Circulating tumor DNA as a molecular blood-borne biomarker to predict tumor response in lung cancer patients treated with immunotherapy

Ed Schuuring

Department of Pathology, UMCG Groningen
e.schuuring@umcg.nl
Disclosures

Consultant/Advisory Board:
AstraZeneca, Bayer, BMS, Roche, Pfizer, Novartis, Amgen, BioCartis, Illumina, Agena Biosciences, CC Diagnostics, Janssen Cilag (Johnson & Johnson), Diaceutics, QCMD, ESP, IQNPATH, Cancer-ID

Speaker’s fee:
Bio-Rad, Abbott, Novartis, Roche, Biocartis, Agena Biosciences, Illumina, Pfizer, Astrazeneca

Grants/Sponsoring:
Pfizer, Biocartis, Cancer-ID, BMS, Bio-Rad, Roche, Agena Biosciences, Promega, Qiagen, CC Diagnostics, Boehringer Ingelheim

Stock/Royalties:
None

Transferred to UMCG-account
The Nobel Prize in Physiology or Medicine 2018

James P. Allison
Prize share: 1/2

Tasuku Honjo
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."
Treatment response in NSCLC patients treated with immune modulating therapy

- Immune checkpoint inhibitors changed the landscape of lung cancer treatment
- Many patients do NOT benefit from this new powerful treatment strategy
- **Critical need to develop biomarkers that evaluate the efficacy of immunotherapy treatment (early and late response)**

Checkmate 17 study (squamous)
Potential biomarkers to predict response to immunotherapy in NSCLC

- PD-L1/PD-1 IHC of tumors
- Mutations associated with resistance (e.g. STK11, KEAP1)
- Hypermutation analysis of tumors (neo-antigens, TMB)
- Microsatellite instability
- Copy-number-alterations
- Imaging with PD-L1 tracers
- Serum profiling (e.g. interleucines)
- Immunogram (immune cell profile)
- Immune cell infiltration
- Expression profiling microbiome
- Monitoring circulating tumor DNA (Tumor Volume)

Today no optimal predictive markers for response to immunotherapy
Liquid biopsies represent molecular pattern of cancer
**Liquid biopsies for predictive molecular testing**

Cell-free plasma contains:

* DNA and circulating tumor DNA (ctDNA)
* RNA and circulating tumor RNA (ctRNA)
* Proteomic/immunological tumor markers
* Pharmacokinetics ([drug])

Leukocytes and circulating tumor cells (CTC)
Platelets and tumor RNA

Routine EDTA-tube after centrifugation
Aim of our pilot/feasibility study:

- Monitor the response to immunotherapy in ctDNA (tumor-specific mutations) using cell-free plasma
- Compare ctDNA data with response of treatment determined by routine Tumor Volume changes (RECIST)
Standard of care for advanced-stage NSCLC in the Netherlands (2019):

> biopsies with druggable mutation (~20%): targeted therapy
> biopsies without druggable mutation: eligible for immunotherapy
Standard of care for advanced-stage NSCLC in the Netherlands (2019):

> biopsies with druggable mutation (~20%): targeted therapy
> biopsies without druggable mutation: eligible for immunotherapy

French SEQ-data of ~20,000 samples:

- In ~80% adca/scc NSCLC: no targeted therapy (including KRAS-mutations)
29 NSCLC patients

- Non targetable mutation confirmed by NGS
- treated with immunotherapy
- KRAS-mutation in pretreatment biopsy

**Design of pilot/feasibility study**

**CT scan:**
Baseline & every 6 weeks
RECIST v1.1. analysis

**Liquid biopsy & ddPCR**
Baseline, 1, 2, 4, 6 and thereafter every 12 weeks

KRAS-mutation as surrogate predictive markers for monitoring response to immunotherapy
Droplet digital PCR technology (ddPCR)

A standard ddPCR-assay: primer-set with HEX-probe for mutation and FAM-probe for wt
KRAS-mutation ddPCR test (example KRAS G12V)

High analytical sensitivity

- Mutant only
- 1% mutant
- 0.1% mutant
- 0.01% mutant
- 0.001% mutant
- 0% mutant

Readout: mutant and wt copies/ul (not only FA)
KRAS-mutation as surrogate predictive markers for monitoring response to immunotherapy

**Droplet digital PCR assays**

- Bio-Rad ddPCR™ *KRAS* G12/13 screenings assay

  **Mutation**
  - G12A
  - G12C
  - G12D
  - G12R
  - G12S
  - G12V
  - G13D

The Bio-Rad droplet digital (ddPCR)-*KRAS* G12/G13 screenings assay covers ~85% of all *KRAS* mutations in NSCLC

- Bio-Rad ddPCR™ *KRAS* Q61H screening kit
- BRAF G469A (LDT-IDT)
- BRAF G469V (LDT-IDT)
ctDNA detection in KRAS mutated NSCLC using ddPCR
(preliminary data)

responder
ctDNA detection in KRAS mutated NSCLC using ddPCR (preliminary data)

non-responder
ctDNA detection in KRAS mutated NSCLC using ddPCR (preliminary data)

Partial-responder

Hiltermann, Groen, Miedema, ter Elst, Schuuring – confidential – do not post
ctDNA detection in KRAS mutated NSCLC using ddPCR (preliminary data)

Non-responder
(ctDNA undetectable at baseline)
First analysis of KRAS ddPCR testing of cfDNA in NSCLC treated with immunotherapy (preliminary data)

<table>
<thead>
<tr>
<th>ctDNA pattern</th>
<th>Description of ctDNA pattern</th>
<th>No Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mutant copies were high at baseline and/or progressively increased over time</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Mutant copies decreased rapidly over time to undetectable levels</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Mutant copies were below the detection limit at baseline and remained undetectable at week 2-6</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>
Analysis of KRAS ddPCR testing of cfDNA in NSCLC treated with immunotherapy
First analysis of KRAS ddPCR testing of cfDNA in NSCLC treated with immunotherapy (preliminary data)

**Preliminary results of this pilot study:**
Decrease ctKRAS-mutant levels (at baseline/T0 vs T2-6 wk) associated with early and durable response

**Perspectives:**
- Confirmation by large numbers of cases
- What is optimal time-point? Only one time-point during treatment?
- Association between early response, durable response, clinical response

**Solutions/planning (ongoing):**
- Bristol-Myers Squibb sponsored Clinical trial (CA209-759) including 40 patients
- KRAS/BRAF/other non-targeted tumor mutations in another 40 ICI-treated patients
- Mutations are monitored with BioRad-ddPCR (standard) and compared to others (Idylla-Biocartis)
- Decision-modeling analysis to reliably predict early and durable response
Tumor-specific mutants (non-KRAS) as potential biomarkers to detect response to immunotherapy in advanced-stage NSCLC

French SEQ-data of ~20,000 samples:
- In ~80% adca/scc: no targeted therapy

TP53, KRAS, KEAP1 and STK11:
- In >90% adca/scc

Barlesi Lancet Oncol 2016

CGARN Nature 2014
Most “older” ctDNA-NGS assay have sensitivity of ~1-5%

Novel assays with iDES (integrated digital error suppression):
> combining UMI (unique-molecular-identifier) and polishing
> Sensitivity 0.1-1%

ctNGS-panel with 77 genes, fusions

* down to 0.1%
Pilot study for detecting circulating tumour DNA in advanced NSCLC using Avenio-NGS analysis and treatment response

- Baseline plasma samples = T0
- Follow up plasma sample = T1 after 4-6 weeks after start immunotherapy
- Outcome in tumour response measured with RECIST v1.1, progression-free survival (PFS) and overall survival (OS).
- Paired blood samples at two time points (7 patients)

Ellen Heitzer, Ed Schuuring, Menno Tamminga, Sanne de Wit, Sabrina Weber Michel Speicher, Leon Terstappen, Harry Groen
Collaboration between University Medical Center Groningen, University Twente and University Graz
Pt 952: 51 yr old female, PS1, smoker
- Adenocarcinoma with KRAS c.38G> A (G13D), PD-L1 ND
- Tumour response: PD; PFS 8 days; OS 59 days
- CTC T0= 46; CTC T1= 85

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant (cds)</th>
<th>Variant (protein)</th>
<th>Variant Description</th>
<th>Baseline</th>
<th>TP1 (2w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>c.622G&gt;A</td>
<td>p.Asp208Asn</td>
<td>Missense variant</td>
<td>19,52%</td>
<td>27,03%</td>
</tr>
<tr>
<td>TP53</td>
<td>c.592G&gt;T</td>
<td>p.Glu198*</td>
<td>Stop gained</td>
<td>15,48%</td>
<td>24,66%</td>
</tr>
<tr>
<td>KEAP1</td>
<td>c.1264G&gt;C</td>
<td>p.Asp422His</td>
<td>Missense variant</td>
<td>15,08%</td>
<td>25,88%</td>
</tr>
<tr>
<td>KRAS</td>
<td>c.38G&gt;A</td>
<td>p.Gly13Asp</td>
<td>Missense variant</td>
<td>14,74%</td>
<td>21,71%</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>c.1352A&gt;T</td>
<td>p.Lys451Ile</td>
<td>Missense variant</td>
<td>14,53%</td>
<td>19,34%</td>
</tr>
<tr>
<td>FLT4</td>
<td>c.3628G&gt;T</td>
<td>p.Ala1210Ser</td>
<td>Missense variant</td>
<td>0,78%</td>
<td>0,65%</td>
</tr>
<tr>
<td>EGFR</td>
<td>c.2024G&gt;A</td>
<td>p.Arg675Gln</td>
<td>Missense variant</td>
<td>0,36%</td>
<td>0,26%</td>
</tr>
<tr>
<td>TSC2</td>
<td>c.658A&gt;C</td>
<td>p.Thr220Pro</td>
<td>Missense variant</td>
<td>0,34%</td>
<td>ND</td>
</tr>
<tr>
<td>RET</td>
<td>c.2539A&gt;C</td>
<td>p.Thr847Pro</td>
<td>Missense variant</td>
<td>0,30%</td>
<td>ND</td>
</tr>
<tr>
<td>TP53</td>
<td>c.838A&gt;G</td>
<td>p.Arg280Gly</td>
<td>Missense variant</td>
<td>0,22%</td>
<td>ND</td>
</tr>
<tr>
<td>BRCA1</td>
<td>c.3255A&gt;T</td>
<td>p.Arg1085Ser</td>
<td>Missense</td>
<td>ND</td>
<td>0,24%</td>
</tr>
<tr>
<td>CDK4</td>
<td>c.820-2A&gt;T</td>
<td>splice acceptor variant &amp; intron var</td>
<td>ND</td>
<td>0,20%</td>
<td></td>
</tr>
</tbody>
</table>

**MET amplification**: TRUE, TRUE
Non-responders show increase of ctDNA

- **1.3 fold increase**
  - Tumour response: PD; PFS 8 days; OS 59 days

- **2.5 fold increase**
  - Tumour response: PD; PFS 45 days; OS 53 days

- **2 fold increase**
  - Tumour response: PD; PFS 43 days; OS 405 days
Responders show Decrease of ctDNA

Tumour response: PR; PFS >450 d; OS NDNo event (progression or death) after 450 days

- 1.4 fold decrease

Tumour response: CR; PFS NR; OS NR No event (progression or death) after 576 days

- 1.5 fold decrease

Tumour response: PR after ipi/nivo; PFS 245 days; OS NR (Alive after 289 days)

- 1.5 fold decrease

Confidential – do not post

Ellen Heitzer, Ed Schuuring, Menno Tamminga, Sanne de Wit, Sabrina Weber Michel Speicher, Leon Terstappen, Harry Groen

Cancer-ID is a project funded by the Innovative Medicines Initiative Joint Undertaking (IMI JU).
Preliminary conclusions

- Detection of allele frequencies with Avenio-NGS in plasma samples from advanced NSCLC is feasible.

- Concordance between durable tumour response on immunotherapy in terms of PFS and OS and Avenio ctDNA is very high in 6/7 samples.

Ellen Heitzer, Ed Schuuring, Menno Tamminga, Sanne de Wit, Sabrina Weber Michel Speicher, Leon Terstappen, Harry Groen
Clinical study: ctDNA as predictor of treatment response in NSCLC patients treated with immune modulating therapy

Main hypotheses
- Changing levels of ctDNA are an early predictor of treatment response
- The baseline level of ctDNA is an indicator for response to immune therapy

Secondary hypothesis
- The extent of changes in ctDNA levels informs about the duration of response
- Changing levels of ctDNA are a better predictor of response than CTC or tdEV
- The number of mutations identified in plasma DNA is predictive for response to ICI

Other considerations:
- Optimal blood drawn (in number and optimal time-points)
- Cost-effectiveness for clinical-care compared to other ctDNA-tests, e.g. ddPCR and MassARRAY/UltraSEEK (COIN-consortium-19Dec2018)
- Harmonisation/standardisation of ctDNA testing in standard of care (COIN and Cancer-ID consortium)

* Paired analysis of 180 NSCLC UMCG patients (t=0, t=4wk, PBC)
* Outcome in tumour response measured with RECIST v1.1, PFS and OS is well documented

Ellen Heitzer, Jeroen Hiltemann, Menno Tamminga, Sabrina Weber, Michel Speicher, Harry Groen, Ed Schuuring(Roche/CancerID-study)
ctDNA as a biomarker for early tumor response assessment in lung cancer patients treated with immunotherapy

**conclusion**

- Decrease in KRAS/BRAF specific ctDNA levels using ddPCR during immunotherapy predicts early and durable tumor response in NSCLC
  Comparable findings using BRAF/NRAS ddPCR in melanoma (Lee JAMA Oncol 2018, Xi CCR 2016, Gray Oncotarget 2015)

- Using Avenio NGS to detect ctDNA is feasible in cell free plasma of all NSCLC treated with immunotherapy
  NGS/ctDNA associated with response in NSCLC (Goldberg CCR 2018, Chaudhuri Cancer Disc 2017)

- Monitoring of ctDNA during treatment with immunotherapy is a promising approach to predict tumor response

- Predictive molecular profiling using liquid biopsies is a feasible, less-invasive and promising new method in Molecular Diagnostics of Lung Cancer
Detection of mutations in circulating tumor DNA in cell free plasma finding the “abnormal” hay in the haystack

Thanks for your attention

GALLOP-study (GIST): P Boonstra, M Tibbesma, A ter Elst, E Schuuring, A Reyners
Lung Cancer studies: M Aguirre, P van der Leest, L Bosman, A Miedema, A ter Elst, J Hiltermann, L v Kempen, A vd Wekken, H Groen, E Schuuring
International EQA/SOP-ctDNA consortia: IQNPath (ESP, AIOM, EMQN, UK NEQAS) (Schuuring), Cancer-ID (E Schuuring, E Heitzer)
ESP-EQA-lung-team: E Dequeker, C Keppens, V Tack, K Dufraing, J von der Thusen, E Schuuring
International Cancer-ID: E Schuuring, H Groen, E Heitzer

Supported by BMS, Biocartis, BioRad, Roche, Agena Biosciences and Cancer-ID