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Mocetinostat for patients with previously treated, locally advanced/metastatic urothelial carcinoma and inactivating alterations of acetyltransferase genes

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Demiana Faltaos and **Diane Potvin**: data curation, formal analysis, writing – reviewing and editing

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James Christensen: conceptualization, methodology, formal analysis, writing – reviewing and editing, visualization, and project administration

Abstract

Background: We evaluated mocetinostat (Class I/IV histone de-acetylase [HDAC] inhibitor) in urothelial carcinoma harboring inactivating mutations or deletions in *CREBBP* and/or *EP300* (histone acetyltransferase genes) in a single-arm, open-label Phase II study.
Methods: Eligible patients with platinum-treated, advanced/metastatic disease received oral mocetinostat (70 mg three times per week [TIW] escalating to 90 mg TIW) in 28-day cycles in a 3-stage study (NCT02236195). The primary endpoint was objective response rate

(ORR).

Results: Genomic testing was feasible in 155/175 patients (89%). Qualifying tumor mutations were: *CREBBP* (15%), *EP300* (8%), and both *CREBBP* and *EP300* (1%). Seventeen patients were enrolled into Stage 1 (ITT population); no patients were enrolled in subsequent stages. One partial response was observed (11% [1/9; efficacy evaluable population which comprised 9/15 planned patients]); activity was deemed insufficient to progress to Stage 2 (null hypothesis: ORR ≤15%). All patients experienced ≥1 adverse event, most commonly nausea (77% [13/17]) and fatigue (71% [12/17]). Median treatment duration was 46 days; treatment interruptions (82% [14/17]) and dose reductions (29% [5/17]) were common. Mocetinostat exposure was lower than anticipated (dose-normalized C_{max} following TIW dosing 0.2 ng/mL/mg).

Conclusions: To our knowledge, this study represents the first clinical trial using genomic-based selection to identify patients with urothelial cancer likely to benefit from selective HDAC inhibition. Mocetinostat was associated with significant toxicities that impacted drug exposure and may have contributed to modest clinical activity in these pretreated patients. The efficacy observed was considered insufficient to warrant further investigation of mocetinostat as a single agent in this setting.

Key words: mocetinostat, urothelial carcinoma, CREBBP, EP300, histone deacetylase

Running head: Mocetinostat in advanced urothelial cancer

Condensed abstract:

Following genomic-based selection of urothelial cancer patients with inactivating mutations/deletions in the histone acetyltransferase genes *CREBBP* and/or *EP300*, single-agent mocetinostat was associated with significant toxicities that limited drug exposure. This may have contributed to limited activity (response rate 11%) in the heavily pretreated platinum-refractory patients in this Phase II study.

Introduction

Urothelial carcinoma of the upper urinary tract and bladder results in 165,000 deaths annually worldwide [1]. Most patients with metastatic disease progress despite platinum-based chemotherapy, and salvage chemotherapy has only modest efficacy [2, 3]. Recently, five immune checkpoint inhibitors were approved for platinum-refractory urothelial carcinoma, and while the anti-programmed death protein-1 (PD1) agent, pembrolizumab has improved overall survival (OS) versus chemotherapy in this setting, many patients do not benefit from such therapy [4]. Consequently, new treatment options are needed.

Dysregulated histone acetylation is implicated in the pathogenesis of several cancers, including urothelial carcinoma. Acetylation of chromatin by histone acetyltransferases (HATs) is generally associated with elevated transcription, while deacetylation, mediated by histone de-acetylases (HDACs), is associated with repressed transcription [5, 6]. Histone acetylation can become dysregulated through the upregulation of HDACs, and/or genetic inactivation of HATs, resulting in silencing of tumor supressor and other genes [5, 6]. Inhibition of HDAC1 and HDAC2 resulted in antitumor activity in urothelial carcinoma *in vitro*, while in urothelial carcinoma patients elevated HDAC1 is linked with poor prognosis [7, 8]. HDAC inhibitors have shown promise in clinical trials across a range of tumor types and several are approved by the FDA including vorinostat for cutaneous T-cell lymphoma (CTCL), romidepsin for CTCL and peripheral T-cell lymphoma (PTCL), belinostat for PTCL, and panobinostat for multiple myeloma [9].

Mocetinostat is an investigational HDAC inhibitor that targets Class I and IV HDACs (isoforms 1, 2, 3, and 11) [10], and has demonstrated anti-tumor activity in patients with hematologic malignancies [11-13]. *In vivo*, mocetinostat induces cell cycle arrest, apoptosis and inhibits tumor growth [10]. Furthermore, a HAT inactivation signature associated with muscle-invasive bladder cancer was inversely influenced by mocetinostat in breast cancer cells [14]. Mocetinostat also demonstrated preferential activity in *CREBBP*- and *EP300*-mutated (HAT genes) xenograft models and solid tumor cell lines, including urothelial cell carcinoma (Supplementary Tables S1, S2; Supplementary Figure S1). Thus, we hypothesized that treating urothelial carcinoma harboring inactivating mutations in *CREBBP* and *EP300* with selective HDAC inhibitors may restore expression of tumor suppressor genes, resulting in anti-tumor responses.

This Phase II study investigated single-agent mocetinostat in patients with locally advanced or metastatic urothelial carcinoma previously treated with platinum-based chemotherapy and inactivating tumor mutations or deletions in *CREBBP* and/or *EP300*.

Methods

Patients and study design

This Phase II, open-label, single-arm, 3-stage, multicenter study was conducted between November 2014 and July 2016 (ClinicalTrials.gov, NCT02236195). Patients with histologically confirmed, locally advanced, unresectable or metastatic urothelial (transitional cell) carcinoma who progressed following platinum-based chemotherapy were recruited. Eligible patients had adequate bone marrow, hepatic and renal function and an inactivating mutation or deletion (homozygous or hemizygous) in *CREBBP* and/or *EP300* (see supplementary appendix). Genomic prescreening of tumor tissue (primary or metastatic, archival tissue was permitted if a fresh biopsy was not available) was performed centrally using next-generation sequencing (NGS; Foundation Medicine; Cambridge, MA, USA) or a Sponsor-approved, local sequencing platform (FoundationOne®, MSK-IMPACT™) or NGS (Oncopanel, Center for Advanced Molecular Diagnostics, Brigham and Women's Hospital, Boston, MA, USA) capturing the full coding regions for *CREBBP* and *EP300*. Key exclusion criteria included prior or current treatment with an HDAC inhibitor and symptomatic or uncontrolled brain metastases.

Oral mocetinostat (Mirati Therapeutics, Inc. San Diego, US) was administered in continuous 28-day cycles at a starting dose of 70 mg three times per week (TIW) for Stage 1. Escalation to 90 mg TIW on Cycle 2 Day 1 was planned for patients without treatment-related Grade ≥3 adverse events (AEs), and 90 mg TIW was the planned starting dose for the Stage 2 and 3 cohorts. Mocetinostat was continued until disease progression or unacceptable AEs.

The protocol was approved by the Institutional Review Boards at each institution, and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients provided written, informed consent.

Study endpoints and assessments

The primary endpoint was objective response rate (ORR: complete response [CR] and partial response [PR] per RECIST v1.1). Secondary endpoints included duration of response (DoR), progression-free survival (PFS: overall and at month 4), OS, 1-year survival rate, safety and pharmacokinetics.

CT scans for tumor evaluation were performed at baseline, 8-week intervals for the first 12 months, and 12-week intervals thereafter. AEs were graded per NCI CTCAE Version 4.03.

Plasma concentrations of mocetinostat were determined using high performance liquid chromatography and tandem mass spectrometry during Stage 1 (pre-dose and 1 h post-dose on day 1 of cycles 1 and 2) with more timepoints planned for Stage 2.

Tumor total mutation burden (TMB) was estimated retrospectively in the 322 target genes included in FoundationOne for patients with central testing (see supplementary appendix).

Statistical analyses

The primary endpoint (ORR) was assessed using an exact test for single proportion (two-sided α=5%; ORR ≤15% [H_0] vs >15% [H_1]) in a three-stage study design to include 15, 18, and 67 patients, respectively, in the efficacy evaluable population (patients meeting the entry criteria who received mocetinostat and had at least baseline and one on-study disease assessments; see supplementary appendix). Safety was assessed in patients receiving ≥1 dose of mocetinostat. Pharmacokinetics were evaluated in all patients with sufficient data. Time-to-event efficacy endpoints were estimated using Kaplan-Meier methodology (see supplementary appendix).

Results

Patient disposition and baseline disease characteristics

Of 175 patients consenting to genomic screening, testing was feasible for 155 (89%; sample quantity/quality was insufficient for 20 patients). Frequently altered genes included *TP53, ARID1A, MLL2 (KMT2D), KDM6A, MLL3 (KMT2C), RB1*, and *CDKN2A/B* (Figure 1).

Thirty-three (21%) patients had ≥1 of the 40 qualifying tumor mutations in *CREBBP* or *EP300* identified: 27 *CREBBP* mutations among 23 patients (15%); 13 *EP300* mutations among 12 patients (8%); mutations in both genes in 2 patients (1%). Each qualifying mutation was observed only once within the study. Qualifying *CREBBP* alterations were most commonly nonsense (8 [5%]), frameshift (7 [5%]) or missense (5 [3%]) mutations. *EP300* mutations were most commonly missense mutations (5 [3%]). Non-qualifying mutations in *CREBBP* and *EP300* (putative passenger mutations) were detected in 18 (12%) individuals (see Supplementary Table S3).

Seventeen of 33 patients with qualifying mutations were enrolled into Stage 1 (Figure 2); baseline demographic and disease characteristics of the enrolled patients are shown in Table 1. Twenty-two qualifying mutations were identified in these 17 patients: 14 *CREBBP* mutations in 12 patients and 8 *EP300* mutations in 7 patients; two patients had qualifying mutations of both *CREBBP* and *EP300* (Supplementary Table S3). Sixteen patients with qualifying mutations were not enrolled, most commonly because they were receiving an earlier line of therapy (Figure 2). The patients received a median (range) of 2 (1–5) prior systemic therapies (Table 1) and all had discontinued mocetinostat at the time of analysis, most due to disease progression (53%) or AEs (24%; Figure 2). Based on Sponsor decision, the study was closed after enrolment of 17 patients, including 9 patients in the efficacy evaluable population (8 patients stopped mocetinostat treatment prior to on-study disease assessment: 4 due to AEs, 3 due to symptomatic deterioration and 1 withdrew consent); Stages 2 and 3 were not recruited.

Efficacy

One objective response was observed (efficacy evaluable population). This PR lasted 3.9 months and occurred in a 67-year old man with disease restricted to lymph nodes. His primary tumor contained two qualifying *EP300* missense mutations (G1347E and P925T) and other mutations (truncating mutations in *ARID1A*, *MLL2* [*KMT2D*], and *CHEK2*; missense

mutation in *ATM*; and amplification of *TERC* and *PRKCI*). The ORR (95% CI) of 11% (0.3, 48%) was not statistically significant (null hypothesis of ≤15% could not be rejected, *P*=1.00). Stable disease lasting 3.5 months and 3.8 months was reported in two patients (22%) and progressive disease was reported in 6 patients (67%; Supplementary Figure S2). Median PFS (95% CI) was 57 days (23, 117 days) in the efficacy evaluable population. Estimated PFS at 4 months was 10% (0, 40%); PFS at 1 year could not be estimated. Median OS (95% CI) was 3.5 months (2.1, 15.7 months) and 1-year survival was 30% (10, 60%) in the ITT population (all patients receiving study medication). Similar efficacy results were observed in the efficacy evaluable and ITT populations.

Safety

Median (range) duration of mocetinostat therapy was 46 days (8, 225 days), and the cumulative median dose (range) administered was 930 mg (280, 7,730 mg). Median (range) relative dose intensity was 99% (37, 117%) during cycle 1 and 84% (14, 117%) in subsequent cycles. Eleven of the 17 enrolled patients initiated ≥2 treatment cycles. Mocetinostat dose was escalated from 70 mg TIW to 90 mg TIW in 9 patients (4 received ≤1 full cycle of mocetinostat 90 mg TIW). Five patients (29%) underwent dose reductions due to AEs (n=3, 18%) or other reasons (n=2, 12%), and 14 patients (82%) had at least 1 dose interruption, most commonly due to AEs (n=11, 65%).

All patients experienced ≥1 treatment-emergent (all causality) AE, and most (n=14, 82%) experienced ≥1 treatment-related AE. The most frequent treatment-emergent AEs were nausea (n=13, 77%), fatigue (n=12, 71%), decreased appetite (n=8, 47%) and diarrhea (n=8, 47%; Table 2); these events were also the most frequent treatment-related AEs. Grade ≥3 treatment-related AEs were fatigue and hyponatremia (n=2, 12%, each). Twenty-one treatment-emergent serious adverse events (SAEs) were reported in 10 patients (59%), including vomiting, lower gastrointestinal hemorrhage, abdominal pain and pericardial effusion (n=2, 12%, each). One SAE of pericardial effusion was assessed as related to mocetinostat (both pericardial effusion events resolved). Ten patients died during the study, all due to their underlying disease.

Pharmacokinetics

Due to the limited blood sampling schedule for Stage 1, the 1 h post-dose sample was considered representative of maximum serum concentration (C_{max}) based on data from prior studies (see supplementary appendix), and pharmacokinetic analyses were restricted to C_{max} and time to C_{max} (t_{max}).

Following a single 70 mg dose of mocetinostat, mean C_{max} was 105 ng/mL. Mean dose-normalized C_{max} was 1.2 ng/mL/mg and inter-subject variability (coefficient of variation, geometric mean) was 90%. Following multiple TIW doses of mocetinostat 50 mg and 90 mg, mean C_{max} was 41 ng/mL and 39 ng/mL, respectively (Supplementary Table S4). The mean dose-normalized C_{max} was 0.2 ng/mL/mg and inter-subject variability was 423%.

Discussion

Inactivating alterations of *CREBBP* and *EP300* are relatively frequent (~13% and ~15%, respectively) in urothelial carcinoma [14-16] and are implicated in dysregulation of key acetylation pathways and oncogenesis [17, 18]. Based on promising findings in urothelial carcinoma cell lines and tumor models (Supplementary Tables S1, S2 and Supplementary Figure S1), we postulated that patients with urothelial carcinoma and inactivating alterations in *CREBBP* and/or *EP300* could be treated by Class I HDAC inhibition via a mechanism of increased histone acetylation leading to an open chromatin state with decreased transcriptional repression of tumor suppressor genes. While the maximum tolerated dose of mocetinostat as a single agent was determined to be 110 mg TIW in other tumor settings, a lower recommended dose of 90 TIW was considered for this study based on prior observations of pericardial infusion and balancing pharmacodynamic and clinical data as well as regulatory guidance [19]. However, single-agent mocetinostat at doses up to 90 mg TIW showed only modest activity in this cohort of heavily pretreated patients with factors indicative of poor prognosis. The ORR of 11% and of 9 evaluable patients only 1 patient (with lymph node-only disease and multiple genomic alterations) remaining alive and progression-free for 4 months was not consistent with meaningful clinical activity. While mocetinostat-related AEs, including gastrointestinal events and fatigue, were consistent with the safety profiles reported in other settings [11, 12, 20], frequent dose

interruptions and reductions were required. Mocetinostat exposure (mean dose-normalized C_{max} 0.2 ng/mL/mg) was lower than in prior mocetinostat TIW trials (0.8 to 1.6 ng/mL/mg). It is feasible that underlying disease and prior treatments may have contributed to limited functional reserve, resulting in poor tolerability. These findings underscore the limitations of preclinical models in predicting clinical activity and toxicity issues related to anticancer treatments. Further evaluation of mocetinostat at lower doses may be useful to guide dose reduction guidance in future study protocols in order to maximize each patient's exposure to treatment.

Studies of other HDAC inhibitors in urothelial carcinoma patients reported mixed results, with responses seen with single-agent vorinostat but not when vorinostat was combined with doxorubicin or docetaxel [21-23]. An ORR of 20% was reported in a small study of belinostat or panobinostat, and prolonged stable disease in one of 3 patients with urothelial carcinoma treated with entinostat plus 13-cis retinoic acid [24, 25]. These data suggest that HDAC inhibitors can be active in urothelial carcinoma, but predictive biomarkers are needed for patient selection. To our knowledge, data regarding genomic predictors of response to HDAC inhibitors are limited. In a Phase II study of panobinostat in patients with relapsed diffuse large B-cell lymphoma, mutations in *MEF2B* were associated with response, while 14 genes including *TOX4*, *PSMD$_{13}$* and *CCNK* were associated with resistance to vorinostat based on a study of human colon cancer cell lines [26, 27]. To our knowledge, this is the first clinical trial using genomic-based selection to identify patients with urothelial carcinoma for treatment with selective HDAC inhibition. This study demonstrates the feasibility of this approach while also providing genomic tumor characterization for this population.

There was considerable genomic variation in *CREBBP* and *EP300*, with each qualifying mutation observed only once in this study. Interestingly, the patient with a confirmed PR harbored two *EP300* mutations in trans, P925T and G1347E, suggesting biallelic loss of function in this pathway could be therapeutically meaningful; however this patient had lymph node-only metastasis, a favorable prognostic factor. It is feasible that mocetinostat activity might be greater as an earlier line of therapy when a longer treatment duration may be feasible and potentially confer meaningful disease-modifying activity. Furthermore, we hypothesized a mechanism of action of mocetinostat to reactivate transcription of tumor suppressor genes, but a relatively high frequency of inactivating alterations in the tumor

suppressor genes *TP53*, *CDKN2A*/*B*, and *RB1* may have limited the potential of epigenetic modulation by mocetinostat to induce tumor response. Potential future treatment strategies could include combining mocetinostat with an inhibitor of the PD1/ programmed death 1 ligand (PD-L1) to take advantage of the former's potential immunomodulatory effects. Indeed, mocetinostat has been shown to increase expression of PD-L1 and augment PD-1/PD-L1 checkpoint blockade immunotherapy [28]. Other combination partners could be considered in the appropriate molecular context.

In summary, single-agent mocetinostat was associated with significant toxicities and limited activity in heavily pretreated patients with advanced/metastatic urothelial carcinoma and poor prognostic factors. Few patients received the intended dose of 90 mg TIW which may have compromised efficacy. Nevertheless, the clinical activity observed does not warrant further investigation as single agent in this setting. Mocetinostat is currently being investigated in other tumors and in combination with immunotherapy.

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Tables and figures

Table 1. Patient demographics and disease characteristics (ITT population)

Patient characteristic	**Mocetinostat** (N=17)
Age, years; median (range)	67 (35–83)
Male gender, n (%)	15 (88)

Race, n (%)	
White	15 (88)
Asian	1 (6)
Black	1 (6)
Smoking history, n (%)	
Past smoker	8 (47)
Never smoker	7 (41)
Current smoker	2 (12)
AJCC/UICC TNM Stage[a]	
IVA	1 (6)
IVB	16 (94)
ECOG PS, n (%)	
0	5 (29)
1	10 (59)
2	2 (12)
Bellmunt scores, n (%)[b]	
0	5 (29)
1	7 (41)
2	5 (29)
Baseline albumin (g/dL), median (range)	4.1 (3.1, 4.7)
Baseline hemoglobin (g/dL), median (range)	12.5 (9.0, 14.5)
Time from diagnosis of urothelial carcinoma, months (range)	26.4 (4.3–95.5)
Location of disease, n (%)[c]	
Lung	13 (77)

Liver	6 (35)
Lymph node[d]	15 (88)
Bladder	3 (18)
Bone	4 (24)
Other	8 (47)
Prior systemic therapy, n (%)	17 (100)
Number of prior regimens, median (range)	2 (1–5)
Patients with prior neoadjuvant/adjuvant regimens, n (%)	10 (59)
Patients with prior advanced disease regimens, n (%)	12 (71)
Patients who completed prior systemic therapy ≤3 months before starting study treatment, n (%)	7 (41)
Prior radiotherapy, n (%)	6 (35)
Prior surgery, n (%)[b]	15 (88)
Cystectomy	10 (59)
Transurethral resection of bladder tumor	9 (53)
Urethrectomy	4 (24)
Other	4 (24)

AJCC/UICC TNM, American Joint Committee on Cancer / Union for International Cancer Control (T) tumor, (N) lymph nodes (M) metastasis; ITT; intent-to-treat (all patients receiving study medication); ECOG PS, Eastern Cooperative Oncology Group Performance Status

[a]Disease subsite (bladder, ureter, or renal pelvis) and disease stage were not specifically collected in this study; disease stage using definitions for bladder cancer were assessed retrospectively

[b]Bellmunt Scores were assessed retrospectively [29]

[c]Patients may have more than 1 disease location or surgery

[d]Baseline disease was confined only to lymph nodes in two patients

Table 2. Treatment-emergent (all causality) adverse events occurring in at least 3 patients (safety population)

MedDRA preferred term **n (%)**	**All grade** **(N=17)**	**Grade 3/4** **(N=17)**
Nausea	13 (77)	1 (6)
Fatigue	12 (71)	3 (18)
Decreased appetite	8 (47)	N/R
Diarrhea	8 (47)	N/R
Hyponatremia	6 (35)	3 (18)
Vomiting	6 (35)	1 (6)
Abdominal pain	5 (29)	2 (12)
Anemia	5 (29)	2 (12)
Back pain	5 (29)	N/R
Constipation	5 (29)	N/R
Hypoalbuminemia	5 (29)	N/R
Hematuria	4 (24)	N/R
Muscular weakness	4 (24)	N/R
Alkaline phosphatase increased	3 (18)	N/R
Chills	3 (18)	N/R
Cough	3 (18)	N/R
Creatinine increased	3 (18)	N/R
Dehydration	3 (18)	1 (6)
Dizziness	3 (18)	N/R

Dysgeusia	3 (18)	N/R
Hypocalcemia	3 (18)	N/R
Lymphocyte count decreased	3 (18)	1 (6)
Pain	3 (18)	1 (6)
Urinary tract infections	3 (18)	N/R

MedDRA, Medical Dictionary for Regulatory Activities; N/R, not reported

Figure 1. Oncoprint of genetic alterations of 150 of the 155 patients in whom genetic profiling of tumor was feasible.

Alterations include truncating mutations, gene amplifications, homozygous deletions, annotated recurrent missense mutations, and missense variants of uncertain significance (variants of unknown significance are excluded from the main study analysis) present in ≥10% of the population. The 150 patients included 144 patients tested centrally at Foundation Medicine and 6 patients tested at local institutions. An arrow (↓) denotes a patient enrolled in the clinical trial (reports from 5 patients tested locally were not available, including 4 patients pre-screened using FoundationOne® testing and including two enrolled patients).

[a]In cases of *CCND1* amplification, this co-occurred with *FGF3*, *FGF4*, or *FGF19* amplification in >80% of cases. In addition, a significant correlation for the co-occurrence of *RB1* and *TP53* mutations and *CREBBP* and *STAG2* mutations and the mutual exclusivity of *CDKN2A* homozygous deletion and *TP53* mutation or *MDM2* amplification and *TP53* mutation was observed.

Figure 2. Patient disposition.

[a]Safety population and ITT population include all patients who received at least one dose of study medication

[b]Efficacy evaluable population includes all ITT patients who met prespecified entry criteria and have at least baseline and one on-study disease assessments adequate for evaluation using RECIST v1.1

ITT, intent to treat

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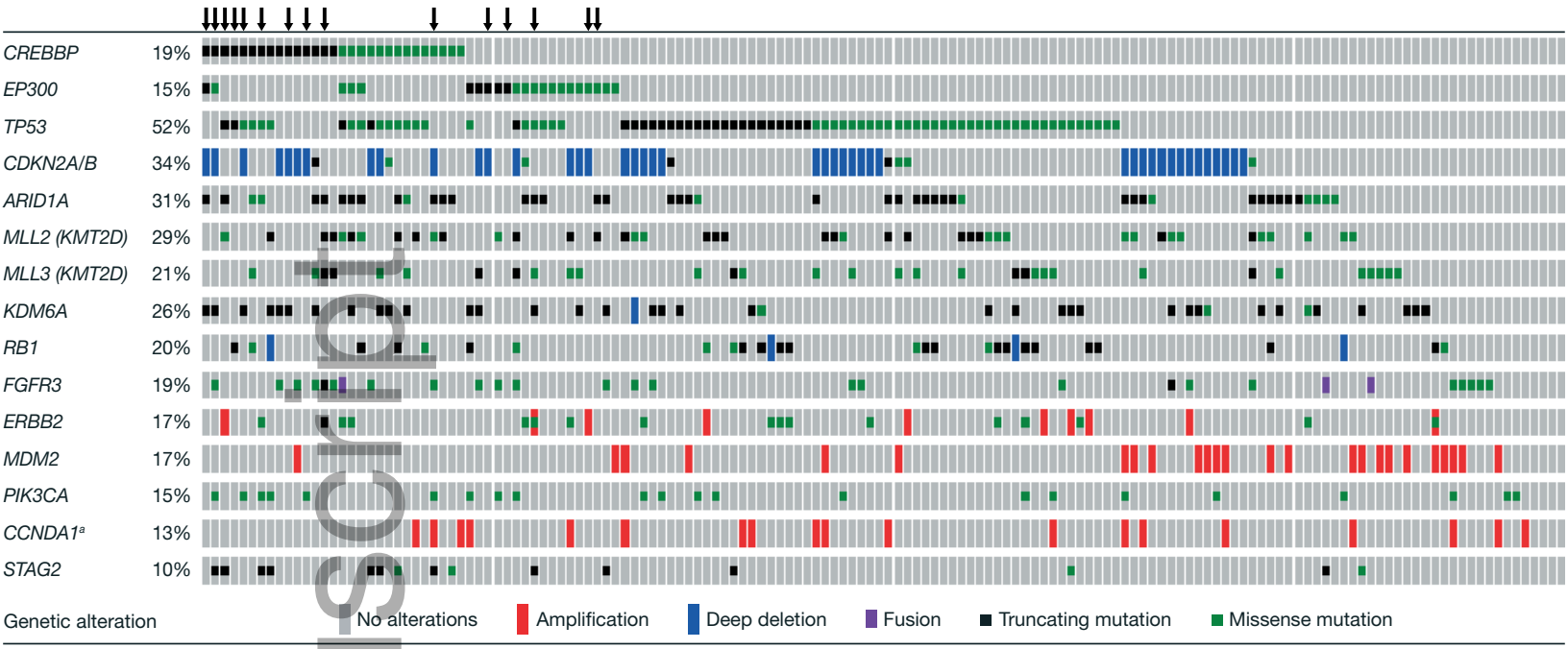

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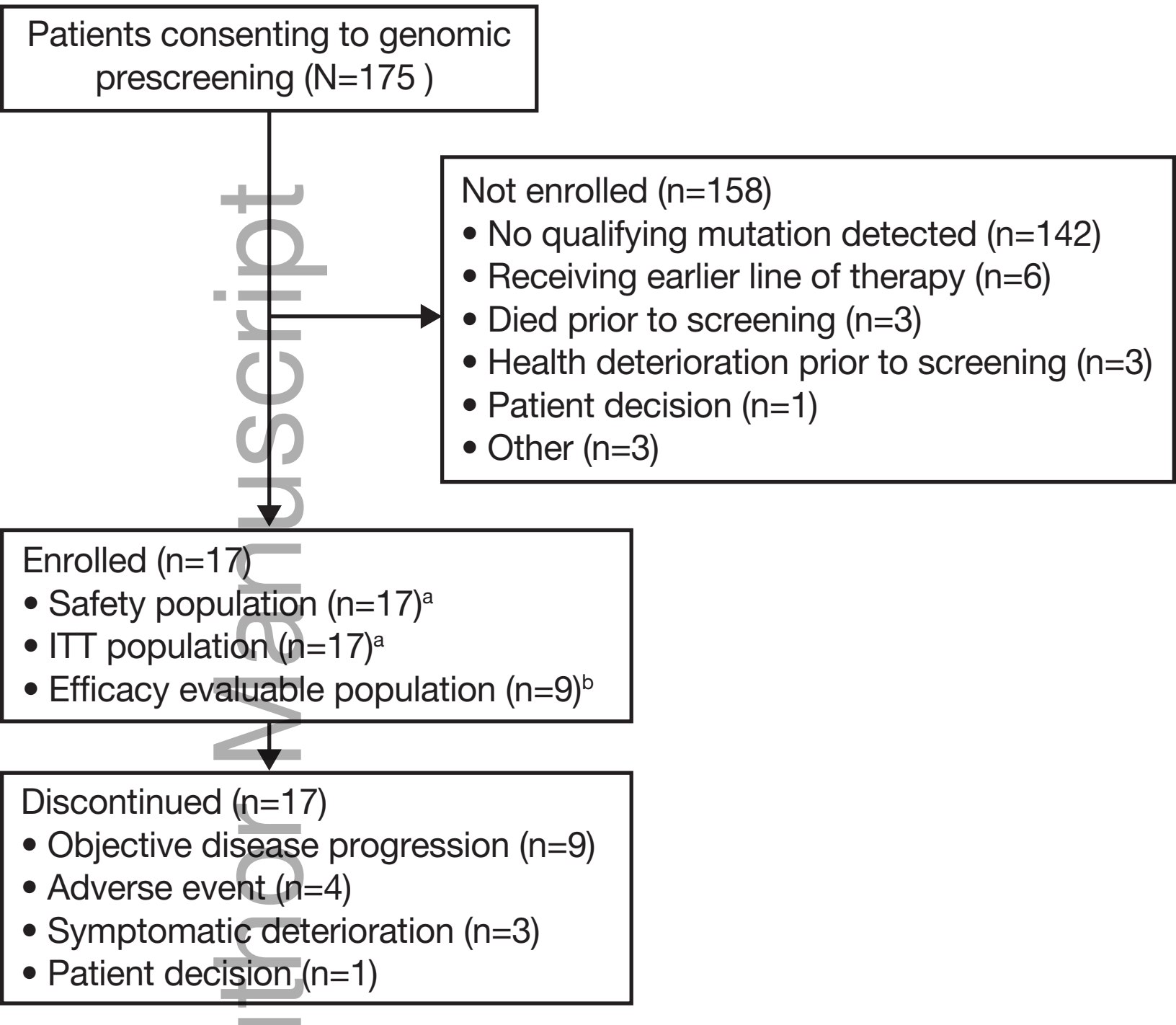

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