PREDICTIVE BIOMARKERS

THE HOLY GRAIL OF PERSONALISED MEDICINE FOR CANCER PATIENTS

Themes to discuss

1. Predictive vs prognostic biomarkers. Trial design - Placebo controls

2. Standardized biomarker assays implemented to Good Clinical Practice

3. Minimally invasive vs tissue biomarkers.
   cf Nucleic acids, Disseminated Tumour cells in bone marrow
   Circulating tumour cells. Longitudinal monitory of drug resistance and disease relapse.
Mazzoni et al – do SNPs in ERCC1 and RRM1 predict response of NSCLC to chemotherapy

Impact:
To spare patients toxic therapy that they are resistant to.
To give chemotherapy to patients most likely to respond

Followed a body of work pioneered by Jean Charles Soria where protein or RNA levels were measured in tumours and a general finding that low levels of ERCC1 and RRM1 is associated with better outcome. Response measured by RECIST.

Is there a less invasive way to predict chemotherapy response?

PCR on DNA purification from a blood sample.
Rapid turnaround and cheap.
Assumed SNPs would correlate with protein expression in tumour
Low ERCC1 expression correlates with prolonged survival after Cisplatin + Gemcitabine Chemotherapy in NSCLC

Lord et al 2002

ERCC1 levels in mRNA isolated from FFPE tumour blocks from n=56 patients

Lower ERCC1 is associated with better outcome
Conclusions: Patients with completely resected NSCLC and ERCC1 –negative Tumours appear to benefit from adjuvant cisplatin based chemotherapy whereas patients with ERCC1 positive tumours do not.

In 2011 They also showed that ERCC1 protein expression and mRNA expression were correlated, but there are inconsistencies in some cases. Fiboulet et al Clin Cancer Research

Later this group showed that the predictive utility of DNA repair enzymes co-segregates with Squamous Cell Carcinomas Pierceall et al Ann Oncol 2012

Latest work focussed on role of p38MAPK and relationship to ERCC1 expression in Light or never smokers with NSCLC. Planchard et al Cancer 2012
Prospective collection of dedicated tumour biopsy for determination of RRM1 and ERCC1 gene expression by real-time quantitative RTPCR

85 recruited, 55 usable data

Double-agent chemotherapy consisting of carboplatin, gemcitabine, docetaxel, and vinorelbine was selected based on gene expression

“Therapeutic decision making based on RRM1 and ERCC1 gene expression in advanced NSCLC I feasible and promising for improvement of patient outcome”
Randomized Phase III trial of gemcitabine-based chemotherapy with *In Situ* RRM1 and ERCC1 protein levels for response prediction in NSCLC

Reynolds et al 2009 JCO

Randomly assigned

n=170

n=85 Gemcitabine

n=85 Gemcitabine + Carboplatin

Lower levels of ERCC1 = longer PFS

By 4 months+ out lower levels of RRM1 = longer PFS

“Quantitative analysis of RRM1 and ERCC1 in routinely collected tumour specimens in community oncology practice is predictive of response to chemotherapy”
ERCC1 and RRM1 in the international adjuvant lung trial by automated quantitative *in Situ* analysis

Belper et al 2011 Am J Path

Distribution of ERCC1 & RRM1 *in situ* protein expression scores in n=730 patients

Problems with skewed data based on contributing centre
Future trials need *prospective collection, standardised protocols*, to better reveal impact....
ERCC1 expression in CTCs using a novel detection platform correlates with PFS in patients with metastatic NSCLC receiving platinum chemotherapy

Das et al 2012

ERCC1 expression was measured in 17 metastatic NSCLC patients who received platinum-based therapy and had ≥2 intact CTCs with acceptable ERCC1 expression assay results.
CUSTOMIZED FIRST LINE CHEMOTHERAPY ACCORDING TO ERCC1 AND RRM1 SNPs IN ADVANCED NON-SMALL-CELL LUNG CANCER (NSCLC) PATIENTS: A PHASE II STUDY.


°Medical Oncology – AOU Careggi, Florence; ^LAB Genetic diagnosis - AOU Careggi, Florence; *Medical Oncology Unit - Versilia Hospital, Viareggio; ”Medical Oncology Unit - Pontedera Hospital, Pontedera; #Medical Oncology Unit - Azienda Sanitaria 10, Florence; §Biostatistic Unit – ITT and AOU Careggi, Florence; Italy.

Citologic or Histologic diagnosis of NSCLC
Stage IIIIB-IV, TNM 6th ed.
Age ≥18 and ≤72
ECOG PS ≤ 1
Chemo Naive pts

ERCC1 and RRM1 SNPs analysis by PCR and Expression Levels Assessment

ERCC1
codon
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<th>ERCC1 SNPs</th>
<th>ERCC1 Expression Level</th>
<th>Cisplatin sensitivity</th>
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<tr>
<td>118 T&gt;C</td>
<td>C/C</td>
<td>LOW</td>
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<td>T/C</td>
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| 8029 C>A   | C/C                   | LOW                  | RESPONDER           |
|            | C/A                   | HIGH                 | NO RESPONDER       |

| -37 C>A    | C/C                   | LOW/NORM             | RESPONDER           |
|            | A/C                   | HIGH                 | NO RESPONDER       |

| 524 T>C    | T/T                   | LOW/NORM             | RESPONDER           |
|            | T/C                   | HIGH                 | NO RESPONDER       |
|            | C/C                   | HIGH                 | NO RESPONDER       |

RRM1
codon
<table>
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| 8029 C>A   | C/C                   | LOW                    | Gemcitabine 1250 gg1,8q21 |
|            | C/A                   | HIGH                   | Gemcitabine 1250 gg1,8q21 |
|            | A/A                   | HIGH                   | Gemcitabine 1250 gg1,8q21 |

| -37 C>A    | C/C                   | LOW/NORM              | Gemcitabine 1250 gg1,8q21 |
|            | A/C                   | HIGH                  | Gemcitabine 1250 gg1,8q21 |

| 524 T>C    | T/T                   | LOW/NORM              | Gemcitabine 1250 gg1,8q21 |
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* - Pts receive up to 6 cycles of CHT;
- Response evaluation is performed with CT scan every 2 cycles (RECIST 1.0);
- The CT scan performed after the 4° cycle (or after the last cycle if early discontinuation of therapy occurs) is considered to define treatment response.
Conclusions (1):

• The trial met the **primary end-point** with 55% RR in the ITT population, which compares favourably with the 20-40% RR that we observe with chemotherapy as delivered in clinical practice.

• The treatment related **G3-G4 toxicity** occurred in the 42% of the ITT population and this configures an acceptable toxicity profile, also assuming that there were no treatment related deaths.

• **Median PFS (5.3 months)** and **OS (8.0 months)** are consistent with what we could aspect for stage IIIB-IV NSCLC pts, unselected for biomolecular or histological features.
Conclusions (2):

- The trial supports the correlation between \textit{ERCC1} /\textit{RRM1} SNPs and \textit{ERCC1}/\textit{RRM1} expression levels which are supposed to influence \textit{Cisplatin} and \textit{Gemcitabine chemosensitivity} respectively.
- The trial generates the hypothesis that \textit{pts with squamous histotype} could benefit mostly from this customized approach (71.4% RR and 85.72% DCR in squamous pts vs 46.2% RR and 69.23% DCR in non squamous pts).
- \textit{ERCC1}/\textit{RRM1} SNPs are assessed by PCR after DNA purification from a blood sample. This is a \textit{rapid and relatively cheap assay} which could be reasonably adopted in our clinical practice. Nevertheless further studies are required to confirm the results of our experience.

Next Steps?

Assay validation to GCP?

Cross site validation of data?

Cuzick et al – Prediction of late recurrence by the ROR (PAM50) score in postmenapausal women in the transATAC cohort.

**Impact:**
ER +ve tumours (70-80%)
To identify those at risk or late relapse (~50%) provide extended adjuvant therapy and spare those not at risk the drug toxicities associated with prolonged treatment. Clear economic impact also.

Usually extended adjuvant therapy given for > 5 years only if there there axillary lymph node involvement.

**For non breast cancer experts:** Complex set of data leading up to this study

**PAM50** – affymetrix 50 gene signature Perou >10years ago.
Defines luminal A, B, Basal and Her2 subtypes - prognostic

**OncoDx** 21 gene signature – prognostic and predictive for endocrine treatment in ER +ve cancer

**IHC4** – ER, PR, Ki67, HER2 equivalent to OncoDx for prognosis

**ROR** = OncoDx plus tumour size

**Question** Which of all these scores performs best to predict time to distant relapse.

**Issue** Dissecting predictive vs prognostic biomarkers?
• **Background:** 4 immunohistochemical markers (ER, PgR, Ki67, HER-2), both alone and combined into the IHC4 score are significantly correlated with time to distant recurrence (TTDR)

• ROR provides significantly more prognostic information in endocrine treatment than the Oncotype Dx Recurrence Score (RS)

• **Objective:** To investigate the relationship between IHC4, RS and ROR (based on PAM50 and tumour size) score in predicting distant recurrence in years 0-5 and years 5-10 separately

• **Methods:** 940 postmenopausal women were included in this analysis
• Univariate and multivariate analyses were performed to determine prognostic value of all scores in the **0-5 year period** and **5-10 year period** separately
• To compare the IHC4 score with all other scores, the previously described sample splitting procedure was used for all four scores
Conclusions
Overall, all scores provided significant additional information in the period between 5 and 10 years with CTS being the strongest prognostic factor in all time periods.

IHC4 was the strong prognostic factor in the multivariate model for all patients and node negative patients in years 0-5.

None of the scores provided any significant prognostic information in years 0-5 for node positive patients in the multivariate model, but some added prognostic information is seen in the late period.

CTS and ROR are the only scores that added clinically significant prognostic information in years 5 to 10. The results presented here may be used to select patients who may benefit from hormonal treatment beyond 5 years. Is this going to be taken up by clinicians? Or is more evidence required?

What next? Placebo controlled trials?

What about DTCs? Pantel and colleagues in Germany 1 DTC in the bone marrow carries adverse prognosis? Would women take this test? Monitor DTCs or CTCs with time? HER2 measured in CTCs?
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