Comparison of large genes panel versus targeted NGS sequencing in the molecular profiling of Non-Small Cell Lung Cancer

M. Schiappacassi[1], S. D'Andrea[1], R. Doliana[1], A. Bearz[2], E. De Carlo[2], A. Del Conte[2], V. Canzonieri[3], F. Bucciotti[1], S.V. Buxa-Kleeberg[4], D. Verderio[4], X. Wang[4], Y. Yu[5], P. Ariyaratne[5], E.Canzian[1], A.Selva[1], V. Da Ros[6], G. Baldassarre[1]

(1) Division of Molecular Oncology Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, National Cancer Institute, Aviano (PN), 33081, Italy, (2) Oncology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, National Cancer Institute, Aviano (PN), 33081, Italy, (3) Pathology Unit, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, National Cancer Institute, Aviano (PN), 33081, Italy, (4) Vela Diagnostics Germany GmbH - Germany, (5) Vela Research Singapore Pte Ltd - Singapore, (6) Clinical Trial Office, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, National Cancer Institute, Aviano (PN), 33081, Italy .

Background

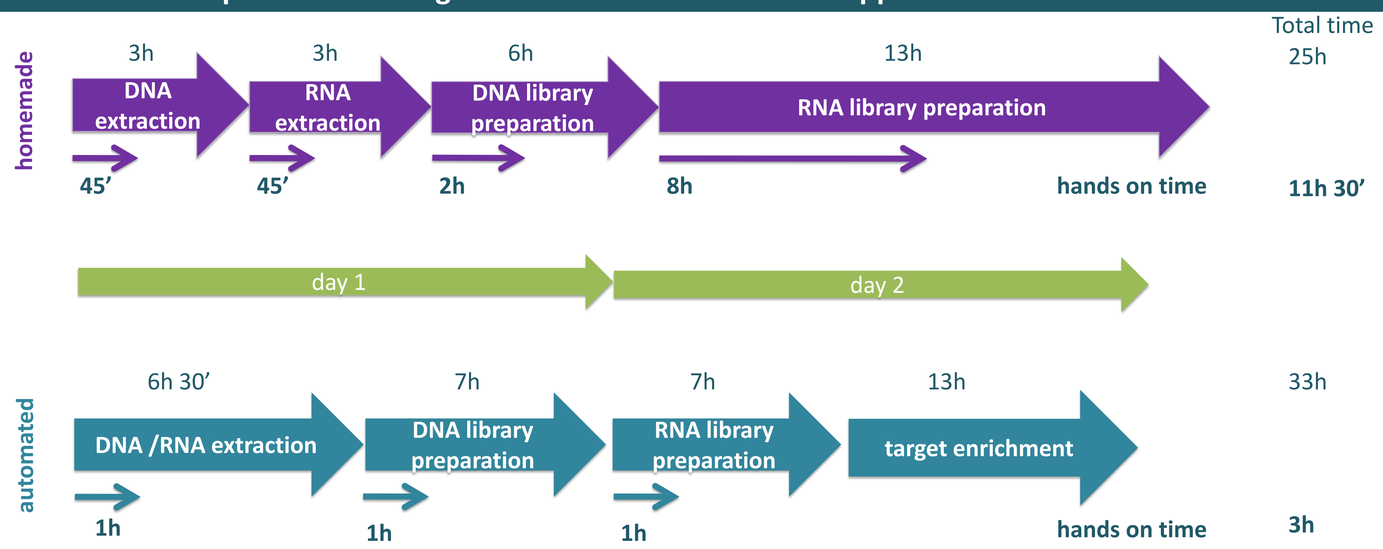
- ✓ **A big debate is underway among the oncology community to define which is the best and more clinically useful approach to profile human cancers in the clinical practice.**
- ✓ **The majority of labs uses either DNA/RNA based small hotspots targeted panels or commercially available Pan-Cancer Panels.**
- ✓ **Here we compare a targeted home-made, fully validated, NGS sequencing approach with a commercially fully automated multi genes panel (Oncokey SL 60 Plus Panel by Vela Diagnostics) that allows determining the mutational status in 60 genes for SNVs, CNVs, Indels, fusions and the tumor MSI status in one assay using DNA and RNA.**
- ✓ **We tested the hypothesis that adding more information on the genetic profile of Non-Small Cell Lung Cancer (NSCLC), respect to the ones requested by clinicians, could unveil possible vulnerabilities and/or predict the response/resistance to targeted treatments, using an already diagnosed retrospective cohort.**
- ✓ **As a secondary aim we compared the quality of NGS outputs and the man hours necessary to perform the analyses with the home made and the fully automated approaches to establish which one is preferable in a small/medium size diagnostic lab.**

1. OncoKey™SL 60 Plus Panel targets

AKT16x	AKT2	AKT3	ALK	ARID1A	ATM
BRAF	BRCA1	BRCA2	CCNE1	CDKN2A	CTNNB1
DICER1	EGFR	ERBB2	ERBB3	ESR1	ETV6
EWSR1	FBXW7	FGFR1	FGFR2	FGFR3	GNA11
GNAQ	HRAS	IDH1	IDH2	KDM6A	KIT
KRAS	MAP2K1	MAP2K2	MET	MLH1	MSH2
MSH6	NRAS	NRG1	NTRK1	NTRK2	NTRK3
PALB2	PDGFRA	PIK3CA	PMS2	POLD1	POLE
PTCH1	PTEN	RAD51B	RAD51C	RAD51D	RAD54L
RET	ROS1	SMARKB1	STK11	TERT	TMPRSS2
TP53	MSI (100 SITES)	HBV	HCV	Merkel Cell Polyomavirus	EBV
HHV-B	HPV	*Helicobacter pylori*	*Salmonella typhi*	*Streptococcus gallolyticus*	*Chlamydia pneumoniae*

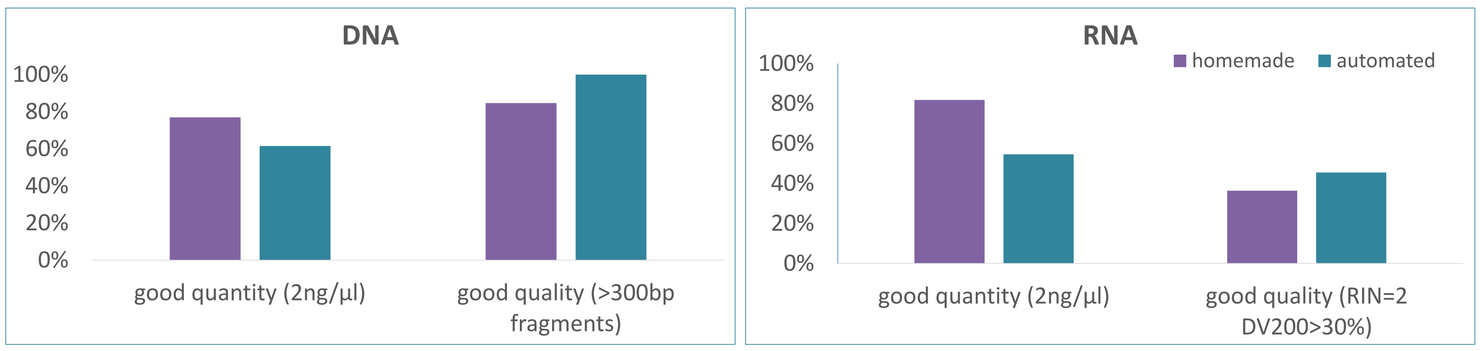
The table summarizes the genes and the cancer-related pathogens covered by the automated system used. Red circles highlight the mutated genes identified by the 60 genes panel and not requested/diagnosed in the previous analysis.

2. Workflow: comparison of timing in homemade vs automated approach



The automated method requires less "hands on" time compared to the manual one, even if the total time is slightly longer.
It is worthy of note that the used automated system works with 16 samples/run: this limit is extremely useful in a large laboratory but can result of difficult management when applied to a small/medium sized one.

3. Concentration and quality of nucleic acid extracts



Considering that 2ng/µl nucleic acid concentration is recommended, the homemade protocol performed better in obtaining the desired DNA/RNA concentration. Conversely, the quality of extracted nucleic acids the automated system was slightly superior.

4. Discordant results

VD6	VARIANT 3a/b EML4 6a/b:ALK 20	FAILED FUSION DETECTION
VD8	EGFR p.(745-750del) + IVS13-(53-54) dupCT cMET	EGFR p.(G719S)
VD12	FAILED FUSION DETECTION	CD47-MET fusion

We observed 3 out of 15 discordant cases as reported in the table on the left.

homemade automated

Case1	cMET ERBB2	EGFR WT	NO MUTATIONS	EGFR p.(E746_A750de)	CONFIRMED	TIER I
Case 2	EGFR BRAF KRAS cMET ERBB2		NO MUTATIONS	PIK3CA p.(E545K)	CONFIRMED	TIER I
Case 3	EGFR BRAF KRAS		NO MUTATIONS	cMET amplification	CONFIRMED	TIER II

Analysis requested PCR based method homemade automated TIER Classification

5. Clinical relevance

In the scheme we summarize the analysis of three cases that took advantage of large NGS panel in our study.
Case 1 Analyses requested were performed in a local hospital with a PCR-based method and no EGFR or cMET variants were detected. The automated NGS panel on the same sample revealed the deletion p.(E746_A750del) in exon 19 of EGFR that is a TIER I variant, then confirmed by our validated protocol.
Case 2 Analyses requested did not include the evaluation of PIK3CA gene variants, but the 60 genes panel identified a PIK3CA TIER I variant that was confirmed by our validated protocol.
Case 3 Analyses requested did not include the cMET amplification that was highlighted using the automated large panel. In this case the TIER II variant was confirmed by our validated protocol.
In 3 out of 15 cases (20%) considered in this work the highest complexity of the NGS panel allowed to unveil clinically relevant variants which may imply an improvement in patient treatment and/or quality of life.

- ✓ **We compared randomly selected NSCLC samples from an already diagnosed cohort that included both high- and low-quality paraffin embedded samples.**
- ✓ **We observed that the 60 genes panel effectively identified already confirmed genes variants in 80% and has discordant results in 20% of the cases.**
- ✓ **In three out of 15 cases the Oncokey SL 60 panel identified previously undisclosed variants and/or copy number alterations that could had a clinical impact on patient management.**
- ✓ **We conclude that the use of automated large NGS genes panel is feasible and potentially useful in a small/medium size diagnostic lab.**

Abstract #N11