Exo-ALK proof of concept: Exosomal analysis of ALK alterations in advanced NSCLC patients

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Background

A subset of NSCLCs (approx. 5%), present alterations in the ALK gene. This produces abnormal ALK proteins that induce cells to grow and spread. Different generation of ALK inhibitors are available for targeted therapy and their indication depends on the detection of ALK alterations in the tissue. Thus, it is mandatory to develop new techniques that allow us to demonstrate ALK alterations in peripheral blood. The purpose of this study is to analyze the feasibility to determine ALK alterations in exosomes (Exo-ALK) in NSCLC patients and determine the sensitivity and specificity of the technique.

Methods

This study is performed in blind in a cohort of 19 NSCLC with and without known alterations of ALK in tumoral tissue. ALK-positive tissue samples were identified by FISH and patients were included independently of stage and time of disease. Exosomal RNA is isolated by exoRNeasy Serum/Plasma (Qiagen) and retrotranscripted by ProtoScript II First Strand cDNA Synthesis kit. The ALK gene present in the exosomes was determined by NGS and bio-informatic analysis by OncoDNA. Samples were provided by the Biobank of the University of Navarra, the UZA Biobank and by the University of Naples Federico II. Samples and data were processed following standard operating procedures approved by the local Ethical and Scientific Committees.

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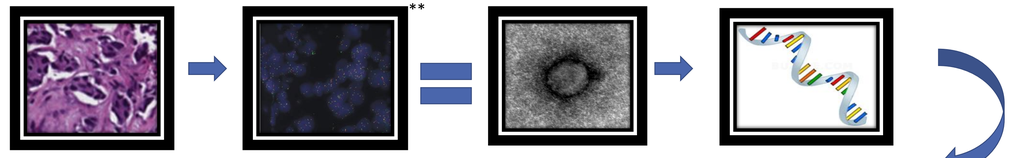
Results

The analyzed samples have been 16 ALK-EML4 tissue positive patients and 3 ALK-EML4 tissue negative, defined in this case by FISH. After analysis, we have been able to detect 9 positive ALK-EML4 patients, 8 negative samples and 2 samples where the RNA was degraded. Looking at the clinical data, the 9 positive samples detected in the exosomal RNA were positive also for ALK-EML4 translocation in the tissue, and comparing the 8 negative samples, 3 were tissue negative and 5 tissue positive. These data show a sensitivity of 64% and a specificity of 100%. No correlation has been found comparing treatment-naïve and pretreated patients.



**Taken from "ALK and crizotinib: after the honeymoon…what else? Resistance mechanisms and new therapies to overcome it" by Rolfo, C. et al. 2014

		ALK-EML4 FISH detection in Tissue (n=19)	
		Positive (16)	**Negative (3)**
ALK-EML4 Exosomal RNA detection. OncoDNA/UZA (n=17)*	**Positive (9)**	9	0
	Negative (8)	5	3
		Sensitivity 64%	**Specificity 100%**

*2 RNA (positive) samples get degraded during the delivery

Conclusion

Exosomes are raising as one of the most promising tools to understand the tumor due to their stability in the blood and their similarity to the cells of origin. Our preliminary results show a high specificity for a proof of concept analysis. Further studies with a larger number of patients and a cross-validation analysis are required, but as we present in this abstract, exosomes can represent an important tool for the clinical management of this specific NSCLC population.