ACELARIN: Validating the RNAscope for molecular profiling of key biomarkers associated with gemcitabine resistance

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BACKGROUND

Gemcitabine & Biomarkers

- Mainstay of pancreatic cancer treatment
- Flawed prodrug: <10% response in patients
- Three key drug resistance mechanisms associated with poor survival prognosis (Table 1)
- No reliable assays for detection of these biomarkers available

Table 1. Impact of Cancer Resistance Mechanisms and Biomarkers

Biomarker	Cancer Resistance Mechanism	Outcome	Incidence
hENT1 (human equilibrative nucleoside transporter 1)	Low expression of membrane transporters	Poor uptake into the cancer cells	50%
dCK (deoxycytidine kinase)	Low expression of activating enzymes	Low levels of the active agent	35%
CDA (cytidine deaminase)	High levels of metabolising enzymes	Rapid degradation of the drug	60%

Innovative Biomarkers Detection: RNAscope®

- *In situ* hybridization technology for RNA detection
- Superior sensitivity, specificity, visualisation and quantification (Figure 1)
- Rapid turn around: assay completed within a single day

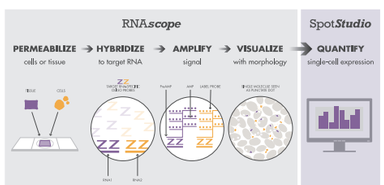

Figure 1. RNAscope procedure completed in less than 24h

Acelarin®: The First Anti-Cancer ProTide

- New generation anti-cancer agent: ProTide (NucleoTide Analogue)
- Overcomes key resistance mechanisms associated with gemcitabine
- Impressive and durable disease control in 88% of evaluable patients across 13 solid tumour types
- Generates 12x higher intracellular levels active agent dFdCTP
- Well tolerated with no unexpected adverse events
- Ongoing combination studies with carboplatin for ovarian cancer
- Phase III global studies planned in ovarian, biliary and pancreatic cancers

Mode of Action

- Acelarin is designed to overcome the key cancer resistance mechanisms (Figure 2)
  - Efficient uptake: hENT1-independent cellular uptake
  - Pre-activated: dCK-independent activation
  - Greater Stability: CDA degradation resistant

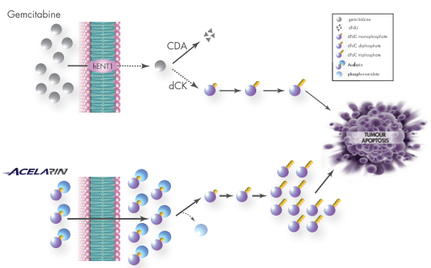

Figure 2. Acelarin overcomes the key gemcitabine resistance pathways

OBJECTIVES

- Assess RNAscope technology as reliable tool for biomarker detection
- Evaluate SpotStudio™ technology as accurate method for biomarker quantification
- Measure key biomarkers associated with gemcitabine resistance
- Initiate the development of an FDA compliant molecular diagnostic tool
- Target optimal patient population for Acelarin treatment

PROCESS & METHODS

- Process for RNAscope/SpotStudio validation (Figure 3)
  - 3 Companies
  - 2 Cancer Research Institutes
  - 2 Technologies

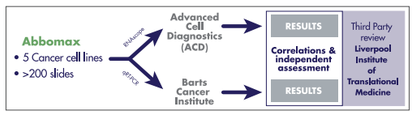

Figure 3. RNAscope technology assessment overview

- Formalin-fixed paraffin-embedded slides: 45 slides / cell line
- Cancer cell lines
  - Pancreatic cancer: BxPC-3; MiaPaCa-2; Panc-1
  - Lung cancer: H1703
  - Ovarian Cancer: OVCAR-3
- RNAscope
  - Biomarkers signal visualisation in morphological context at 20x magnification (distinguishes tumour from healthy tissue in biopsies)
  - Specific Z probes to detect hENT1, dCK and CDA biomarkers
- SpotStudio
  - Spots per cell automated biomarkers signal quantification (ACD & Definiens)
- qRT-PCR
  - Industry gold standard in-solution method for RNA quantification
  - Large dynamic range, high sensitivity and specificity
  - Challenges:
    - Lacks morphological context
    - Limited data reproducibility

RESULTS

RNAscope specifically reveals biomarkers signals in morphological context

- Specific hENT1, dCK and CDA *in situ* hybridization (Figure 4)
- Strong and clear signal: low background

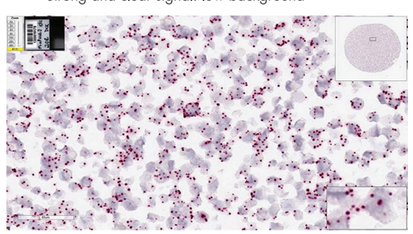

Figure 4. RNAscope staining of dCK in MiaPaCa-2 pancreatic cancer cells

SpotStudio rapidly and reproducibly measures biomarkers expressions

- dCK and hENT1 were expressed at medium to high levels: >5-25 spots/cell across cell lines (Figure 5)
- CDA was expressed at low levels: <5 spots/cell across cell lines

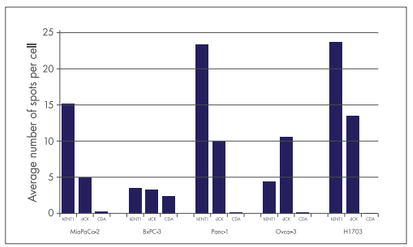

Figure 5. SpotStudio quantification of hENT1, dCK and CDA biomarkers in cancer cell lines

qRT-PCR quantification lacks morphological context

- Used as a reference methodology for biomarkers quantification and comparison with SpotStudio
- hENT1, dCK and CDA specific oligonucleotides were designed & validated
- hENT1 and dCK mean relative expression were medium to high (0.64 to 1.16 fold over GAPDH) across cell lines (Figure 6)
- CDA mean relative expression was low (0.34 fold over GAPDH) across cell lines

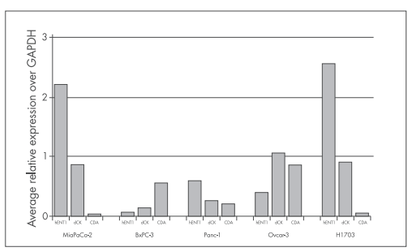

Figure 6. qRT-PCR quantification of hENT1, dCK and CDA biomarkers in cancer cell lines

Relationship between RNAscope/SpotStudio and qRT-PCR quantifications

- Third party independent assessment of data sets (Liverpool Institute of Translational Medicine)
- Results overlay showed a trend in biomarkers expression within individual cell lines (Figure 7)
- A positive correlation was observed between the two data sets for dCK (Figure 8)

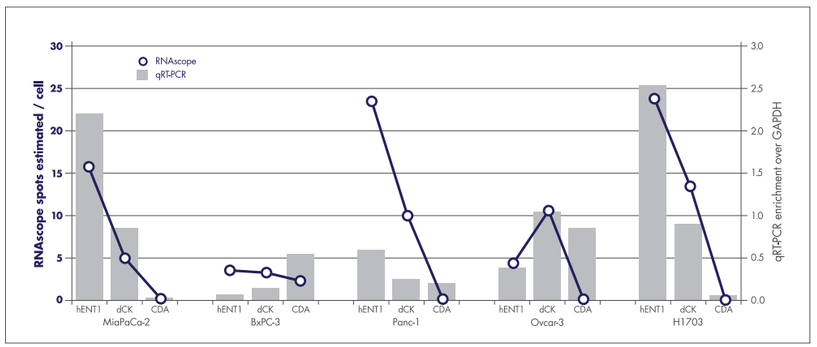

Figure 7. RNAscope and qRT-PCR biomarkers quantifications overlay

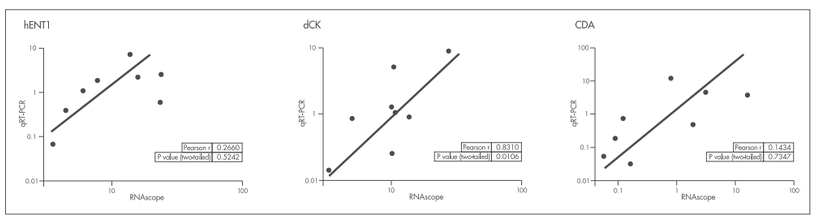

Figure 8. RNAscope and qRT-PCR results correlation for hENT1, dCK and CDA

CONCLUSIONS

- **RNAscope is a promising assay for contextual detection of key RNA biomarkers**
- **SpotStudio is a reliable technology for biomarker quantification**
- **Specific RNAscope probes for hENT1, dCK and CDA biomarkers have been validated**
- **Expression levels for hENT1, dCK and CDA by SpotStudio have been measured in multiple cancer cell lines**
- **Predictive clinical thresholds are currently being evaluated for each biomarker**
- **This molecular diagnostic assay may facilitate patient selection for treatment with Acelarin**

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