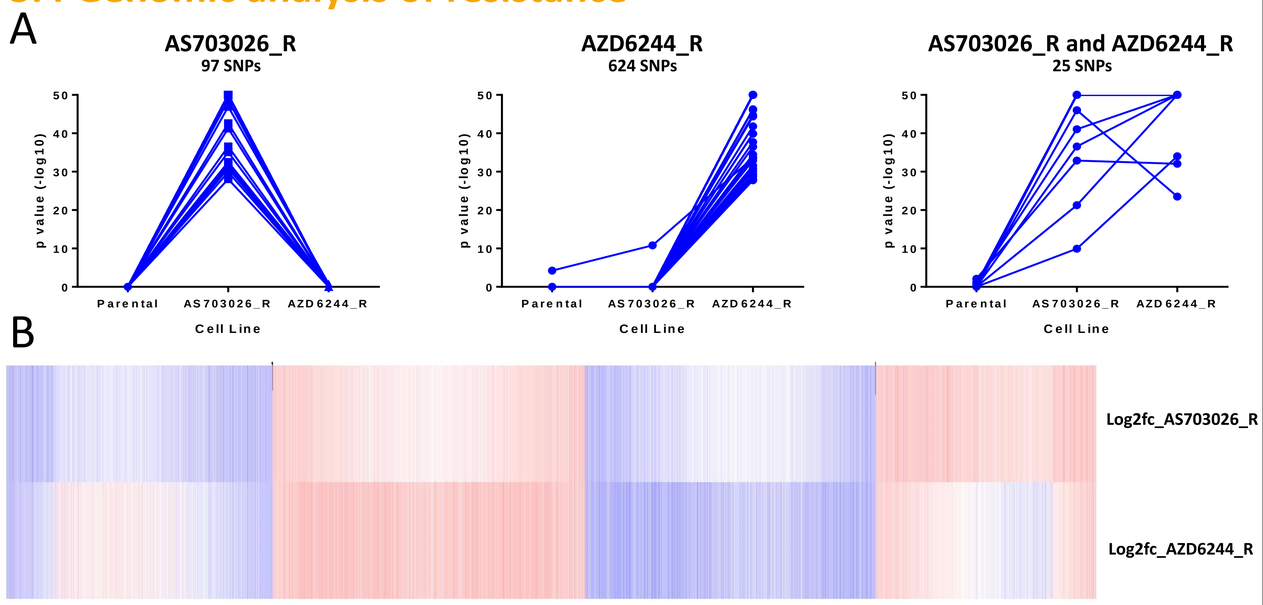


**Figure 3. Genomic analysis of MEK inhibitor resistance. A)** THP1 parental, AS703026_R and AZD6244_R were subjected to whole exome sequencing. Fuzzy clustering identified enriched SNPs where a p-value threshold of $10^{-9}$ was set for significant enrichment. SNPs were classified according to presence in THP1-AS703026_R alone, AZD6244_R alone or present in both AS703026_R and AZD6244_R. **B)** DESeq2 (version 1.8.1) was used to analyse RNAseq data and hierarchical clustering revealed strong concordance of gene expression between AS703026_R and AZD6244_R.

**Table 3. Snapshot of genes identified as mutated in THP1 MEK inhibitor resistant cell lines and causally related to cancer (COSMIC Gene Census database; accessed 08012015)**

| **Cell Line** | **Chromosome** | **Start** | **End** | **Reference nucleotide** | **Alternate nucleotide** | **Mutation type** | **Ensembl gene name** |
|---|---|---|---|---|---|---|---|
| AS703026_R | 19 | 4110558 | 4110558 | G | T | snp | MAP2K2 |
| | 1 | 51829622 | 51829622 | C | T | snp | EPS15 |
| | 17 | 5035634 | 5035634 | G | T | snp | USP6 |
| | 17 | 5035641 | 5035641 | C | T | snp | USP6 |
| AZD6244_R | 10 | 43606856 | 43606856 | G | A | snp | RET |
| | 11 | 119156193 | 119156193 | C | T | snp | CBL |
| | 12 | 56493492 | 56493492 | G | A | snp | ERBB3 |
| | 13 | 32920978 | 32920978 | C | T | snp | BRCA2 |
| | 17 | 7579542 | 7579542 | C | G | snp | TP53 |
| | 19 | 17948009 | 17948009 | G | A | snp | JAK3 |

## 4.0 Conclusions

- *RAS* mutations do not delineate AML sensitivity to MEK inhibitors *in vitro*
- Resistance to MEK inhibitors is rapid and characterised by acquisition of large numbers of SNPs
- Only 3.4% of SNPs are common to both resistance models despite strong concordance in gene expression
- JAK3 or MAP2K2 mutations may drive resistance

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