**MARIA SISSA PEREIRA SANT'ANA**

# STATUS DE MUTAÇÃO *BRAF* p.V600E EM DIFERENTES ÁREAS MICROSCÓPICAS DO AMELOBLASTOMA

**Faculdade de Odontologia**
**Universidade Federal de Minas Gerais**
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Dissertação apresentada ao Colegiado de Pós-graduação da Faculdade de Odontologia da Universidade Federal de Minas Gerais como requisito parcial à obtenção do grau de Mestre em Odontologia – área de concentração em Estomatologia.

**Orientador:** Prof. Felipe Paiva Fonseca

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# FOLHA DE APROVAÇÃO

**STATUS DE MUTAÇÃO BRAF p.V600E EM DIFERENTES ÁREAS MICROSCÓPICAS DO AMELOBLASTOMA**

## MARIA SISSA PEREIRA SANT'ANA

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Mestre, área de concentração Estomatologia.

Aprovada em 21 de julho de 2020, pela banca constituída pelos membros:

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Coordenadora do Colegiado de Pós-Graduação
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“Não há segredo nenhum, e o teu sonho, a mão do amor traz...”

Oswaldo Montenegro

## RESUMO

*BRAF* p.V600E é a mutação mais comum em ameloblastomas convencionais e ameloblastomas unicísticos. Sabe-se que os cistos odontogênicos não apresentam esta mutação e representam um dos principais diagnósticos diferenciais dos ameloblastomas, em especial de sua variante unicística. Muitos casos de ameloblastoma unicístico e de alguns cistos odontogênicos, como o cisto dentígero e o cisto inflamatório radicular, compartilham semelhanças clínicas e radiográficas, podendo exibir características histológicas semelhantes em biópsias incisionais ou em tecidos muito inflamados, dificultando o diagnóstico preciso da lesão. As diferentes áreas morfológicas encontradas em um ameloblastoma e as características moleculares de cada uma destas regiões devem ser investigadas com o objetivo de determinar se a avaliação molecular ajudaria a estabelecer o diagnóstico correto de ameloblastoma independente de sua apresentação microscópica, contribuindo para a escolha do tratamento mais apropriado pelo cirurgião bucomaxilofacial. Portanto, o objetivo deste estudo foi verificar o status de mutação do gene *BRAF* em regiões histológicas de ameloblastomas que exibiam aspectos microscópicos distintos. Foram analisados cinco casos de ameloblastomas, sendo três casos classificados como Ameloblastoma unicístico e dois casos classificados como Ameloblastoma (convencionais), porém com grandes áreas de degeneração cística. Duas ou três regiões exibindo diferentes características microscópicas foram selecionadas de cada caso e enriquecidas por meio de microdissecção manual. Reações de qPCR ou de sequenciamento de Sanger foram realizadas para determinar a presença da mutação *BRAF* p.V600E. Observamos que quatro casos exibiram a mutação *BRAF* p.V600E em todas as diferentes áreas microscópicas avaliadas, enquanto que o único caso negativo para mutação em *BRAF* também demonstrou negatividade em todas as regiões microscópicas avaliadas. Nossos resultados sugerem que o ameloblastoma exibe um perfil homogêneo em relação à presença da mutação *BRAF* p.V600E ao longo de regiões histológicas com diferentes características microscópicas.



**Palavras-chave:** Ameloblastoma. BRAF. BRAF p.V600E. Tumor odontogênico.

## ABSTRACT

### *BRAF* p.V600E status in epithelial areas of ameloblastoma with different histological aspects

*BRAF* p.V600E is the most common mutation in conventional ameloblastomas and unicystic ameloblastomas. It is known that odontogenic cysts do not present this mutation and that they represent an important differential diagnosis for ameloblastomas, especially its unicystic variant. Several unicystic ameloblastoma and some odontogenic cysts, such as dentigerous and radicular cysts, share clinical and radiographic features, and may exhibit similar histological characteristics in incisional biopsies or in severely inflamed tissues, making it difficult to accurately diagnose the lesion. Different morphological areas found in ameloblastomas and the molecular characteristics of each of these regions should be investigated in order to determine whether molecular evaluation would help to establish the correct diagnosis, contributing to select the most appropriate treatment by oral surgeons. Therefore, the aim of this study was to verify *BRAF* mutational status in histological areas of ameloblastomas presenting different microscopic features. Five cases of ameloblastoma were analysed, with three cases classified as unicystic ameloblastoma and two cases classified as (conventional) ameloblastoma with large areas of cystic degeneration. Two or three regions exhibiting different microscopic characteristics were selected from each case and enriched by manual microdissection. qPCR or Sanger sequencing were performed to determine *BRAF* p.V600E status. We observed that four cases exhibited *BRAF* p.V600E in all different areas evaluated, while the only negative case for *BRAF* mutation also demonstrated negativity in all microscopic regions analyzed. Our results suggest that ameloblastomas appear to exhibit a homogeneous profile regarding *BRAF* p.V600E along histological regions of the tumor presenting different microscopic appearance.



**Keywords:** Ameloblastoma. BRAF. BRAF p.V600E. Odontogenic tumour.


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## LISTA DE ABREVIATURAS E SIGLAS

MAPK Mitogen Activated Protein Kinases

OMS Organização Mundial da Saúde

**SUMÁRIO**

## 1 CONSIDERAÇÕES INICIAIS

Tumores odontogênicos representam um grupo de lesões derivadas de restos epiteliais associados à odontogênese que afetam predominantemente os ossos gnáticos, mas também tecidos moles adjacentes como a gengiva, apresentando comportamento clínico e características histopatológicas bastante variáveis (MEDEIROS *et al.*, 2018; SILVA *et al.*, 2016). De acordo com a última classificação de Neoplasias de Cabeça e Pescoço da Organização Mundial da Saúde (OMS), os tumores odontogênicos são classificados em: tumor odontogênico epitelial, quando originado do epitélio odontogênico sem a presença de ectomesênquima; tumores odontogênicos mesenquimais, quando não há o envolvimento de epitélio odontogênico; e tumores odontogênicos mistos, quando epitélio odontogênico e ectomesênquima odontogênico estão envolvidos (EL-NAGGAR *et al.*, 2017).

Dentre os tumores odontogênicos, excetuando-se os odontomas, o ameloblastoma representa a neoplasia mais comum, correspondendo a cerca de 18% de todos os casos diagnosticados (KREPPEL *et al*., 2018). Geralmente apresenta-se como uma lesão de crescimento lento e contínuo, assintomática, localmente invasiva, podendo exibir comportamento clínico agressivo. Afeta igualmente homens e mulheres, com idade variável de acordo com cada subtipo, porém sendo mais comumente diagnosticado em adultos jovens, e com maior frequência na região posterior de mandíbula (EFFIOM *et al.*, 2018). De acordo com a OMS (2017) os ameloblastomas são classificados em: ameloblastoma (anteriormente denominado ameloblastoma sólido convencional ou multicístico), ameloblastoma unicístico e ameloblastoma periférico ou extraósseo (EL-NAGGAR *et al.*, 2017).

Ameloblastomas (convencionais) ocorrem principalmente entre a quarta e quinta décadas de vida. Quando em estágios avançados, seu comportamento agressivo pode levar ao aparecimento de diversos sinais e sintomas como expansão óssea, limitação de abertura da boca, dificuldade de alimentação e assimetria facial (EFFIOM *et al.,* 2018). Ao exame radiográfico é possível observar uma imagem radiolúcida uni ou multilocular, comumente descrita como bolhas de sabão ou favos de mel. Tais lesões apresentam-se microscopicamente como ilhas ou cordões de tecido epitelial contendo células basais colunares, semelhantes a pré-ameloblastos, exibindo núcleo hipercromático e polarização invertida e vacuolização citoplasmática, estando dispostas em paliçada. Nas regiões centrais destas ilhas

neoplásicas, as células epiteliais estão organizadas mais frouxamente, semelhante ao retículo estrelado do órgão do esmalte (ALMEIDA *et al*., 2016). Microscopicamente, o ameloblastoma (convencional) ainda pode ser categorizado em algumas variantes, como os subtipos folicular, plexiforme, acantomatoso, de células granulares, desmoplásico e de células basais (EL-NAGGAR *et al.*, 2017)

Descrito por Robinson e Martinez em 1977, o ameloblastoma unicístico representa aproximadamente 5%-22% dos casos de ameloblastoma e é considerado uma variante menos agressiva do tumor (AGANI *et al.*, 2016; REICHART *et al*., 1995). Ocorre em pacientes mais jovens, geralmente na segunda década de vida. Radiograficamente, apresenta-se como uma imagem radiolúcida unilocular e bem delimitada que pode assemelhar-se com inúmeros outros tumores e cistos que acometem os ossos gnáticos. Além disso, sua frequente associação com a coroa de um terceiro molar incluso faz com que a semelhança com os cistos dentígeros seja muito forte, tornando este cisto seu principal diagnóstico diferencial. O exame histopatológico revela um epitélio ameloblástico como o descrito anteriormente que reveste parcial ou totalmente uma única cavidade cística, e apresenta uma cápsula fibrosa (PEREIRA *et al*.,2016).

Ameloblastomas unicísticos podem ser classificados em: luminal (limitante epitelial restrito à superfície luminal); intraluminal (apresentando proliferações epiteliais em direção ao lúmen, sem invasão da cápsula fibrosa) e mural (quando observamos ilhas de epitélio ameloblástico invadindo a cápsula de tecido conjuntivo). Quanto a este último subtipo, existe uma discussão em relação à sua natureza realmente unicística e à abordagem terapêutica da lesão, levando em consideração seu comportamento clínico mais agressivo que as demais variantes do ameloblastoma unicístico, assemelhando-se ao ameloblastoma (convencional) (AGANI *et al.*, 2016; DANDRYIAL *et al.*, 2011; JAIN *et al.*,2017).

A abordagem terapêutica padrão para o ameloblastoma é a ressecção cirúrgica com margem de segurança, devido ao potencial infiltrativo e de recorrências locais que ocorrem em cerca de 15% dos casos (KREPPEL *et al*., 2018). Entretanto, esse manejo cirúrgico pode resultar em significativa deformidade facial e alto grau de morbidade, afetando diretamente a qualidade de vida do paciente. A enucleação neoplásica seguida da utilização de solução de Carnoy e/ou osteotomia periférica, é uma opção de tratamento menos agressiva, mais utilizada no contexto do ameloblastoma unicístico (BROWN *et al.*, 2014; KRUPPA *et al.*,

2014; PEREIRA *et al.*, 2016). Casos que recebem esta abordagem estão associados a índices mais elevados de recidivas, porém, com menor morbidade (ALMEIDA *et al*., 2016).

*BRAF* é um gene da via de sinalização celular das proteínas quinases ativadas por mitógeno (MAPKs) que controla diversos processos celulares como proliferação, diferenciação e sobrevivência, através de uma cascata de fosforilações. Quando ocorre a mutação p.V600E, *BRAF* é ativado e desencadeia a sinalização MEK/ERK da via, resultando em aumento da proliferação e sobrevivência celular (BROWN AND BETZ, 2015DHILLON et al., 2007). Atualmente, diversos grupos de pesquisa têm desenvolvido estudos relacionados à biologia molecular do ameloblastoma a fim de esclarecer sua patogênese. De acordo com estudos recentes, a mutação *BRAF* p.V600E tem sido demonstrada como um evento molecular frequente em casos de ameloblastoma (convencional) (BROWN *et al.*, 2014; FREGNANI *et al*., 2017; KRUPPA *el al.*, 2014; SWEENEY *et al.*, 2014) e unicístico (DINIZ *et al.*, 2015; HEIKINHEIMO *et al.*, 2019; PEREIRA *et al.*, 2016). Apesar desta mutação também estar presente em outras neoplasias odontogênicas como o carcinoma ameloblástico e o carcinoma odontogênico de células claras, ela não parece estar ocorrendo em cistos odontogênicos como o cisto dentígero e o cisto inflamatório radicular (BRUNNER *et al*., 2015; DINIZ *et al*., 2015).

Recentemente, inibidores seletivos de *BRAF* tem sido usados para o tratamento de pacientes afetados por outros tipos de neoplasias que também apresentam a mutação p.V600E, como o melanoma, o câncer de pulmão e o câncer colorretal. Entretanto, apesar do tratamento aumentar a sobrevida de alguns pacientes, um grupo de indivíduos acometidos por estas doenças não parece responder bem à quimioterapia com inibidores de BRAF e em alguns casos o tumor adquire resistência ao tratamento (SANZ-GARCÍA *et al*., 2017).

Kaye *et al*. (2015),Tan *et al*. (2015), Faden *et al*. (2017) e Fernandes et al. (2018) relataram o uso de inibidores de BRAF em casos individuais de ameloblastoma que não apresentaram boas respostas clínicas após a realização de terapias cirúrgicas convencionais. Os dois primeiros estudos utilizaram dabrafenibe, durante 16 semanas e 12 meses, respectivamente; e o terceiro utilizou vemurafenibe por 11 meses como tratamento paliativo da paciente. Todos os autores relataram diminuição do tamanho da lesão, com resposta contínua à medicação, mesmo após

um longo período de tempo. Essa resposta prolongada e satisfatória a um único fármaco pode indicar uma característica de homogeneidade molecular do ameloblastoma (FADEN *et al*., 2017; TAN *et al*., 2015).

Quando há falha na resposta ao tratamento, dentre outras possíveis causas, esta pode estar relacionada à heterogeneidade genética exibida pelo tumor. O desenvolvimento de uma neoplasia se dá através de um dano ao DNA de uma única célula que durante a replicação repassa a mutação para seus clones. A contínua proliferação dessas células resulta em uma diversidade clonal que contribui para a heterogeneidade do tumor e consequentemente, possível resistência à terapia (CROCKFORD *et al.*, 2014; DAGOGO-JACK & SHAW, 2017; GOMES *et al.*, 2014). No contexto das neoplasias que apresentam a mutação BRAF p.V600E, estudos recentes têm demonstrado a presença de uma variabilidade genética em diferentes áreas de uma lesão. Yancovitz *et al*. (2012) demonstraram haver uma heterogeneidade na presença de *BRAF* p.V600E tanto inter quanto intratumoral em melanomas primários e metastáticos, apesar de Boursault *et al*. (2013) e Riveiro-Falkenbach *et al*. (2015) observarem em seus estudos que esta mutação ocorreria de forma homogênea nos melanomas. Em relação ao câncer de pulmão, a heterogeneidade intratumoral no que se refere à presença da mutação em BRAF também pôde ser encontrada, como demonstrado em todos os casos investigados por Tatematsu *et al*. (2015).

A possível relação entre a presença da mutação *BRAF* p.V600E e a apresentação microscópica do ameloblastoma já foi discutida anteriormente na literatura. Tan *et al*. (2015) sugeriram em seu relato de caso que após a utilização dos inibidores de *BRAF* houve uma modificação no aspecto microscópico da lesão que se apresentou com mais áreas escamosas. Entretanto, no estudo que desenvolvemos avaliando o potencial prognóstico da mutação em uma ampla amostra de ameloblastomas, não foi possível identificar associações entre a ocorrência de *BRAF* p.V600E e os subtipos microscópicos da neoplasia (FRAGNANI *et al*., 2016). A possível relação entre a presença da mutação e diferentes aspectos microscópicos do ameloblastoma torna-se clinicamente importante ao considerarmos que em muitas biópsias incisionais de lesões císticas ou predominantemente císticas, não é possível realizar uma diferenciação diagnóstica segura e confiável entre ameloblastomas e outros cistos odontogênicos como o cisto dentígero e o cisto

inflamatório radicular, comprometendo a escolha terapêutica mais apropriada pela equipe de cirurgia buco maxilofacial (DUNSCHE *et al*., 2003; PEREIRA *et al*., 2016).

Portanto, considerando a complexidade biológica do ameloblastoma e suas características clínicas de maior agressividade local, ao mesmo tempo que a apresentação microscópica do tumor muitas vezes não permite a realização de um diagnóstico seguro em biópsias incisionais pequenas e altamente inflamadas, a identificação de biomarcadores moleculares como a mutação *BRAF* p.V600E poderia representar uma ferramenta diagnóstica auxiliar nestes casos borderline, entendendo, porém, que esta mutação não está presente em 100% dos casos de ameloblastoma. Entretanto, é necessário investigar se a presença ou ausência da mutação nos ameloblastomas ocorre de forma consistente em toda extensão neoplásica, independentemente da existência de áreas microscópicas com aspectos histopatológicos distintos, como sugerido por Gomes et al.(2014). Por fim, esta discussão também poderá trazer evidências iniciais quanto ao perfil de heterogeneidade genética que esta neoplasia odontogênica pode possuir, no contexto da mutação *BRAF* p.V600E.

## 1.1 Objetivos da pesquisa

### 1.1.1 Objetivos gerais

Avaliar o status de mutação do gene *BRAF* em ameloblastoma (convencional) e ameloblastoma unicístico.

### 1.1.2 Objetivos específicos

a) Investigar a presença da mutação *BRAF* p.V600E em áreas tumorais exibindo diferentes aspectos microscópicos.

b) Verificar a utilidade da identificação da mutação *BRAF* p.V600E para o diagnóstico de ameloblastoma independente dos aspectos microscópicos exibidos pelo tumor.

c) Determinar se o ameloblastoma possui evidências de heterogeneidade genética quanto à presença da mutação *BRAF* p.V600E.

# 2 METODOLOGIA EXPANDIDA

## 2.1 Seleção da amostra

Cinco casos de ameloblastoma fixados em formalina e embebidos em parafina (FFPE) foram obtidos dos arquivos do Departamento de Patologia Oral da Universidade Federal de Minas Gerais (Belo Horizonte/Brasil) e dos arquivos de Patologia Oral da Universidad Autónoma de Nuevo León (Monterrey/México). Todos os casos apresentavam dados clínicos, radiográficos, macroscópicos e histológicos completos, e os diagnósticos foram confirmados por dois patologistas orais (FPF e RSG), seguindo as diretrizes da Organização Mundial de Saúde (OMS) para classificação de tumores de cabeça e pescoço (VERED *et al*., 2017). Os tumores consistiam em três ameloblastomas unicísticos e dois ameloblastomas (convencionais) exibindo grandes áreas de degeneração cística. Resumidamente, os ameloblastomas unicísticos apresentavam macroscopicamente e histologicamente uma única cavidade cística envolvida por epitélio ameloblástico. Enquanto que ambos os casos de ameloblastoma (convencional) apresentavam pelo menos uma área de grande degeneração cística envolvida por epitélio ameloblástico, bem como ilhas e ninhos de epitélio ameloblástico no estroma de tecido conjuntivo fibroso

Este estudo foi desenvolvido de acordo com os padrões éticos e foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (CAAE: 97428718.5.0000.5149).

## 2.2 Dissecção manual

Para cada amostra de ameloblastoma unicístico, duas áreas microscopicamente distintas foram selecionadas e obtidas por dissecção manual. Área 1: epitélio odontogênico neoplásico apresentando características microscópicas típicas ou muito sugestivas de ameloblastoma, isto é, presença de células epiteliais colunares semelhantes a pré-ameloblastos, dispostas em paliçada com núcleos hipercromáticos, e mais superficialmente, células dispostas frouxamente, semelhantes ao retículo estrelado do órgão do esmalte. Área 2: epitélio odontogênico neoplásico que não apresentava características de epitélio

ameloblástico, como por exemplo, áreas de epitélio atrófico, hiperplásico, ausência de organização de células em paliçada, dentre outras.

Para as duas amostras de ameloblastoma (convencional), a Área 1 e a Área 2 foram selecionadas a partir do revestimento epitelial das grandes degenerações císticas seguindo os critérios microscópicos descritos acima. Além disso, a Área 3 compreendia ilhas ou ninhos de epitélio ameloblástico presentes na cápsula do tumor.

As áreas de interesse foram selecionadas e delimitadas a partir da análise da lâmina de H&E sobreposta ao bloco de parafina correspondente de cada amostra. Após a seleção, essas áreas foram dissecadas manualmente a 7-10µm usando um Micrótomo Rotary 820 Spencer Type (American Optical Company), para posterior extração de DNA.

## 2.3 Extração de DNA

Fragmentos dissecados de áreas selecionadas foram desparafinizados com Solução Desparafinizadora (QIAGEN, Hilden, Alemanha) e digeridos com proteinase K (QIAGEN, Hilden, Alemanha) a 56° C overnight. O DNA genômico (gDNA) foi isolado da parafina usando o kit QIAamp DNA FFPE Tissue (QIAGEN, Hilden, Alemanha) de acordo com as instruções do fabricante. A qualidade do gDNA foi analisada por espectrofotometria no equipamento NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, EUA) considerando a razão de absorbância. A quantificação do DNA foi realizada com o fluorímetro Qubit 4 (Thermo Fisher Scienti fi c Inc). O gDNA extraído foi armazenado a -20 ° C.

## 2.4 Reação em Cadeia de Polimerase Quantitativa (qPCR)

O status de mutação foi avaliado pela reação em cadeia da polimerase quantitativa específica do alelo competitivo TaqMan (qPCR), que é um ensaio altamente específico e sensível que pode detectar pequenas quantidades de DNA mutado. As reações foram realizadas usando sondas TaqMan específicas (BRAF_476_mu e BRAF_rf) para detectar a transversão T> A na posição c.1799 (Applied Biosystems®, Foster City, EUA). As reações foram realizadas através do equipamento StepOne Plus (Applied Biosystems) usando o protocolo universal de

termociclagem para detecção de mutação (95 ° C por 10 min; 5 ciclos: 92 ° C por 15 se 58 ° C por 1 min; 40 ciclos: 92 ° C por 15 se 60 ° C por 1 min).

## 2.5 Detecção da mutação

O status da mutação foi determinado usando o software Taqman Mutation Detector ™, versão 2.0 (Life Technologies Corporation, Carlsbad, EUA). O limite delta Ct (Ctmutado - Ctselvagem; limiar 0,2) foi 9 (Lang et al., 2011) e os valores de Ct acima de 37,0 foram desconsiderados.

## 2.6 Sequenciamento de Sanger

Em relação à amostra em que qPCR não funcionou, o sequenciamento de Sanger foi usado para investigar a presença ou ausência da mutação. A PCR convencional foi realizada, seus produtos foram purificados usando o Reagente de Limpeza de Produtos ExoSAP-IT ™ PCR (Life Technologies) e o sequenciamento do DNA foi realizado usando o Big Dye Terminator v3. 1 Kit de seqüenciamento de ciclo (Applied Biosystems) e executado em um analisador de DNA ABI 3730 (Applied Biosystems). Foi realizado o sequenciamento unidirecional e os cromatogramas foram analisados manualmente no aplicativo Snap Gene® Viewer usando as sequências de referência para comparação**.**

# 3 ARTIGO

Os resultados foram escritos em língua inglesa na forma de artigo científico.

## 3.1 Artigo Científico

Artigo submetido ao periódico internacional: **Journal of Oral Pathology and Medicine** (Qualis A2 – Fator de impacto: 2.03, em 2020).

**_BRAF_ p.V600E status in epithelial areas of ameloblastoma with different histological aspects**

**Running title: BRAF p.V600E status in different areas of ameloblastoma**

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**Conflict of interest:** None.

## ABSTRACT

**Background:***BRAF* p.V600E is present in up to 80% of conventional and unicystic ameloblastomas. Meanwhile, it is known that some odontogenic cysts do not present this mutation, but are considered important differential diagnoses for ameloblastomas, especially for the unicystic variant. Many cases of unicystic ameloblastomas and some odontogenic cysts, such as dentigerous and radicular cysts, may share clinical and radiographic features, possibly exhibiting similar histological characteristics in incisional biopsies, hampering an accurate diagnosis in many cases. Microscopic areas of ameloblastomas presenting different histological appearance must be investigated with regards to their molecular characteristics in order to determine whether the evaluation of molecular features would reliably help to establish the diagnosis independent of the tumour microscopic variability. Therefore, this study aimed to verify *BRAF* mutational status in microscopic regions of ameloblastomas presenting different histological aspects. **Methods:** Five cases of ameloblastoma were analysed. Two or three regions exhibiting different microscopic characteristics were selected from each case and manually dissected. TaqMan allele-specific qPCR or Sanger sequencing were performed to determine *BRAF* p.V600E status. **Results:** We observed that four cases exhibited *BRAF* p.V600E in all different areas evaluated, while the only negative case for the *BRAF* mutation also showed negative mutation status in all microscopic regions analysed. **Conclusion:** Results demonstrate that ameloblastomas appear to exhibit a homogeneous profile regarding the *BRAF* p.V600E no matter what histological feature is observed under light microscopy, suggesting that this molecular test may contribute to establish the correct diagnosis in cases that this odontogenic tumour microscopically resembles other odontogenic cysts.


**Keywords:** ameloblastoma; BRAF; BRAF p.V600E; odontogenic tumour.

## Introduction

Odontogenic tumours represent a group of gnathic lesions with variable clinical behaviour and histopathological features. Ameloblastoma is the most common odontogenic neoplasm, with locally aggressive clinical behaviour associated with frequent recurrences and high morbidity rates.[1] Although rare, the malignant transformation of ameloblastoma into ameloblastic carcinoma may also occur.[2] Treatment approaches vary, but surgical resection is the standard treatment for ameloblastomas; however, it frequently results in facial deformity and functional impairment.[3]

According to the World Health Organization Classification of Head and Neck Tumours, ameloblastomas is classified into ameloblastoma (formerly called solid or multicystic ameloblastoma), unicystic ameloblastoma and peripheral/extraosseous ameloblastoma. Conventional ameloblastoma is the most common subtype, accounting for approximately 90% of all cases, while unicystic ameloblastoma represents 5 to 22%. Moreover, ameloblastomas commonly demonstrate different architectural histological patterns in one single case.[4]

Although ameloblastoma microscopic diagnosis is usually a straightforward approach in routine pathology workflow, small biopsies of unicystic ameloblastomas and those with severe inflammatory infiltrate may cause significant difficulties because they may resemble other odontogenic lesions like dentigerous and inflammatory cysts. Therefore, in this scenario a reliable microscopic diagnosis may be very difficult to be obtained, potentially leading to incorrect clinical managements. The search for new tools that may contribute to a more reliable diagnosis in these challenge circumstances remains desirable.[5,6]

Recently, *BRAF* p.V600E was shown to be present in approximately up to 80% of conventional ameloblastomas[7-10] and unicystic ameloblastomas, [6,11,12] whereas other odontogenic cysts like keratocysts, dentigerous cyst and inflammatory cyst were shown to be

devoid of this molecular alteration.[6,13] *BRAF* is a gene of the mitogen-activated protein kinase (MAPKs) cell signaling pathway that controls various cellular processes such as cell proliferation, differentiation and survival. When the p.V600E mutation occurs, *BRAF* is constitutively activated and triggers MEK/ERK signalling pathway, resulting in increased cell proliferation and survival, and consequent neoplastic transformation.[14]

Selective *BRAF* inhibitors, such as vemurafenib and dabrafenib, have been approved for the treatment of mutation-positive cancers, like melanoma and colorectal carcinoma[15,16] and have already been used in recurrent *BRAF* p.V600E positive ameloblastomas non-responsive to primary surgical approaches.[17-20] However, although the use of these drugs is associated with increased survival for some patients affected by melanoma, other groups of patients does not seem to benefit from this therapy.[21] Still, colorectal cancer has a more complex and heterogeneous biology, leading to resistance to targeted treatment.[16] This failure in treatment response is partly attributed to genetic heterogeneity of tumours, which may demonstrate different patterns of gene expression in one same tumour.[22]

Although *BRAF* p.V600E has already been investigated in conventional and unicystic ameloblastomas during the last years, there was no attempt to determine whether tumour areas with different microscopic appearance in the same neoplasm would present distinct mutational status, as proposed by Gomes et al. (2014),[23] which may significantly impact the diagnostic reliability of this mutated gene in scenarios where microscopic appearance of ameloblastic epithelium is not representative of the tumour and resemble other odontogenic lesions. Therefore, the aim of this study is to investigate the *BRAF* p.V600E status in microscopic areas of ameloblastomas presenting different histological aspects under light microscopy.

## Materials and Methods

### *Ethical issues*

This study was developed in accordance with ethical standards and was approved by the institutional review board and the Ethics Committee of the Universidade Federal de Minas Gerais (CAAE: 97428718.5.0000.5149).

### *Sample selection*

Five formalin-fixed, paraffin-embedded (FFPE) ameloblastomas were retrieved from the Oral Pathology files of the Universidade Federal de Minas Gerais (Belo Horizonte/Brazil) and from the Oral Pathology files of the Universidad Autónoma de Nuevo León (Monterrey/Mexico). All cases contained full clinical, radiographic (**Figure 1**) and pathologic data available, and the diagnoses were confirmed by two oral pathologists (FPF and RSG) following the latest World Health Organization (WHO) classification of Head and Neck Tumours guidelines. The tumours consisted of three unicystic ameloblastomas and two conventional ameloblastomas. Areas of ameloblastic epithelium and neoplastic odontogenic epithelium that did not present ameloblastomatous features were observed in all samples. Briefly, unicystic ameloblastomas presented one single cystic cavity surrounded by ameloblastic epithelium, while both cases of conventional ameloblastoma presented at least one large cystic degeneration surrounded by ameloblastic epithelium as well as neoplastic cells in the tumour capsule. Gross surgical specimens were available for all tumours to support our diagnoses.

### *Tumor tissue selection and manual dissection*

For each unicystic ameloblastoma sample, two different areas were selected according to histopathological characteristics and obtained using manual dissection. *Area 1*: neoplastic

epithelium presenting microscopic features typical or very suggestive of ameloblastoma, i.e., presence of basal columnar pre-ameloblast-like epithelial cells, arranged in palisade with hypercromatic nuclei and overlying loosely arranged cells. *Area 2*: neoplastic odontogenic epithelium that did not present ameloblastomatous features. For conventional ameloblastoma samples, *Area 1* and *Area 2* were selected from the epithelial lining of the large cystic degenerations following the microscopic criteria described above. Additionally, *Area 3* comprised ameloblastic islands present in the tumour capsule (**Figures 2 and 3**).

The areas of interest were delimited and selected using H&E slide superimposed to its corresponding paraffin block for each sample. After selection, these areas were manually dissected at 7-10μm using a Microtome Rotary 820 Spencer Type (American Optical Company) for posterior DNA extraction.

*DNA extraction*

Dissected fragments from selected areas were deparaffinized with Deparaffinization Solution (QIAGEN, Hilden, Germany), and digested with proteinase K (QIAGEN, Hilden, Germany) at 56°C overnight. Genomic DNA (gDNA) was isolated from FFPE sections using QIAamp DNA FFPE Tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The gDNA quality was analyzed by spectrophotometry NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) considering the absorbance ratio. gDNA quantification was performed with Qubit 4 Fluorometer (Thermo Fisher Scientific Inc). The extracted gDNA was stored at -20°C, until it was used.

*BRAF p.V600E assessment by TaqMan allele-specific qPCR*

The *BRAF* p.V600E mutation status was assessed by TaqMan allele-specific quantitative polymerase chain reaction (qPCR), which is a highly specific and sensitive assay that can detect rare amounts of mutated DNA in a background of wild-type DNA. The

reactions were performed using specific TaqMan probes (BRAF_476_mu and BRAF_rf) to detect the T>A transversion at position c.1799 (Applied Biosystems®, Foster City, USA). The reactions were run on a StepOne Plus instrument (Applied Biosystems) using the universal mutation detection thermocycling protocol.The mutation status was determined using Taqman Mutation Detector™ Software, version 2.0 (Life Technologies Corporation, Carlsbad, USA). Following the manufacturer's recommendation.

*Sanger Sequencing*

For one case in which the allele-specific qPCR reaction was not considered technically satisfactory, Sanger sequencing was applied to investigate whether mutated *BRAF* was present or not in the tumour regions selected. Conventional PCR was performed and its products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Life Technologies). DNA sequencing was performed using Big Dye Terminator v3. 1 Cycle Sequencing Kit (Applied Biosystems) and run on an ABI 3730 DNA Analyzer (Applied Biosystems). Chromatograms were manually inspected in the Snap Gene® Viewer app using the reference sequence for comparison.

**Results**

The mean age of individuals in the selected cases was 22 years (range from 14 to 29 years-old), most of them males (3 males:2 females). All lesions were primary tumours located in the mandible, mainly in posterior region. All patients were treated with surgical excision. Areas of characteristic ameloblastic epithelium and neoplastic odontogenic epithelium that did not present ameloblastomatous features were observed in the histopathologic analyses of all samples.

All five ameloblastoma cases showed homogeneous *BRAF* mutational status in distinct epithelial areas within the same tumour (**Table 1**). When tested by allele-specific qPCR, four samples were *BRAF* p.V600E positive. In only one case (UA1) the *BRAF* mutational status could not be determined by the allele-specific qPCR assay and therefore, Sanger sequencing was carried out. This case showed homogenous *BRAF* p.V600E negativity, since the mutation was not identified in any analysed areas (**Figure 4**).

**Discussion**

Ameloblastoma pathogenesis remains to be better understood, but a series of studies has recently demonstrated the importance of *BRAF* mutation for the development of this neoplasm.[7,8,9] Although *BRAF* p.V600E is mostly present in ameloblastomas, some other odontogenic tumours with ameloblastomatous component also harbour this molecular event.[6,11,24] According to the literature, over than 46% of ameloblastomas are positive for *BRAF* p. V600E, including the unicystic variant.[6,11,12,25] Notably, Heikinheimo et al. (2019)[12] found that 94% of mandibular unicystic ameloblastomas were *BRAF* p.V600E positive.

The importance of *BRAF* mutation for ameloblastoma clinical behaviour is still unclear and very difficult to be determined given the lack of standardized parameters to determine ameloblastoma aggressiveness. A small number of studies suggested an association between the presence of *BRAF* p.V600E and a more aggressive behaviour in ameloblastomas,[10] whereas others did not find an association between the presence of the mutation and clinicopathological features.[25] Despite the paucity of association between *BRAF* p.V600E and clinicopathological parameters in many studies, the high frequency of this mutation in ameloblastomas suggests that it could be eligible for targeted therapy with the currently available *BRAF* inhibitors already approved for clinical management of melanomas and colon cancer.[23,26]

Previously, different research groups reported single cases of recurrent ameloblastomas treated with the *BRAF* inhibitors dabrafenib and trametinib combined;[17] vemurafenib[20] and dabrafenib only,[18,19] describing positive therapeutic effects, including resolution of previous associated symptoms and reduction of the lesion size, even after a long period of follow-up. Replacing radical surgery by targeted therapy for patients affected by ameloblastomas would significantly decrease morbidity and improve their quality of life. However, these drugs have significant side effects such as nausea, skin toxicity, arthralgia,

cutaneous squamous cell carcinoma and hepatic abnormalities, that must be considered in order to choose the best therapy for each patient individually.[27]

Recent studies have demonstrated the occurrence of an important genetic variability regarding the presence of *BRAF* p.V600E, both in one single case and among different cases in human cancers that frequently exhibit this mutation such as melanoma, colorectal cancer and lung cancer.[28,29] In the present study we also attempted to investigate if there would be any genetic heterogeneity in terms of the presence or absence of *BRAF* p.V600E in histological regions of ameloblastomas exhibiting different microscopic aspects. This possible genotype-phenotype correlation in the context of *BRAF* mutation and ameloblastoma was briefly discussed by Tan et al. (2016)[18] that described a morphological change in their ameloblastoma case when the patient was submitted to a *BRAF* p.V600E inhibitor therapy, although several studies failed to identify any association between this mutation and ameloblastoma microscopic subtypes.[10] We observed that 4 out of 5 cases (80%) investigated in our research were positive for *BRAF* p.V600E, corroborating the frequency of occurrence of the mutation observed by previous.[7,12,25]

Moreover, the four cases positive for the mutation also demonstrated its presence in all morphological regions investigated. In the only case where qPCR reaction was not satisfactory, we performed Sanger sequencing that demonstrated the absence of *BRAF* p.V600E in all different areas investigated. The latter result should be seen with caution, as long as direct sequencing is a less sensitive technique that might not identify mutations in a low percentage frequency.[30] Our findings support that *BRAF* p.V600E expression in ameloblastoma is homogeneous in the different areas from the same tumor.

Unicystic ameloblastoma may share clinical and radiographic similarities with other odontogenic lesions like dentigerous and inflammatory radicular cysts. Moreover, unicystic and (conventional) ameloblastomas with large cystic degenerations may display microscopic

features that may strongly resemble both cysts, which is a special concern in small incisional biopsies and in cases with severe inflammatory infiltrate.[5,6] Therefore, although *BRAF* p.V600E is not present in all cases of ameloblastomas, its molecular assessment could be helpful to determine the correct diagnosis in some challenging cases (approximately 80% in some series as described above), which is supported by our previous results demonstrating that these dentigerous and inflammatory cysts do not harbour *BRAF* mutation.[6] However, the occurrence of the mutation in all regions of the neoplastic epithelium, including in those areas that do not resemble ameloblastoma and that may cause some insecurity in terms of its reliable microscopic representativeness, would be mandatory for using the molecular approach as a diagnostic auxiliary, which was demonstrated in our study. Performing this differential diagnosis is very important to determine patients' treatment, since dentigerous and inflammatory cysts demand a more conservative management, not being associated with recurrences or significant local morbidity, whereas ameloblastomas, even its less aggressive unicystic variant, require larger surgical approaches and the potential for local recurrences must be kept in mind by surgeons and clinicians .[3]

In conclusion, our study contributes to the molecular characterization of ameloblastomas by showing that they harbour *BRAF* p.V600E in microscopic areas demonstrating different histological aspects in one same neoplasm. Therefore, molecular detection of *BRAF* p.V600E may be supportive in the differential diagnosis of ameloblastomas with other odontogenic cysts in microscopic challenging cases, making molecular tests a possible complementary approach to clinical, radiographic and histopathological examination.

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**Figure legends**

**Figure 1.** Radiographic images. **A)** Panoramic radiography of case UA1 exhibits a radiolucent image in the right posterior region of the mandible, involving an impacted third molar. **B)** Radiolucent area affecting the posterior region of the mandible, left side. Correspondent to case UA3. **C)** AM4 radiographic image showing a well-defined radiolucent area, involving an impacted third molar.

**Figure 2.** Macroscopic and histopathological aspects of selected UAs. **A; D; G)** Gross aspect of the specimens after surgical removal, exhibiting a single cavity. **B; E; H)** Selected areas which shows characteristics of an ameloblastic epithelium, such as ameloblast-like cells with hyperchromatic nuclei, arranged in palisade with overlaying loosely arranged cells (H&E; 200X). **C; F; I)** Appearance of neoplastic epithelium that does not resemble ameloblastoma, due to lack of ameloblastic features (H&E; 200X).

**Figure 3.** Macroscopic and histopathological aspects of selected AMs. **A; E)** Conventional ameloblastomas showing large cystic degenerations. **B; F)** Selected areas which shows characteristics of an ameloblastic epithelium, such as ameloblast-like cells with hyperchromatic nuclei, arranged in palisade with overlaying loosely arranged cells (H&E; 200X). **C; G)** Appearance of neoplastic epithelium that does not resemble ameloblastoma, due to lack of ameloblastic features (H&E; 200X). **D; H)** Microscopic regions that represent the solid area of the lesions, exhibiting a follicular pattern.

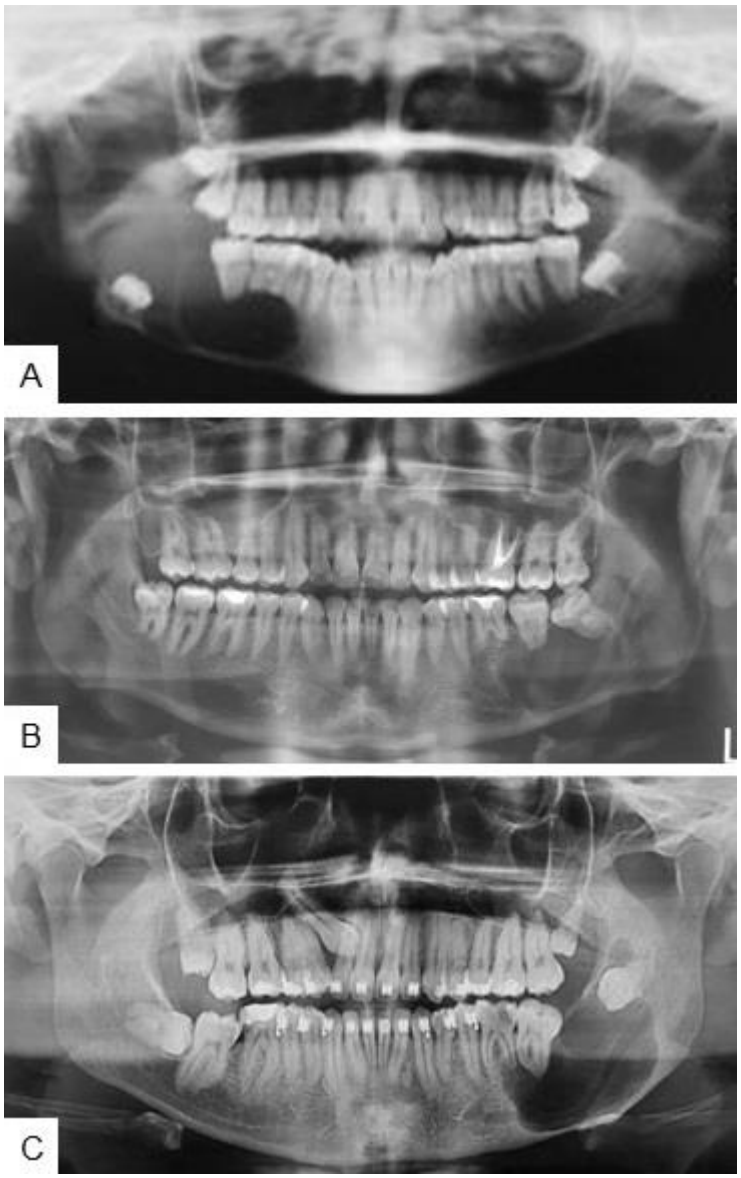
**Figure 4.** **A)** Amplification plot obtained from allele-specific qPCR assay showing the mutation detection. The yellow and red colored curves correspond to amplification of reference gene and mutant allele, respectively. **B)** Sanger Sequencing result. Screen shot of the chromatogram showing the wild type sequence for *BRAF* codon 600.

**Table 1. Clinical features and *BRAF* p.V600E status in each selected area of ameloblastoma cases**

| Case number | Clinical data | | | *BRAF* status | | |
|---|---|---|---|---|---|---|
| | Age (years) | Sex | Location | Representative area | Non representative area | Solid area |
| UA 1 | 14 | F | Mandible | Wild type* | Wild type* | NE |
| UA 2 | 22 | M | Mandible | Mutant | Mutant | NE |
| UA 3 | 21 | M | Mandible | Mutant | Mutant | NE |
| AM 4 | 24 | M | Mandible | Mutant | Mutant | Mutant |
| AM 5 | 29 | F | Mandible | Mutant | Mutant | Mutant |

UA, unicystic ameloblastoma; AM, conventional ameloblastoma; F, female; M, male; NE, not evaluated.

*Results obtained by Sanger Sequencing

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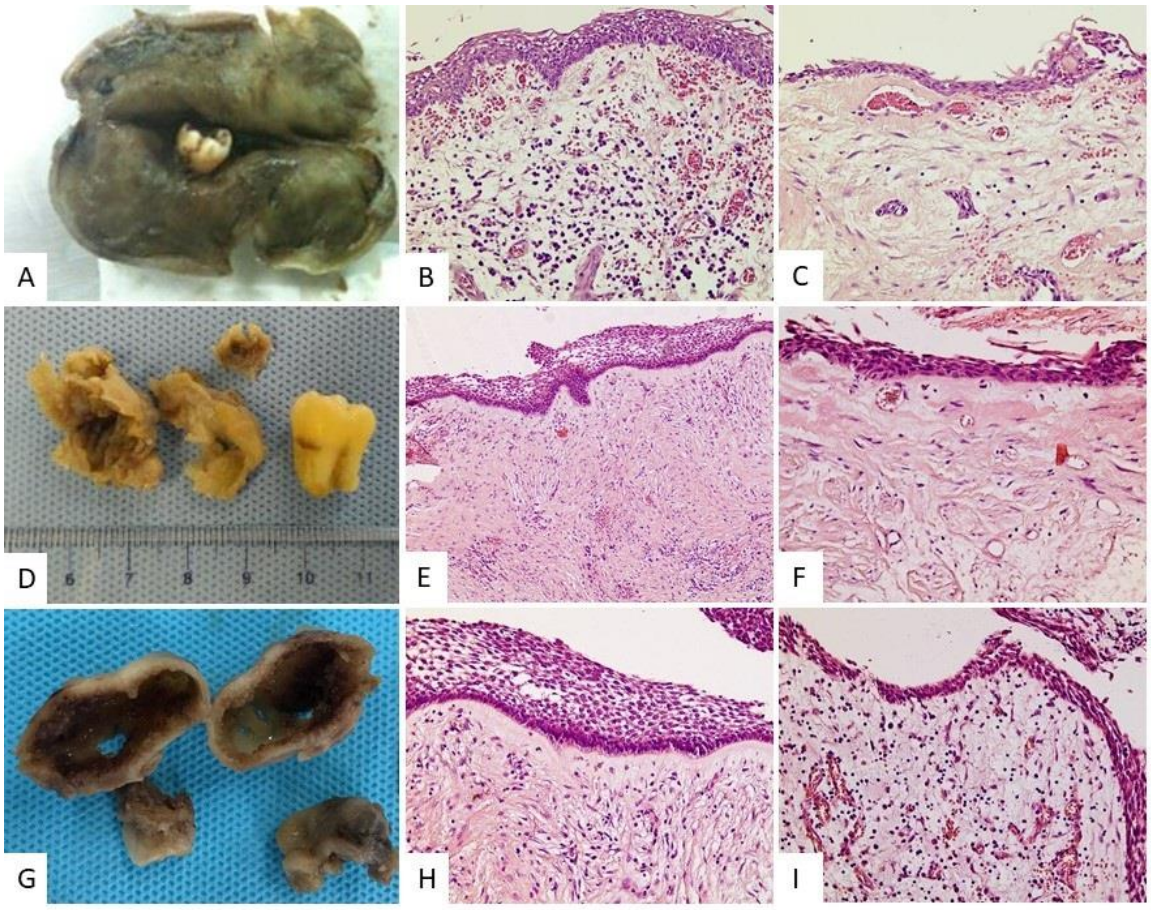


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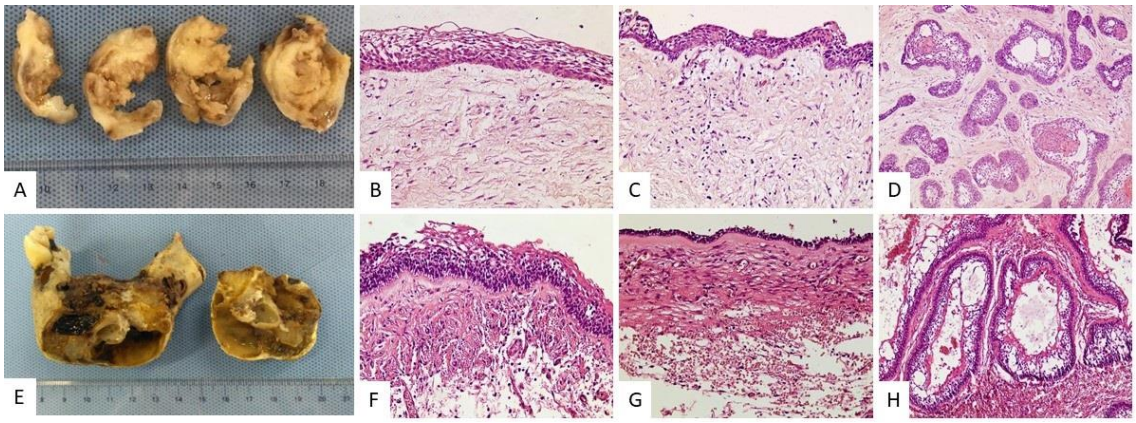


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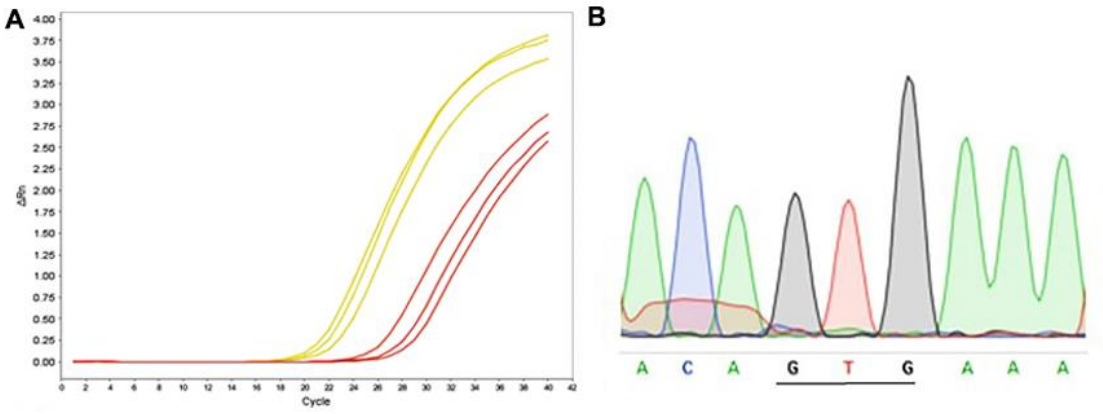


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## 4 CONSIDERAÇÕES FINAIS

O presente estudo contribui com informações relacionadas à biologia molecular dos ameloblastomas. Observou-se a presença da mutação *BRAF* p.V600E nas diferentes áreas histológicas analisadas, tanto no ameloblastoma (convencional) quanto no ameloblastoma unicístico. A presença da mutação em áreas microscópicas não representativas do tumor indica que a análise do status de mutação do gene BRAF pode ser utilizada de forma confiável como auxiliar no diagnóstico da lesão em casos cujo diagnóstico microscópico não é claramente evidente, o que ocorre com maior frequência em biópsias incisionais pequenas ou muito inflamadas. No entanto, a detecção da mutação deve ser analisada em conjunto com as características clínicas, radiográficas e histológicas, porque *BRAF* p.V600E não está presente em 100% dos casos de ameloblastoma. Além disso, a presença da mutação em todas as áreas das amostras analisadas reforça a ideia de que o ameloblastoma seja uma lesão com perfil genético homogêneo, o que poderia facilitar o desenvolvimento e implementação de terapias utilizando inibidores de *BRAF*.

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# ANEXO A - Aprovação do comitê de ética em pesquisa

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

## PARECER CONSUBSTANCIADO DO CEP

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** Análise do perfil metabolômico do Ameloblastoma
**Pesquisador:** Felipe Paiva Fonseca
**Área Temática:**
**Versão:** 3
**CAAE:** 97428718.5.0000.5149
**Instituição Proponente:** UNIVERSIDADE FEDERAL DE MINAS GERAIS
**Patrocinador Principal:** Universidade Federal de Minas Gerais

**DADOS DO PARECER**

**Número do Parecer:** 3.059.929

**Apresentação do Projeto:**
Trata-se de um projeto das grandes áreas de Ciências da Saúde e Biológicas, cuja proposta é realizar um estudo metabolômico de ameloblastoma. Coforme o proponente descreve, o ameloblastoma é uma neoplasia odontogênica epitelial originada do aparato dentário embrionário, cuja patogênese envolve mutações em genes que regulam a proliferação celular. O tumor apresenta formas clínicas e histopatológicas diversas, comportamento clínico agressivo e potencial para sofrer malignização. A metabolômica é uma técnica bioquímica que utiliza a espectrometria de massa e a bioinformática para identificar e quantificar, em uma amostra biológica, os produtos finais das atividades bioquímicas teciduais: os metabólitos. Metabólitos fornecem uma boa correlação com o fenótipo tecidual. Desta maneira, o estudo do perfil metabólico produzido por tumores odontogênicos pode contribuir para o entendimento da patogenia destas lesões, visto não haver a aplicação da metabolômica neste contexto, podendo os resultados ajudar no aprimoramento do manejo clínico destes tumores. Serão utilizadas 14 amostras de ameloblastoma e 6 amostra de tecido odontogênico morfologicamente normal obtidos de casos diagnosticados como capuz pericoronário obtidas de blocos de parafina arquivados no Laboratório de Patologia Bucomaxilofacial da Faculdade de Odontologia da Universidade Federal de Minas Gerais (FO-UFMG). Os diagnósticos serão revisados e confirmados por meio da análise de novos cortes histológicos corados em hematoxilina e eosina e revisados por um patologista oral experiente de acordo com os critérios presentes na última classificação de tumores odontogênicos da

Organização Mundial de Saúde (OMS, 2017). Como critérios de inclusão serão selecionados casos de pacientes com diagnóstico de ameloblastoma confirmados e que possuam tecido parafinizado disponível para os ensaios laboratoriais.

**Objetivo da Pesquisa:**

Como objetivo primário é descrito avaliar o perfil metabolômico de ameloblastoma. Como objetivos secundários são descritos Identificar quais as vias metabólicas mais ativas e quais os metabólitos mais presentes no ameloblastoma, além de identificar possíveis alterações, e comparar com tecido odontogênico morfologicamente normal.

**Avaliação dos Riscos e Benefícios:**

Riscos relacionados à quebra de confidencialidade dos participantes. Benefícios: Compreensão a respeito da etiopatogenia do ameloblastoma e identificação de possíveis biomarcadores

**Comentários e Considerações sobre a Pesquisa:**

Pesquisa relevante, com abordagem inovadora que poderá trazer contribuições para o manejo da neoplasia odontológica estudada. É um pesquisa de risco baixo, por não envolver intervenções diretas ao paciente, mas que prevê o uso de material biológico humano, no caso em questão, de biópsias incluídas em parafina. Também envolve consulta a prontuário dos pacientes. Previsão de término em 2019.

**Considerações sobre os Termos de apresentação obrigatória:**

Foram apresentados Folha de Rosto assinado pela vice-diretora da Faculdade de Odontologia, Parecer consubstanciado aprovado pela Câmara Departamental do Departamento de Clínica, Patologia e Cirurgias Odontológicas, Formulário de Informações Básicas, Projeto Completo, Termo de Constituição de Biorrepositório. Apresentados TCLE, TCLE para responsável e TALE, além de Carta resposta.

**Recomendações:**

Corrigir, no TCLE para responsável, a linguagem do trecho correspondente a autorização de uso de material biológico ( ONDE SE LÊ: ( ) Concordo que o meu material biológico seja utilizado somente para esta pesquisa. ( ) Concordo que o meu material biológico possa ser utilizado em outras pesquisa,..." CORRIGIR PARA " ( ) Concordo que o material biológico do(da) menor sob minha responsabilidade seja utilizado somente para esta pesquisa. ( ) Concordo que o meu material biológico do(a) menor sob minha responsabilidade possa ser utilizado em outras pesquisa,..."

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**Conclusões ou Pendências e Lista de Inadequações:**

O proponente atendeu as solicitações da diligência.

SMJ, o projeto Título da Pesquisa: Análise do perfil metabolômico do Ameloblastoma do pesquisador Felipe Paiva Fonseca, está aprovado. Tendo em vista a legislação vigente (Resolução CNS 466/12), o COEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um suma rio dos resultados do projeto (relatório final).

**Considerações Finais a critério do CEP:**

Tendo em vista a legislação vigente (Resolução CNS 466/12), o CEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

**Este parecer foi elaborado baseado nos documentos abaixo relacionados:**

| Tipo Documento | Arquivo | Postagem | Autor | Situação |
|---|---|---|---|---|
| Informações Básicas do Projeto | PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1193716.pdf | 18/11/2018 14:00:48 | | Aceito |
| Outros | Carta_Resposta2.pdf | 18/11/2018 14:00:24 | André Myller Barbosa Silva | Aceito |
| Projeto Detalhado / Brochura Investigador | Projeto_de_Mestrado_com_alteracoes_COEP2.pdf | 18/11/2018 13:59:59 | André Myller Barbosa Silva | Aceito |
| TCLE / Termos de Assentimento / Justificativa de Ausência | TALE.pdf | 18/11/2018 13:59:28 | André Myller Barbosa Silva | Aceito |
| TCLE / Termos de Assentimento / Justificativa de Ausência | TCLE_responsavel.pdf | 18/11/2018 13:59:16 | André Myller Barbosa Silva | Aceito |

**Endereço:** Av. Presidente Antônio Carlos,6627 2º Ad Sl 2005
**Bairro:** Unidade Administrativa II **CEP:** 31.270-901
**UF:** MG **Município:** BELO HORIZONTE
**Telefone:** (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

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Continuação do Parecer: 3.059.929

| | | | | |
|---|---|---|---|---|
| TCLE / Termos de Assentimento / Justificativa de Ausência | TCLE_paciente.pdf | 18/11/2018 13:58:55 | André Myller Barbosa Silva | Aceito |
| Outros | Termo_constituicao_biorrepositorio.pdf | 18/10/2018 14:46:42 | André Myller Barbosa Silva | Aceito |
| Folha de Rosto | Folha_de_Rosto.pdf | 23/08/2018 12:40:46 | André Myller Barbosa Silva | Aceito |
| Declaração de Instituição e Infraestrutura | Carta_de_anuencia_local_de_pesquisa.pdf | 23/08/2018 12:40:07 | André Myller Barbosa Silva | Aceito |
| Outros | Curriculo_Lattes_Andre_Myller_Barbosa_Silva.pdf | 23/08/2018 09:49:06 | André Myller Barbosa Silva | Aceito |
| Outros | Curriculo_Lattes_Ricardo_Santiago_Gomez.pdf | 23/08/2018 09:44:37 | André Myller Barbosa Silva | Aceito |
| Outros | Curriculo_Lattes_Felipe_Paiva_Fonseca.pdf | 23/08/2018 09:41:33 | André Myller Barbosa Silva | Aceito |
| Declaração de Instituição e Infraestrutura | Parecer_consubstanciado_com_aprovacao_da_camara.pdf | 23/08/2018 09:39:56 | André Myller Barbosa Silva | Aceito |

**Situação do Parecer:**
Aprovado

**Necessita Apreciação da CONEP:**
Não

BELO HORIZONTE, 05 de Dezembro de 2018

**Assinado por:**
**Eliane Cristina de Freitas Rocha**
**(Coordenador(a))**

**Endereço:** Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
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# ANEXO B - Informações da revista

Revista: Journal of Oral Pathology and Medicine - A2

Editor-in-chief: Peter Brennan

## Author Guidelines

**Sections**



### 1. SUBMISSION

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

**Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at https://mc.manuscriptcentral.com/jopm**

**Click here** for more details on how to use ScholarOne.

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**Preprint policy**

**Please find the Wiley preprint policy here**.

This journal accepts articles previously published on preprint servers.

*Journal of Oral Pathology & Medicine* will consider for review articles previously available as preprints. Authors may also post the submitted version of a manuscript to a preprint server at any time. Authors are requested to update any pre-publication versions with a link to the final published article.

For help with submissions, please contact: **JOPM.office@wiley.com**

## 2. AIMS AND SCOPE

*Journal of Oral Pathology & Medicine* publishes manuscripts of high scientific quality representing original clinical, diagnostic or experimental work in oral pathology and oral medicine. The journal does not usually consider papers on periodontal or related diseases. Papers advancing the science or practice of these disciplines will be welcomed, especially those which bring new knowledge and observations from the application of techniques within the spheres of light and electron microscopy, tissue and organ culture, immunology, histochemistry, immunocytochemistry and molecular biology.

Review papers on topical and relevant subjects will receive a high priority and articles requiring rapid publication because of their significance and timeliness will be included as brief reports not exceeding three printed pages.

All submitted manuscripts falling within the overall scope of the Journal will be assessed by suitably qualified reviewers, but manuscripts in an incorrect format will be returned to the author without review.

## 3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

### i. Original Research Articles

*Journal of Oral Pathology & Medicine* welcomes Original Research Articles of high scientific quality representing original clinical, diagnostic or experimental work in oral pathology and oral medicine. Papers advancing the science or practice of these disciplines will be welcomed, especially those which bring new knowledge and observations from the application of techniques within the spheres of light and electron microscopy, tissue and organ culture, immunology, histochemistry, immunocytochemistry and molecular biology.

*Word limit:* 3,000 words maximum, excluding abstract and references.

*Abstract:* 250 words maximum; must be structured, under the sub-headings: Background, Methods, Results, Conclusion. Should not contain abbreviations.

*References:* Maximum of 30 references.

*Figures/Tables:* Total of no more than 6 figures and/or tables.

*Main Text Structure:* should be divided into introduction, material and methods, results and discussion:

- ***Introduction:*** should clearly state the purpose of the article. Give only strictly pertinent references. Exhaustive literature reviews are inappropriate.
- ***Materials and Methods:*** must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced. As a condition of publication, authors are required to make materials and methods used freely available to academic researchers for their own use. This may for example include antibodies etc. Other supporting data sets must be made available on the publication date from the authors directly.
  Please see the Editorial Policies and Ethical Considerations section for requirements related to Clinical Trials, Experimental Subjects and Suppliers.
- ***Results:*** Present your results in logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables, illustrations, or both: emphasize or summarize only important observations.
- ***Discussion:*** Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat in detail data given in the Results section. Include in the Discussion the implications of the findings and their limitations and relate the observations to other relevant studies.

### ii. Review Articles

*Journal of Oral Pathology & Medicine* commissions review articles and also welcomes uninvited reviews. Reviews are subject to peer-review.

*Word limit:* 3,000 words maximum, excluding abstract and references.

*Abstract:* 250 words maximum. Should not contain abbreviations. Please choose headings appropriate for the article.

*References:* Maximum of 50 references.

*Figures/Tables:* Total of no more than 6 figures and/or tables.

*Main Text* must comprise an introduction and a running text structured in a suitable way according to the subject treated. A final section with conclusions may be added.

**iii. Systematic Reviews**

A systematic review is a comprehensive high-level summary of primary research on a specific research question that attempts to identify, select, synthesise and appraise all (high-quality) evidence relevant to that question. A meta-analysis uses statistical methods to quantitatively evaluate pooled data from single studies. Many pathological reviews are likely not to have sufficient data on clinical outcomes to warrant a meta-analysis.

While the content of a systematic review will be partly determined by the topic and evidence, as a minimum the review should:

- Clearly state the purpose of the review
- Determine inclusion and exclusion criteria to generate a PRISMA flowchart that includes identification of studies, screening, eligibility and inclusion data (See Liberati A, Altman DG, Tetzlaff J et al, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009: 339: b2700)
- Determine the primary end point of the review ie. acceptance or rejection of the null hypothesis
- Clearly describe the search methodology [databases (preferably more than one), search terms]
- Describe the process of data extraction
- Undertake statistical assimilation if appropriate
- Evaluate the quality and/or risk of bias of the studies included preferably using a standard assessment tool (See Guyatt, GH, Oxman AD, Vist GE et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008; 336: 924-926
- Provide recommendations for future researches

*Word limit:* 3,000 words maximum, excluding abstract and references.

*Abstract:* 250 words maximum. Should not contain abbreviations. Please choose headings appropriate for the article.

*References:* Maximum of 50 references.

*Figures/Tables:* Total of no more than 6 figures and/or tables.

*Main Text* must comprise an introduction and a running text structured in a suitable way according to the subject treated. A final section with conclusions may be added.

Systematic reviews must be registered in the **PROSPERO** (International Prospective Register of Systematic Reviews) database and the registration number provided in the text.

**iv. Brief Reports**

Original research material requiring rapid publication because of their significance and timeliness will be included as Brief Reports.

*Word limit:* 1,000 words maximum, excluding abstract and references.

*Abstract:* 250 words maximum. Should not contain abbreviations. Please choose headings appropriate for the article.

*References:* Maximum of 10 references.

*Figures/Tables:* Total of no more than 3 figures and tables.

**v. Letters to the Editor**

Letters, if of broad interest, are encouraged. Letters should not be confused with Brief Reports. Letters may deal with material in papers published in *Journal of Oral Pathology & Medicine* or they may raise new issues, but should have important implications.

*Word limit:* 750 words maximum, excluding abstract and references.

*References:* Maximum of 5 references.

*Figures/Tables:* Total of no more than one figure and table.

**Case Reports: Please note that *Journal of Oral Pathology & Medicine* no longer accepts submissions of case reports. The journal also does not accept case reports with an extensive literature review.**

**4. PREPARING THE SUBMISSION**

**Cover Letters**

Cover letters are not mandatory; however, they may be supplied at the author's discretion.

**Parts of the Manuscript**

The manuscript should be submitted in separate files: main text file; figures.

**Main Text File**

The text file should be presented in the following order:

i. A short informative title containing the major key words. The title should not contain abbreviations (see Wiley's **best practice SEO tips**);
ii. A short running title of less than 40 characters;
iii. The full names of the authors;
iv. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
v. Acknowledgments;
vi. Abstract and keywords;
vii. Main text;
viii. References;
ix. Tables (each table complete with title and footnotes);
x. Figure legends;
xi. Appendices (if relevant).

Figures and supporting information should be supplied as separate files.

**Authorship**

Please refer to the journal's authorship policy the **Editorial Policies and Ethical Considerations section** for details on eligibility for author listing.

**Acknowledgments**

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

**Conflict of Interest Statement**

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the section 'Conflict of Interest' in the **Editorial Policies and Ethical Considerations section** below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

**Abstract**

Abstracts and keywords are required for some manuscript types. For details on manuscript types that require abstracts, please refer to the 'Manuscript Types and Criteria' section.

**Keywords**

Please provide 2-5 keywords. Authors are encouraged to choose keywords from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser list at **www.nlm.nih.gov/mesh**.

**References**

All references should be numbered consecutively in order of appearance and should be as complete as possible. In text citations should cite references in consecutive order using Arabic superscript numerals. For more information about AMA reference style please consult the **AMA Manual of Style**

Sample references follow:

*Journal article*

1. King VM, Armstrong DM, Apps R, Trott JR. Numerical aspects of pontine, lateral reticular, and inferior olivary projections to two paravermal cortical zones of the cat cerebellum. J Comp Neurol 1998;390:537-551.

*Book*

2. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990. 1223 p.

*Internet document*

3. American Cancer Society. Cancer Facts & Figures 2003. http://www.cancer.org/downloads/STT/CAFF2003PWSecured.pdf Accessed March 3, 2003

**Tables**

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

**Figure Legends**

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

**Figures**

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

**Click here** for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

**Color Figures.** Figures submitted in color may be reproduced in colour online free of charge. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white.

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Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

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Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

### General Style Points

The following points provide general advice on formatting and style.

- **Abbreviations:** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only. Use only standard abbreviations. Useful is Baren DN, ed. Units, symbols, and abbreviations. A guide for biological and medical editors and authors. 4. ed. London: Royal Society of Medicine.
- **Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website for more information about SI units.
- Use no roman numerals in the text.
- In decimals, a decimal point, and not a comma, will be used.

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graphical abstract design – so you can submit your manuscript with confidence. Also, check out our resources for **Preparing Your Article** for general guidance about writing and preparing your manuscript.

***Guidelines for Cover Submissions:*** If you would like to send suggestions for artwork related to your manuscript to be considered to appear on the cover of the journal, please follow these **general guidelines**.

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