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Brief Communications

MET D1228N and D1246N are the Same Resistance Mutation in *MET* Exon 14 Skipping

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Abstract

Comprehensive genetic profiling using next-generation sequencing technologies has become an integral part of precision oncology. Variant annotation requires translating the DNA findings into protein level predictions. In this article we highlight inconsistencies in variant annotation for the MET D1228N exon 19 resistance mutations. MET D1228N and D1246N represent the same resistance mutation in MET exon 14 skipping alterations annotated on different transcripts. Additional examples of relevant variants annotated on different transcripts emphasize the importance of avoiding erroneous interpretation when realizing precision oncology. ***The Oncologist*** 2021;26:e2297–e2301

Comprehensive genetic profiling using next-generation sequencing technologies has become an integral part of precision oncology [1]. The new wealth of genetic information underscores the need for accurate interpretation and clear communication between laboratory professionals and clinicians. Recent studies have shown marked variability in the interpretation of the pathogenicity of genomic alterations [2]. Although there are numerous reasons contributing to the complexities of interpreting genetic data, one underlying cause of propagating inconsistencies in variant annotation is the need to translate the DNA findings into protein-level predictions. Paralleling the central dogma of molecular biology (DNA → RNA → protein), inconsistencies in protein prediction arise when mutations are named using different transcript templates resulting in different amino acid positions that represent the same DNA-level variant.

Although there are numerous examples of inconsistencies in variant annotation, one of the most common *MET* resistance alterations represents a particularly interesting case study. We recently encountered inconsistencies in the annotation for the *MET* D1228N exon 19 resistance mutation. In our analyses, we have observed a discrepancy of the described mutation annotation in prior publications (Fig. 1A) [3–13]. Specifically, this *MET* point mutation confers resistance to inhibitors targeting *MET* exon 14 skipping mutants and can be annotated as D1228N or D1246N depending on the transcript used (Fig. 1A) [6, 7]. The D1246N annotation is based on NM_001127500.3 (*MET* transcript, variant 1; Fig. 1B), whereas the D1228N annotation is based on the 18 amino acid–shorter transcript, NM_000245.4 (*MET* transcript, variant 2; Fig. 1C).

For bioinformaticians, the genomic coordinates of the mutation is considered the ground truth and self-explanatory—although the version (so-called assembly) of the reference genome must also be noted for an unmistakable annotation (e.g., genome version hg19=GRCh37 from 2009 vs. hg38=GRCh38 from 2013). Translating the nucleotide alteration to the protein level enables immediate recognition of its clinical significance. For example, most oncologists readily identify *BRAF* V600E, *EGFR* L858R, and *KRAS* G12C as oncogenic driver alterations for which Food and Drug Administration–approved targeted therapies are available. In this *MET* example, the genomic alteration is hg19 Chr:7 Pos:116423407 G > A, and although it is unambiguous, the syntax is less useful than *MET* D1228N, the terminology used most commonly in the clinical literature. The D1228N mutation has been originally described as a relevant resistance mutation arising in lung cancers with *MET* exon 14 skipping mutations after treatment with MET tyrosine kinase inhibitors [14]. However, the original description of the variant years earlier used the longer transcript (D1246N). Although D1228N has become part of the clinical lexicon as a key resistance mutation, genomic standards have since evolved to annotate the longest transcript, resulting in D1246N. Thus, currently both *MET* D1228N and D1246N coexist, for example, when reports are received from two laboratories using different transcript isoforms for mutation annotation.

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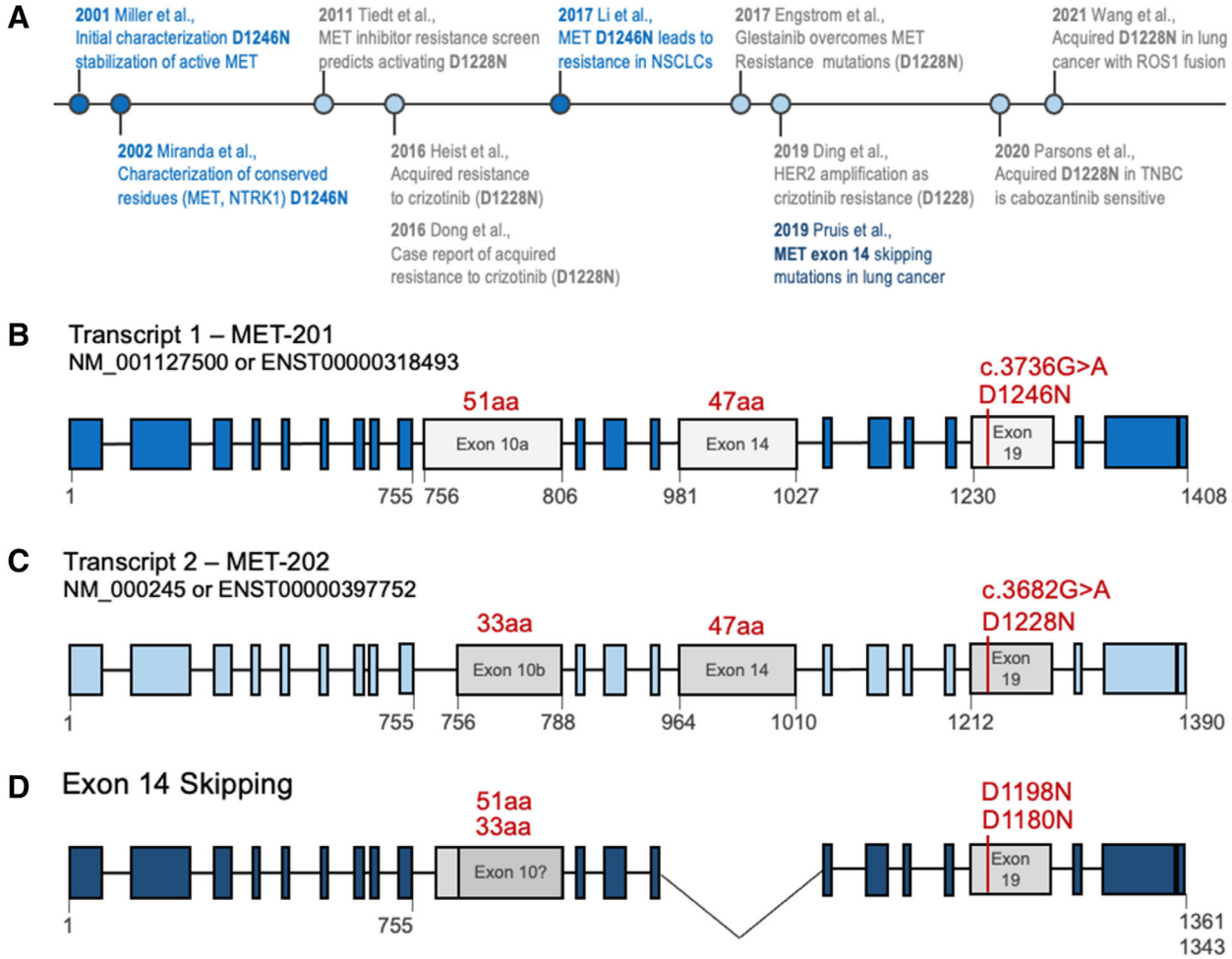

Figure 1. The *MET* D1246N and D1228N are identical variants annotated on different transcript. **(A):** Timeline of literature usage of *MET* D1246N (blue) or D1228N (gray) with key findings; references are in supplemental online Table 1. **(B):** Schematic of *MET* transcript 1, which is 6,876 nucleotides (1,408 amino acids) and uses exon 10a (gray). The exon 19 resistance mutation is denoted D1246N (c.3736G>A) (red). The formal annotation for hg19 Chr:7 Pos:116423407 Ref:G Alt:A is Ensembl: ENST00000318493: c.3736G>A; ENSP00000317272: p.Asp1246Asn, National Center for Biotechnology Information (NCBI): NM_001127500. Exon lengths in amino acids are shown in red. Relevant amino acid residues are shown in gray below exons. **(C):** Schematic of *MET* transcript 2, which is 6,822 nucleotides (1,390 amino acids) and uses the 18 amino acid–shorter exon 10b (gray). The exon 19 resistance mutation is denoted D12228N (c.3682G>A) (red). The formal annotation for hg19 Chr:7 Pos:116423407 Ref:G Alt:A is Ensembl: ENST00000397752: c.3682G>A; ENSP00000380860:p.Asp1228Asn, NCBI: NM_000245. Exon lengths in amino acids are shown in red. Relevant amino acid residues are shown in gray below exons. **(D):** *MET* exon 14 skipping transcript. In *MET* exon 14 skipping, the 47 amino acids encoded by exon 14 are missing, resulting in a shorter protein. This results in a shift of the C-terminal amino acid numbering; the resistance mutations are shown in red. It is currently unknown whether all *MET* exon 14 skipping cases use exon 10a or 10b—therefore, the assumed position of the resistance mutation is annotated as D1198N (exon 10a) or D1180N (exon 10b). Relevant amino acid residues are shown in gray below exons.
Abbreviations: aa, amino acids; MET-201, *MET* transcript 1; MET-202, *MET* transcript 2; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer.

There are additional aspects further complicating this discrepancy in the setting of *MET* exon 14 skipping mutations. First, because of exon 14 skipping during RNA splicing, the pathogenic transcript isoform is 47 amino acids shorter. Second, it is unclear whether this transcript uses exon 10a or 10b (Fig. 1D); the exact exon makeup is currently unclear. Third, the 11-kb genomic distance between *MET* exon 14 skipping mutations and the D1228N/D1246N resistance mutation in exon 19 (~11 kb) poses unique technical challenges in delineating the specific transcript isoform used. Thus, in tumors containing *MET* transcripts missing exon 14, neither D1246N nor D1228N truly represents the actual position at the protein level (Fig. 1D). The precise annotation on a mutant-specific transcript, although biologically accurate, is technically problematic because of current sequencing limitations. Therefore, selection of a standard wild-type reference transcript is necessary for consistency across laboratories.

Although we focus here on the specific *MET* D1246N/D1228N example, this issue is readily generalizable, as there are several other examples that share similar discrepancies (Table 1). However, there are also several precedents for successful nomenclature changes; for example, *BRAF* V600E (formerly reported as *BRAF* V599E) and *H3F3A* K28M/G35

Table 1. Examples of relevant mutations and their preferred clinical and selected inconsistent variant annotations

Gene symbol	Amino acid alteration	Nucleotide alteration Transcript ID(gene):coding DNA position (AA change)	Genomic COORDINATES Chromosome:position (assembly)	Clinical relevance/context
ABL1	T315I	NM_005157.6(ABL1):c.944C>T (p.Thr315Ile)	Chr9:130872896C>T(GRCh38) =Chr9:133748283C>T (GRCh37)	Imatinib resistance mutation MANE selected
	T334I (same as T314I)	NM_007313.2(ABL1):c.1001C>T (p.Thr334Ile)	Same as above	
ABL1	M351T	NM_005157.6(ABL1):c.1052T>C (p.Met351Thr)	Chr9:130873004T>C(GRCh38) =Chr9:133748391T>C (GRCh37)	Imatinib resistance mutation MANE selected
	M370T (same as M351T)	NM_007313.2(ABL1): c.1109T>C (p.Met370Thr)	Same as above	
ABL1	E236K	NM_005157.6(ABL1):c.706G>A (p.Glu236Lys)	Chr9:130862919G>A(GRCh38) =Chr9:133738306G>A (GRCh37)	Imatinib resistance mutation MANE selected
	E255K (same as E236K)	NM_007313.2(ABL1):c.763G>A (p.Gly255Lys)	Same as above	Imatinib resistance mutation
BRAF	V640E*	NM_001374258.1(BRAF): c.1919T>A (p.Val640Glu)	Chr7:140753336T>A(GRCh38) =Chr7:140453136T>A (GRCh37)	Targetable *BRAF* mutation MANE selected
	V600E	NM_001378468.1(BRAF): c.1799T>A (p.Val600Glu)	Same as above	Targetable *BRAF* mutation
	V599E (same as V600E)	NM_001378468.1(BRAF): c.1799T>A (p.Val599Glu)	Same as above	Initial numeration was disregarding the first methionine; not in use anymore
EGFR	A289T	NM_005228.5(EGFR):c.865G>A (p.Ala289Thr)	Chr7:55221821G>A(GRCh37) =Chr7:55154128G>A(GRCH38)	Hotspot variant (used in COSMIC)
	A244T (same as A289T)	NM_001346897.2(EGFR): c.730G>A (p.Ala244Thr)	Same as above	Hotspot variant (used in TCGA)
EGFR	L858R	NM_005228.5(EGFR):c.2573T>G (p.Leu858Arg)	Chr7:55191822T>G(GRCh38) =Chr7:55259515 T>G (GRCh37)	Targetable *EGFR* hotspot mutation MANE selected
	L813R (same as L858R)	NM_001346897.2(EGFR): c.2438T>G (p.Leu813Arg)	Same as above	Targetable *EGFR* hotspot mutation
	L805R (same as L858R)	NM_001346900(EGFR): c.2414T>G (p.Leu805Arg)	Same as above	Targetable *EGFR* hotspot mutation
	L591R (same as L858R)	NM_001346941(EGFR): c.1772T>G (p.Leu591Arg)	Same as above	Targetable *EGFR* hotspot mutation
EGFR	T790M	NM_005228.5(EGFR):c.2369C>T (p.Thr790Met)	Chr7:55181378C>T(GRCh38) =Chr7:55249071C>T(GRCh37)	*EGFR* resistance/targetable mutation
	T745M (same as T790M)	NM_001346897.2(EGFR): c.2234C>T (p.Thr745Met)	Same as above	*EGFR* resistance/targetable mutation
ERBB2	V777L[b]	NM_004448.3(ERBB2): c.2329G>T (p.Val777Leu)[b]	Chr17:39724747(GRCh38) =Chr17:37881000(GRCh37)	Oncogenic signaling No MANE selection
	V777L[b]	NM_004448.3(ERBB2): c.2329G>C (p.Val777Leu)[b]	Same as above	Different nucleotide change converges at amino acid level
	V747L (same as V777L)	NM_001005862.2:c.2239G>T (p.Val747Leu)	Same as above	Oncogenic signaling
	V762L (same as V777L)	NM_001289936.1:c.2284G>T (p.Val762Leu)	Same as above	Oncogenic signaling
FGFR2	N549H	NM_00141.5(FGFR2):c.1645A>C (p.Asn549His)	Chr10:121498522A>C(GRCh38) =Chr10:123258036A>C (GRCh37)	Crouzon syndrome MANE selected
	N550H (same as N549H)	NM_001144913.1(FGFR2): c.1648A>C (p.Asn550His)	Same as above	Crouzon syndrome
	N437H (same as N549H)	NM_001144914.1(FGFR2): c.1309A>C (p.Asn437His)	Same as above	Crouzon syndrome

(continued)

Table 1. (continued)

Gene symbol	Amino acid alteration	Nucleotide alteration Transcript ID(gene):coding DNA position (AA change)	Genomic COORDINATES Chromosome:position (assembly)	Clinical relevance/context
H3 (F3A)	K28M	NM_002107.6(H3F3A):c.83A>T (p.Lys28Met)	Chr1:226064434(GRCh38) =Chr1:226252135(GRCh37)	Diagnostic biomarker for midline glioma
	K27 (same as K28)	NM_002107.6(H3F3A):c.83A>T (p.Lys27Met)	Same as above	Initial numeration was disregarding the first methionine; not in use anymore
H3F3A	G35R	NM_002107.6(H3F3A):c.100G>C (p.Gly35Arg)	Chr1: 226064451G>C(GRCh38) =Chr1:226252152G>C (GRCh37)	Diagnostic biomarker for midline glioma
	G34R (same as G35R)	NM_002107.6(H3F3A):c.100G>C (p.Gly34Arg)	Same as above	Initial numeration was disregarding the first methionine; not in use anymore
MET	T992I	NM_000245.4(MET):c.2975C>T (p.Thr992Ile)	Chr7:116771936C>T(GRCh38) =Chr7:116411990C>T (GRCh37)	SNP/activating germline variant MANE selected
	T1010I (same as T992I)	NM_001127500.3(MET): c.3029C>T (p.Thr1010Ile)	Same as above	Single nucleotide polymorphisms Activating germline variant
MET	D1228N	NM_000245.4(MET): c.3682G>A (Asp1228Asn)	Chr7: 116783353G>A(GRCh38) =Chr7:116423407G>A (GRCh37)	Resistance mutation MANE selected
	D1246N (same as D1228N)	NM_001127500.3(MET): c.3736G>A (Asp1246Asn)	Same as above	Resistance mutation

[a]*BRAF* V640E is currently MANE selected (https://www.ncbi.nlm.nih.gov/clinvar/variation/13961/, accessed July 7, 2021).
[b]Different nucleotide changes can converge at the amino acid level.
Abbreviations: AA, amino acids; COSMIC, Catalogue of Somatic Mutations in Cancer; MANE, Matched Annotation from NCBI and EMBL-EBI; SNP, single nucleotide polymorphism; TCGA, The Cancer Genome Atlas.

(formerly reported as K27/G34) [15]. The reason for these one-number amino acid discrepancies was that initial papers disregarded the first methionine, as it is cleaved in an early post-translational state [16]. Variant annotation at the protein level, however, is insufficient for portraying accurate amino acid level changes, as these vary depending on the transcript used. For example, *EGFR* p.L858R could be annotated differently when using other isoforms. *EGFR* p.L858R (NM_005528) is the same variant as *EGFR* p.L813R (NM_001346899), *EGFR* p.L805R (NM_001346900), and *EGFR* p.L591R (NM_001346941). Other pertinent examples are highlighted in Table 1. At a minimum, the transcript ID or RefSeq ID should be included for variant annotations in clinical reports. Many clinical laboratories do report all the relevant information (nucleic acid change, transcript ID, and amino acid change); however, the synthesis of all this information is unwieldly, especially as transcript IDs are less recognizable and unlikely to be memorized.

As our knowledge of clinically relevant specific mutation increases, the field is collectively tasked to converge annotations on one transcript. Large-scale efforts are under way to unify and maintain harmonized transcript nomenclatures (curated independently by the National Center for Biotechnology Information and the European Molecular Biology Laboratories–European Bioinformatics Institute) by merging the annotations of biologically similar transcripts [17]. Importantly, in this case, the MANE Select version 0.93 transcript is NM_000245.4, which would result in D1228N (rather than D1246N).

The confusion caused by variant numbering due to alternative exons is likely only the beginning. As many of the common DNA-level variants have been established and RNA sequencing technologies are now emerging as robust clinical diagnostics, the relevance of isoform-specific alterations will become more apparent. For certain cancer types this has already happened. Aside from *MET* exon 14 skipping mutations as an emerging biomarker in lung cancer, other relevant examples include the androgen receptor splice variant ARv7 (aberrant splicing to cryptic exon 3) in castration-resistant prostate cancer [18] and the *EGFR* variant III (EGFRvIII; deletion of exons 2–7) in glioblastoma [19]. These transcripts are defined by their specific exon composition, and as we identify additional oncologically relevant alternative transcripts, a harmonized transcript nomenclature becomes a foundational building block for reliable annotation and efficient integration into clinical practice.

The practical realization of precision oncology is nuanced, and accurate *MET* transcript annotation represents one relevant challenge. Until one reference wild-type transcript is widely accepted and laboratories consistently follow these principles, it should be noted that

D1228N and D1246N are the same variant. As we move precision oncology forward, these details will be imperative to avoid confusion in publications, avoid erroneous interpretation as a diagnostic inaccuracy, and facilitate clear communication between molecular pathologists and oncologists to improve patient care.

Disclosures

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