10 DUAL USE OF MALIGNANT EFFUSIONS FOR GENOTYPING AND DRUG SCREENING IN PERSONALIZED THERAPY OF LUNG CANCER PATIENTS

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BACKGROUND

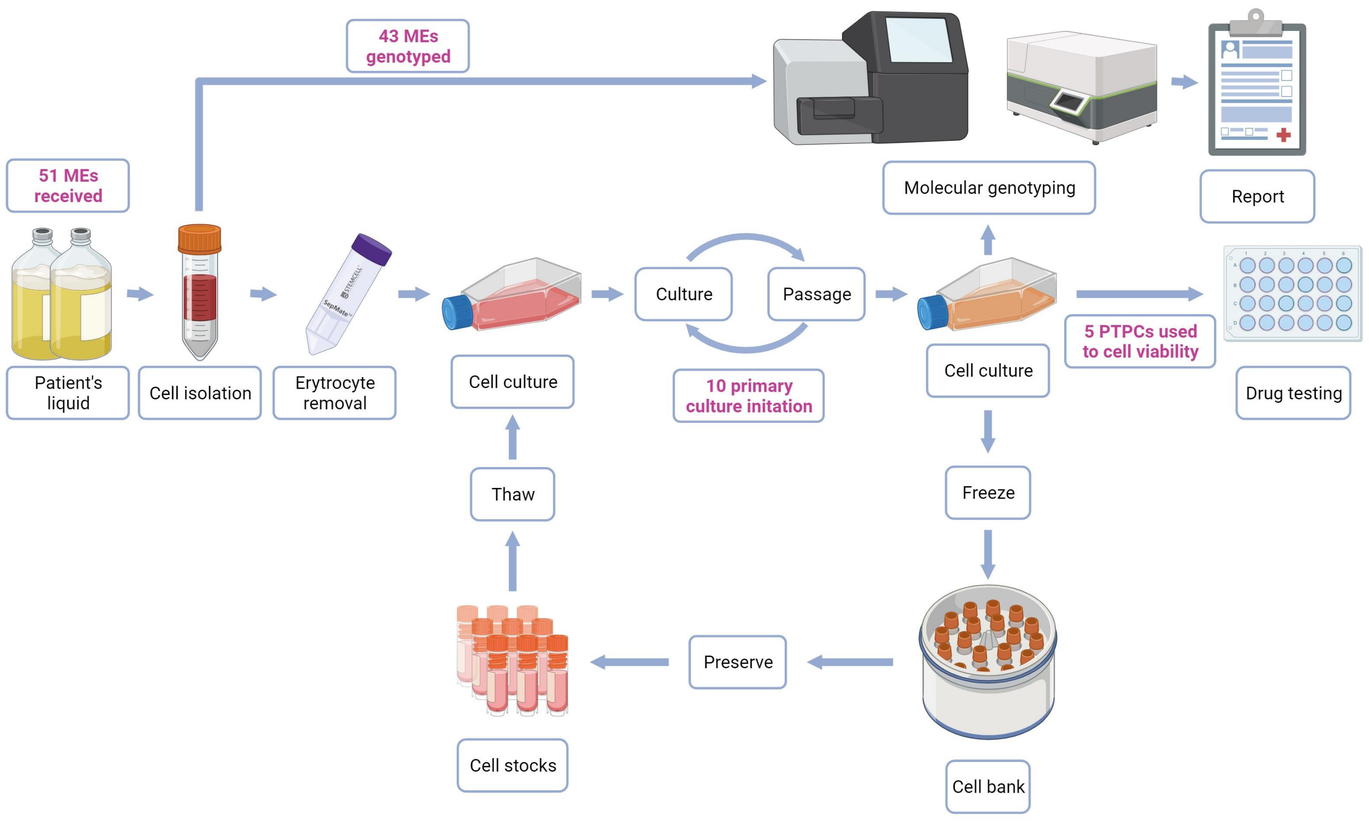
Malignant effusions (MEs) such as ascites, pleural or pericardiac effusions are common in many tumors, either at diagnosis and/or at progression to therapies and have been associated with a poorer prognosis in lung cancer. MEs contain circulating free DNA/RNA (cfDNA/RNA), representing an excellent source of material for tumor genotyping. In addition, pure tumor primary cultures (PTPCs) can be established from the tumor cells present in MEs. These cultures allow the in vitro testing of antitumor drugs and, due to their similarity with the patient's tumor, might be of help in treatment selection.

OBJECTIVES

- In this study, we aimed to incorporate MEs collection from lung cancer (LC) patients into the routine clinical practice in order to determine clinically relevant alterations and to initiate PTPCs to be used in drug testing.

METHODS

MEs from LC patients of the hospitals participating in the study were systematically collected, volumes ranged from 5 to 5,000 mL. Cells were isolated by centrifugation of the entire MEs volume if needed, erythrocytes removed and the remaining cells cultured. PTPCs and ME supernatants were genotyped using a 30-gene NGS panel and gene expression was analyzed using a commercial panel containing 770 mRNA hybridization probes. Cells were treated with specific drugs depending on their molecular alterations, viability was determined by MTT.



RESULTS

Figure 1: Heatmap of alterations found in MEs genotyped. Mutations, gene amplifications or fusions were detected in 38/43 (84.4%). In red are the patients from which we have paired tissue samples, in all the cases we had coincident alterations.

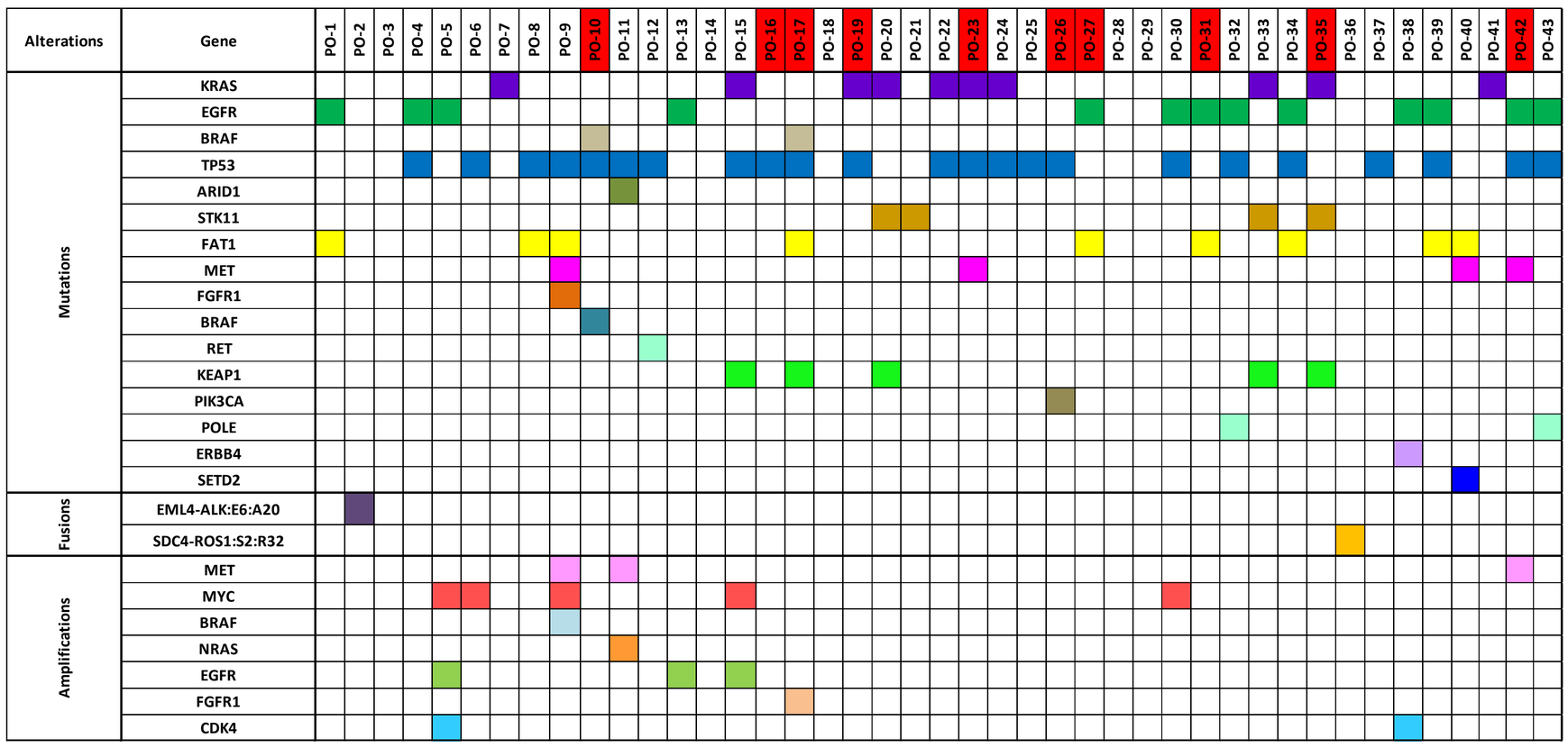


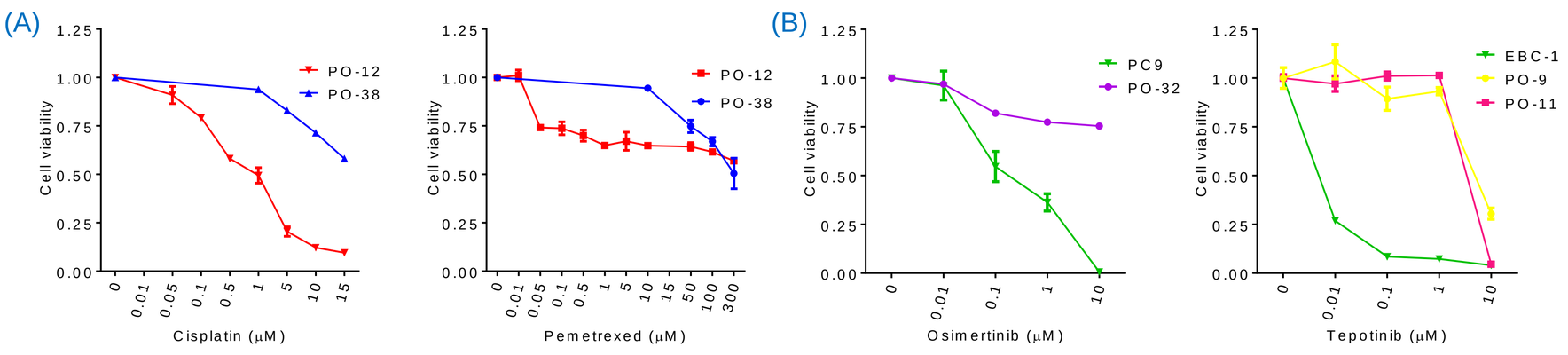
Table 1: Characteristics of the fluids analyzed in the project.

Fluid Characteristics	Total of samples n=43	PTPCs n=10
Type of fluid		
Pleural effusion (MPE)	40 (93 %)	10 (100.0 %)
Ascites	3 (7 %)	0 (0.0 %)
Cytology (presence of tumor cells)		
Positive	6 (14.0 %)	3 (30 %)
Negative	0 (0.0 %)	0 (0.0 %)
ND	37 (86.0%)	7 (70.0 %)
Histology		
Adenocarcinoma	42 (97.7 %)	10 (100.0 %)
Squamous Cell Carcinoma	1 (2.3 %)	0 (0.0 %)
Paired blood or FFPE samples		
Yes	10 (23.3 %)	7 (70.0 %)
No	33 (76.7 %)	3 (30 %)
Collection time		
Baseline	17 (39.5 %)	5 (50.0 %)
Progression	17 (39.5 %)	4 (40.0 %)
ND	9 (20.9 %)	1 (10.0 %)
Clinically relevant alterations		
Detected	38 (88.4 %)	9 (90.0 %)
Not detected	5 (11.6 %)	1 (10.0 %)

Table 2: Characteristics and cell viability of the 5 PTPCs tested by MTT.

Sample	Fluid	Primary tumor	Basal/Progression	Progression to	Alterations	IC_{50} (µM)
PO-12	Pleural	Lung ADC	Basal	–	MET polisomy	0.8 (cisplatin) > 300.0 (pemetrexed)
PO-38	Pleural	Lung ADC	Basal	–	EGFR p.S768_D770dup	15.0 (cisplatin) 300.0 (pemetrexed)
PO-9	Pleural	Lung ADC	Progression	Tepotinib	MET amp MET p.D1228N/H and p.Y1230H	4.7 (tepotinib)
PO-11	Pleural	Lung ADC	Progression	Tepotinib	MET amp; NRAS amp	4.5 (tepotinib)
PO-32	Pleural	Lung ADC	Progression	Osimertinib	EGFR p.L858R	>10.0 (osimertinib)

Figure 2: Dose-response plots of the PTPCs in Table 2 to selected drugs. (A) Baseline PTPCs treated with chemotherapeutic agents (B) PTPCs at progression treated with the targeted therapies received by the corresponding patients, PC9 and EBC-10 has been used as controls.



PO-12 was treated with **carboplatin** and **pemetrexed** with **partial response** during 6 months.
PO-38 was treated with **carboplatin** and **pemetrexed** with **stable disease** from July 2023.

Figure 3: Micrographs of primary cell cultures. (A) PTPCs from patients, PO-12 and PO-38, at baseline and (B) PTPCs derived from patients PO-9 and PO-32 who progressed to treatment.

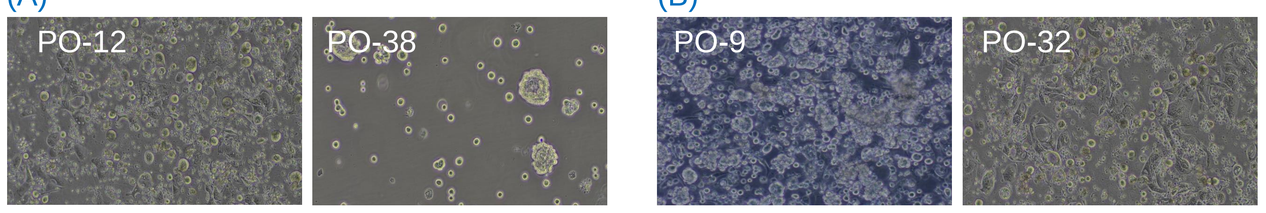
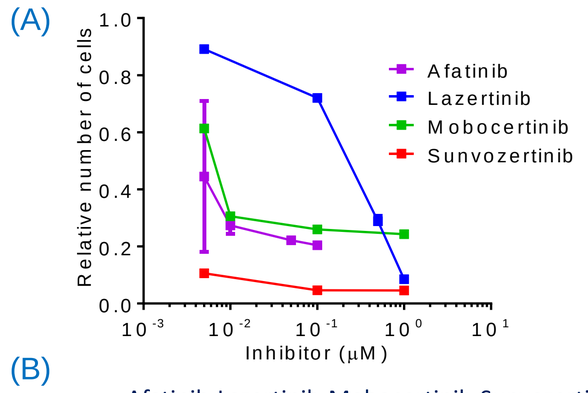


Figure 4: Cell viability of PO-38 treated with possible treatments to be used after chemotherapy progression. (A) dose-response plots and (B) IC_{50} values of PO-38 to selected inhibitors.



(B)

	Afatinib	Lazertinib	Mobocertinib	Sunvozertinib
IC_{50} (µM)	< 0.005	0.300	0.007	< 0.005

CONCLUSIONS

MEs can be prospective collected in the clinical setting and used for a dual purpose. cfDNA and cfRNA can be isolated from the fluid fraction and employed for genotyping, while cells can be cultured and antitumor drugs tested.