# ACELARIN: A novel nucleotide analogue that overcomes the key cancer resistance mechanisms associated with poor survival

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## BACKGROUND

### ProTides

A new generation of chemotherapeutic agents (nucleotide analogues)

- Innovative phosphoramidate chemistry
- Designed to overcome the key cancer resistance mechanisms
- Broad clinical utility to benefit the majority of cancer patients
- Superior efficacy and safety profile

### Acelarin – The First Anti-Cancer ProTide

Overcomes all key resistant mechanisms associated with gemcitabine:

- Activation is independent of deoxycytidine kinase (dCK)
- Cellular uptake is independent of nucleoside transporters (hENT1)
- Avoids breakdown by cytidine deaminase (CDA)

### Mode of Action / Metabolism

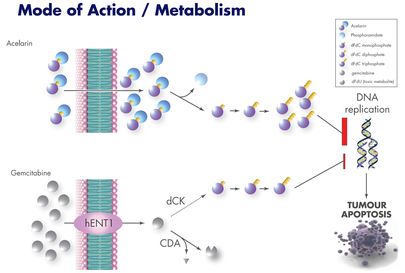



*Figure 1. Acelarin overcomes all key gemcitabine resistance*

## METHODS

### Cytotoxic Activity Studies

- Using multiple cancer cells, including gemcitabine resistant lines
- Utilising inhibitors 2T2D and NBTI to mimic cancer resistance mechanisms by blocking dCK and hENT1, respectively
- Determining $IC_{50}$ values and intracellular levels of the active moiety dFdCTP

### Stability Study

- Cytidine deaminase assay: UV spectrum recorded from 220-350nm

### *In vivo* Mouse Xenograft Studies

- Pancreatic MiaPaCa-2 and BxPC-3 human tumours

### Formal Toxicology Study

- Beagle dogs

### Phase I Study (ProGem1)

- Dose escalation study to determine the RP2D, safety, PK and anti-tumour activity of Acelarin
- Patients with advanced, rapidly progressing solid tumours

## RESULTS

### Acelarin Cytotoxicity

- Greater potency than gemcitabine (2 to 4 fold) in partially resistant and resistant pancreatic cell lines

*Table 1. $IC_{50}$ values of Acelarin against pancreatic cancer cell lines (μM)*

| | MiaPaCa-2 | BxPC-3 |
|---|---|---|
| **Acelarin** | 0.44 ± 0.06 | 0.15 ± 0.04 |
| **Gemcitabine** | 1.04 ± 0.71 | 0.67 ± 0.34 |

### Acelarin Overcomes Cancer Resistance

- Cellular activation is independent of dCK
  - Acelarin more cytotoxic than gemcitabine in RT112 bladder cancer cells and retains activity despite dCK inhibition (using 100μM deoxycytidine as a substrate competitor)

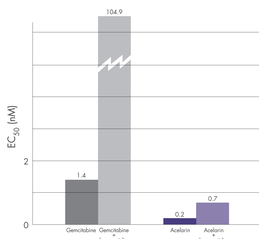



*Figure 2. Gemcitabine activity blocked after dCK inhibition. Acelarin activity independent of dCK*

- Cellular uptake is independent of nucleoside transporters
  - Acelarin baseline activity 3x superior to gemcitabine in PANC-1 cells and maintained effective cytotoxic activity despite hENT1 inhibition using dipyridamole

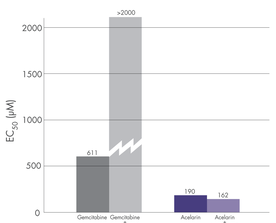



*Figure 3. Gemcitabine activity blocked after hENT1 inhibition. Acelarin activity independent of hENT1*

- Acelarin is resistant to cytidine deaminase degradation
  - Acelarin UV spectrum unchanged at 30 minutes whereas gemcitabine degraded after just 2 minutes

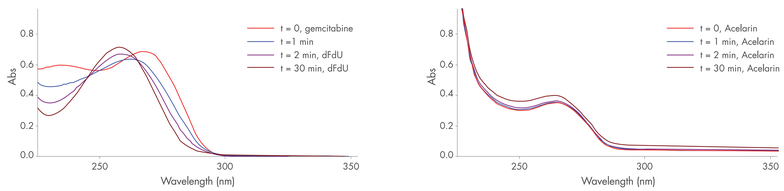



*Figure 4. Cytidine deaminase degrades gemcitabine within 2 minutes. Acelarin is completely stable*

### Acelarin Achieves High Intracellular Active Moiety Levels

- Gemcitabine is converted to its active triphosphate form (dFdCTP) after phosphorylation by dCK
- Inhibition of dCK by 2T2D reduces gemcitabine conversion to dFdCTP
- Acelarin is independent of dCK and produces high levels of dFdCTP in the presence of dCK inhibitors
- Inhibition of hENT1 by NBTI dramatically reduces gemcitabine conversion to dFdCTP
- Acelarin is independent of hENT1 and produces high levels of dFdCTP in the presence of hENT1 inhibitors

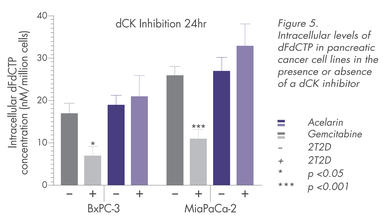



*Figure 5. Intracellular levels of dFdCTP in pancreatic cancer cell lines in the presence or absence of a dCK inhibitor*

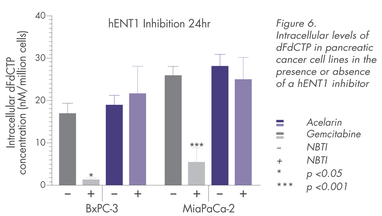
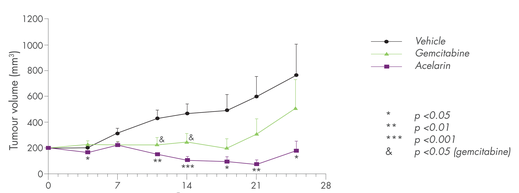



*Figure 6. Intracellular levels of dFdCTP in pancreatic cancer cell lines in the presence or absence of a hENT1 inhibitor*

### Acelarin's Superior Inhibition of Tumour Growth

- Acelarin achieved significantly greater reduction in tumour volume than gemcitabine in xenografts of MiaPaCa-2 human pancreatic cancer cells

*Figure 7. Acelarin exhibits greater reduction in tumour volume than gemcitabine in the pancreatic xenograft MiaPaCa-2*




### Acelarin is Better Tolerated Than Gemcitabine

- Acelarin's MTD is 4x higher than gemcitabine in toxicology studies with Beagle dogs

### Acelarin's Clinical Pharmacokinetics

- Plasma
  - Acelarin plasma half life is more favourable than gemcitabine (7.9 hours versus 1.5 hours, respectively)
- Intracellular dFdCTP
  - $C_{max}$ reached at 30 minutes EOI
  - Long half life ($t_{1/2}$ = 11 hours)
  - Even at 24 hours EOI Acelarin achieves levels of dFdCTP higher than reported for gemcitabine at its $C_{max}$ at 2 hours

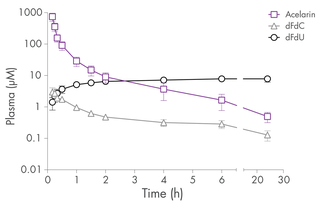



*Figure 8. Mean plasma concentrations (±SD) of Acelarin, gemcitabine (dFdC) and deaminated gemcitabine (dFdU) in patients*

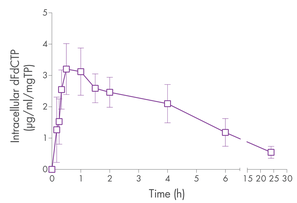



*Figure 9. Mean intracellular concentrations (±SD) of dFdCTP achieved by Acelarin in patients*

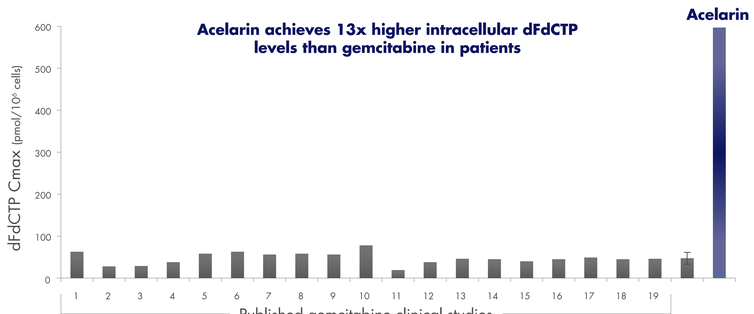



*Figure 10. Intracellular dFdCTP levels achieved by Acelarin compared with gemcitabine (equimolar dosing)*

### ProGem1: Phase I Study

- Ongoing: 42 patients recruited to date
- Acelarin is well tolerated
- Acelarin is achieving high disease control across a variety of solid tumors

## CONCLUSION

- Acelarin overcomes all the key cancer resistance mechanisms associated with gemcitabine
- Acelarin generates 13x higher intracellular dFdCTP levels than gemcitabine
- Acelarin is better tolerated than gemcitabine
- Acelarin is being developed for patients with pancreatic, biliary, ovarian and NSCL cancers

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