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

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8/12/2014
DISCLOSURES

• Nothing to disclose
Summary: FLT3 signalling

- 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

Annexin/PI apoptosis induction

* p<0.05
** p<0.001
*** p<0.0001
Medium term: adherent ITD+ cells inhibited by 14 day culture

Day 0

AML  Pacritinib/DMSO

MS5

D7

Suspension cells removed
Wash stromal layer $\rightarrow$ fresh media

D14

Suspension cells removed
Trypsinize stroma
Flow & PCR

AML cells

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

AMS  Pacritinib/DMSO

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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<sup>int</sup> cells
- Seeded onto 96 well plates
- Pacritinib treatment 5-6 weeks

**p<0.0001

Dose dependent reduction in colony formation
Pacritinib inhibits pSTAT5

Western blot

Flow cytometry

Stat5
pStat5
Pacritinib targets stromal mediated pStat5 survival pathway

Basal pStat5 levels

**pStat5 3 hrs**

- AML
- Suspension
- Adherent

**pStat5 24hrs**

- AML
- Suspension
- Adherent

* P<0.05

**UT** 300 1000

**pSTAT5**

**STAT5**

**Actin**
Pacritinib targets stromal mediated β-catenin levels

Basal active β-catenin levels

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

![Graphs showing active β-catenin levels at 3hrs and 24hrs with different conditions.]
Synergy

Synergy Pacritinib:AraC

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: Pacritininib 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

• Joanna Zabkiewicz
• Steve Knapper
• Caroline Alvares
• Gareth Edwards
• Alan Burnett
• Michelle Lazenby
• Marie Gilmour
• Carol Guy
• Amanda Gilkes
• Michelle Doyle
• Sarah Baker
• Paul White
• Sian Edwards
• Jack Singer
• Suliman Al-Fayoumi
• Patients and families