Detection of MET alterations at the DNA, RNA and protein levels in NSCLC patients progressing on ALK and ROS1 targeted therapies

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BACKGROUND

ALK and *ROS1* fusion-positive NSCLC patients derive clinical benefit from tyrosine kinase inhibitors (TKIs) but ultimately relapse. Acquired resistance mechanisms include on-target secondary mutations or copy number gains and activation of bypass signaling. Although *MET* amplification has been described as a bypass resistance mechanism to ALK and ROS1 inhibitors, there are limited data on *MET* gene and protein overexpression.

OBJECTIVES

(i) detection of MET alterations at the DNA, mRNA and protein levels in *ALK*, *RET* and *ROS1* fusion-positive NSCLC patients progressing on TKIs
(ii) establishment of primary cultures using samples from those patients.

METHODS

MET alterations were analyzed in 31 patients after progression on TKIs; 17 *ALK, 5 RET* and 9 *ROS1* fusion-positive. Informed consent was obtained from all patients. In tumor tissue samples, NGS and FISH were used to detect resistance mutations and amplifications, *MET* mRNA expression levels were determined by nCounter and total and phospho-MET levels were assessed by IHC and Western blotting. Liquid biopsy samples were analyzed exclusively by NGS to determine mutations and copy number variations.

CONCLUSIONS

-MET alterations were found in 3/7 tumor biopsies of fusion-positive patients after progression to TKIs. Two of them showed *MET* amplification and one MET protein overexpression and activation in the absence of *MET* copy number gains.

-Despite the small size of the cohort, our results suggest that testing at the RNA and protein levels can discover amplification-negative patients with MET activation who may derive benefit from a MET targeted therapy.

-Consequently, tumor tissue should be preferred over liquid biopsies to determine MET status in patients in progression to TKIs.

REFERENCES

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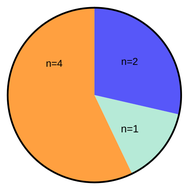
RESULTS

-A total of 39 samples at progression were available from 31 patients (p.) treated with fusion-specific TKIs, including tumor biopsies (n=7), plasma (n=26), pleural effusions (n=3) and cerebrospinal fluids (n=3).
-In the case of tumor tissue biopsies, MET alterations were detected in 3/7 p. (43%), 2 *ALK* fusion-positive p. progressing to lorlatinib and one *ROS1* fusion-positive p. progressing to crizotinib. The two *ALK* fusion-positive p. had *MET* amplification by FISH and NGS, mRNA upregulation by nCounter, protein overexpression by IHC and receptor activation as detected by IHC with a pMET antibody. For one of these p., *MET* amplification was not detected in the availabe pretreatment biopsy. In contrast, the *ROS1* positive p. showed MET overexpression and activation by nCounter and IHC but was not amplified by FISH at progression. The same MET profile was observed in a pretreatment biopsy
-Primary cultures were established from the three p. showing MET alterations. MET positivity was maintained in all cases, particularly if the cells were cultured in presence of an fusion-specific TKI. *ALK* and *ROS1* fusions were also maintained. Sensitivity of the primary cultures to MET inhibitors is currently being tested
-Regarding liquid biopsies, *MET* amplification by NGS was present in 2/24 p. (8%). Both were *ALK*-positive p. at progression to lorlatinib and *MET* amplification was detected in blood or pleural effusion. Other possible mechanisms of resistance detected were secondary mutations in *ALK* (n=5), *ROS1* (n=1) and *EGFR* (n=2) and *FGFR1* amplification (n=1)

Fig 1: Genetic alterations in patients progressing to fusion-specific TKIs

Patients (n=31)	
Type of fusion	
ALK	17
ROS1	9
RET	5
Progression to	
Lorlatinib	13
Alectinib	11
Ceritinib	2
Selpercatib	1
Crizotinib	4
No. samples (n=39)	
Type of sample	
Tissue	7
Blood	26
LCR	3
Pleural effusion	3
No.samples/patient	
1 sample	18
≥2	21



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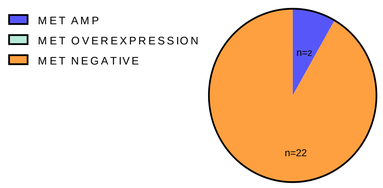
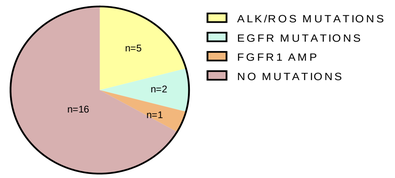


LIQUID BIOPSY=24

RESISTANCE MUTATIONS
ALK
ALK p.G1269A
ALK:p.G1202fs*56
ALK: p.I1171N
ALK: p.L1196M
ALK: p.C1156Y
ROS1
p.G2026R



LIQUID BIOPSY=24
RESISTANCE MUTATIONS

Fig 2: FISH, IHC MET, IHC p-MET and clinical evolution of a patient with MET activation at progression to lorlatinib

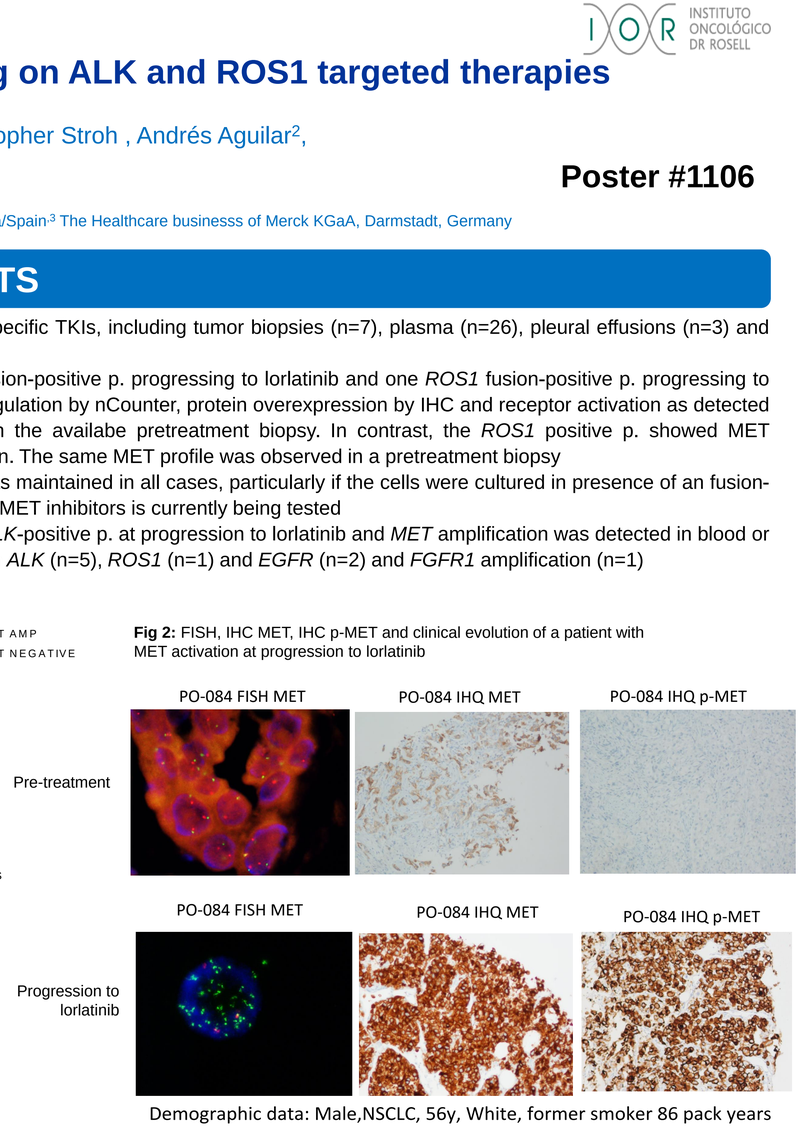
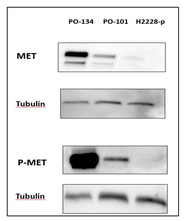
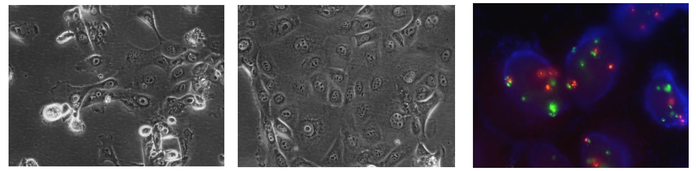


Fig 3. Primary cultures from fusion-positive patients progressing to TKIs with MET alterations. Western blotting (left), micrographs (middle) and FISH (right)





Primary culture treated with Lorlatinib 5 µM +HGF(left) / - HGF(right) 1ng/ml