La Ricerca Traslazionale nelle neoplasie polmonari: stato dell’arte ed esperienze di ricerca
Parma, 27 novembre 2019

FARMACOGENOMICA nelle neoplasie polmonari

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Stat Rosa pristina nomine, nomina nuda tenemus

"The ancient Rose remains by its name, naked names (are all that) we have"
Pharmacogenetics & pharmacogenomics

- No universally accepted definitions of either
- Often used interchangeably
- The term pharmacogenomics coined in connection with the Human genome project
Pharmacogenetics is the study or clinical testing of specific genetic variations that give rise to differing drug response, including metabolism & disposition, and tolerability & efficacy.

Pharmacogenomics is the study of the role of all the genome in drug response.

Adapted from Ann Intern Med 2006

Adapted from J Clin Oncol 2005
Pharmacogenetics focuses on the association of one gene or several genes with drug activity, while pharmacogenomics considers the whole genome, through the broader application of new genomic technologies.

However, in oncology pharmacogenetics is often considered as concerning the individual patient’s features and pharmacogenomics as those of the tumour.
Pharmacogenetics & pharmacogenomics

Goals

- Maximize drug efficacy
- Minimize drug toxicity
- Predict patients who will respond
- Aid in new drug discovery/development
- Decrease in costs of health care
The Precision Medicine Initiative

“an unprecedented effort to accelerate a new era of medicine, focused on delivering more tailored treatment and preventive strategies”

President’s 2015 State of the Union address
Pharmacogenetics of NSCLC

“A number of pharmacogenetic studies have been carried out in non-small-cell lung cancer (NSCLC) to identify and characterize genes involved in chemotherapy activity. However, the results obtained so far are controversial and no reliable biomarker is currently used to predict clinical benefit from platinum-based chemotherapy” - Toffalorio et al., 2018

The most advanced biomarker to predict cisplatin efficacy seems ERCC1 expression
We were unable to validate the predictive effect of immunostaining for ERCC1 protein. The discordance in the results of staining for ERCC1 suggested a change in the performance of the 8F1 antibody.

The “Guardian of the genome” – an old key to unlock the ERCC1 issue. Friboulet et al., commentary to Heyza et al., CCR Feb 2019.
Pharmacogenetics of gemcitabine

Several studies evaluated polymorphisms of CDA

Ciccolini et al.,
Nat Rev Cancer 2011

http://gutemol.sourceforge.net/
by Dr. M Tarini, CNR Pisa
Evaluating CDA to predict gemcitabine activity

- Pharmacogenetics
- Pharmacodynamics
- Pharmacokinetics

Difluorodeoxycytidine (dFdC)

Liver

CDA

dFdU

dFdC

Gemcitabine triphosphate

Gemcitabine HCl
...from a genotype to a phenotype biomarker

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PR+SD</th>
<th>PD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDA Lys\textsuperscript{27} Gln</td>
<td>25 (92.6)</td>
<td>2 (7.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>22 (75.9)</td>
<td>7 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>5 (55.6)</td>
<td>4 (44.4)</td>
<td></td>
</tr>
</tbody>
</table>

Tibaldi et al., CCR 2008

Discussion on:
Ciccolini et al., J Clin Oncol 2010
Giovannetti et al., J Clin Oncol 2010

P=0.028
Advanced NSCLC patients (N=132*) treated with gemcitabine-cisplatin, from Livorno (N=76) and Parma Civic Hospital (N=56)
*Genotype/Phenotype data in 126 patients

No significant association with:
• Response/ Clinical benefit
Trend toward significance with
• TTP (P=0.053)
Significant association with OS

Multivariate analysis

<table>
<thead>
<tr>
<th>Covariates for OS</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.628 (0.353–1.118)</td>
</tr>
<tr>
<td>Male</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0.619 (0.358–1.068)</td>
</tr>
<tr>
<td>Epidermoid carcinoma</td>
<td>1 (ref.)</td>
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<tr>
<td>CDA A79C</td>
<td></td>
</tr>
<tr>
<td>AA-AC</td>
<td>0.357 (0.163–0.786)</td>
</tr>
<tr>
<td>CC</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>CDA C435T</td>
<td></td>
</tr>
<tr>
<td>CC-CT</td>
<td>0.794 (0.335–1.881)</td>
</tr>
<tr>
<td>TT</td>
<td>1 (ref.)</td>
</tr>
<tr>
<td>CDA activity</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.512 (0.310–0.846)</td>
</tr>
<tr>
<td>High</td>
<td>1 (ref.)</td>
</tr>
</tbody>
</table>

Tibaldi et al, Ann Oncol 2012

SELECTION OF THE BEST BLOOD COMPARTMENT TO MEASURE CYTIDINE DEAMINASE ACTIVITY TO STRATIFY FOR OPTIMAL GEMCITABINE OR CYTARABINE TREATMENT
Nucleosides, Nucleotides and Nucleic Acids 2012

Godefridus J. Peters,1 Richard J. Honeywell,1 Marie Maulandi,1,2 Elisa Giovannetti,1 Nienke Losekoot,1 Marie-Christine Etienne-Grimaldi,3 Gerard Milano,3 Cindy Serdjebi,2 and Joseph Ciccolini,2 for the EORTC-Pharmacology and Molecular Mechanism Group
1Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
2Transfer Oncology Laboratory, Aix-Marseille University, Marseille, France
3Laboratoire d’Oncopharmacologie, Centre Antoine Lacassagne, Nice, France
A prospective GOIRC study

The Cox proportional hazards regression model used for multivariate analysis confirmed CDA enzymatic activity as independent prognostic factor for progression and survival

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DF</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>Chi-square</th>
<th>Pr &gt; Chisq</th>
<th>Hazard ratio</th>
<th>95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>-0.01623</td>
<td>0.01491</td>
<td>1.1847</td>
<td>0.2764</td>
<td>0.984</td>
<td>0.956-1.013</td>
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<td>Sex: female</td>
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<td>0.55047</td>
<td>0.28231</td>
<td>3.8020</td>
<td>0.0512</td>
<td>1.734</td>
<td>0.997-3.016</td>
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<tr>
<td>ECOG PS 1</td>
<td>1</td>
<td>0.50064</td>
<td>0.23169</td>
<td>4.6691</td>
<td>0.0307</td>
<td>1.650</td>
<td>1.048-2.598</td>
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<tr>
<td>ECOG PS 2</td>
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<td>1.20025</td>
<td>0.46382</td>
<td>6.6964</td>
<td>0.0097</td>
<td>3.321</td>
<td>1.338-8.243</td>
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<td>Stage IIIB</td>
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<td>-0.49090</td>
<td>0.27448</td>
<td>3.1987</td>
<td>0.0737</td>
<td>0.612</td>
<td>0.357-1.048</td>
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<tr>
<td>CDA high &gt; 7.2</td>
<td>1</td>
<td>0.47659</td>
<td>0.22985</td>
<td>4.2993</td>
<td>0.0381</td>
<td>1.611</td>
<td>1.026-2.527</td>
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<tr>
<td>Panel B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>-0.01091</td>
<td>0.01466</td>
<td>0.5536</td>
<td>0.4569</td>
<td>0.989</td>
<td>0.961-1.018</td>
</tr>
<tr>
<td>Sex: female</td>
<td>1</td>
<td>0.18483</td>
<td>0.25073</td>
<td>0.5434</td>
<td>0.4610</td>
<td>1.203</td>
<td>0.736-1.967</td>
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<tr>
<td>ECOG PS 1</td>
<td>1</td>
<td>0.64678</td>
<td>0.22667</td>
<td>8.1206</td>
<td>0.0044</td>
<td>1.909</td>
<td>1.224-2.979</td>
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<tr>
<td>ECOG PS 2</td>
<td>1</td>
<td>0.98242</td>
<td>0.41952</td>
<td>5.4839</td>
<td>0.0192</td>
<td>2.671</td>
<td>1.174-6.078</td>
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<tr>
<td>Stage IIIB</td>
<td>1</td>
<td>-0.81121</td>
<td>0.28671</td>
<td>8.0236</td>
<td>0.0046</td>
<td>0.444</td>
<td>0.253-0.779</td>
</tr>
<tr>
<td>CDA high &gt; 7.2</td>
<td>1</td>
<td>0.58208</td>
<td>0.22099</td>
<td>6.9376</td>
<td>0.0084</td>
<td>1.790</td>
<td>1.161-2.760</td>
</tr>
</tbody>
</table>

ECOG Eastern Cooperative Oncology Group, PS performance status, CDA cytidine deaminase

Large-scale validation in a phase-ii biomarker-trial, including a matched cohort of patients treated with another regimen, is needed to further verify the predictive potential of CDA

Future studies should also evaluate the modulation of CDA activity in inflammatory conditions

Tibaldi et al, Br J Cancer 2018
Expanding “precision oncology” studies to embrace complexity

“Precision Oncology: Between Vaguely Right and Precisely Wrong”

- Precision Oncology relies on indiscriminate sequencing of genomes in biopsies that barely represent the heterogeneous mix of tumor cells

- so-called "driver mutations" are not actual omnipotent "drivers" but represent, in the simplest scheme, molecular lesions that are causatively necessary but not sufficient for pathogenesis

- Nongenetic heterogeneity is a critical consideration as cells respond to broad, environmental perturbations and drug treatments by converting to many other cell states, including stem-like, resistant cell phenotypes

new technologies for studying genetic and nongenetic cell population heterogeneity, tumor cell plasticity, and intercell communication are warranted
New –omics approach: in (some) liquid biopsies RNA may outperform DNA

- The “information density” of DNA might be insufficient
- Static (DNA) vs. dynamic (RNA) snap shot of individual or tumor
- RNA useful for re-arrangements, splice variants, expression patterns, etc.
- Therapy resistance via pathway activation, not by mutation per se

**Nucleic acid platforms**

1) **Plasma**: low quality mRNA, high quality ctDNA
2) **Extracellular vesicles**: low quality mRNA, low quality ctDNA
3) **CTCs**: complex isolation, low CTC mRNA yield
4) **Platelets**: easy isolation, high quality mRNA
Platelets contain tumor-derived RNA biomarkers

Nilsson et al, Blood 2011
TEP for detection, diagnostics and prevention

[Thanks to ThromboDX, Prof. Wurdinger]
Patients and Healthy donors

Training cohort: Cancer n=136
Validation cohort: Cancer n=92
A total of **5003 significantly differential RNAs in TEPs** were identified between cancer and healthy controls
Self-learning algorithm

Binary SVM procedure Training and Validation

All samples (n = 228)

Training (n = 175)  Validation (n = 108)

Legend

- Healthy donors
- Cancer patients

Algorithm training
- Feature selection
- Parameter tuning

Sample prediction

Classify samples

Performance evaluation
- Accuracy / Sensitivity / Specificity

Legend

- Healthy donors
- Cancer patients
Results in NSCLC

Legend
- PAAD
  AUC Training: 0.999
  AUC Validation: 1.000
- CRC
  AUC Training: 0.996
  AUC Validation: 1.000
- GBM
  AUC Training: 0.979
  AUC Validation: 0.926
- BrCa
  AUC Training: 0.977
  AUC Validation: 1.000
- NSCLC
  AUC Training: 0.986
  AUC Validation: 0.977
- HBC
  AUC Training: 1.000
  AUC Validation: 0.990

Random classifiers

A

NSCLC (n = 60) HD (n = 55)

B

Training LOOCV

Validation

Sensitivity

100 - Specificity

Legend
- PAAD
  AUC Training: 0.999
  AUC Validation: 1.000
- CRC
  AUC Training: 0.996
  AUC Validation: 1.000
- GBM
  AUC Training: 0.979
  AUC Validation: 0.926
- BrCa
  AUC Training: 0.977
  AUC Validation: 1.000
- NSCLC
  AUC Training: 0.986
  AUC Validation: 0.977
- HBC
  AUC Training: 1.000
  AUC Validation: 0.990
Cancer Cell

RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics

Authors
Myron G. Best, Nik Sol, Irsan Kooi, ..., Bakhos A. Tannous, Pieter Wesseling, Thomas Wurdinger

Download Best of Cancer Cell 2015
AGE might influence mRNA
Pre- and post-analytical and bioinformatics artifacts
Effects of inflammatory diseases, benign tumors
...with a new algorithm

Swarm intelligence-enhanced algorithm: Nature-inspired programming recipes

Best et al.
August 2017

The validation study showed accurate TEP-based detection of early- (n=106) and late- (n=518) stage NSCLC independent of age, smoking, whole-blood storage time, and various inflammatory conditions.
**Conclusions**

Despite pharmacogenetics/omics and precision oncology straightforward rationale, concerns about their effectiveness are mounting.

In some cases (eg, CDA for gemcitabine/platinum treated NSCLC) the phenotype performs better than the genotype.

Validation of methodologies and prospective trials are needed.

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"Precision" Must Not Mean "Narrow"

With new technologies for studying genetic and nongenetic cancer cell population heterogeneity, and tumor cell plasticity, we can achieve a new "precision" and hopefully guide better diagnostic/prognostic/predictive and preventive studies.

Tumor-educated platelets (TEPs) can function as blood-based biosource for (early/differential) cancer diagnostics (including NSCLC), with 98% accuracy.

Ongoing studies are evaluating the prognostic and predictive value of TEP...
“Show not what has been done, but what can be. How beautiful the world would be if there were a procedure for moving through labyrinths”
And the next meeting you should participate ;)

"PAMM is quite unique. A friendly and collaborative annual meeting of translational cancer researchers, and a perfect forum for young scientists to make their first international conference debut."

Andrew Westwell
Board member of the EORTC-PAMM and invited chairman
(Cardiff University, United Kingdom)