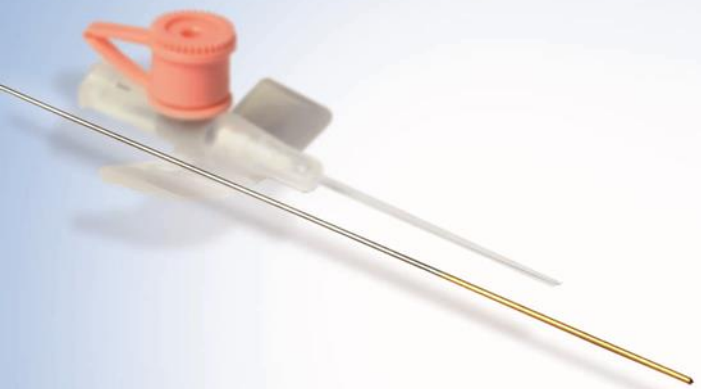

An innovative technology for *in vivo* isolation of circulating tumor cells in non-small cell lung cancer (NSCLC) patients and immunofluorescent detection of ALK protein

B. Dlugaszewska[1], L. Gasiorowski[2], S. Herold[1], B. Nowack[1], G. Dworacki[3], K. Luecke[1], W. Dyszkiewicz[2]

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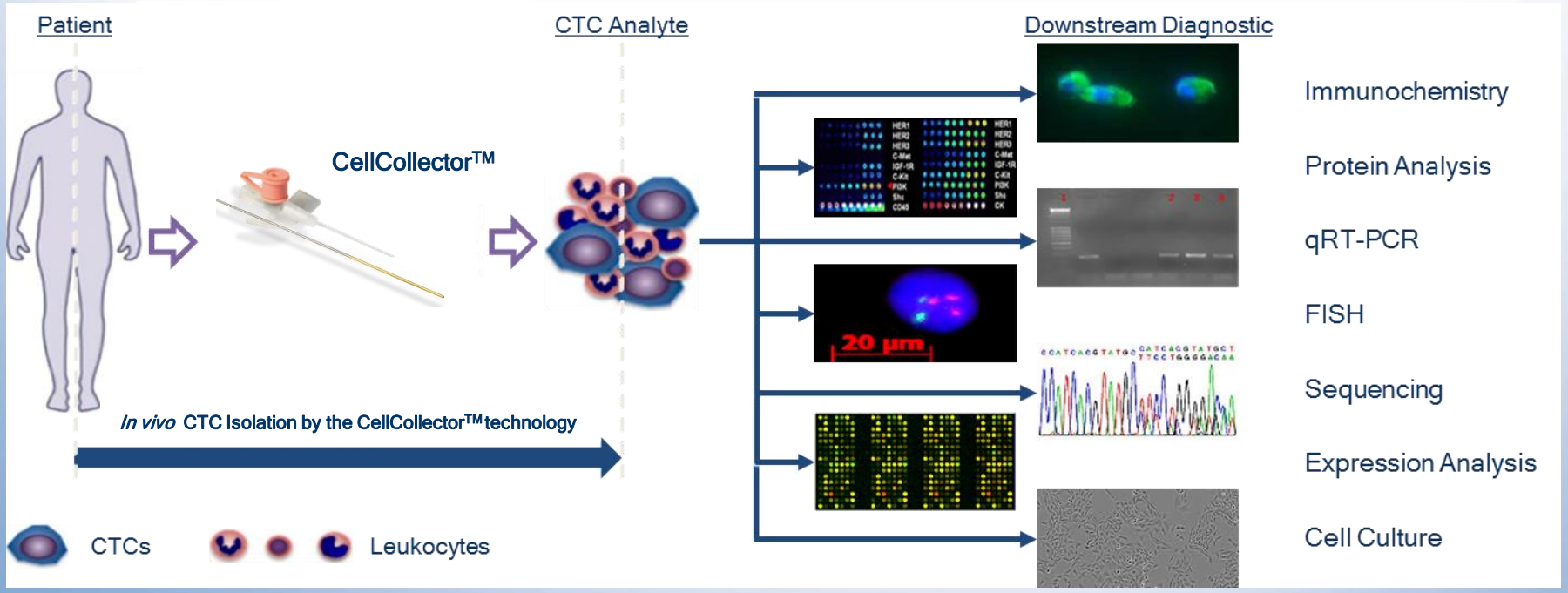
elcc EUROPEAN LUNG CANCER CONFERENCE
Geneva, Switzerland 26-29 MARCH 2014

Background

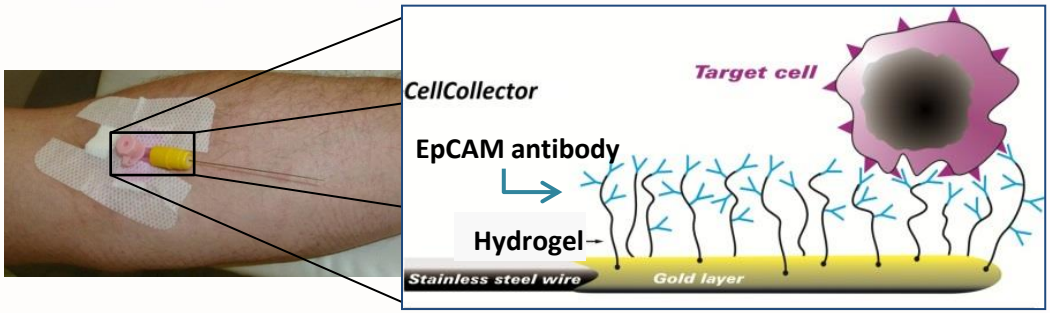
- CTCs isolation → liquid biopsy:
  - Prognostic and predictive biomarker
  - Treatment efficacy assessment
  - Comprehensive biomarker analysis
  - Lung cancer screening auxillary tool?
- Blood sample is limited in terms of sensitivity
- *In vivo* alternative increases access to CTCs



Materials and methods

GILUPI NANOMEDIZIN

60 subjects
48 NSCLC + 12 controls



Insertion of the GILUPI CellCollector™ into a peripheral arm vein of the patient (30 min. exposure time)

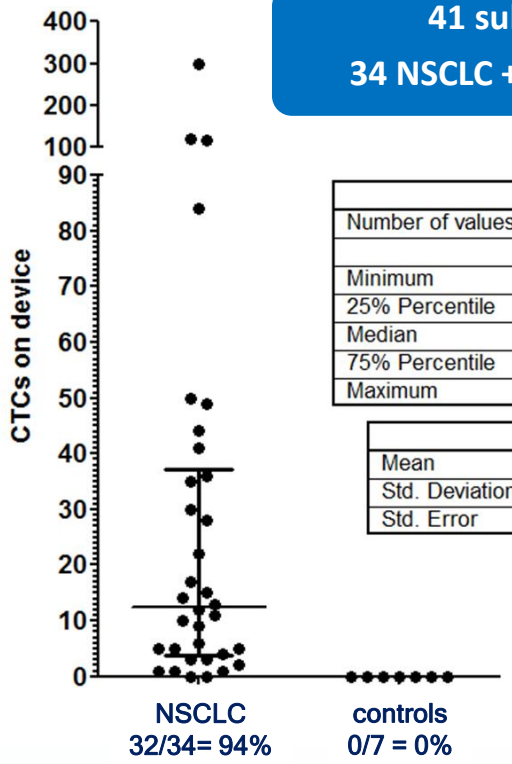
41 subjects
34 NSCLC + 7 controls

Immunocytochemical staining and enumeration of CTCs

Results

- Application of the GILUPI CellCollector™ was well tolerated with no side effects in 100% of the patients; the procedure was comparable to blood withrawal
- GILUPI CellCollector™ sensitivity was 94% (32/34), similar for early and late stage NSCLC patients
- In paired samples, the CellSearch® method allowed to detect CTCs in 1 patient only

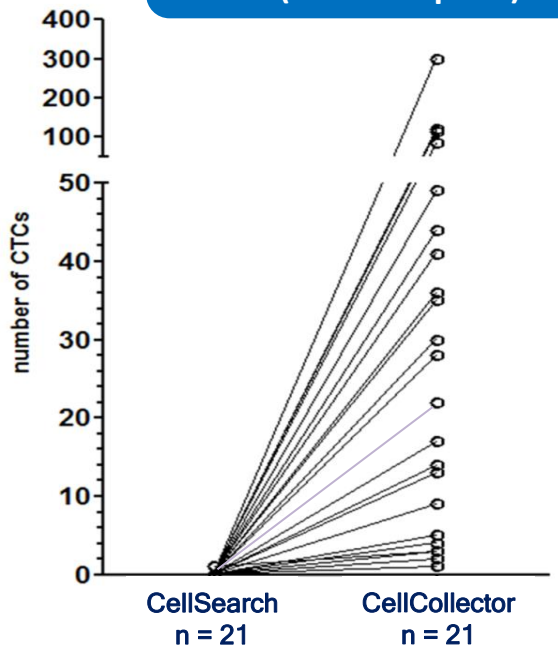
41 subjects
34 NSCLC + 7 controls



	cancer	no cancer
Number of values	34	7
Minimum	0.0	0.0
25% Percentile	3.8	0.0
Median	13	0.0
75% Percentile	37	0.0
Maximum	300	0.0

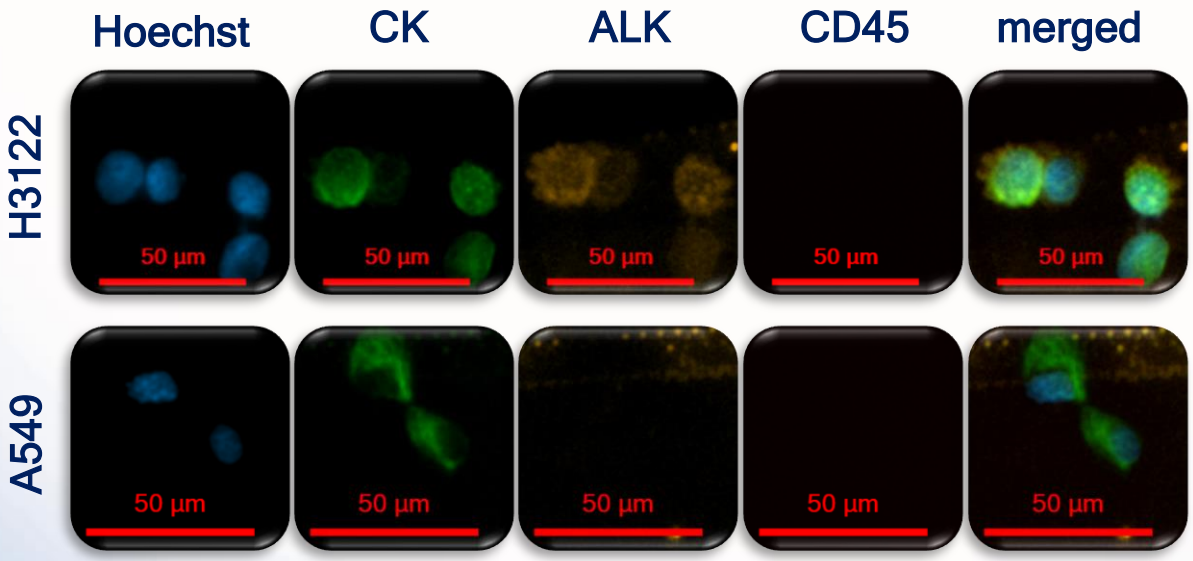
	cancer	no cancer
Mean	32	0.0
Std. Deviation	56	0.0
Std. Error	9.6	0.0

21 NSCLC
CellSearch vs. CellCollector
(matched pairs)



Results

ICC analysis of CTCs on the GILUPI CellCollector™ was improved to identify the anaplastic lymphoma kinase protein (ALK)



Lung cancer cells were captured by the GILUPI CellCollector™ in spiking experiments. Cell characterisation was achieved by immunofluorescence with antibodies against epithelial markers (cytokeratins) and possible therapeutic targets (ALK). Nuclei were counterstained with Hoechst.

Conclusions

- In NSCLC patients CTC detection rate with the GILUPI CellCollector™ was higher than with the CellSearch® analysis
- High sensitivity for early NSCLC → concept of an early shedding of CTCs into the circulation is now well established
- The GILUPI CellCollector™ method offers the possibility of an early detection of CTCs years before the onset of overt metastasis, contributing to an improved identification of patients in need of additional systematic anticancer therapy
- ALK gene rearrangements in NSCLC patients are an indication for targeted therapy with crizotinib
- The GILUPI CellCollector™ offers an alternative for ALK FISH or IHC on tumor tissue

An innovative technology for *in vivo* isolation of circulating tumor cells in non-small cell lung cancer (NSCLC) patients and immunofluorescent detection of ALK protein

B. Dlugaszewska et al. Poster 26PD

- Strong points:
  - Novel device does require large blood collection for CTC isolation
  - CTC isolation compares favorably with the CellSearch® method
  - Promising data using NSCLC cell lines

An innovative technology for *in vivo* isolation of circulating tumor cells in non-small cell lung cancer (NSCLC) patients and immunofluorescent detection of ALK protein

B. Dlugaszewska et al. Poster 26PD

- Strong points:
  - Novel device does require large blood collection for CTC isolation
  - CTC isolation compares favorably with the CellSearch® method
  - Promising data using NSCLC cell lines
- Suggestions for additional work:
  - Identification of circulating cells as tumor cells
  - Performance of FISH on isolated CTCs
  - Isolation of sufficient cell numbers for DNA extraction and analyses
  - Correlation of CTCs with the known molecular features of the patient's NSCLC (use of EGFR-mutation specific antibodies?)
  - Comparison with NSCLC mutation detection in free plasma DNA

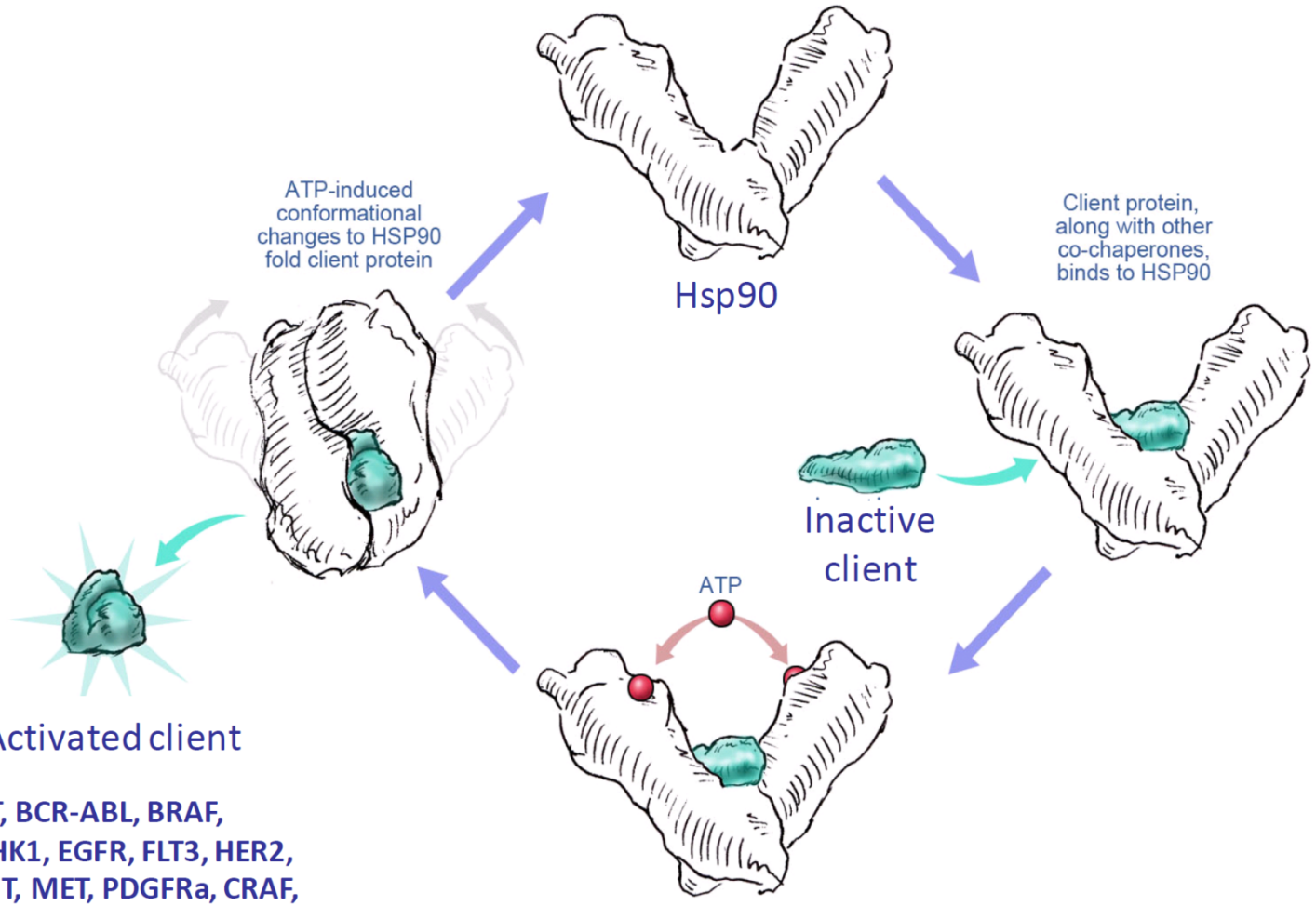
Ultra-deep sequencing of circulating free DNA to identify predictive, mutated HSP90 clients in GALAXY-1 (NCT01348126), a randomized phase 2b study of ganetespib plus docetaxel versus docetaxel alone in 2nd line advanced NSCLC

Dean Anthony Fennell[1], Alexey Antonov[2], Callum Rakhit[2], Jacqui Shaw[1], Iman El-Hariry[4], Vienna Reichert[3], Vojo Vukovic[3], L. Miguel Martins[2],

[1]University of Leicester, Leicester, United Kingdom, [2]MRC Toxicology Unit, Leicester, United Kingdom, [3]Synta Pharmaceuticals, Lexington, USA

ELCC 2014
Abstract number 39PD

Hsp90 Drives Malignant Growth by Activating Multiple Cancer Promoting Client Proteins



ALK, AKT, BCR-ABL, BRAF, CDK4, CHK1, EGFR, FLT3, HER2, HIF1a, KIT, MET, PDGFRa, CRAF, SRC, VEGFR, AR, ER …

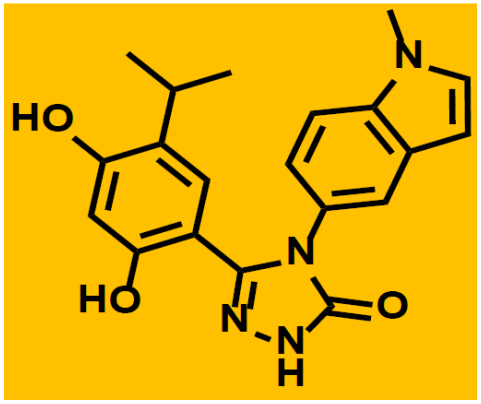
L. Neckers, 2012

Introduction

A next-generation Hsp90 inhibitor

Well tolerated with acceptable safety profile

- **Over 1000 patients treated to date**
- **Diarrhea is a class effect; Grade 1,2, manageable with prophylactic treatment**



Signal of activity with ganetespib single agent in lung and metastatic breast cancer

In the GALAXY-1 trial, ganetespib and docetaxel combination improved PFS and OS in 2nd line advanced NSCLC adenocarcinoma

Circulating free DNA (cfDNA) is present at low levels in plasma of healthy individuals allowing detection of somatic mutations by deep sequencing.

The aim of this work was to determine the mutational spectrum of patients enrolled into the GALAXY-1 trial using this liquid biopsy strategy.

Methodology & Results

- Here we report the results of targeted sequence analysis of plasma cfDNA from the first 105 patients enrolled in the GALAXY-1 Trial; patient baseline clinical characteristics are shown in **Table 1**.
- cfDNA was isolated from 1ml of plasma collected at baseline (pre-dose, C1D1) using Qiagen kits and quantified by AQ real-time PCR as described previously (Page et al. 2011). As only 3 of the 105 samples met the desired cut-off of 1.7ng/µl cfDNA (10ng template) for direct sequencing, samples were concentrated by SpeedVac®; 36 samples met the requirement of 10ng template DNA, while the remaining samples had <10 ng template DNA.
- Semiconductor sequencing with the Ion AmpliSeq™ Cancer Panel was first performed in calibration standards (**Figures 1 and 2**) and then extended to these samples together with 3 samples that did not require concentration.

Total = 39 cfDNA samples sequenced

Methodology & Results (cont.)

- DNA from the total plasma cfDNA pool was enriched using a PCR-based approach (Ion AmpliSeq™ Cancer Hotspot Panel v2, Life Technologies Ltd, Paisley, UK). Pooled PCR products were used for DNA library preparation using the Ion Xpress™ Plus Fragment Library Kit (Life Technologies Ltd, Paisley, UK). Adaptor-ligated products were then size-selected by gel purification and amplified to obtain genomic DNA libraries that were suitable for sequencing on the Ion PGM System. Sequencing reads were aligned to the reference human genome (release hg19). Mutations were detected using the Torrent Server Variant Caller plug-in (tvc 3.6-39, 62687) with parameters set to "Somatic – High Stringency". An overview of sequencing results, showing percentages of mutation positive patients in each arm is shown in **Figure 3**.
- Presence of KIT and PTEN mutations appears to correlate with tumor response **(Figure 4)**.

Table 1. Baseline Clinical Characteristics (N = 105)

	Ganetespib + Docetaxel N=51	Docetaxel N=54
Median Age (range)	59.6 (41, 75)	58.3 (42, 86)
Gender		
Male	26 (51)	33 (61)
Female	25 (49)	21 (39)
Smoking Status		
Never	10 (20)	13 (24)
Ever	41 (80)	41 (76)
ECOG Status		
0	28 (55)	26 (48)
1	23 (45)	28 (52)
Interval since dx advanced disease		
≤ 6 months	21 (41)	18 (33)
>6 months	30 (59)	36 (67)

Figure 3. Percentages of mutation positive patients in the two study arms of the GALAXY-1 clinical trial

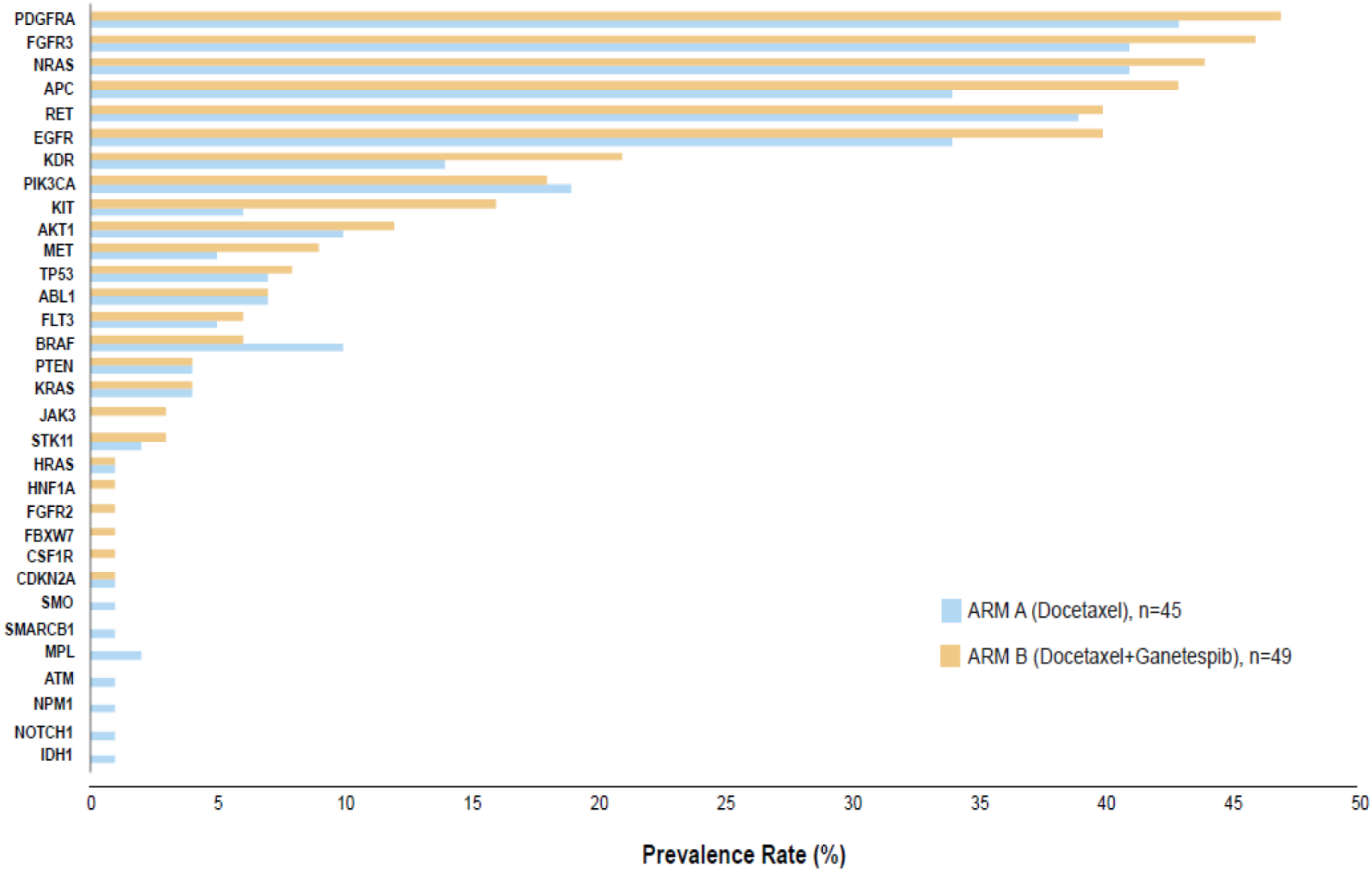
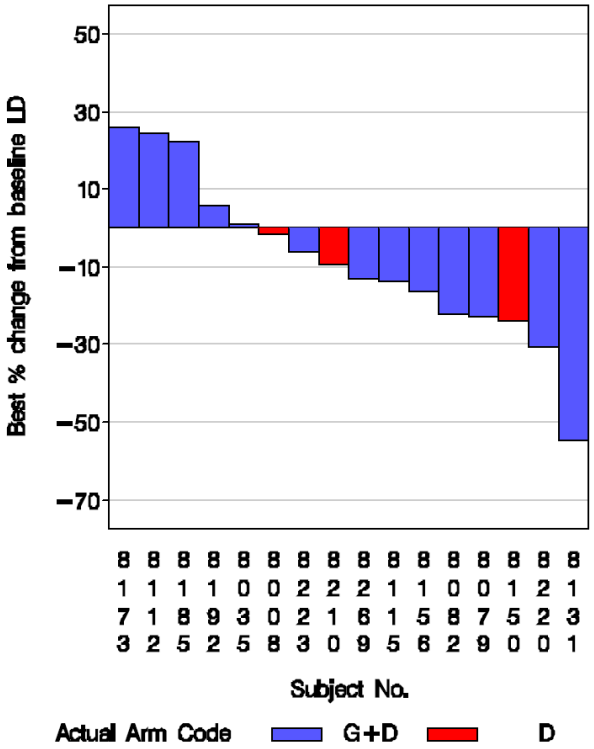
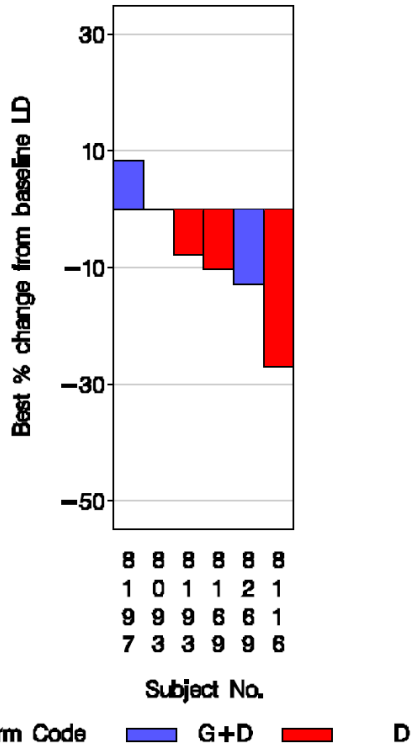


Figure 4. Waterfall plots for patients with KIT or PTEN mutations

All patients with KIT mutations (COSM28026)*



All patients with PTEN mutations (COSMIC5816)*



*Two patients with KIT mutations and 1 patient with PTEN mutation were non-evaluable for best % change from baseline LD.

Summary

- To our knowledge, this is one of the largest series of patients to be profiled for somatic mutations in cfDNA
- The investigation of somatic mutations in cfDNA using Ion Torrent technology identified Single Nucleotide Variants (SNV) in several of the genes commonly mutated in NSCLC (e.g. EGFR, PIK3CA, MET), as well as germline mutations and SNVs in genes not commonly associated with NSCLC pathogenesis (e.g. KIT).
- Preliminary analysis suggests that PTEN and KIT mutations may have prognostic potential in advanced NSCLC patients
- Next steps
  - Full cfDNA analysis on remaining approximately 200 samples (both baseline and post-treatment) is in progress
  - Tumor tissue mutational analysis is currently ongoing. Correlation analysis with plasma cfDNA results will performed
- The results presented suggest that cfDNA mutational analysis has the potential to be used to study tumor heterogeneity and mechanisms of resistance

Ultra-deep sequencing of circulating free DNA to identify predictive, mutated HSP90 clients in GALAXY-1 (NCT01348126), a randomized phase 2b study of ganetespib plus docetaxel versus docetaxel alone in 2nd line advanced NSCLC

D. A. Fennell et al. Poster 39PD

- Strong points:
  - Solid rationale for trial of Hsp90 inhibitor in NSCLC given prominent role of mutant kinases in this cancer, many of which require Hsp90 for folding/stabilization/activity
  - Prospective collection of blood for isolation cfDNA built into clinical trial

Ultra-deep sequencing of circulating free DNA to identify predictive, mutated HSP90 clients in GALAXY-1 (NCT01348126), a randomized phase 2b study of ganetespib plus docetaxel versus docetaxel alone in 2nd line advanced NSCLC

D. A. Fennell et al. Poster 39PD

- Strong points:
  - Solid rationale for trial of Hsp90 inhibitor in NSCLC given prominent role of mutant kinases in this cancer, many of which require Hsp90 for folding/stabilization/activity
  - Prospective collection of blood for isolation cfDNA built into clinical trial
- Suggestions for additional work:
  - Increase amount of blood collected for plasma DNA extraction
  - Collect data on NSCLC histologic subtypes and tumor mutation status
  - Ultra-deep sequencing without matched normal DNA makes it difficult or impossible to distinguish somatic mutations from germline polymorphisms and Ion Torrent sequencing artifacts
  - Do subset analyses based on variant allele percentages or run sequencing analyses in duplicate? Low variant allele percentages (<5%) show lower reproducibility.
  - Separate analysis of canonical driver mutations in EGFR and KRAS
  - Somatic KIT mutations are very rare in NSCLC; specific KIT variants associated with responses should be examined.

Molecular heterogeneity and response to Gefitinib of *EGFR* mutant advanced lung adenocarcinoma

Emilio Bria[1], Sara Pilotto[1], Eliana Amato[2], Matteo Fassan[2], Silvia Novello[3], Tiziana Vavalà[3], Luisella Righi[3], Isabella Sperduti[4], Aldo Scarpa[2,5], Giampaolo Tortora[2].

[1]Medical Oncology, Azienda Ospedaliera Universitaria Integrata, University of Verona, Verona, Italy; [2]ARC-NET Applied Research on Cancer Center; [3]University of Torino, Department of Oncology, A.O.U. San Luigi, Orbassano (Torino), Italy; [4]Biostatistics, Regina Elena National Cancer Institute, Rome; [5]Department of Pathology and Diagnostics, University of Verona, Verona, Italy.

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Geneva, Switzerland
26-29 MARCH 2014

Background

- Tyrosine-Kinase Inhibitors (TKIs) of the *EGFR* pathway for patients carrying the sensitizing gene mutations do provide a striking benefit in delaying the progression of advanced lung adenocarcinoma.
  - Nevertheless, 25-30% of such patients rapidly progress during treatment.
- In this regard, the assessment of **molecular heterogeneity** may help identify this clinically relevant subgroup.
- **Multigene next generation sequencing (NGS)** may help to concurrent screen for potential genetic abnormalities deputed to drive cancer prognosis or therapeutic opportunities.

Bria E et al, ELCC 2014

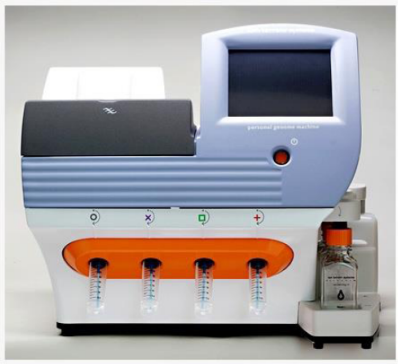
Methods
[Patients' Inclusion Criteria]

- *EGFR* mutant patients receiving 1st line Gefitinib for advanced lung adenocarcinoma were analyzed for mutations in 22 genes.
- Patients were retrospectively grouped according to the treatment resistance to Gefitinib and the Time to Progression:
  - **Poor responders** (progression at 1st assessment)
  - **Intermediate responders** (progression within 12 months).
  - **Good responders** (progression after 12 months/treatment ongoing).

Bria E et al, ELCC 2014

Methods
[Deep Sequencing]

- Mutational status of ***22 cancer-associated genes*** using multiplex PCR amplification of DNA from microdissected paraffin embedded tissues and the ***Ion Lung and Colon Cancer Panel (Life Technologies)*** :
  - *AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, KRAS, MAP2K1, MET, NOTCH1, NRAS, PIK3CA, PTEN, SMAD4, STK11, TP53*
- Data Validation was accomplished with Sanger sequencing and IHC analysis (TP53).



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Methods
[Aims]

- To correlate the Gefitinib activity (in terms of treatment resistance and Progression-Free Survival, PFS) with:
  - **The rate of cells carrying the EGFR mutation (the Rate of Mutated Cells, RMC)**
    - **Quantitative correlation**
  - **The presence of Additional Coexisting Mutations [ACM] (other than EGFR mutation)**
    - **Qualitative correlation**

Bria E et al, ELCC 2014

Results
[Patients' Charateristics]

- **Eighteen patients were gathered in 2 institutions:**
  - Median age: 71 yrs (range: 37-83)
  - Median Follow-Up: 8 months (range: 1-33)
- **Events:**
  - Progressions: 14
  - Deaths: 7.

Patients' Characteristics	[n] (%)
Sex	
Male	6 (33.3)
Female	12 (66.7)
ECOG Performance Status	
0	14 (77.8)
1	4 (22.2)
Smoking status	
Current	6 (33.3)
Never smokers	12 (66.7)
EGFR mutation	
Exon 19 Deletion	13 (72.2)
L858R	5 (27.8)
Response to treatment	
Objective Response	12 (66.7)
No response	6 (33.3)

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Results
[Deep Sequencing]

- **Findings according to groups:**
  - Smokers were significantly more represented in the poor group than in the others **[66.7% vs 10.0%, *p[Fisher]=0.04*]**.
  - *TP53* mutations (median RMC 45%) were exclusively documented among poor/intermediate than in good responders **[66.7% vs 0%, *p[Fisher]=0.01*]**.

Bria E et al, ELCC 2014

Patients' Characteristics	[n] (%)
TP53 mutation	
No	11 (61.1)
Yes	7 (38.9)
CTNNB1 mutation	
No	16 (88.9)
Yes	2 (11.1)
SMAD4 mutation	
No	17 (94.1)
Yes	1 (5.6)
MET mutation	
No	17 (94.1)
Yes	1 (5.6)
KRAS mutation	
No	16 (88.9)
Yes	2 (11.1)
PIK3CA mutation	
No	17 (94.1)
Yes	1 (5.6)

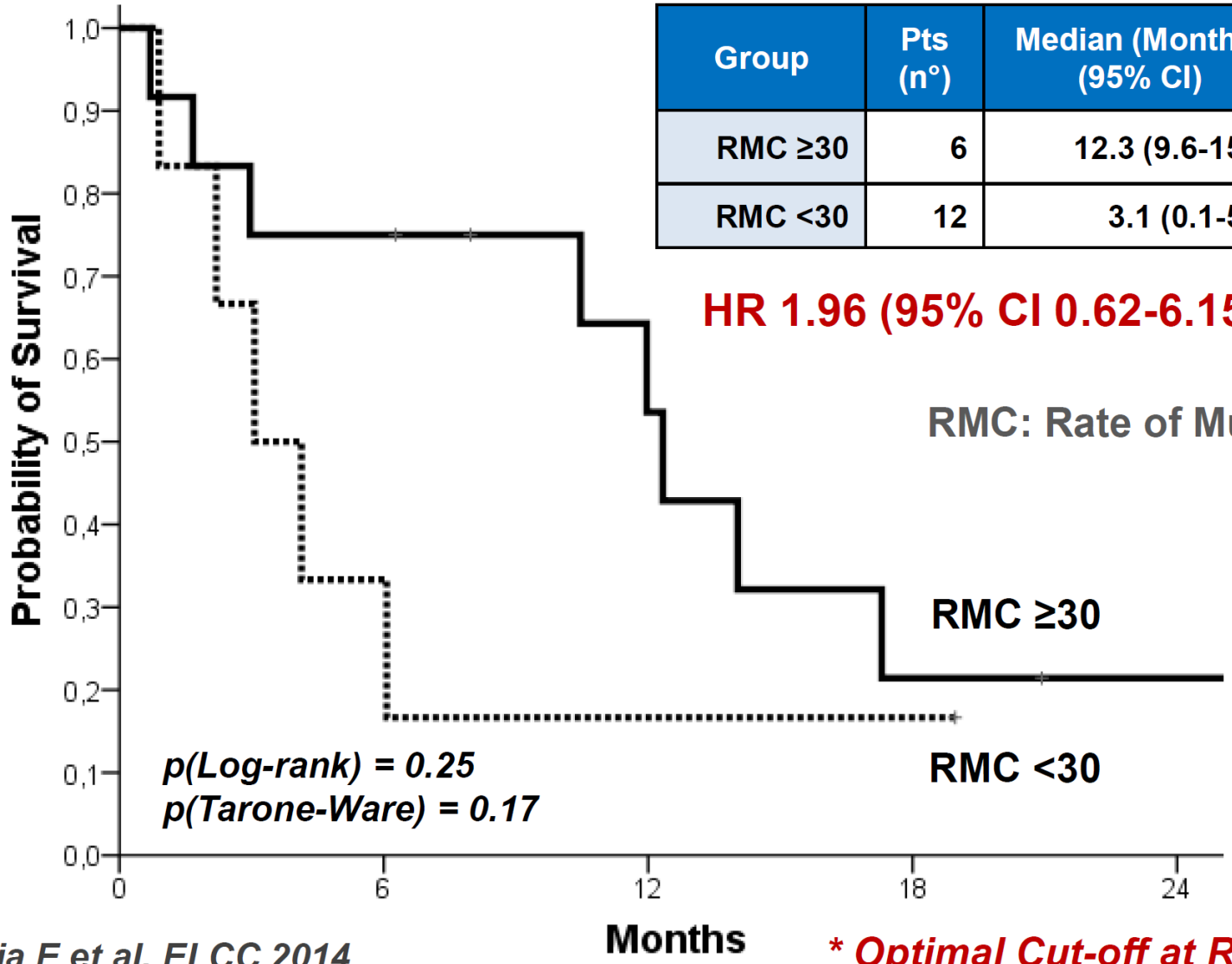
Results

[PFS according to EGFR-RMC (Cut-off 30%*)]

Group	Pts (n°)	Median (Months) (95% CI)	2-yr (%)
RMC ≥30	6	12.3 (9.6-15.0)	21.4%
RMC <30	12	3.1 (0.1-5.9)	16.7%

HR 1.96 (95% CI 0.62-6.15), p=0.25

RMC: Rate of Mutated Cells



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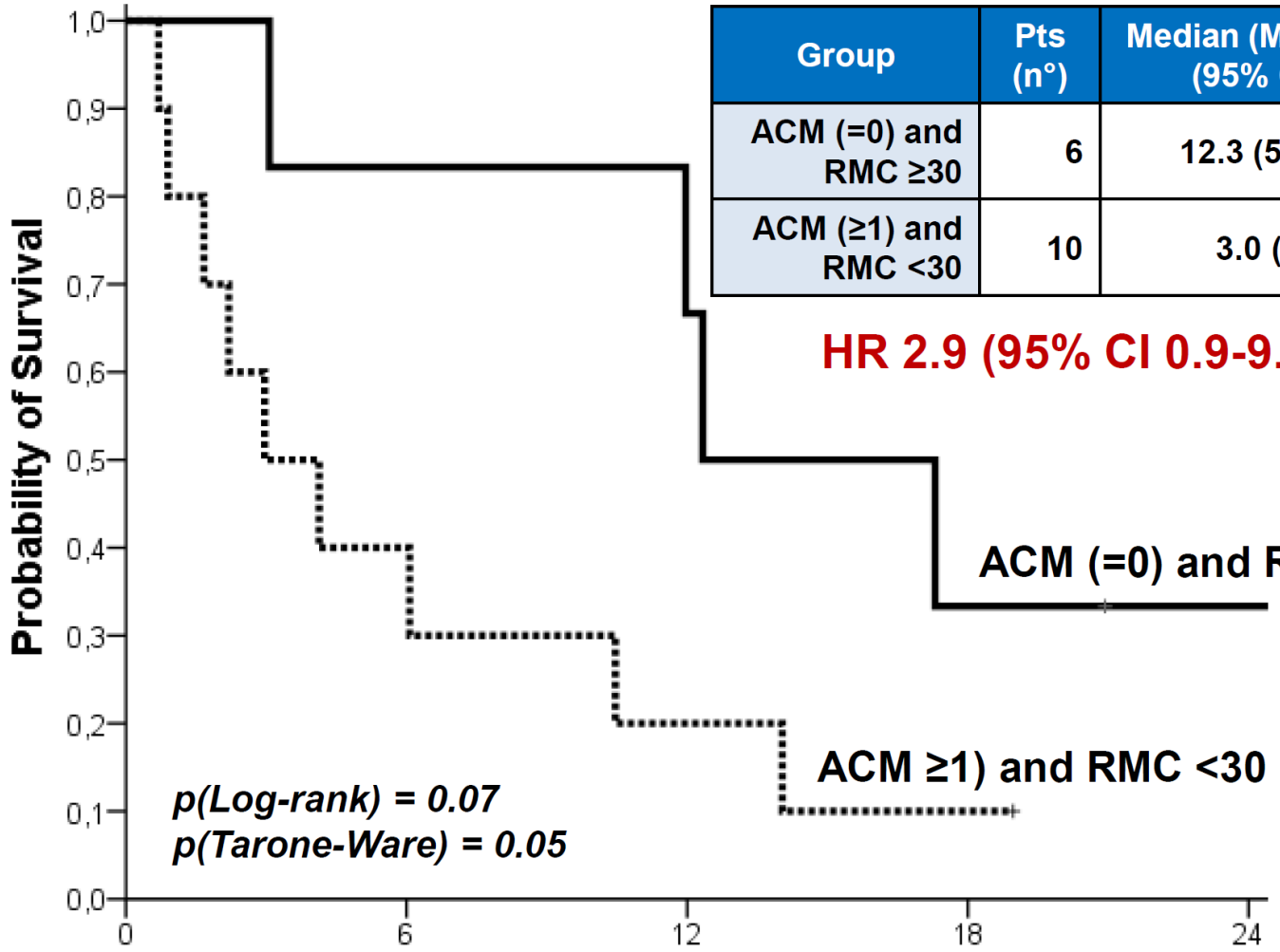
** Optimal Cut-off at ROC analysis*

Results

[PFS according to ACM and EGFR-RMC]

Group	Pts (n°)	Median (Months) (95% CI)	2-yr (%)
ACM (=0) and RMC ≥30	6	12.3 (5.9-18.7)	33.3%
ACM (≥1) and RMC <30	10	3.0 (0.1-5.9)	10.0%

HR 2.9 (95% CI 0.9-9.6), p=0.08



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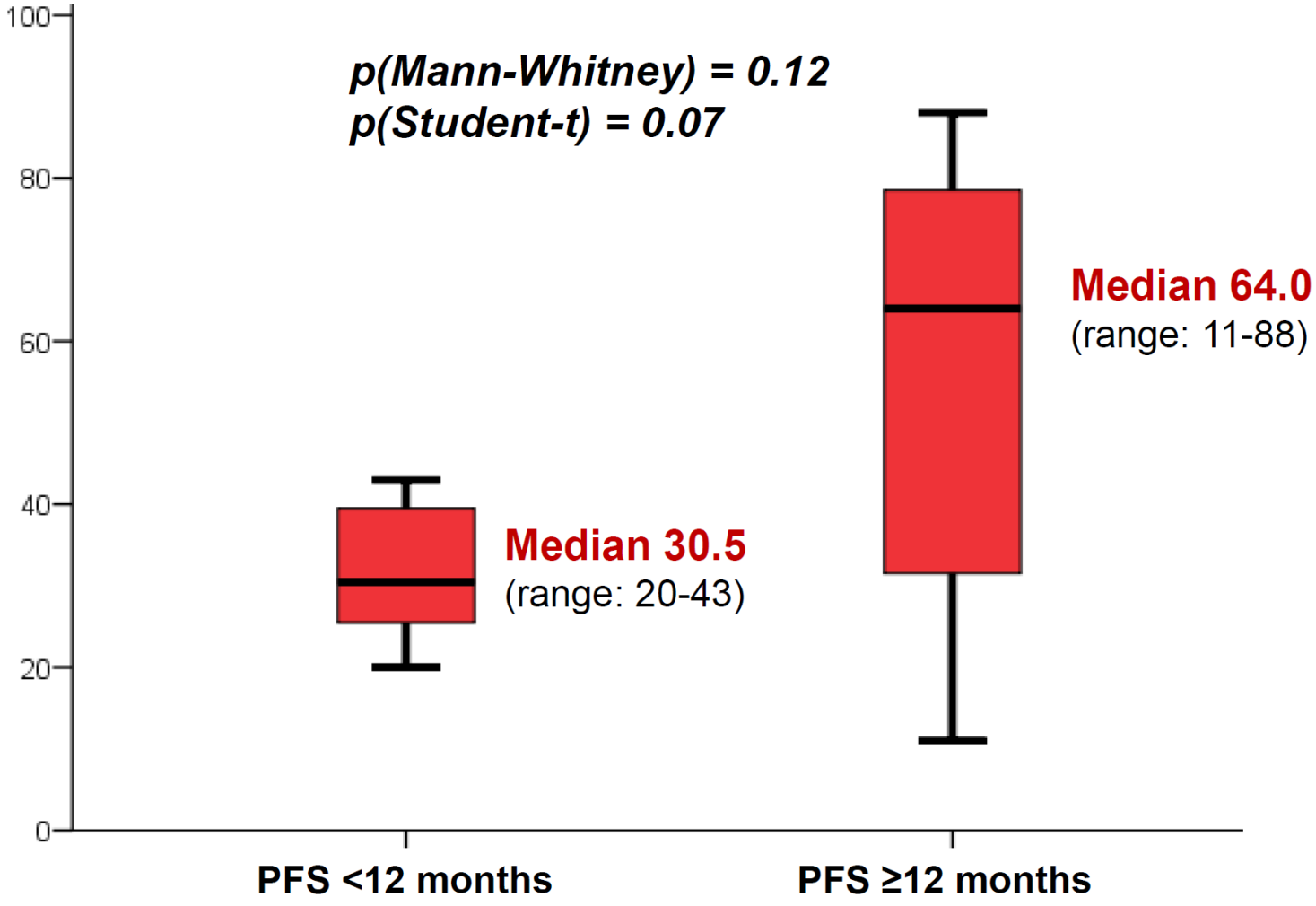
ACM: Additional Coexisting Mut.

Results

[EGFR Rate of Mutated Cells [RMC] according to PFS]



Bria E et al, ELCC 2014

Conclusions

- Although exploratory and unpowered for conclusive results, these data support the role of **NGS technology to improve the *EGFR* mutant patients' selection** to be addressed to receive Gefitinib (or TKIs in general) as a:
  - **Qualitative tool** ***(presence of additional mutations)***
  - **Quantitative tool** ***(rate of EGFR-mutated cells)***.
- Both features may indicate the presence of the **Tumor Heterogeneity** of a diagnosed disease
- A larger validation is ongoing.

Support: Italian Association for Cancer Research (AIRC-MFAG 14282, IG 11930, 5X1000 12182 and 12214).

AIRC

ARC NET APPLIED RESEARCH ON CANCER RICERCA APPLICATA SUL CANCRO

emilio.bria@ospedaleuniverona.it

Molecular heterogeneity and response to Gefitinib of EGFR mutant advanced lung adenocarcinoma

Bria E. et al. Poster 95PD

- Strong points:
  - Addresses important question of why 20-25% EGFR-mutated lung adenocarcinomas do not respond to first generation EGFR TKIs
  - Use of Ion Torrent sequencing allows efficient analysis of concurrent mutations (Additional Coexisting Mutations [ACM]) in clinical tumor samples

Molecular heterogeneity and response to Gefitinib of EGFR mutant advanced lung adenocarcinoma

Bria E. et al. Poster 95PD

- Strong points:
  - Addresses important question of why 20-25% EGFR-mutated lung adenocarcinomas do not respond to first generation EGFR TKIs
  - Use of Ion Torrent sequencing allows efficient analysis of concurrent mutations (Additional Coexisting Mutations [ACM]) in clinical tumor samples
- Suggestions for additional work:
  - Increase study size (currently n=18)
  - Consider results in the context of the generally accepted concept that the major EGFR mutations are typically clonal (present in every tumor cell)
  - Incorporate tumor content estimations into the analyses of the rate of cells carrying the EGFR mutation (the Rate of Mutated Cells RMC)
  - Examine possible relationship of histologic level of differentiation to RMC (better differentiated tumors may have lower proportion of tumor cells)
  - Instead of combining all Additional Coexisting Mutations [ACM] for survival analysis, perform separate analyses for major ACM such as P53, etc…
  - Define features of ACM: do they appear at the same variant allele frequencies as the EGFR mutation or do they appear subclonal? Are they all at canonical sites or are some at novel or rare sites (e.g. within KRAS, MET, etc…)?