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EORTC-SPECTA Arcagen study, comprehensive genomic profiling and treatment adaptation of rare thoracic cancers

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Summary

Tables and Figures

ABSTRACT

Introduction: Arcagen (NCT02834884) is a European prospective study aiming at defining the molecular landscape of rare cancers for treatment guiding. We present data from the cohort of rare thoracic tumors.

Methods: Patients with advanced pleural mesothelioma (PM) or thymic epithelial tumors (TET) underwent genomic profiling with large targeted assay [>300 genes, tumor mutational burden (TMB), microsatellite instability (MSI) status] on formalin-fixed paraffin embedded (FFPE) or plasma samples. EORTC molecular tumor board (MTB) advised for biomarker-guided treatments.

Results: 102 patients recruited from 8 countries between July 2019-May 2022 were evaluable: 56 with PM, 46 with TET (23 thymomas, 23 thymic carcinomas). Molecular profiling was performed on 70 FFPE samples (42 PM, 28 TET), and 32 cases on ctDNA (14 PM, 18 TET), within a median turnaround time of 8 days from sample reception. We detected relevant molecular alterations in 66 out of 102 patients (65%; 79% PM, 48% TET), 51 of 70 FFPE samples (73%; 90% PM, 46% TET) and 15 of 32 plasma samples (47%; 43% PM, 50% TET). The most frequently altered genes were *CDKN2A/B, BAP1, MTAP* in PM and *TP53, CDKN2A/B, SETD2* in TET. The TMB was low (mean 3.2 Muts/MB), 2 PM had MSI-high status. MTB advised for molecular-guided treatment options in 32 situations, for 17 PM and 15 TET patients (75% clinical trial option, 22% off label drug or compassionate use, 3% early access program).

Conclusions: Molecular testing and MTB discussion are feasible for patients with rare thoracic cancers and allowed broadening of treatment options for 30% of the cases.

INTRODUCTION

Pleural mesothelioma (PM) and thymic epithelial tumors (TET) are rare aggressive thoracic malignancies. There are 30.000 new PM cases per year worldwide, with an incidence in Europe of <1 per 100.000 per year.[1] It is related to asbestos exposure, with clinical onset occurring after decades of latency. Histological classification into three major subtypes - epithelioid, sarcomatoid, and biphasic - is important in terms of prognosis and treatment.[2] The most recent therapeutic regimens consist of combination with immunotherapy targeting PD-(L)1 and CTLA-4, and antiangiogenics, bringing the median overall survival of patients with unresectable PM up to 1.5 years.[3–5] However, when progressing, there is a lack of effective treatment options. The annual incidence of TET (thymomas and thymic carcinomas together) is 1.3-3.2 cases per million worldwide.[6] Thymomas are subdivided into sub-types, and tumor stage and resection status are fundamental prognostic factors.[7] Multimodal treatments including radiotherapy, surgery and chemotherapy are key to disease control. Multi-tyrosine kinase inhibitors, biological agents and immunotherapy may have a role in the metastatic recurrent setting,[2] although effective predictive biomarkers are lacking[8]. The low incidence of these diseases hindered the running of randomized trials for new therapeutic strategies. Rare cancers are also under-represented in genomic profiling programs. All this contributes to the scarcity of targeted therapies available for PM and TET.[2]

Arcagen is a European Organisation for Research and Treatment of Cancer (EORTC)-SPECTA prospective multicohort and multicenter study aiming at performing genomic profiling, associated with a molecular tumor board (MTB) for treatment recommendation, for patients with rare cancers across Europe. Here we present the first results of the cohort of rare thoracic malignancies.

METHODS

Patients older than 12 with a diagnosis of locally advanced or metastatic mesothelioma of pleura and pericardium, thymoma, thymic carcinoma, regardless of the number of treatment lines received, were recruited. A recent tumor specimen was requested for analysis (less than 2-year-old). If non available, or failed quality control at the central biobank, a liquid biopsy was performed. Clinical-pathological data were collected according to the EORTC-SPECTA protocol (NCT02834884).[19] All patients provided written informed consent for sample collection and genomic analysis. The SPECTA Arcagen study was approved by ethical committees: - Commissie voor Medische Ethiek ZNA (Belgium), reference 4881, date 10/03/2021 - Comissao de Etica para a Saude de CHLO (Portugal), reference 2150, date 28/06/2021 - Eticka komise pro Multicentricka Klinicka Hodnoceni, Fakultini Nemocnice v Motole (Check Republic), reference 017/08, date 01/12/2021. This study has been conducted in accordance with international standards of ethical principles for medical research (Declaration of Helsinki, Good Clinical Practice guidelines).

Comprehensive genomic profiling was performed with a targeted next generation sequencing (NGS) test (FoundationOne®CDx) of >300 genes, including MSI and TMB, using DNA isolated from FFPE tumor tissue specimens. If tumor tissue was not available or inadequate, the analysis was performed by FoundationOne®Liquid CDx on ctDNA isolated from plasma. FoundationOne®CDx and FoundationOne®Liquid CDx have high accuracy and analytical sensitivity, although there may be some differences between the two tests due to the nature of the samples they analyze. They also depend on the specific genetic alterations being analyzed, tumor heterogeneity, and the quality of the sample. No TMB calculation was reported at the beginning of the project using FoundationOne®Liquid CDx.

Each individual genomic report was reviewed and discussed by the EORTC SPECTA multidisciplinary MTB, which met monthly, composed of experienced oncologists, biologists, bioinformatics and geneticists, expert in molecular medicine and thoracic cancers management.

Its primary objective was to analyze the NGS report to suggest personalized treatment strategies in the context of the patient pathology and status. This process involved evaluating, in addition to what was concluded in the FoundationOne®CDx or FoundationOne®Liquid CDx report, the pathogenicity and the targetability of individual molecular alterations according to the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Only pathogenic or likely pathogenic variants were considered. Matched therapies and targeted agents were recommended where possible based on existing preclinical and clinical data, available within clinical trials not restricted to EORTC trials, or outside a trial.

RESULTS

Characteristics of the study cohort

122 patients from 8 different countries were registered between July 2019 and May 2022 (Supplementary Figure 1 and Supplementary Table 1). 102 patients (84%) were evaluable, with adequate samples for molecular profiling: 56 PM and 46 TET (23 thymic carcinomas and 23 thymomas). Patient and disease characteristics are summarized in Table 1. At study enrollment, PM patients were older than TET (72 and 60 years, respectively). There was a prevalence of men among PM patients (63.7%) and of woman in the TET cohort (54.3%). 58% of the patients had been exposed to tobacco (61% PM, 54% TET). All histologic subtypes of MP and thymomas were represented. Most of the patients were pretreated in the advanced setting at the time of the MTB discussion (up to 5 previous lines of therapy) (Table 1).

Table 1: Patient and disease characteristics

	All cancers, n=102 N (%)	**Mesothelioma, n=56 N (%)**	**Thymic epithelial tumors, n=46 N (%)**
Age, median [IQR]	70 [55-74]	72 [67-75]	60 [43-70]
Sex, female	37 (36.3%)	12 (21.4%)	25 [54.3%]
Smoking status Never Former Current NA	39 (38%) 48 (47%) 11 (11%) 4 (4%)	21 (38%) 29 (52%) 5 (9%) 1 (2%)	18 (39%) 19 (41%) 6 (13%) 3 (7%)
Histology		• Epithelioid: 33 (59%) • Sarcomatoid: 4 (7%) • Biphasic: 5 (9%) • Others: 4 (7%) • NA: 10 (18%)	Thymic carcinoma: 23 (50%) Thymoma: 23 (50%) • A: 1 (4%) • AB: 1 (4%) • B1: 1 (4%) • B1/B2: 1 (4%) • B2: 6 (26%) • B2/B3: 4 (17%) • B3: 4 (17%) • NA: 5 (22%)
Localized or locally advanced disease at diagnosis	29 (28.4%)	5 (8.9%)	25 (54.3%)
Number of previous lines of therapy for metastatic disease, median (range)	1 (0-5)	1 (0-5)	1 (0-5)
Biopsy site/specimen Primitive tumor Nodal metastasis Metastasis other Blood	48 (47%) 2 (2%) 20 (20%) 32 (31%)	35 (62%) 2 (4%) 5 (9%) 14 (25%)	12 (26%) / 16 (35%) 18 (39%)
MSI-status High Stable NA	2 88 12	2 51 3	0 37 9
TMB Muts/MB, mean (range)	3.6 (0-61) NA: 13	4 (0-61) NA: 3	3.1 (0-15) NA: 10

Abbreviations: IQR, interquartile range; MB, megabase; MSI, microsatellite instability; NA, not available;

TMB, tumor mutational burden.

Samples analysis

Figure 1 describes the sample workflow and reasons for rescue with liquid biopsy. Considering all tumor types together, the molecular analysis of PM was performed on formalin-fixed paraffin embedded (FFPE) tissue samples in 42 cases (75%), while 14 cases (25%) were analyzed by liquid biopsy. Conversely, the genomic analysis was done on tissue in 28 TET patients (61%), while 18 cases were tested by liquid biopsy (39%). The main reason for rescue was poor FFPE quality (19 cases, 60%), no sufficient FFPE available (22%) or FFPE material older than 2 years (18%). The median turnaround time (TAT) from samples acquisition from each center to the molecular report was 8 days for both analyses (thus excluding the previous steps of patient registration and sample preparation).

Figure 1: Sample workflow and MTB recommendations
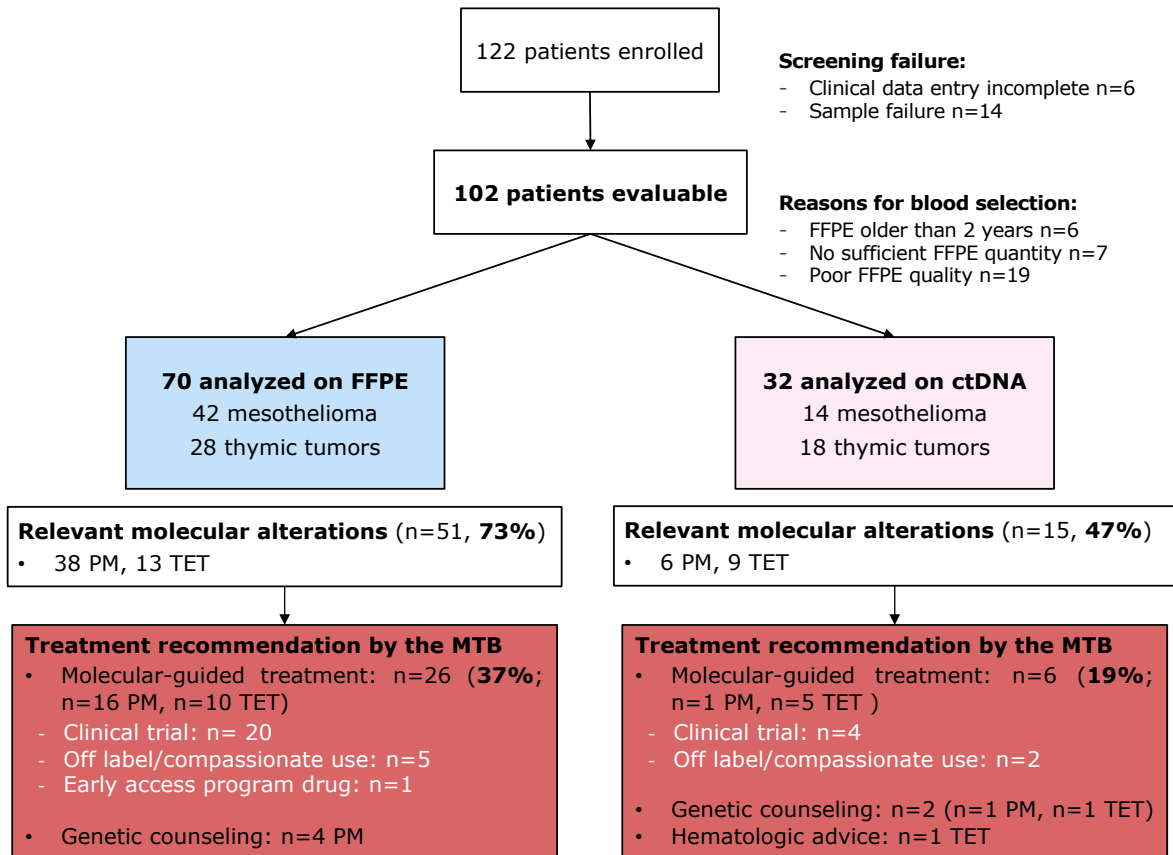
Workflow summarizing the operational settings of the EORTC Arcagen project from patient enrollment to molecular tumor board recommendations. In pink, molecular analysis performed on plasma, in blue on FFPE samples.

Genomic profiling results and MTB recommendations

The oncoplot in Figures 2 reports the 20 most frequently detected alterations in each cancer type. We detected relevant molecular alterations in 66 out of 102 patients (65%). To note, no ESCAT tier 1 or 2 molecular targets were detected. MTB recommendations are summarized in Figure 1.

Pleural mesothelioma

Overall, 79% of PM cases (n=44) harbored at least one molecular alteration. The most frequent aberrations in MP were deletions or rearrangements of *CDKN2A/B*, single nucleotide variants (SNV) or deletion of *BAP1* and *NF2,* deletions of *MTAP* and SNV of *TP53* (Supplementary Figure 2). The detection rate was higher in FFPE with alterations identified in 38 out of 42 samples (90%) and limited to 6 out of 14 plasma samples (43%). The tumor mutational burden (TMB) was generally low (mean 4 Muts/MB), however 2 PM cases had microsatellite instability (MSI)-high status. MTB advised for potential treatment options in 17 situations (30% of the PM cohort). Recommendation for germline testing and eventual genetic counseling to be performed locally was given for 5 patients, mostly due to the detection of pathogenic variants in *BAP1* of suspected germline origin.

Thymic epithelial tumors

In TET, 48% of the cases (n=22) carried at least a molecular alteration. We mostly detected SNV of *TP53*, SNV or deletion of *CDKN2A/B*, SNV of *SETD2* and deletions of MTAP (Supplementary Figure 3). For TET, the detection rate was 50% in plasma samples (9 out of 18 cases) and 46% in FFPE samples (13 out of 28 cases). The TMB was generally low (mean 3.1 Muts/MB). MTB advised for potential treatment options in 15 situations (33%).

One hematologic consultation was suggested due the detection of clonal hematopoiesis in liquid biopsy, associated with an increased risk of developing a hematologic neoplasm.

Combined analysis

Considering PM and TET together, the detection rate of pathogenic or likely pathogenic molecular alterations was higher in FFPE samples than in liquid biopsy (73% vs 47%). MTB advised for treatment options in 32 situations (75% clinical trial option, 22% off label drug or compassionate use, 3% early access program) (Supplementary Figure 4). Hence, a molecular-matched treatment indication as further line of treatment in case of disease progression was given for 31% of the overall population. Overall, tissue and plasma analysis informed the treatment indication in 37% and 19% of the cases, respectively. A detail of MTB treatment indications is reported in Supplementary Table 2. For 11 patients there was no treatment adaptation based on the MTB recommendation due to an early death linked to a disease progression, and in 19 cases the disease had not progressed at time of database lock.

Figure 2: Oncoplot of the 20 most common molecular alterations
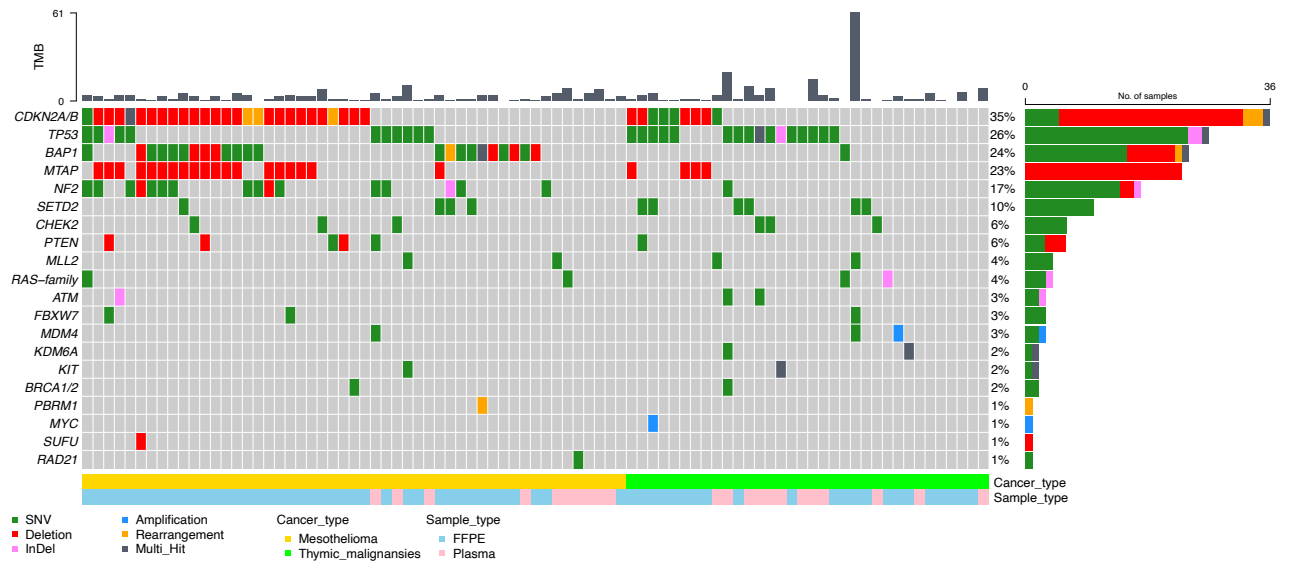
Oncoplot summarizing the main molecular alterations in mesothelioma (yellow squares, left part of the plot) and thymic malignancies (green squares, right part of the plot). TMB is highlighted on the top of the figure. Molecular alterations: SNV in green, deletion in red, InDel in pink, amplification in blue, rearrangement in yellow and multi-hits in dark grey.

DISCUSSION

Comprehensive genomic profiling has emerged as a powerful tool for understanding the biology of rare cancers and identifying potential targets for therapy. Overall, we show that genomic profiling has the potential to inform personalized treatment strategies for 31% of patients with advanced rare thoracic tumors in an efficient manner (TAT of 8 days from receipt of the sample), with treatment recommendation discussed during a pan-Europe MTB. The low failure rate (less than 20%) and successful operational set-up of the project highlight the feasibility of systematic screening for rare cancers.

Previous studies have explored the molecular landscape of PM and TET using a variety of genomic profiling techniques. Results of our analysis are in line with literature reports on PM,[9–13] which have identified a number of recurrent genetic alterations and signaling pathways that may contribute to the development and progression of PM. Some of the key findings include frequent mutations in tumor suppressor genes such as *CDKN2A/B, TP53, BAP1 and NF2,* as well as in genes involved in chromatin remodeling, such as *SETD2*. Mutations in DNA damage response genes were also represented. We confirmed that also TET exhibit a diverse range of genomic alterations involving cell cycle and chromatin regulation.[8,14] Most frequently altered genes include *TP53 (*one Y220C mutant, target of future potential drug),[15] loss of *CDKN2A* and MTAP, mutations in *KIT*, *PTEN* and *RAS* family genes. Nevertheless, while genomic profiling has provided valuable insights into the molecular landscape of these cancers, previous studies did not address how this may translate into clinical utility. Indeed, many unanswered questions and major barriers exist on this regard, such as the definition of the clinical relevance of detected molecular alterations, clinical trials availability at the time of tumor profiling and drugs accessibility. The aim of our study was to bypass these limitations, by providing an efficient and clinically relevant molecular profile in support to patients and treating clinicians for improving the management of rare thoracic cancers in Europe. This is also key to better select patients for

clinical trials assessing targeted therapies, considering that the 75% of the MTB recommendations was a clinical trial option (phase I or basket trial in most of the cases).

Limitations to this study include a short follow-up after enrollment, limiting availability of data about the implementation of MTB recommendations, thus possible clinical benefit associated with it. Nevertheless, we noticed that in 34% of cases with a MTB recommendation, death due to disease progression hampered the possibility to adapt the therapy, suggesting that tumor profiling should be performed earlier at cancer diagnosis rather than during treatment for metastatic disease, when clinical conditions could prevent the inclusion in a clinical trial. Another pitfall relies into the intrinsic limitation of using circulating tumor DNA (ctDNA) for tumor genotyping. The detection rate may have been overestimated, particularly in the liquid biopsy cohort, due a misinterpretation of some clonal hematopoiesis variants (e.g., *TP53,* that is non univocally somatic). Nevertheless, previous evidence showed 80% detection of at least one molecular alteration through ctDNA analysis in TET patients,[16] and mutations and rearrangements could be preferentially detected in liquid than tissue biopsy.[17] The inconsistent detection rate in PM and TET could be largely explained by clinical-pathological differences influencing ctDNA shedding.[18]

Overall, the results of this study highlight the role of academic MTB in expanding diagnostic and targeted-treatment options for patients affected with rare thoracic tumors and demonstrate the operational feasibility on a multinational scale. Tumor tissue sequencing remains the best approach for molecular characterization, but liquid biopsy may play an important role for cases with inadequate tissue sample. Access to drugs remains critical for patients with rare cancers.

Data availability Statement

Data can be access in line with EORTC data sharing policy (https://www.eortc.org/app/uploads/2023/06/L-01-POL-01.pdf). The link for data request can be found here: https://www.eortc.be/services/forms/erp/request.aspx

Sequencing data generated in this study were uploaded in the European Genome-phenome Archive (EGA), dataset reference number EGAD50000000168.

Author Contributions

Co-first authors: Tagliamento and Morfouace have contributed equally

Conceptualization: Blay, Morfouace, Robert

Data curation: Tagliamento, Morfouace, Loizides, Poncin, Fernandes

Formal analysis: Tagliamento, Morfouace, Loizides

Funding acquisition: Blay, Morfouace, Robert

Investigation: Oliveira, Greillier, Raimbourg, Toffart, Chatelier, Cloarec, Sullivan, Brasiuniene, Peron, Oselin, Besse, Girard

Methodology: Morfouace, Blay, Besse, Girard

Project administration: Robert, Poncin, Fernandes

Roles/Writing - original draft: Tagliamento, Morfouace, Loizides, Besse, Girard

Writing - review & editing: all the Authors

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NF2 , *CDKN2A* , and *CUL1* in Malignant Pleural Mesothelioma. *Cancer Res.* **75**, 264–269 (2015).

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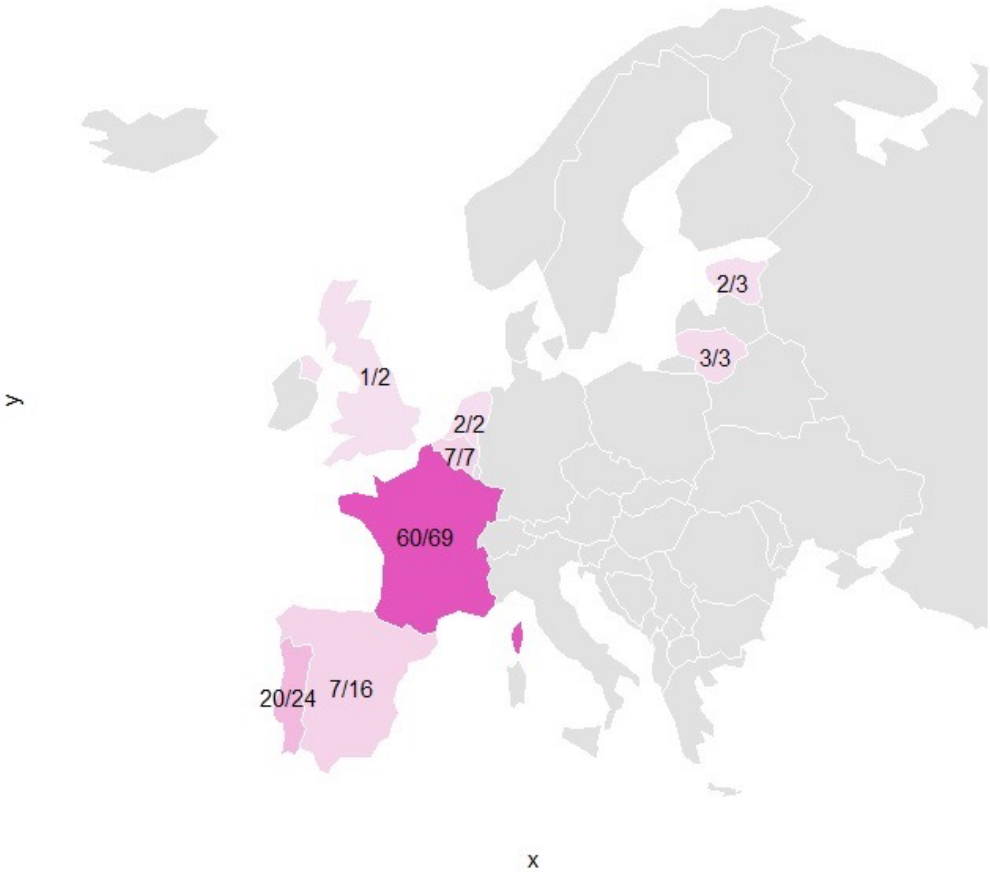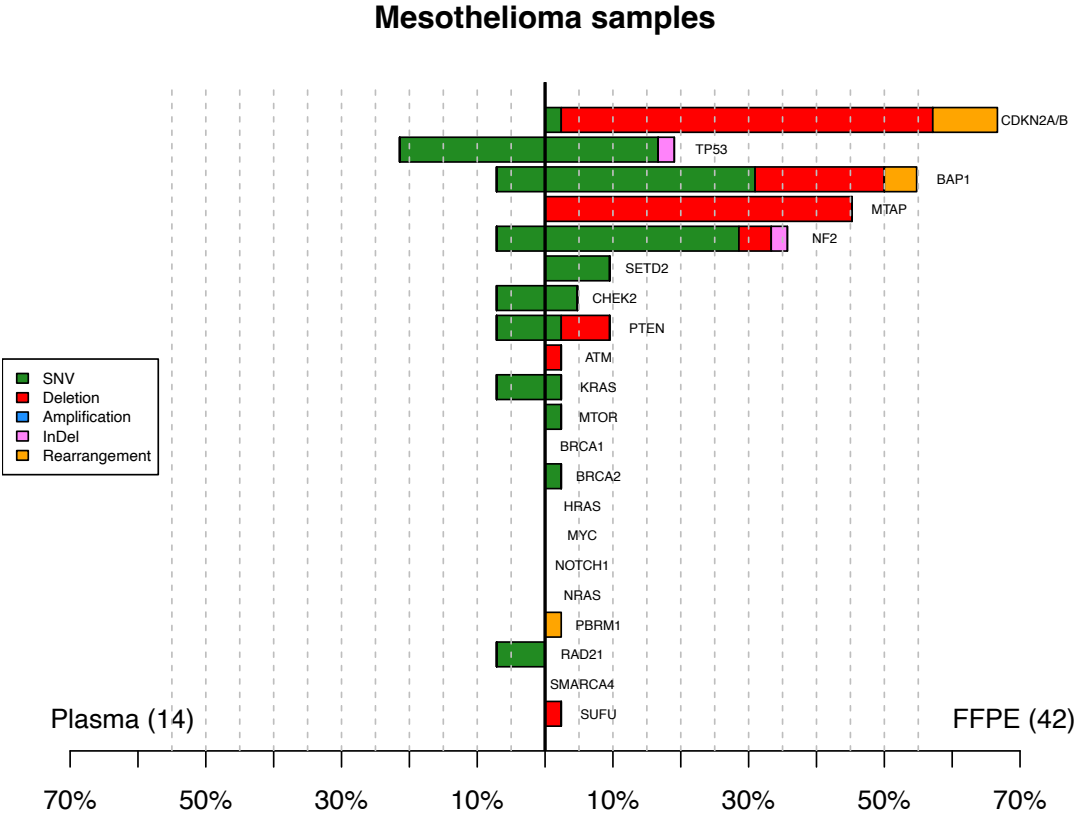
ESMO Open **5**, e001075 (2020).

SUPPLEMENT

Supplementary Table 1: List of participating centers

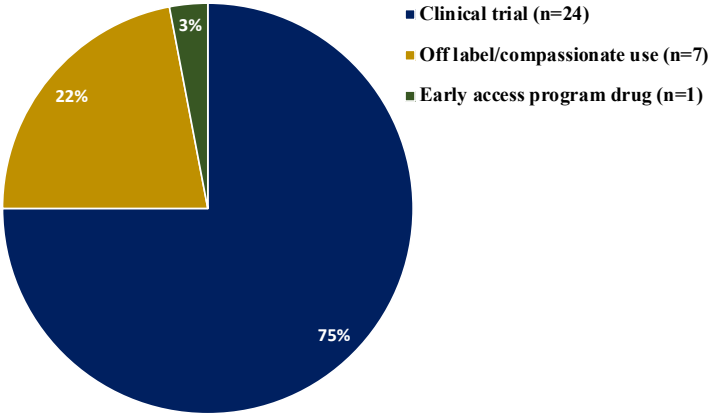| Center name	Country	Registered	Enrolled
Cen.Hospitalar Vila Nova Gaia	PT	2	1
Hospital Universitario Infanta Leonor	ES	4	0
U.Z. Leuven - Campus Gasthuisberg	BE	2	2
CUF hospitals - Hospital CUF Tejo	PT	2	2
C.H.U. Sart-Tilman	BE	5	5
CHU de Lyon - Hopital Lyon Sud	FR	3	3
North Estonia Medical Centre - Mustamäe unit	EE	3	2
CHU de Grenoble - La Tronche - Hopital A. Michallon	FR	11	8
Centre Hospitalier Avignon	FR	7	7
Gustave Roussy	FR	11	9
Centre Oscar Lambret	FR	1	1
Institut de Cancerologie de l'Ouest (ICO) - Institut de Cancerologie de l'Ouest (ICO) - Centre Rene Gauducheau	FR	10	9
Centre Eugene Marquis	FR	1	0
Assistance Publique - Hopitaux de Marseille - Hopital Nord (APHM)	FR	14	13
Academisch Ziekenhuis Maastricht	NL	2	2
Hospital Clinic Universitari de Barcelona	ES	6	3
Hospital De La Santa Creu I Sant Pau	ES	6	4
Lithuanian Oncology Center-Nat. Cancer Inst	LT	3	3
Institut du Cancer de Montpellier	FR	3	2
The Christie NHS Foundation Trust	UK	1	1
Cambridge University Hospital NHS - Addenbrookes Hospital	UK	1	0
Instituto Portugues De Oncologia - Instituto Portugues de Oncologia do Porto	PT	19	17
Clinique Mutualiste de l'Estuaire - Centre d'oncologie	FR	7	7
CUF hospitals - Hospital CUF Descobertas	PT	1	0

Supplementary Figure 1: Map of participating countries

Mesothelioma samples

Thymic_malignansies samples
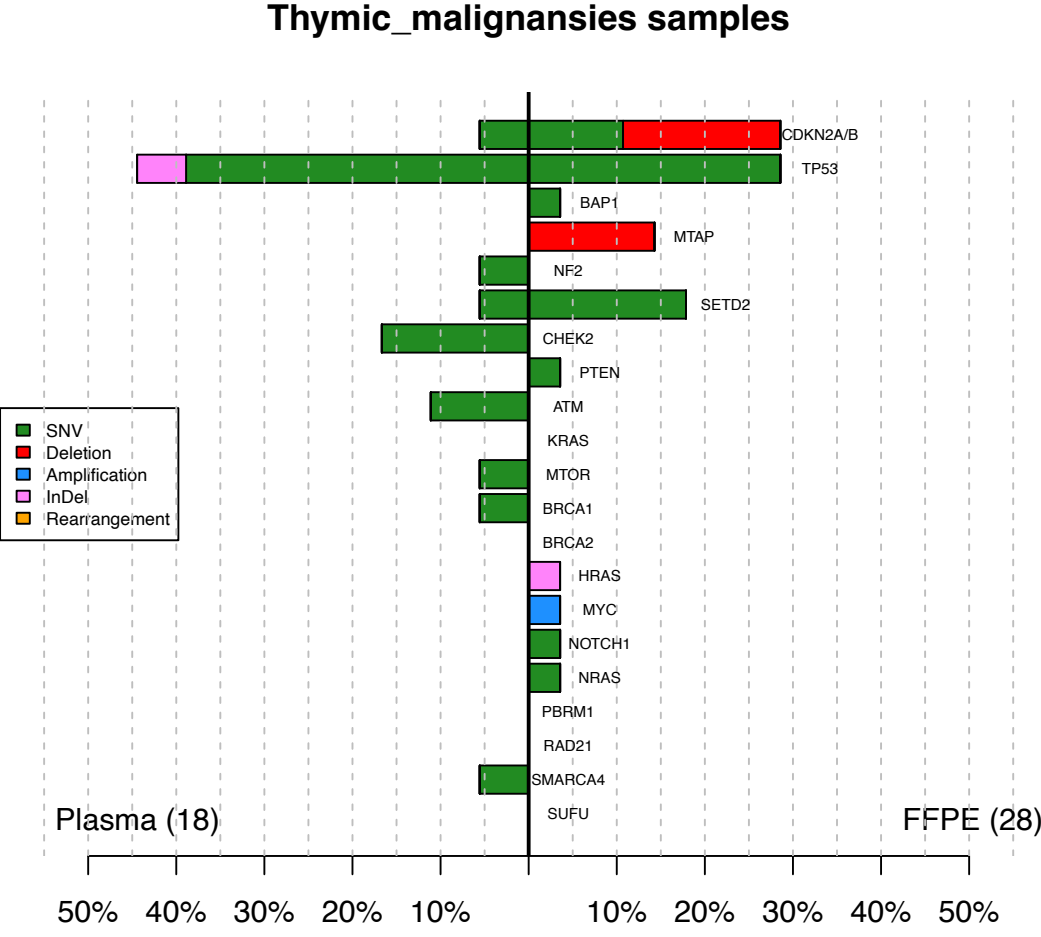
Supplementary Figure 4: Type of TMB treatment orientation

- Clinical trial (n=24)
- Off label/compassionate use (n=7)
- Early access program drug (n=1)

Supplementary Table 2: MTB treatment recommendations

MTB recommendation	n° patients
Investigational MAT2A inhibitor	7
Investigational p53-MDM2 inhibitor + CDK4/6 inhibitor	5
Investigational PARP inhibitor + immune checkpoint blockers	4
Immune checkpoint blockers	4
Investigational AKT inhibitor	3
mTOR inhibitor	2
KRAS G12C inhibitor	1
MEK inhibitor	1
Farnesyltransferase inhibitor	1
MET inhibitor	1
Investigational IGF-1R-targeted drug	1
Imatinib	1
FGFR inhibitor	1
Total	**32**