Epigenetic genes alterations in metastatic solid tumors: results from the prospective precision medicine MOSCATO and MATCH-R trials

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Abstract

Purpose: Although the role of epigenetic alterations in oncogenesis has been well studied, their prevalence in metastatic solid tumors is still poorly described. We therefore aimed at: (i) describing the presence of epigenetic gene alterations (EGA) - defined by an alteration in a gene encoding an epigenetic regulator; and (ii) evaluating their relationship with clinical characteristics and outcome in patients (pts) included in prospective molecular profiling trials.

Patients and methods: On-purpose tumor biopsies from pts with metastatic solid tumors enrolled in the Gustave Roussy-sponsored MOSCATO (NCT01566019) and MATCHR (NCT02517892) trials were molecularly profiled using Whole Exome Sequencing (WES). Alterations in 176 epigenetic genes were assessed and classified as pathogenic variants (PV) or non-pathogenic variants by a molecular tumor board. Clinical characteristics and outcome were collected.

Results: Between Dec 2011 and Oct 2016, WES was successfully performed in 292 pts presenting various solid tumors. We found 496 epigenetic gene alterations in 134 patients (49%), including 237 pathogenic variants in 86 patients; 63 tumor samples (47%) presented ≥3 EGAs. The median number of previous treatment lines was 3 (1 – 10). The most frequently altered genes were *KMT2D* and *KMT2C* (16% each), *ARID1A* and *SETD2* (10% each) and *KMT2A* (8%).; 31% of EGA co-occurred with a driver gene alteration ($p < 0.001$). Outcome was not correlated with the presence of EGA.

Conclusions: Epigenetic alterations occur frequently in metastatic solid tumors. With the current development of epigenetic modifiers, they increasingly represent actionable targets. Such genes should now be systematically analyzed in molecular profiling studies.

Background

Epigenetic dysregulation and chromatin remodeling abnormalities have been widely linked to cancer initiation, progression and resistance to therapies [1–3]. Epigenetic mechanisms refer to changes in gene function or transcription levels that do not result from a change in the primary DNA sequence [1]. The main levels of epigenetic regulation comprise DNA modifications, histone post-translational modifications, chromatin remodeling, RNA post-transcriptional regulation and non-histone protein post-translational modifications [4, 5]. These are ensured by more than 800 epigenetic proteins, which can recognize (“reader”), add (“writer”) or remove (“eraser”) epigenetic marks from their specific targets [6, 7]. While enzymes modifying DNA methylation most often act on their own, chromatin remodelers belong to multiprotein complexes, such as the best characterized SWItch/Sucrose Non-Fermentable (SWI/SNF) or Polycomb complexes [3, 8, 9].

This role of epigenetic dysregulation in cancer supported the development of various small molecule inhibitors targeting epigenetic proteins, such as first- and second- generation histone deacetylase and DNA methyltransferase inhibitors. More recently, a novel wave of third-generation compounds enlarged epigenetic targeting options, including bromodomain and extra-terminal domain inhibitors (BETi), protein

methyltransferase inhibitors (such as protein arginin N-methytransferase inhibitors, PRMT5i) and histone demethylase inhibitors (such as lysine-specific histone demethylase 1, LSD1i) [7]. Clinical results obtained with these therapies have been promising in hematological tumors, and led to the registration of some of these ([10, 11]). By contrast, the efficacy of first- and second-generation epigenetic drugs in solid tumors has been disappointing. However, recent results obtained with histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2) inhibitors in SMARCB1-defective tumors [12], isocitrate dehydrogenase (IDH1) inhibitors in *IDH*-mutant cholangiocarcinoma [13] and PRMT5 inhibitors in adenoid cystic carcinoma [14–16] have challenged this paradigm, and suggested that, like targeted therapies, epigenetic-modifying drugs should be used in a ”precision medicine” approach based on a molecular selection.

The prevalence of alterations in genes encoding a protein involved in epigenetic regulation processes, hereafter defined as “epigenetic gene alteration” (EGA), has however beenpoorly studied in clinical samples. This mainly results from the fact that most epigenetic genes have traditionally not been included in the conventional “cancer gene panels” until recently, and also perhaps because most alterations identified by next generation sequencing (NGS) technologies are still of unknown pathogenic significance, thereby limiting their clinical relevance. The increasing use of whole exome sequencing (WES) is now allowing a comprehensive and systematic characterization of genetic alterations in tumors. Still, most studies have focused on alterations in driver oncogenes or DNA repair genes, and the prevalence of EGA alterations has not been specifically studied in solid tumors.

Here, we provide a comprehensive description of the occurrence of EGAs detected by WES analysis of patients with advanced or metastatic cancers enrolled in the Gustave Roussy molecular profiling trials MOSCATO and MATCH-R, compare it to data from the TCGA cohorts, and evaluate the relationship of the presence of EGAs with clinical characteristics and outcome.

Methods

Study Design and Procedures

We retrospectively analyzed whole exome sequencing (WES) data from patients enrolled between December 2011 and October 2016 within the Molecular Screening for Cancer Treatment Optimization trial (MOSCATO; NCT01566019) or MATCH-R trial (NCT02517892). MOSCATO and MATCH-R are prospective, single-center, precision-medicine trials that are still ongoing and recruiting patients with unresectable, advanced or metastatic solid tumors and hematologic malignancies and refractory to at least one prior line of treatment. Both studies aim at prospectively using tumor molecular profiling to guide treatment decisions and match therapy (off-labeled use of approved drugs or enrolment in an early phase trial) on a personalized medicine basis.

All patients signed an informed consent form for the trial and matched genomic analyses. Both trial protocols were approved by an institutional review committee and done in accordance with the

Declaration of Helsinki.

Sequencing Analyses

Patients were considered to have a successful biopsy assessed by a senior pathologist on a hematoxylin and eosin slide based on tumor cellularity (> 10%), on the quality and quantity of the available genetic material after nucleic acid extraction. Genetic material extraction methods were previously described in *Massard et al* [17]. All tumor samples Molecular profiling was assessed by high-throughput next generation sequencing techniques, targeted next-generation sequencing (panel of 75 oncogenes). In cases with tumor cellularity > 30% RNA sequencing and/or whole exome sequencing (WES) were also performed.

Following alignment with bwa-0.7.12 on hg19, bam files were cleaned according to the Genome Analysis Toolkit recommendations. WES somatic variant calling was performed using GenomeAnalysisTK-4.0.10.1 Mutect2 target on 176 epigenetic modifying genes. Annovar-v2018-04-16 with cosmic70, 1000g2015aug, avsnp150, exac03, esp6500siv2, nci60, dbnsfp33a, kaviar_20150923, clinvar_20170905, intervar_20170202 and hrcr1 were used for annotations, and functional prediction was performed using Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, PROVEAN, VEST3, MetaSVM, MetaLR, M-CAP, SIFT and REVEL. Only SNVs within exons of coding genes or splice sites were kept. Then, variants reported in more than 1% of the population in the 1000 genomes (1000g2015aug), Exome Sequencing Project (esp6500siv2), or Exome Aggregation Consortium (ExAC03) were discarded to filter out polymorphisms. Synonymous variants were subsequently filtered out. Mutation Annotation Format conversion and graphical visualization was done using maftools-1.8.0 R-package.

Epigene Alterations

A list of 176 genes encoding epigenetic-modifying proteins (including chromatin remodelers, DNA methyltransferases and DNA demethylases) was built based on current knowledge. Pathogenicity of the missense variants were determined using Rare Exome Variant Ensemble Learner (REVEL) scoring, following Ensemble basic cut-off [18]. Variant with a score strictly above 0.5 were annotated as “likely pathogenic” and under 0.5 as “likely benign”. Nonsense and frameshift variants were manually classified as pathogenic and annotated as “certainly pathogenic”.

Patients’ characteristics

Patients’ clinical characteristics collected included: age, gender, tumor type, type and number of previous lines of treatment, number and type of metastatic sites, disease progression including clinical and/or - radiological progression (as defined by RECIST 1.1 criteria[19]).

TCGA Analysis

TCGA somatic mutations were retrieved using R data package TCGAmutations-0.2.0, importing data from TCGA MC3 exomes dataset [20]. We focused on the 16 cohorts (BRCA, LGG, GBM, COAD, CHOL, READ, OV, UCEC, UCS, LUAD, LUSC, SARC, BLCA, PRAD, KIRC and HNSC) that fit the best the tumor types included in

our cohort and the same 176 genes encoding epigenetic-modifying proteins. Comparison between TCGA cohorts and ours was done using fisher test on all genes which are mutated in at-least 5 samples in one of the cohorts. Genes with a p-value < 0.1 are shown in a forest plot representing the log odd ratio of those genes between the two compared cohorts.

Statistical Analyses

Descriptive statistics were applied to summarize the data including chi-squared test and Fisher's exact test for categorical data and t-test for continuous data. Time-to-event endpoints such as progression-free survival (PFS) and overall survival (OS) were analyzed by Kaplan–Meier estimates. OS was defined as the time from inclusion to death from any cause. The date of data cut-off was October 2019All the analyses were carried out using STATA statistical software (version 14; STATA, College Station, TX) and R statistical software (R version 3.3.0, http://www.R-project.org/.), the 'survival' R package (version 2.37.4, published by T. Therneau).

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

Study Workflow, patients' and tumors' characteristics

Overall, 1223 and 130 patients were enrolled between December 2011 and October 2016 in the MOSCATO (NCT01566019) and MATCH-R trials (NCT02517892), respectively (Fig. 1). A successful tumor biopsy was performed in 948 patients from the MOSCATO trial and 89 patients from the MATCH-R study, which was characterized either by WES, or by comparative genomic hybridization array and a targeted gene panel. WES was successfully performed and analyzed in 292 patients: 206 patients from the MOSCATO trial and 86 patients from the MATCH-R study.

Among these, 143 (49%) patients displayed at least one alteration in a gene encoding a protein involved in epigenetic regulation (herein referred as "epigenetic gene alteration", EGA). After removal of 12 patients whose clinical data cannot be communicated within the frame of this manuscript, we identified 134 patients (46%) with at least one tumor EGA. Patient characteristics are described in Table 1. Briefly, the median number of previous treatment lines was three (range, 1–10), and 79 patients (59%) received three or more lines of prior therapies, including chemotherapy (121 patients, 90%), targeted therapy (75 patients, 56%) and immunotherapy (19 patients, 14%). The most frequent tumor types were non-small cell lung cancer ([NSCLC] 32 patients; 24%), urological cancer (30 patients; 22%) and breast cancer (18 patients; 8% [Figure 2A]). When comparing these frequencies with the tumor types of patients enrolled in the MOSCATO trial, we noted that EGAs seemed to occur more often in urological and lung tumors, and less frequently in gastrointestinal or head and neck cancers (Fig. 2A).

Table 1
Patient characteristics.

Characteristics	Epigenes mutant patients (N = 134)	All MOSCATO patients (N = 948)
Age - years		
Median	59	57
Range	19–81	19–86
Gender		
Female	71 (53%)	463 (49%)
Male	63 (47%)	485 (51%)
Number of previous lines		
Median	3	4
Range	1–10	0–15
≥ 3 lines	80 (60%)	NA
Prior therapies		
Chemotherapy	121 (90%)	NA
Targeted Therapy	75 (56%)	NA
Immunotherapy	19 (14%)	NA
Number of metastatic sites		
Median	2	2
Range	0–4	0–9
≥ 3 sites	32 (24%)	NA
Driver mutation & matched therapies		
Yes	42 (31%)	199 (21%)*
No	92 (69%)	749 (79%)*

Description of patient characteristics with mutant epigenes from our series and from the MOSCATO study.

Epigenetic gene alterations

WES analysis identified 496 EGAs in the 134 of the above-described samples. After adjustment on tumor cellularity, the median allelic frequency of EGAs was 25% (10% – 97%). When analyzing the coding consequence of EGAs, we found that 361 (73%) were classified as missense mutations, 56 (11%) as nonsense mutations, 65 (13%) as frameshift (42 deletions and 23 insertions), eight (2%) as splice site alterations, and six (1%) as non-frameshift insertions or deletions (3 each; Fig. 2B). We next used the REVEL scoring to evaluate the pathogenicity of missense EGA, and found that 116 (23%) were likely pathogenic variants (LPV), 243 (49%) likely benign variants and 16 (3%) variants of unknown pathogenicity; 121 nonsense and frameshift EGAs (25%) were manually annotated as certainly pathogenic variants (CPV), (Fig. 2C). For further analyses, we therefore grouped the CPV and LPV (n = 237; 48% of EGAs), and herein refer to them as “pathogenic variants” (PV).

When examining the co-occurrence of an EGA with a tumor driver gene mutation (defined as an activating alteration in an oncogene), we found that 41 (31%) of the 134 tumor samples also harbored a known driver alteration. This proportion was significantly higher than in the overall tumors profiled in the whole MOSCATO series (21%; $p < 0.017$, Pearson chi-square test; Fig. 2D). Co-occurring driver gene mutations included *FGFR* translocation/amplification (8 patients, 20%), *EGFR* mutation (7 patients, 17%), *ALK* translocation and *BRAF* mutation (5 patients, 12% each).

The median number of EGA per tumor sample was three (range, 1–19), with 41 (31%), 33 (25%), 60 (44%) patients’ tumors presenting one, two, or three or more EGA (Supplementary Figure S1). Among the 60 samples with three or more EGA, the most frequent tumor type was non-small cell lung cancer (13 cases, 22%), and a co-occurring driver gene mutation was found in eight samples (62%) (Supplementary Figure S2). When comparing these samples with those harboring two or less EGAs, the presence of three or more EGAs was significantly associated with a previous treatment by immunotherapy (19/19 patients; $p = 0.001$, Pearson chi-square test and Bonferonni correction for multiple testing). It was neither correlated to prior treatment by chemotherapy (84/121 patients; $p = 0.83$) or targeted therapy (48/75 patients; $p = 0.17$), nor with the number of prior treatment lines ($p = 0.8$), age ($p = 0.2$), or the presence of a driver gene mutation ($p = 0.2$).

Oncoplot of the distribution of EGAs revealed that most frequently altered genes were *KMT2D* and *KMT2C* (16% each of all patients with EGAs), *ARID1A* and *SETD2* (10% each), *KMT2A* (8%), *CREBBP*, *EP300*, *TET1* and *ASXL3* (7% each; Fig. 2E and Supplementary Figure S3).

Pathogenic variants (PV) in epigenetic genes

Overall, 237 pathogenic variants were identified in 86 patients (64%). The most frequent tumor types with PV were urological (22 EGAs; 26%), lung (17 EGAs; 20%) and gastrointestinal (11 EGAs; 13%) (Fig. 3A), with a median number of two PV in each case (range 1–13, 1–19 and 1–13, respectively) (Fig. 3B). Tumors with two or more PV were more likely to present an oncogenic mutation in a driver gene (14/41, 34%), as compared to tumors with only one PV (6/41, 15%; $p < 0.001$; Pearson chi-square test). The presence of two or more PVs (as opposed to one single PV) associated with previous treatment by immunotherapy (16 patients ; $p = 0.001$, Pearson chi-square test and post-hoc 0.05 correction for multiple

testing), whereas it was not correlated to age ($p = 0.2$), prior chemotherapy (71 patients; $p = 0.83$), prior targeted therapy (48/75 patients; $p = 0.17$), or to the number of prior lines of therapy ($p = 0.8$). The most frequently altered genes were *KMT2D* (11% of all patients with PV), *ARID1A* and *SETD2* (5% each), *EP300* and *CREBBP* (4% each; Fig. 3C and Supplementary Figure S4).

Distribution of altered genes across epigenetic regulation processes

Epigenetic alterations occurred in genes encoding proteins involved in various epigenetic regulation processes, including chromatin remodeling, and post-translational protein modifications or recognition (Supplementary Figure S5). In particular,10% (51/496), 19% (92/496) and 71% (353/496) EGAs were detected in the Polycomb complex, Trithorax complex, or in other epigenetic modifiers, respectively (Supplementary Figure S5). The most frequent EGA occurred in the *KMT* gene family (n = 140, 28% of all EGAs), SWI/SNF complex (n = 54, 11% of all EGAs), *KDM* (n = 50, 10% of all EGAs) and *HDAC* (n = 49, 10% of all EGAs) genes families (Supplementary Figure S5). Among them, the most frequent altered genes were *KMT2D* (n = 32, 6% of all EGAs), *KMT2C* (n = 24, 5% of all EGAs), *TET1* (n = 17, 3% of all EGAs), *SETD2* (n = 15, 3% of all EGAs) and *ARID1A* (n = 14, 3% of all EGAs; Supplementary Figure S6). For the 93 tumors with two or more EGAs, the latter most frequently occurred in distinct chromatin remodeling complexes or in groups of proteins with different functions, rather than in one single complex family or in proteins with similar functions (65% *versus* 35% of cases, respectively; $p < 0.001$; Pearson chi-square test; Supplementary Figure S7).

When considering only PV, mutations occurred in the Trithorax complexes, Polycomb complexes or other epigenetic modifiers in 25% (n = 58/ 237), 6% (n = 15/ 237) and 69% (n = 164/ 237) of the samples, respectively (Fig. 4A). Pathogenic variants were mostly found in *KMT2D* (n = 7, 3% of all PVs), *KMT2C* (n = 16, 7% of all PVs) and *ARID1A* (n = 12, 5% of all PVs; Fig. 4B). Here again, when multiple PVs were present within a single tumor, these seemed to most frequently occur in distinct chromatin remodeling complexes or in proteins with different functions (5% *versus* 18% of cases, respectively; $p < 0.001$; Pearson chi-square test [Figure 4C]).

Patients’ Outcome

None of the included patients received a targeted therapy based on the identification of the epigenetic alteration. Overall, 101 (75%) patients received an anticancer treatment following the MOSCATO/MATCHR biopsy, including targeted therapy, chemotherapy and immunotherapy in 47 (35%), 36 (27%) and 18 patients (13%), respectively. One patient presented a complete response, 24 patients (24%) a partial response, 35 patients (35%) a stable disease, and 40 patients (40%) a progressive disease. The median progression-free survival (PFS) and median overall survival (OS) of the whole series were 3.7 months (CI 95%, 3.1–4.6) and 14.4 months (95% CI, 11.9–16.6), respectively.

We next compared the outcome of the 134 patients whose tumor presented an EGA to the one of the 99 MOSCATO patients whose tumor did not harbor any EGA by WES profiling (herein referred to as “epigene

wild-type”). We found that the PFS of patients was comparable in patients with epigene wild-type and -mutant tumors (HR = 1.26, 95% CI 0.97–1.64; $p = 0.08$ log-rank test; Fig. 5A). Similarly, no difference was observed for OS between both populations (HR = 0.95, 95% CI 0.7–1.3; $p = 0.8$ log-rank test; Fig. 5B).

In order to evaluate whether the functional consequence of the EGA would influence outcome, we next stratified the patients according to the EGA pathogenicity. We first compared the outcome of patients whose tumors presented a PV *versus* those with likely benign variants or variants of unknown pathogenicity, and could neither identify any difference in PFS (HR = 1.05, 95% CI 0.73–1.51; $p = 0.8$ log-rank test; Supplementary Figure S8), nor in OS (HR = 1.54, 95% CI 0.98–2.41; $p = 0.06$ log-rank test; Fig. 5C). We subsequently compared the outcome of patients whose tumors presented a PV *versus* patients with epigene wild-type tumors, and did neither identify a difference in PFS (HR = 1.23, 95% CI 0.92–1.64; $p = 0.15$ log-rank test; Supplementary Figure S9), nor in OS (HR = 0.81, 95% CI 0.58–1.14; $p = 0.23$ log-rank test; Fig. 5D).

We finally interrogated whether the presence of multiple of EGAs or PV would influence outcome, and stratified the patients according to the number of EGAs or PV (with the median number of each group taken as threshold). We could not observe any significant difference in OS according to the presence of three or more EGAs (HR = 0.8, 95% CI 0.53–1.21; $p = 0.28$ log-rank test; Supplementary Figure S10), or two or more PV *versus* a single PV (HR = 0.92, 95% CI 0.56–1.53; $p = 0.76$ log-rank test; Supplementary Figure S11). When looking at the 25 patients whose tumor responded to therapy given after the molecular screening biopsy, we observed that most of them presented a tumor with less than three EGA (17 patients, 68%$p = 0.07$ Pearson chi-square test).

Comparison to The Cancer Genome Atlas (TCGA) dataset

In order to interrogate whether our findings would revalidate in larger cohorts, we used the TCGA WES dataset [20]. We first searched for the presence of an EGA in our pre-defined list of 176 epigenes in the breast (BRCA), lung (LUAD and LUSC), prostate (PRAD), colon (COAD), rectal (READ), bladder (BLCA), ovarian (OV), uterine clear cell (UCEC), renal clear cell (KIRC), and head and neck (HNSC) datasets, as well as in the low-grade glioma (LGG), glioblastoma (GBM), cholangiocarcinoma (CHOL), sarcoma (SARC) and uterine carcinosarcoma (UCS) series. Among the 6461 analyzed samples, at least more than one EGA could be detected in 4391 tumor samples (68%). Similar to what we identified in our series, the most frequently mutated genes were *KMT2D* (664 samples; 15% of tumors with EGA), *KMT2C* (619 samples; 14%) and *ARID1A* (573 samples; 13%); this proportion was comparable to that observed in our series, where the respective EGA frequencies were 16%, 16% and 10% (Fig. 2E). When comparing the frequency of other EGA, we found that *IDH1* and *JARID2* pathogenic variants were significantly more frequent in the TGCA dataset (11% *versus* 4%, $p < 0.0001$ for *IDH1*; 3% versus none, $p < 0.0001$ for *JARID2*), whereas *SEDT1B* was significantly less frequently altered in the TCGA dataset (2% versus 5%; $p < 0.0001$ log-rank test). Most frequent EGAs within TCGA were similar between TCGA and our series including *SMARCC2*, *KDM5B*, *MTA2*, *WHSC1*, *SMARCB1*, suggesting that epigene mutations might overall occur at similar frequencies in earlier stage tumors (Supplementary Figure S12).

When exploring patient outcomes using the whole TCGA series, we could not detect any difference in OS according to the presence or absence of an EGA (HR = 1.06, 95% CI 0.96–1.19; $p = 0.26$ log-rank test; Supplementary Figure S13). When assessing OS according to the presence or absence of an alteration in one of the four most frequently mutated genes, we found that OS was significantly better in patients with *ARID1A*- (HR = 1.52; 95% CI 1.23–1.87, $p < 0.0001$ log rank test)) and *IDH1*- (HR = 1.66; 95% CI 1.33–2.08, $p < 0.0001$ log rank test) mutant tumors as compared to tumors that were not altered in these genes (Figs. 5E, F), while no difference in OS was observed for patients with *KMT2D*- (HR = 1.03; 95% CI 0.86–1.23, $p = 0.73$ log rank test) or *KMT2C*-mutant tumors (HR = 1.05; 95% CI 0.89–1.25, $p = 0.56$ log rank test; Supplementary Figure S14).

Discussion

Epigenetic dysregulation contributes to tumor development and progression [1]. However, the presence of epigenetic alterations has been poorly studied in solid tumors, partly because these genes were not included in most oncology gene panels, and because these alterations were not actionable until recently. In the present study, we report the prevalence of alterations in genes encoding a protein involved in epigenetic regulation, in a variety of metastatic solid tumors. We found that epigenetic alterations were present in half of patients, and that two thirds of them were pathogenic variants which frequently co-occurred with mutations in oncogenic drivers.

Most studies performed so far have focused on the description of alterations in epigenetic genes in a given tumor type or on alterations of a specific epigenetic gene or chromatin remodeling complex across tumor types [21–24]. To our knowledge, this is the first study that describes, across several tumor types, alterations in epigenetic genes. Although our data emerge from a limited single-center dataset, the frequency of alterations and the most frequently mutated genes, including *KMT2D*, *KMT2C* and *ARID1A*, were comparable to identified EGAs in the TCGA dataset and MSK-IMPACT study, suggesting that it may be representative of most tumor series [23, 25]. Interestingly, the most frequent epigenetic gene alterations occurred within the *KMT* gene family or SWI/SNF chromatin remodeling complex subunits, for which therapeutic approaches based on synthetic lethality or epigenetic antagonism have been proposed [8, 9]. For instance, EZH2 inhibitors have shown clinical efficacy in the treatment of multiple SMARCB1-defective diseases[12, 26] and are now approved for advanced epithelioid sarcoma [27]. Similarly, ARID1A deficiency is synthetic lethal with EHZ2 inhibition, PI3K/AKT pathway inhibition, and PARP or ATR inhibition, amongst others [28–30]. Most of these therapeutic approaches are now being evaluated in early proof-of-concept clinical trials (NCT04042831, NCT04065269, NCT03348631), and we can hope that these will soon give promising results that will support the systematic evaluation of epigenetic gene alterations to customize patients' treatment. The recent successes of IDH inhibitors in IDH-mutant acute myeloid leukemia and cholangiocarcinoma [13, 31], or of BET inhibitors in Nut-midline carcinoma [32] further support such precision medicine-based approach, and we can hope that the new generation of epidrugs will bring further therapeutic progresses in the field.

We observed that the median allelic frequency of epigenetic alterations was 25%, and that one third of them co-occurred with a driver mutation. This suggests that most of the detected epigenetic gene alterations are subclonal, and may be best targeted using combinatorial approaches – where one drug would target the driver alteration and the second drug the epigenetic alteration. Noteworthy, such co-occurrence might either represent a biological cooperation, notably when both mutations are found at a high allelic frequency (e.g. PIK3CA and ARID1A in clear cell ovarian carcinoma), or might either reflect the presence an acquired epigenetic resistance mechanism to a targeted therapy directed against the driver alteration, in the case of multiple epigenetic alterations in various chromatin remodeling complexes. Further, two or more epigenetic alterations were observed in approximately 70% of patients from our series. Intriguingly, epigenetic aberrations occurred more frequently in different chromatin remodeling complexes than within the same complex (65% vs 35%; $p < 0.001$). Whether such alterations co-occur (and potentially cooperate) within the same tumor cell, or whether they arise from different subclones would be best addressed using single-cell DNA sequencing.

Our series included various tumor types in which epigenetic alterations have regularly been described, such as lung (24%), urological (22%), breast (13%), and gastrointestinal cancers (10%), and all patients had been heavily pretreated for metastatic disease, with a median of number of three previous therapy lines. For example, 90% of them already received chemotherapy, a factor known to cause random genetic mutations and which alters the epigenetic landscape of cancer cells [33]. Epigenetic modifications, notably in DNA methylation, nucleosome remodeling, or histone post-translational modifications, can also favor chemoresistance [33, 34] Therefore, it is generally admitted that epigenetic alterations tend to be more frequent in late-stage diseases, unless they the exception of particular diseases where they are early driver events, such as rhabdoid tumors or clear cell carcinomas. Although the aim of our study was not to compare dynamic changes of the epigenetic landscape over time, we hypothesized that part of the epigenetic alterations observed our series would results from exposure to prior therapies and/or from the selection pressure favoring clones with increased cellular plasticity resulting from such mutations – for example through the acquisition of the metastatic phenotype. In order to explore this, we compared our results with epigenetic alterations identified on The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga/), which mostly contains genomic profiles of earlier stage tumors, and notably treatment-naïve surgical samples [23]. Surprisingly, the prevalence of alterations in epigenetic genes was similar between the TCGA and our series, as was the case for the most frequently mutated genes, i.e. *KMT2D* (15% vs 16%), *KMT2C* (14% vs 16%) and *ARID1A* (13% vs 10%). If this might suggest that treatments received or disease evolution did not influence the emergence of subclones with epigenetic gene mutations, it may also result from a difference in cohort composition, notably regarding tumor types. Furthermore, we did not have access the allelic frequency of the TCGA alterations, which may differ from the one observed in our series despite this apparent similar prevalence of alterations.

We did not identify any correlation between the presence of an epigenetic gene alteration and outcome in our series although a trend for a shorter survival was observed in patients with pathogenic EGAs ($p = 0.06$). When comparing with TCGA series, mutations in *ARID1A* and *IDH1* were associated with better overall survival in contrast to non-mutant patients. By contrast, previously published studies have

correlated mutations in *ARID1A* and *IDH1* with a poor prognosis [35]. Contrary to our pan-cancer analysis, these assessments were made in specific tumor types, suggesting that the predictive value of such mutations may vary according to the tumor type. Further, the biological impact of molecular alterations in epigenetic genes is still poorly characterized, and uncertainties remain regarding the pathogenicity and functional consequence of some variants. Although the enzymatic activity of epigenetic regulators and chromatin remodeling complex composition are mostly shared between cell types, the resulting signaling consequences are highly cell lineage-specific. Thorough functional studies evaluating the consequence of each mutation in a biologically-relevant cellular context, are therefore highly needed. These will allow to discriminate which tumor types will most benefit from precision medicine-based epigenetic-targeting therapeutic approaches, and to establish the role of such alterations in disease progression and resistance to treatment. For example, the efficacy of EZH2 inhibitors has been inconstant across the spectrum of *SMARCB1*-deficient diseases [36], illustrating the importance of the cellular context – by contrast with NTRK inhibitors for example, which work in virtually all NRTK-fusion driven diseases.

Limitations of our study encompass its retrospective nature, the fact that it was run at a single institution and the small number of patients assessed. In similar precision medicine programs, 10 to 20% of patients were enrolled in a genomically matched clinical trial [17, 25, 37]. Here, none of the patients could benefit from a genomically matched therapy, mostly because of the lack of active drugs with proven benefit in such cases, because of the uncertainty on the functional consequence of some mutations, and because of the complexity of epigenetic regulation – as opposed to oncogenic kinases for example. The fact that 64% of the patients with at least one epigenetic gene alteration in their tumor presented a pathogenic variant, highlights that there is still an important unmet medical need in this era. The advent of new drugs targeting epigenetic regulators will hopefully open novel therapeutic avenues for the targeting of such alterations.

Conclusions

Epigenetic alterations occur in almost half of the patients with advanced solid tumors. However, apart from rare exceptions such as EZH2, IDH or BET inhibitors, there is currently no approved therapy for this patient population which represents an unmet medical need. The current acceleration in epigenetic drug development and epigenetic molecular profiling brings new hopes in the field, and will hopefully soon translate into novel efficient therapeutic strategies, for patients benefit.

Declarations

Ethics approval and consent to participate

MOSCATO and MATCHR trials were approved by the Ethics Committee from Gustave Roussy. All patients consented to participate in the study in MOSCATO and MATCHR trial at Gustave Roussy.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

PMR: Consultant/Advisory fees from Roche and AbilityPharma; CB declares Consulting BMS, GSK, AZ and Funding from BMS; LV reports personal fees from Adaptherapy, is CEO of RESOLVED, non-personal fees from Pierre-Fabre and Servier, grants from Bristol-Myers Squibb, all outside the submitted work; AV is an employee and stakeholder of Astrazeneca since August 2020; JCS received consultancy fees from Relay Therapeutics; was an employee of AstraZeneca 2017–2019; has shares in AstraZeneca, Daiichi Sankyo, Gritstone; he is an employee of Amgen since August, 2021; CM is a consultant/Advisory fees from Amgen, Astellas, Astra Zeneca, Bayer, BeiGene, BMS, Celgene, Debiopharm, Genentech, Ipsen, Janssen, Lilly, MedImmune, MSD, Novartis, Pfizer, Roche, Sanofi, Orion. SPC declares honoraria from Amgen, AstraZeneca, BMS, EISAI, Janssen, MSD, Novartis and Roche, advisory Board from Alderaan Biotechnology, Amgen, AstraZeneca, Avacta, Oncovita, Seagen, UltraHuman and Travel and congress support from AstraZeneca, MSD, Ose Pharma, Roche, Sotio. JMM declares participating in advisory board from BMS - Celgene, GSK, MSD, Roche; As part of the Drug Development Department (DITEP) the authors principal investigator/ sub-Investigator from Therapeutics, Astex Pharmaceuticals, Astra Zeneca Ab, Aveo, Basilea Pharmaceutica International Ltd, Bayer Healthcare Ag, Bbb Technologies Bv, Beigene, BicycleTx Ltd, Bioalliance Pharma, Blueprint Medicines, Boehringer Ingelheim, Boston Pharmaceuticals, Bristol Myers Squibb, Ca, Celgene Corporation, Chugai Pharmaceutical Co, Cullinan-Apollo, Curevarc, Daiichi Sankyo, Debiopharm, Eisai, Eisai Limited, Eli Lilly, Exelixis, Faron Pharmaceuticals Ltd, Forma Tharapeutics, Gamamabs, Genentech, Glaxosmithkline, H3 Biomedicine, Hoffmann La Roche Ag, Imcheck Therapeutics, Innate Pharma, Institut De Recherche Pierre Fabre, Iris Servier, Iteos Belgium SA, Janssen Cilag, Janssen Research Foundation, Kura Oncology, Kyowa Kirin Pharm. Dev, Lilly France, Loxo Oncology, Lytix Biopharma As, Medimmune, Menarini Ricerche, Merck Sharp & Dohme Chibret, Merus, Molecular Partners Ag, Nanobiotix, Nektar Therapeutics, Novartis Pharma, Octimet Oncology Nv, Oncoethix, Oncopeptides, Onyx Therapeutics, Orion Pharma, Oryzon Genomics, Ose Pharma, Pfizer, Pharma Mar, Pierre Fabre Medicament, Plexxikon, Roche, Sanofi Aventis, Seattle Genetics, Sotio A.S, Syros Pharmaceuticals, Taiho Pharma, Tesaro, Turning Point Therapeutics, Xencor; Research Grants from Astrazeneca, BMS, Boehringer Ingelheim, GSK, INCA, Janssen Cilag, Merck, Novartis, Pfizer, Roche, Sanofi; Non-financial support (drug supplied) from Astrazeneca, Bayer, BMS, Boringher Ingelheim, GSK, Medimmune, Merck, NH TherAGuiX, Pfizer, Roche. The other authors declare no potential conflicts of interest.

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Authors' contributions

PMR, LCM, SPV conceived and designed the study. All authors collected and assembled data. SPC, LCM, and PMR developed the tables and figures. SPC and PMR conducted the literature search and wrote the manuscript. All authors were involved in the critical review of the manuscript and approved the final version.

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Figures

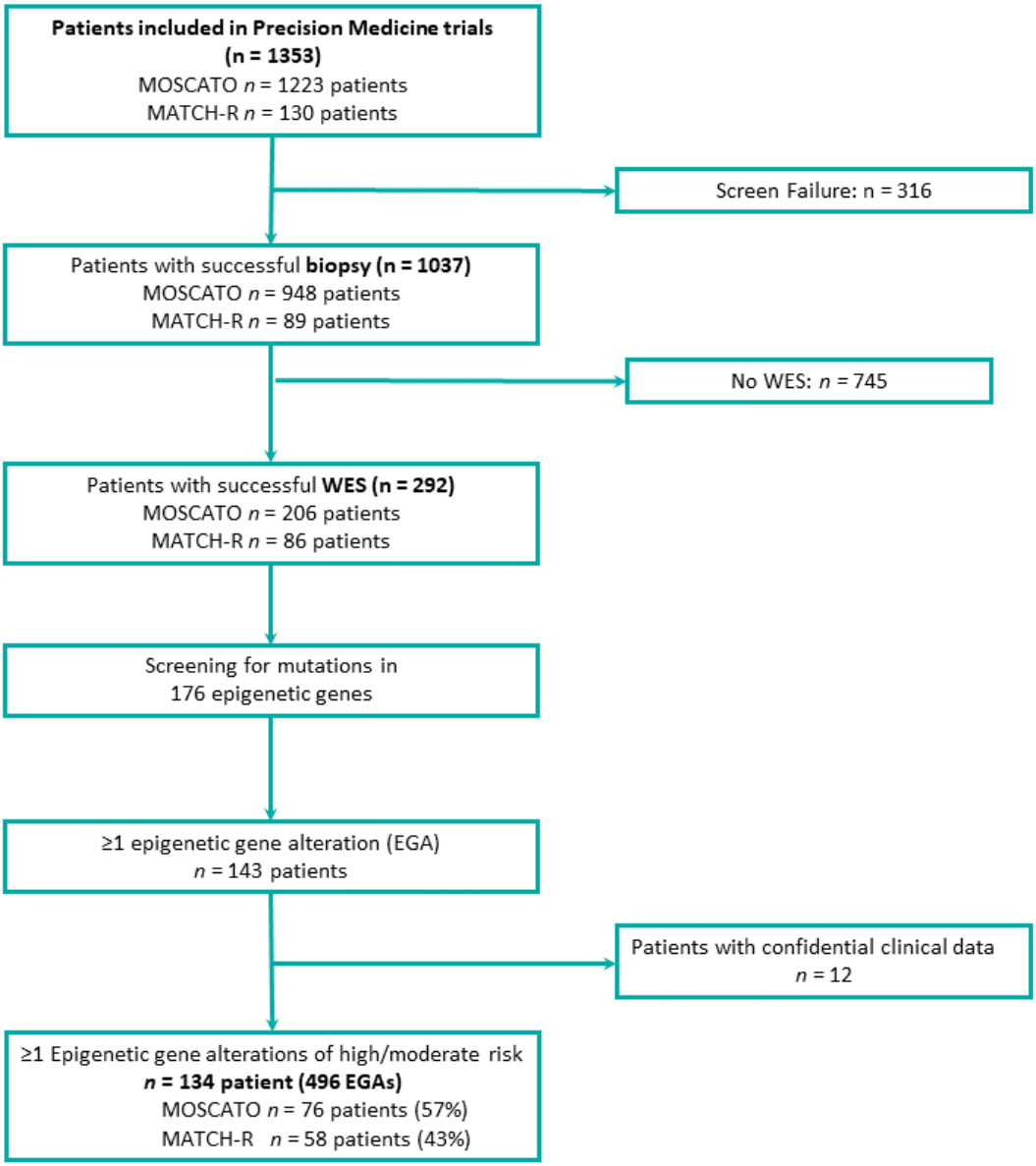

Figure 1

Figure 1

Flowchart of patient selection for the study

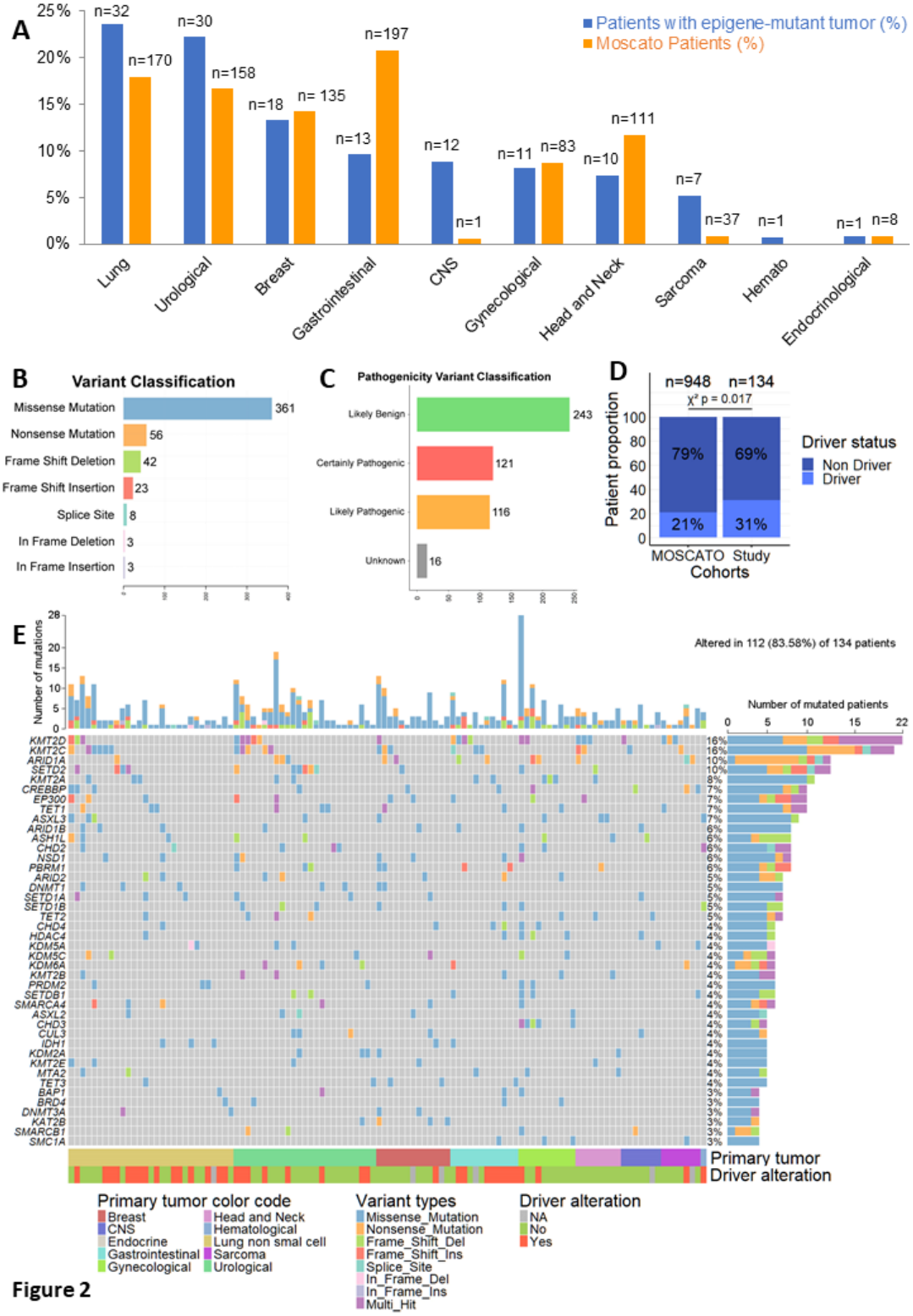

Figure 2

Distribution of patients and epigenetic gene alterations (EGA) identified in the MOSCATO trial. (A) Proportion of patients with EGA classified per tumor type, and compared to the proportion of patients with the similar tumor type enrolled in the MOSCATO trial; the exact corresponding patient number is indicated on the top of each histogram bar; (B, C) Variant classification according to the type of alteration (B) and the pathogenicity assessment according to REVEL scoring (C); (D) Percentage of tumors with or

without driver alteration according to the presence of an EGA; (E) Oncoplot of the most frequent (≥3%) epigenetic alterations; percentages represent the proportion of patients with a given EGA as compared to the total number of patients with EGA.

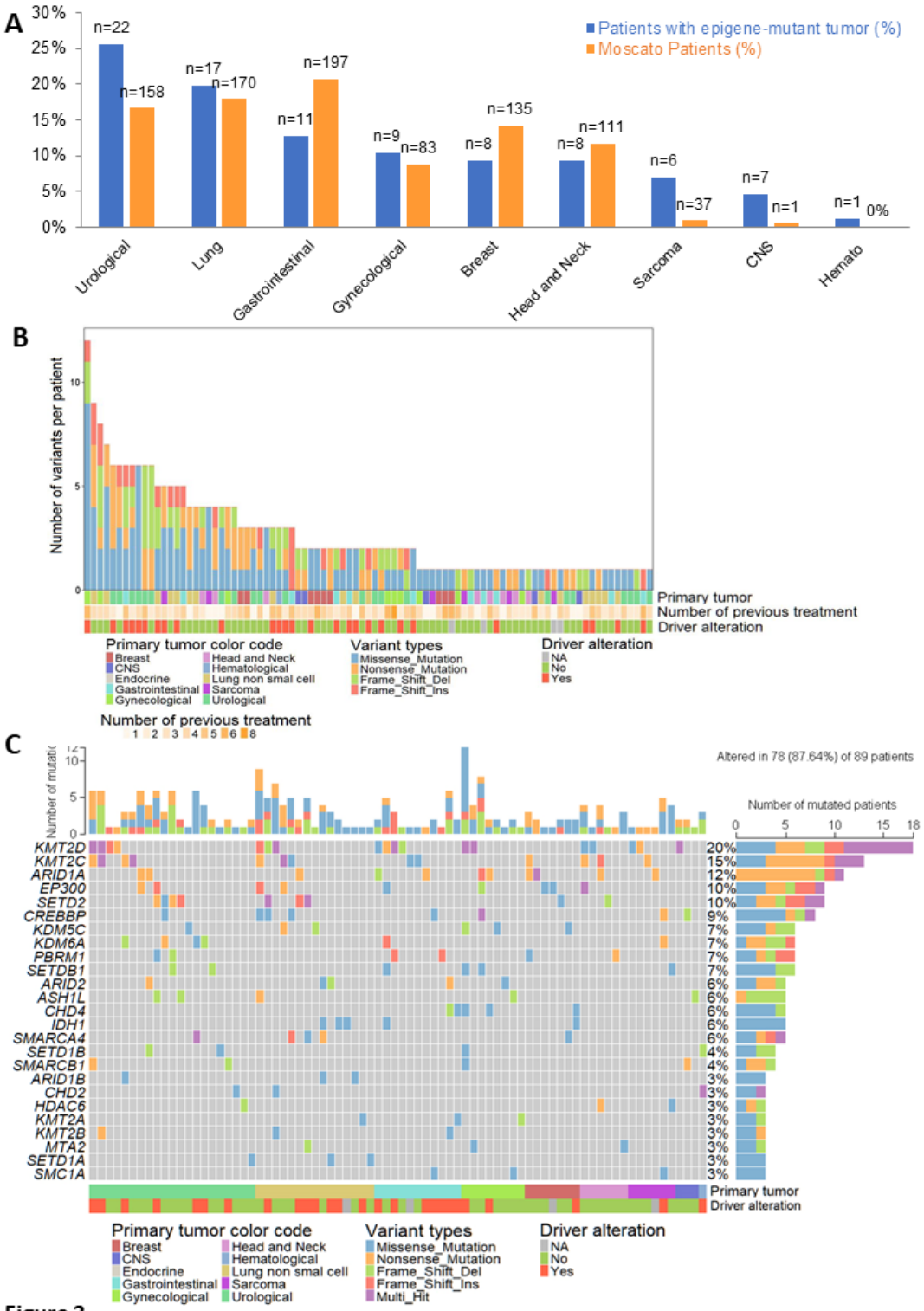

Figure 3

Figure 3

Description of pathogenic variants. (A) Proportion of patients with epigenetic alterations classified per tumor type, and compared to the proportion of patients with the similar tumor type enrolled in the MOSCATO trial; (B) Number of PV per sample; (C) Oncoplot of the PV detected in at least 3% of the samples; percentages represent the proportion of patients with a PV in a given gene, as compared to the total number of patients with PV.

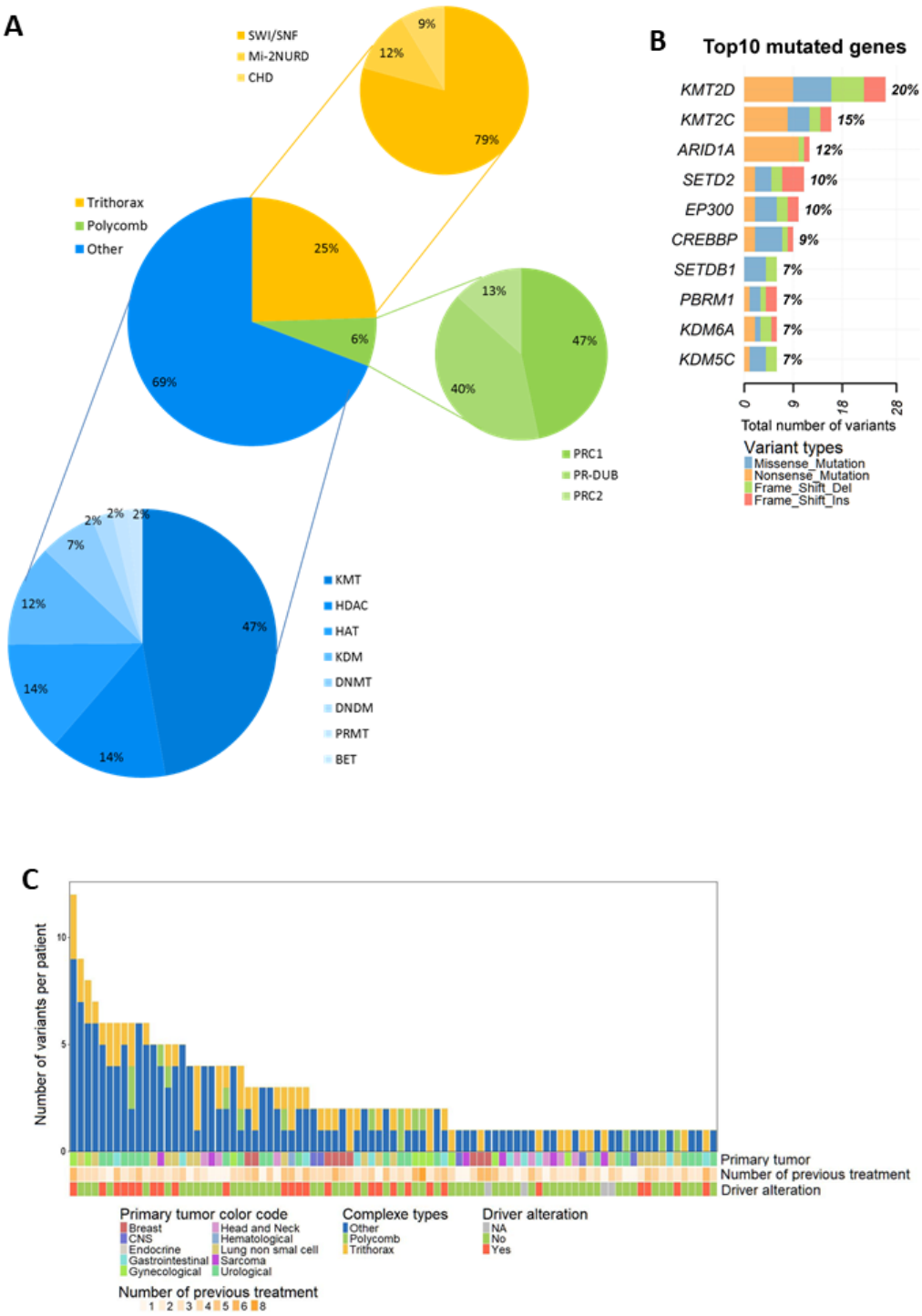

Figure 4

Figure 4

Distribution of the pathogenic variants according to their chromatin remodeling complex family and type of protein alteration (n=237). (A) Distribution of each single PV; **(B)** Top 10 most frequent mutated epigenetic genes presenting at least one PV; percentages are calculated as the proportion of pathogenic patients with at least one mutation in this gene; **(C)** Distribution of PV per tumor sample according to their chromatin remodeling complex family or protein function (percentages are calculated as the proportion of patients in our cohort with at least one mutation in per gene). Abbreviations: BET: bromodomain and extraterminal domain; DNDM: DNA demethylase; DNMT: DNA methyltransferase; HAT: Histone acetyltransferase; HDAC: histone deacetylase; KDM: Histone lysine demethylase; KMT: Histone lysine methyltransferase; Mi-2/NuRD: Mi-2/nucleosome remodeling and deacetylase; CHD: Chromodomain helicase DNA-binding; PRC1: Polycomb repressive complex 1; PRC2: Polycomb Repressive Complex 2; PRMT: Protein arginine methyltransferase; PR-DUB: Polycomb repressive deubiquitinase; SWI/SNF: SWItch/Sucrose Non Fermentable.

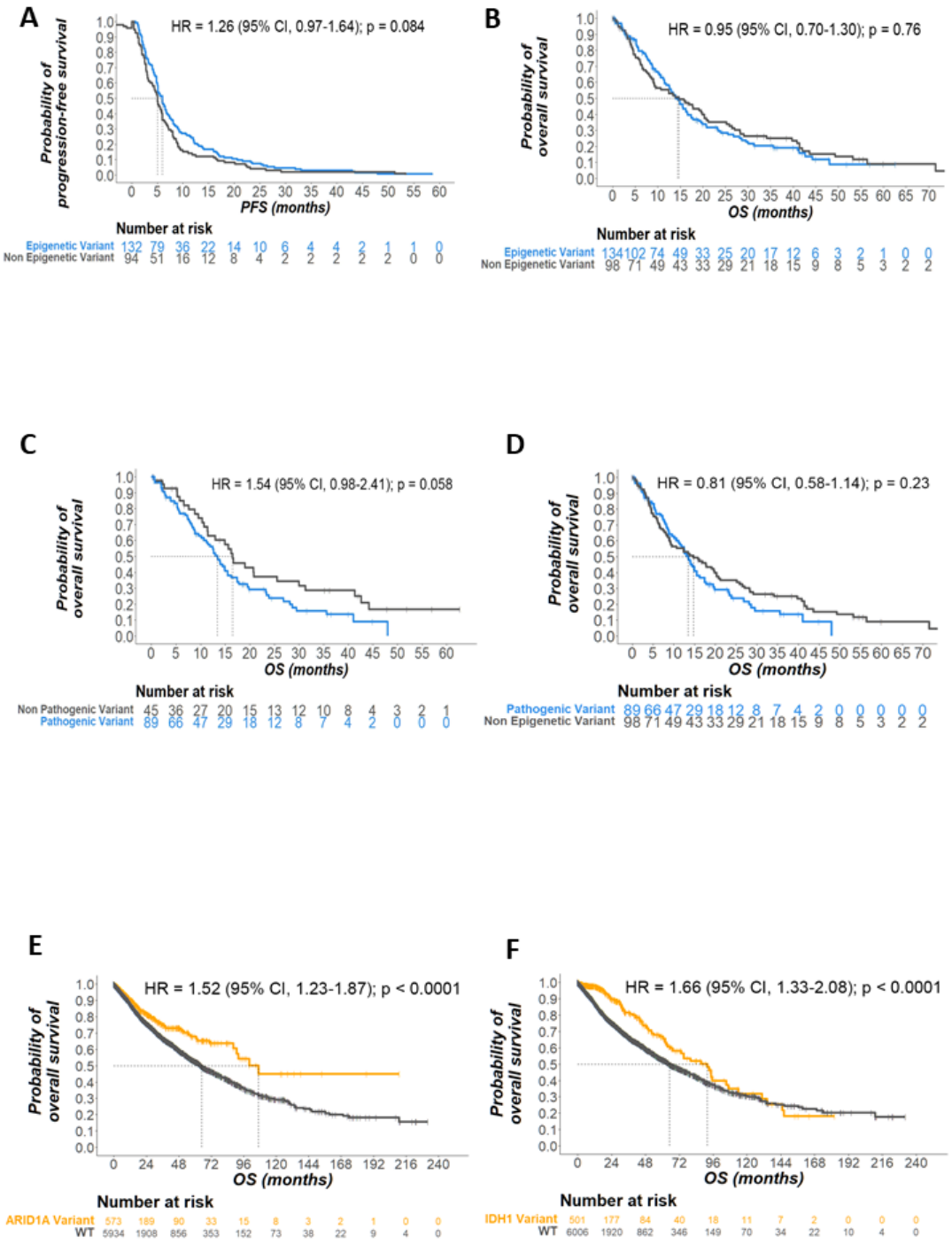

Figure 5

Figure 5

Survival probabilities according to the presence of epigenetic alterations. (A) Progression-Free Survival (PFS) probability according to the presence or absence of EGA; **(B)** Overall Survival (OS) probability according to the presence or absence of EGA; **(C)** OS probability according to the presence of a pathogenic *versus* non-pathogenic variant; **(D)** OS probability according to the presence of a pathogenic

variant *versus* the absence of EGA; **(E, F)** OS probability from TCGA patients according to the presence or absence of alteration within *ARID1A* **(E)** or *IDH1* **(F)**.

Supplementary Files

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