Abstract #270
Pacritinib Suppresses Leukemic Outgrowth from FLT3-ITD Positive Stroma-Adherent Primary AML Cells

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*equal contribution

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DISCLOSURES

- Nothing to disclose

Summary: FLT3 signalling

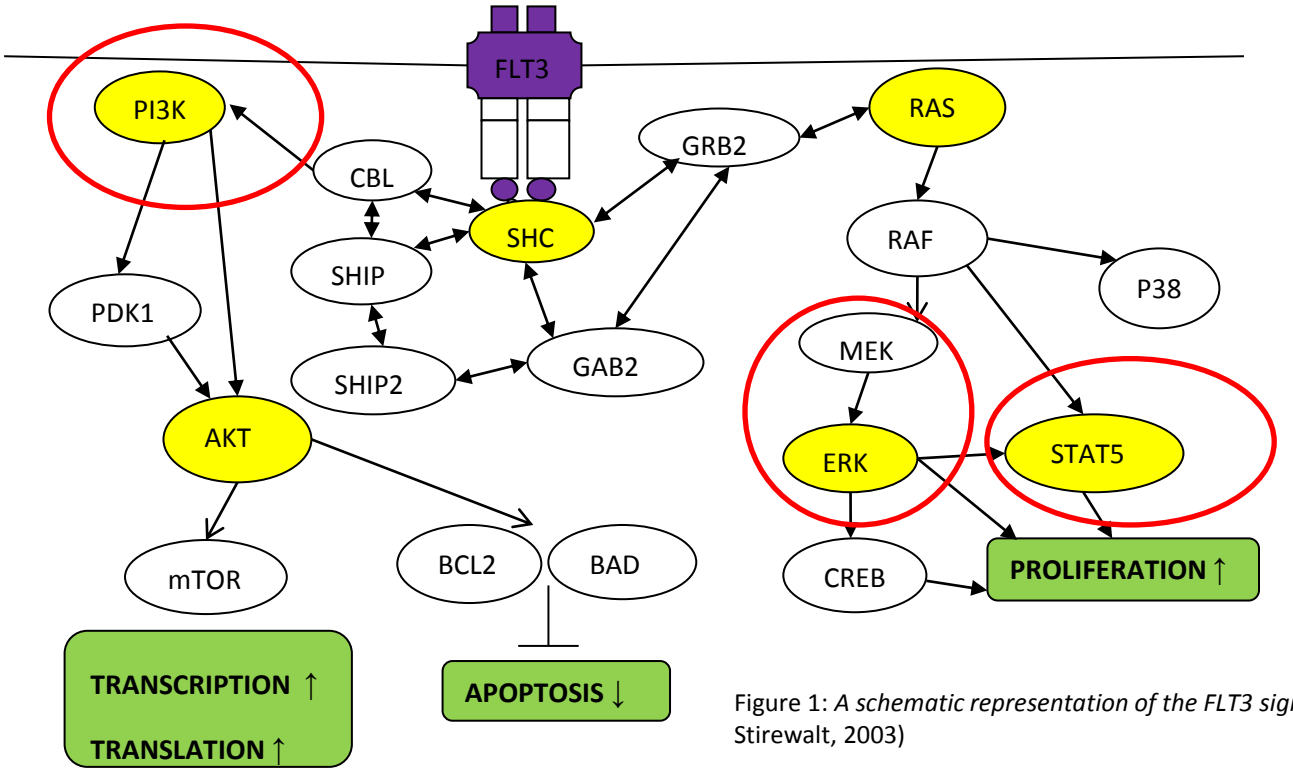


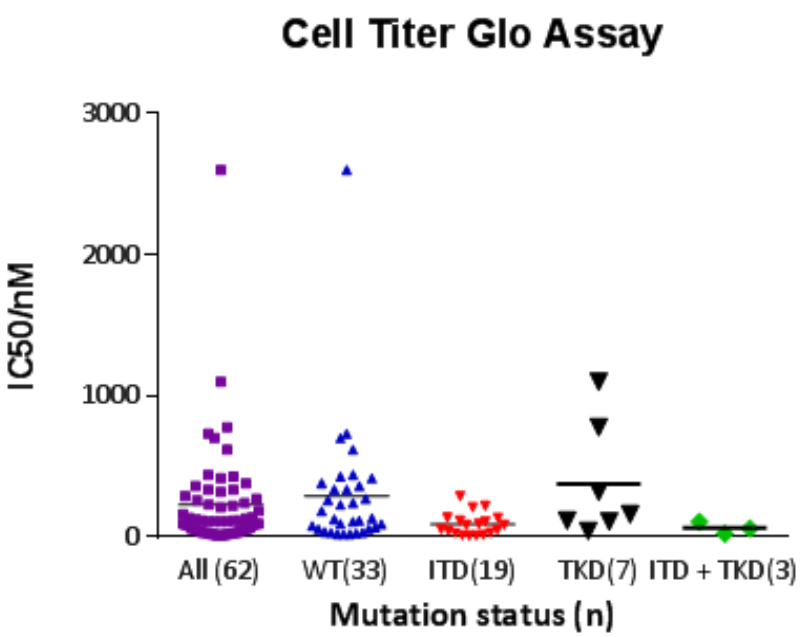
Figure 1: *A schematic representation of the FLT3 signalling cascade* (adapted from Stirewalt, 2003)

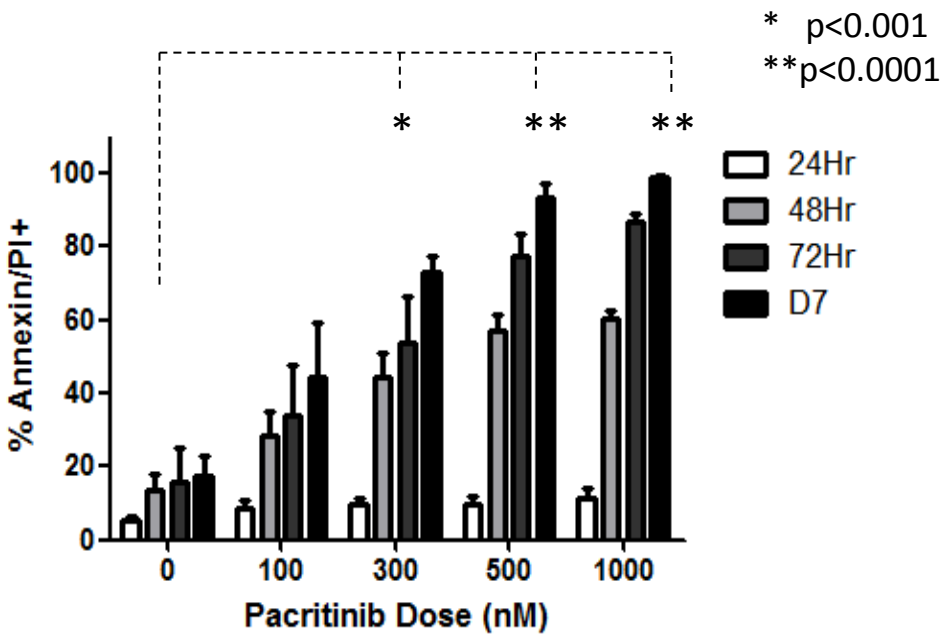
- **FLT3 mutations 30% AML (ITD>TKD)**
- **Receptor activation → proliferation and survival advantage**
- **Many pathways → part of cancer initiating events**

Aims and rationale

- Assess in-vitro properties of Pacritinib in primary AML samples
- Dual FLT3 and JAK2 inhibitor → equipotent activity
- **Target multiple pathways → overcome stromal protection?**
- **Analyse in short, medium and longer term assays**

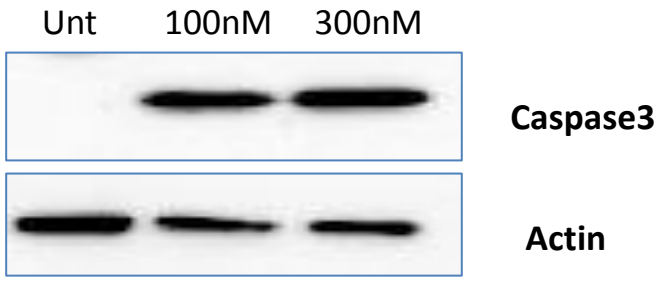
Pacritinib efficacy





- Mean IC_{50}
  ITD 92.3nM vs WT 229.4nM
  p=0.004

- Dose dependent apoptosis
- Cleaved caspase 3



Stromal Co-Culture: 3 Assays used with differing time points

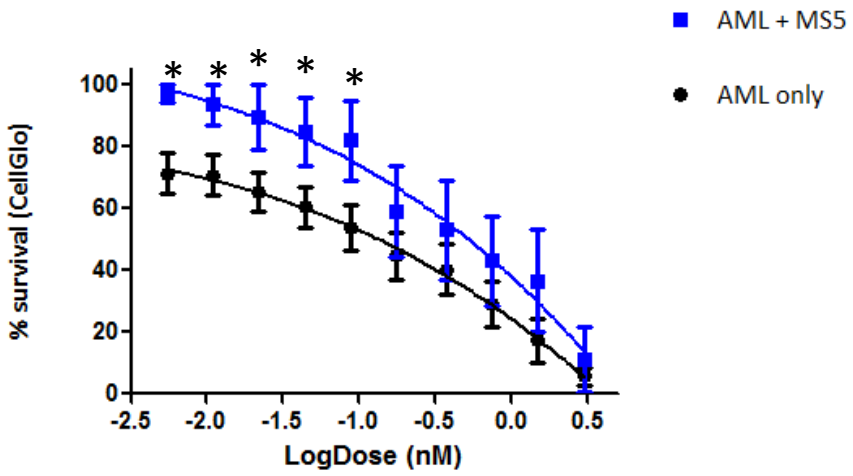
1. **Short Term** - *Cell Glo Assay compare IC_{50s} at 48 hours*
2. **Medium Term** – *7-14 day outgrowth of suspension and adherent cells*
3. **Long Term** – *Cobblestone formation at 5-6 weeks*

Short term data only widely published

Short term culture: stromal protection at 48 hours

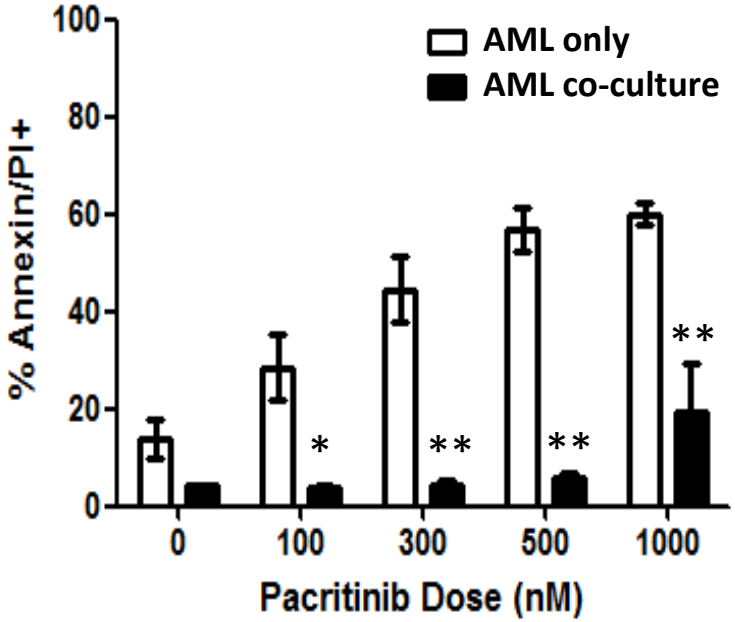
AML protected by stroma at low doses of Pacritinib

Mean dose response curves



* p<0.05

Annexin/PI apoptosis induction



* p<0.001
**p<0.0001

Medium term: adherent ITD+ cells inhibited by 14 day culture

AML

Pacritinib/DMSO

MS5

Suspension cells removed
Wash stromal layer → fresh media

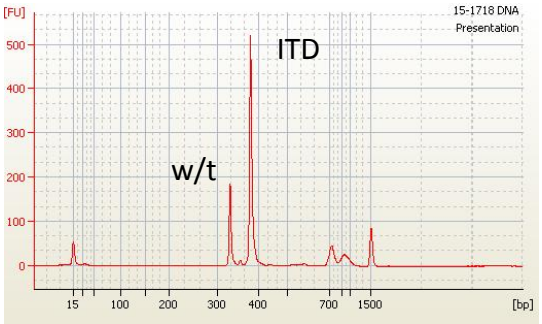
Suspension cells removed

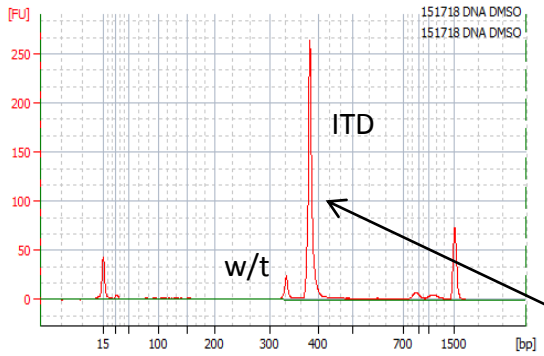
Trypsinize stroma
Flow & PCR

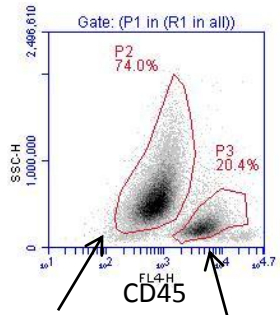
Day 0

D7

D14



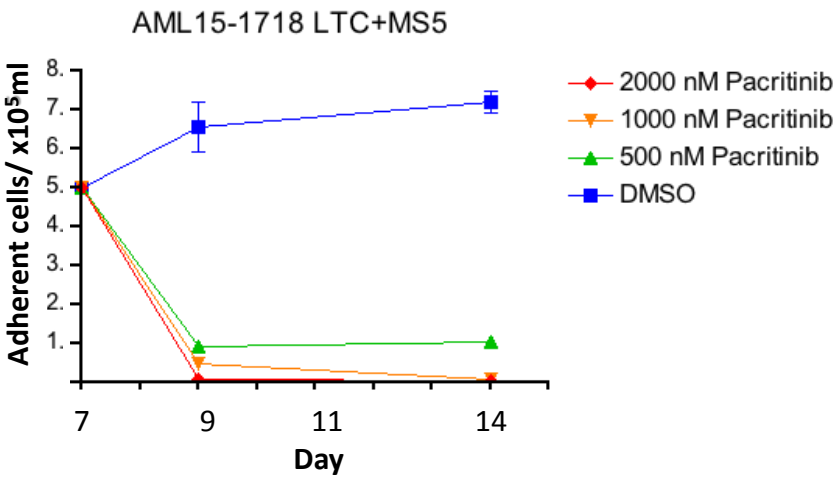




stroma

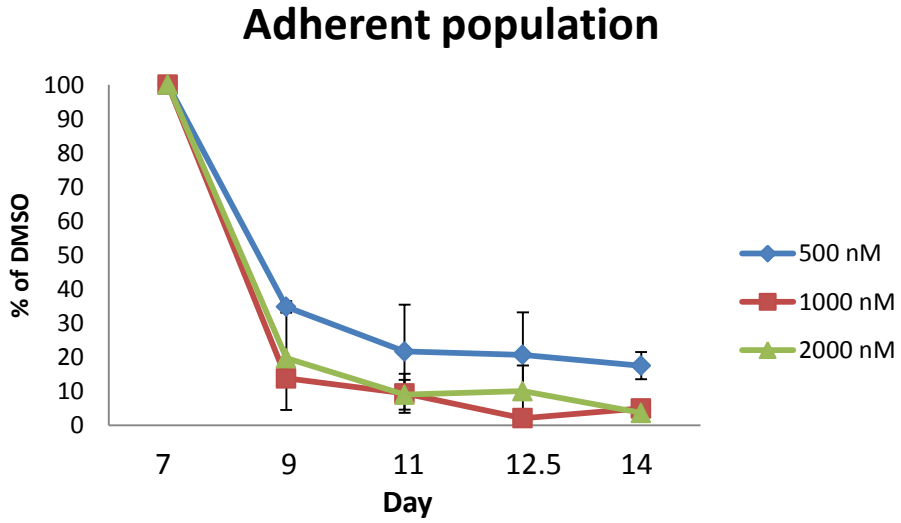
stroma adherent AML cells

Repopulation Assay: Suppression of leukaemic outgrowth from FLT3 ITD+ adherent cells

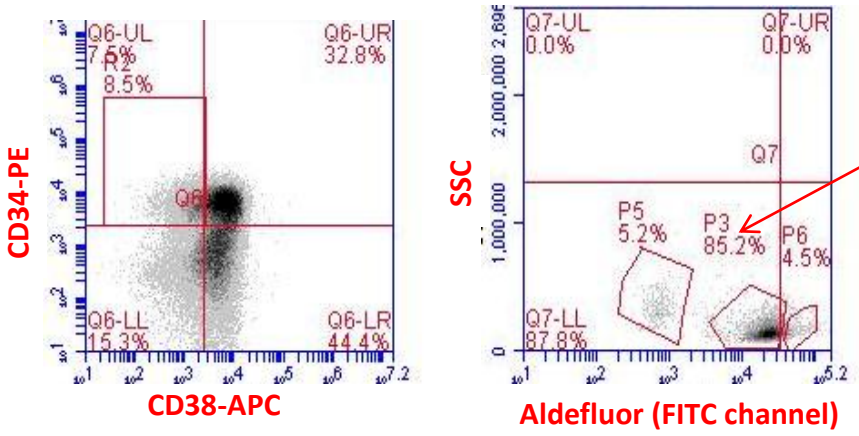


- Day 14 FLOW analysis of adherent cell flasks.

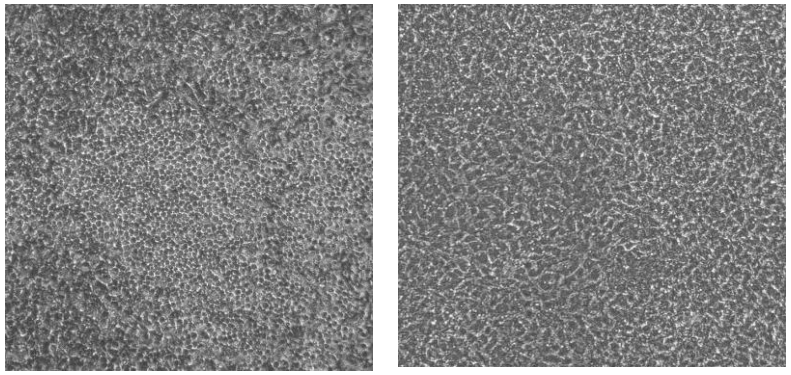
- Pacritinib treatment inhibits re-population



Long Term Assay: Suppression of CAFC formation in CD34+/CD38- population

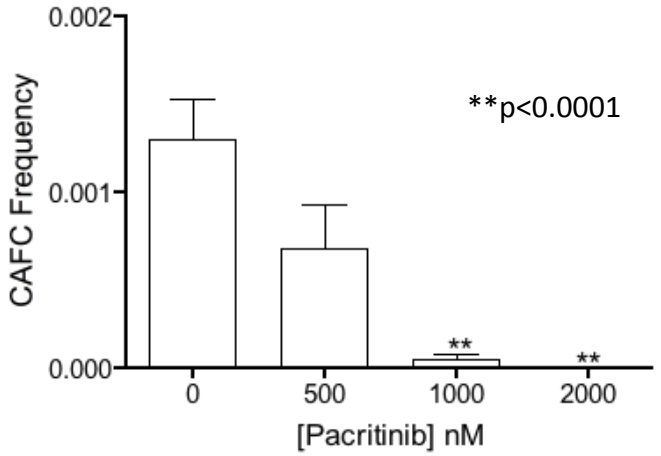


- CD34+, CD38-, ALDH^{int} cells
- Seeded onto 96 well plates
- Pacritinib treatment 5-6 weeks



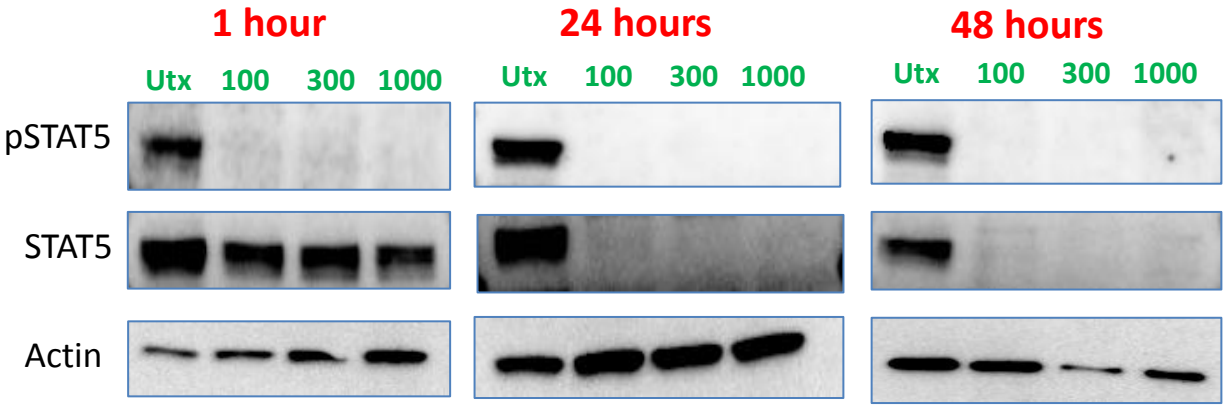
Untreated

2000nM

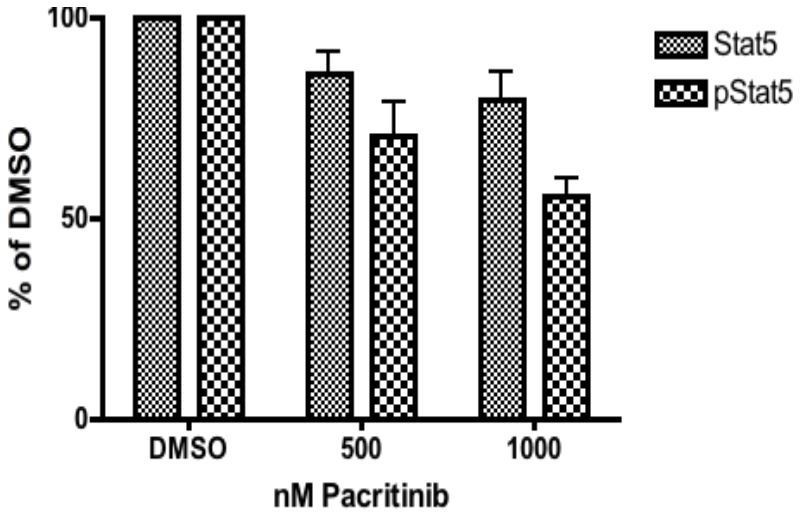


Dose dependent reduction in colony formation

Pacritinib inhibits pSTAT5



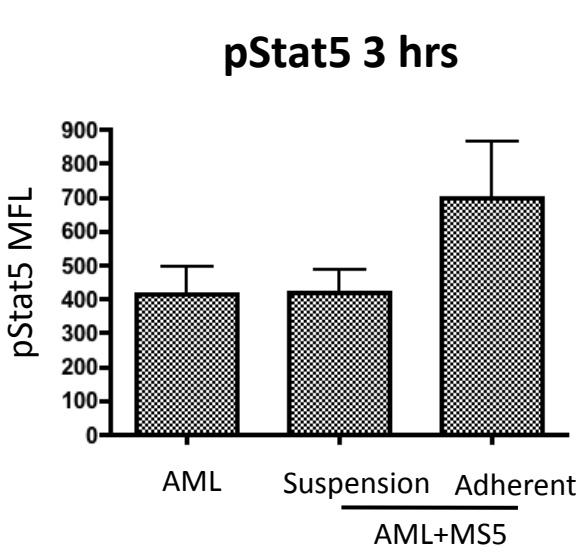
Western blot

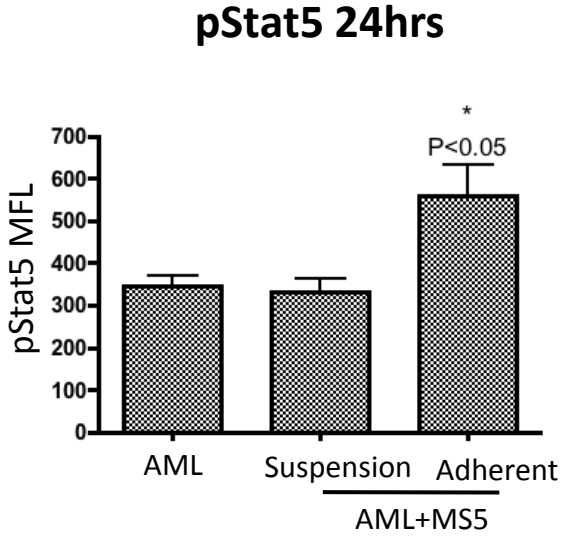


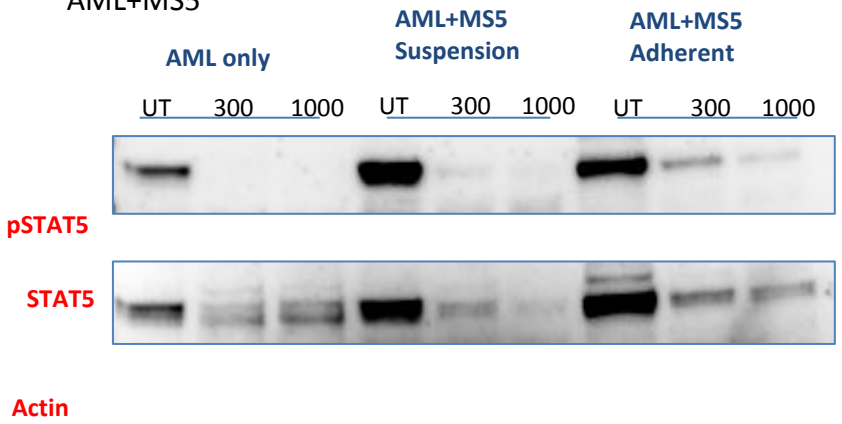
Flow cytometry

Pacritinib targets stromal mediated pStat5 survival pathway

Basal pStat5 levels

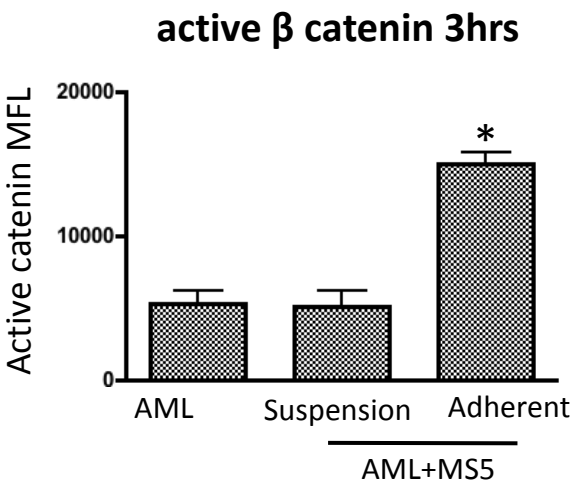


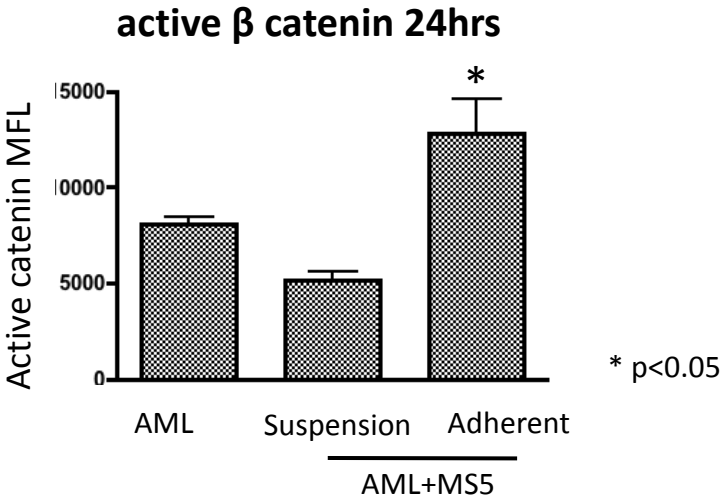




Pacritinib targets stromal mediated β-catenin levels

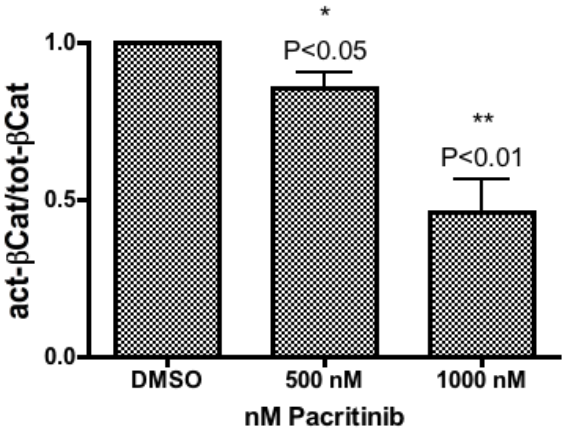
Basal active β catenin levels



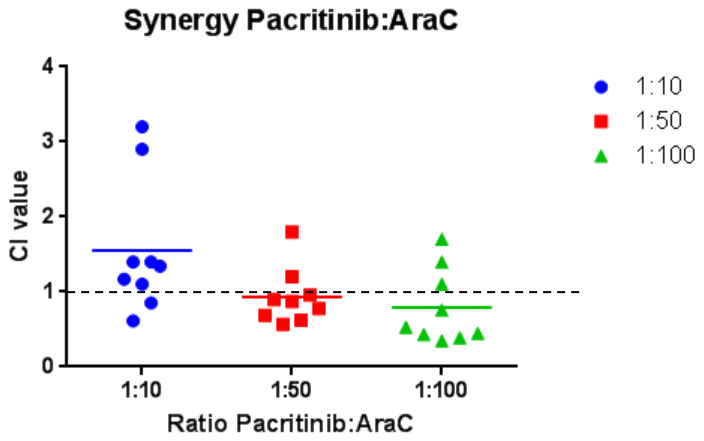


* p<0.05

- Plays a role in self renewal of primitive cells on stroma
- Time dependent reduction of active signalling

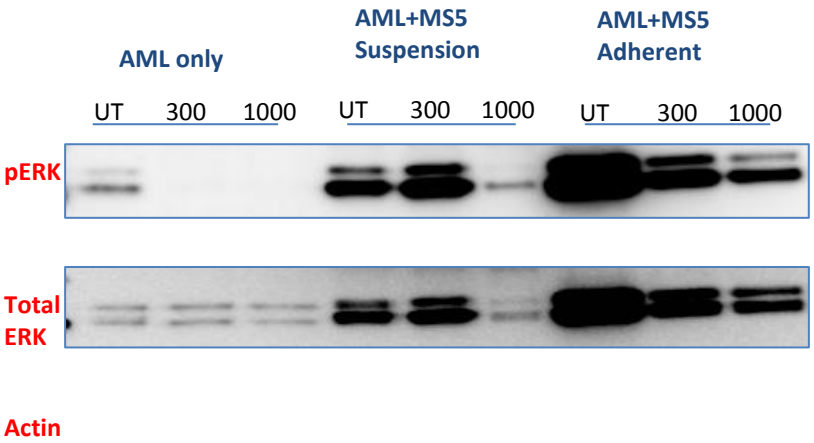


Synergy

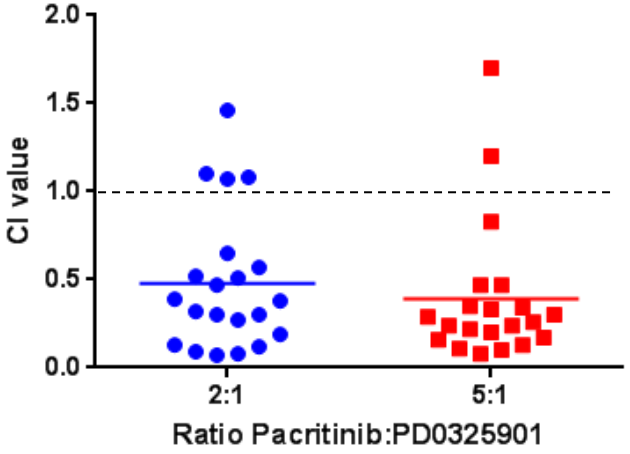


Mean CI: Moderate synergy
1:10=1.6
1:50 =0.9
1:100 =0.8

Stromal induction of pERK



Pacritinib + MEK inhibitor



Mean CI : Good synergy
2:1 = 0.48
5:1 = 0.39

Conclusions: Pacritinib in AML

- Targets ITD mutated samples
- Short term stromal protection at 48 hours
- Medium term suppression of leukaemic outgrowth
- Long term reduction in CAFC formation at 5-6 weeks
- Downstream inhibition of pSTAT5 → attenuated in stroma adherent cells
- Upregulation of pERK/ERK on stroma
- Good synergy with MEK inhibitor
- **Potentially overcome environment mediated resistance**

Acknowledgements

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