



## REVIEW

# Characterization of FGFR signaling pathway as therapeutic targets for sarcoma patients

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### ABSTRACT

The fibroblast growth factor receptor (FGFR) family plays important roles in regulating cell growth, proliferation, survival, differentiation and angiogenesis. Deregulation of the FGF/FGFR signaling pathway has been associated with multiple development syndromes and cancers, and thus therapeutic strategies targeting FGFs and FGFR in human cancer are currently being explored. However, few studies on the FGF/FGFR pathway have been conducted in sarcoma, which has a poor outcome with traditional treatments such as surgery, chemotherapy, and radiotherapy. Hence, in the present review, we provide an overview of the role of the FGF/FGFR pathway signal in sarcoma and FGFR inhibitors, which might be new targets for the treatment of sarcomas according to recent research.

### KEYWORDS

Sarcoma; FGFR signaling pathway; FGFR inhibitors

## Introduction

Sarcomas are tumors of putative mesenchymal origin, which can arise anywhere in the body. They are rare tumors, comprising less than 1% of adult cancers and nearly 21% of children cancers<sup>1</sup>. The vast majority of diagnosed sarcomas are soft tissue sarcomas, while malignant bone tumors make up just over 10% of sarcomas<sup>2</sup>. The rarity of the disease combined with the diverse number of subtypes make sarcomas very difficult to study and treat. Owing to the poor outcome of traditional treatments such as surgery, chemotherapy and radiotherapy, new treatments such as target therapy have been investigated and have shown great success.

The fibroblast growth factor receptor (FGFR) signaling pathway not only plays a ubiquitous role in a variety of biological processes, including cell growth, survival, differentiation and angiogenesis, but has also been implicated in tumor development<sup>3,4</sup>. Identification of the roles and relationships within the fibroblast growth factor (FGF)/FGFR family and their links to tumor growth and progression will

be critical in designing new drug therapies to target FGFR pathways<sup>3</sup>. This review highlights the growing knowledge of FGFs and FGFR in tumor cell growth and survival, and provides an overview of FGF intracellular signaling pathways, the role of FGFR signaling pathway in sarcomas, and current research on FGFR inhibitors in sarcoma.

## FGFs

FGFs are expressed in almost all tissues and play important roles in development, wound healing and neoplastic transformation by promoting mitosis of both epithelial and mesenchymal cell types<sup>5</sup>. To date, 23 different FGFs have been identified in human, among which 18 (FGF1-10 and 16-23) serve as mitogenic signaling molecules that bind to four high-affinity FGFRs (FGFR1-4). FGFs also bind the heparan sulfate glycosaminoglycans (HSGAGs), which facilitates dimerization (activation) of FGFRs and protect the ligands from degradation<sup>6</sup>. Thus, FGF signaling is activated through FGF binding to FGFRs in an HSGAG-dependent manner.

The FGFs are highly dependent on specific FGFR signaling to promote tumor progression. Deregulation of FGFs, such as FGF1, -2, -3, -4, -5, -10, -17, -18, and -19, have been verified in a variety of human tumors<sup>5</sup>. In particular, FGF2 is expressed in many malignant tumors including breast cancer,

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pancreatic cancer, non-small cell lung cancer (NSCLC), bladder cancer, head and neck cancer, prostate cancer, hepatocellular carcinoma (HCC), melanomas and astrocytomas<sup>7-15</sup>. FGF1 is also related with tumorigenesis and epithelial-to-mesenchymal transition, as well as invasion and metastasis<sup>16,17</sup>. In contrast, the expression of other FGFs such as FGF6-9, FGF11 and FGF12-16 in malignant tumors has been less extensively investigated. Recent evidence has shown that FGF7 and FGF10 are involved in the proliferation of ameloblastoma cells through the MAPK pathway<sup>18</sup>, while FGF8 might contribute to the proliferative and metastatic capacity of colorectal cancer (CRC) cells through Yes-associated protein 1 (YAP1)<sup>19</sup>. Sun et al.<sup>20</sup> found that FGF9 secreted by cancer-associated fibroblasts (CAFs) might play an important role in promoting the anti-apoptosis and invasive capability of gastric cancer cells. In cisplatin-resistant HeLa cisR cells, FGF13 plays a pivotal role in regulating resistance to platinum drugs, possibly through a mechanism shared by platinum and copper<sup>21</sup>. FGF15 may contribute to HCC development in the context of chronic liver injury and fibrosis<sup>22</sup>. FGF16 might contribute to the cancer phenotype of ovarian cells and might be a therapeutic strategy for treating invasive ovarian cancers<sup>23</sup>. FGF22 might play a potential pro-oncogenic role in the skin<sup>24</sup>. However, available data were mainly from preclinical work on cell models, and further clinical confirmation is required.

Previous studies also showed that FGF1 (acidic FGF) and FGF2 (basic FGF) and their receptors can promote autocrine and paracrine growth control of cancers<sup>5,25,25</sup>. These autocrine and paracrine loops involving FGFRs and FGFs have been found in NSCLC, HCC, breast and prostate cancer, and CRC<sup>4,26</sup>. Once these loops have become disrupted, resulting from increased release of FGFs in cancer or stromal cells, they can promote the proliferation, survival,

and angiogenesis abilities of cancer cells. FGFs, and especially FGF2, are among the earliest identified pro-angiogenic factors in tumors, and they have a direct effect on tumor angiogenesis<sup>27</sup>. A recent study showed that FGF2 and its receptor FGFR3 as well as apurinic/aprimidinic endonuclease 1 (APE1) play an important role in angiogenesis in human osteosarcoma cells<sup>28</sup>. Another report demonstrated that *in vitro* and *in vivo*, miR-503 can reduce tumor angiogenesis by downregulating FGF2 and vascular endothelial growth factor-A (VEGFA)<sup>29</sup>.

## FGFRs

The FGFRs are receptors that bind to members of the FGF family of proteins. The receptors consist of an extracellular ligand domain with three immunoglobulin-like domains (I-III), a transmembrane domain, and an intracellular tyrosine kinase domain that transmits the signal to the interior of the cell. An acid box, located between the domains Ig-I and Ig-II, plays a role, together with the Ig-I-like domain, in receptor auto-inhibition. Ig-II and Ig-III compose the ligand-binding site<sup>30</sup>. There are only four FGFRs (FGFR1, FGFR2, FGFR3 and FGFR4) in the cell surface with seven isoforms FGFR-(1b, 1c, 2b, 2c, 3b, 3c and 4) owing to alternative splicing in the Ig-III-like domain, with different ligand-binding specificities<sup>6,31</sup> (**Table 1**). Each receptor can be activated by several FGFs, and the FGFs can also activate more than one receptor in many cases. For example, FGF1 can bind all seven principal FGFRs<sup>44</sup>, while FGF7 can only activate FGFR2b<sup>45</sup>.

The gene encoding FGFR5 was recently identified. The FGFR5 protein lacks a cytoplasmic tyrosine kinase domain and one isoform compared with FGFR1-4, and FGFR5 $\gamma$  only includes the D1 and D2 extracellular domains<sup>46</sup>. FGFR5 has been assumed to be a decoy receptor to inhibit ligand-

**Table 1** Ligand specificities of the FGFR family

Receptor	Ligand	
	FGF1-10 <sup>32</sup>	FGFR15-23
FGFR1	FGFR-1b	FGF-1, 2, 3, and 10
	FGFR-1C	FGF-1, 2, 4, 5, and 6
FGFR2	FGFR-2b	FGF-1, 3, 7, and 10
	FGFR-2C	FGF-1, 2, 4, 6, and 9
FGFR3	FGFR-3b	FGF-1 and 9
	FGFR-3C	FGF-1, 2, 4, 8, and 9
FGFR4	FGFR-4	FGF-1, 2, 4, 6, 8, and 9

FGF-1-10 was adapted from reference 32. As FGF-11-14 were intracellular non-secretory factors, these are not shown

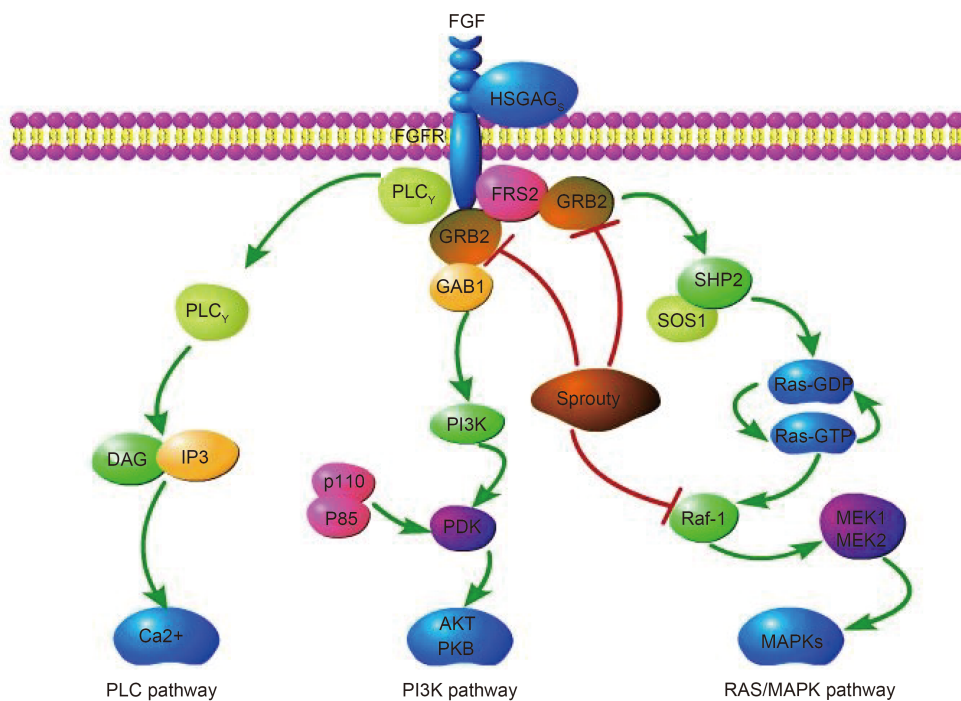
induced responses of other FGFRs. However, researchers demonstrated that FGFR5 does not act as a decoy receptor but directly affects ligand-independent and -dependent mechanisms of extracellular signal-regulated kinase 1/2 (ERK1/2) activity by interacting with known substrate proteins<sup>35,46,47</sup>.

Endothelial cells express FGFR1 more often than FGFR2, while the expression of FGFR3 or FGFR4 has been less frequently reported<sup>3</sup>. Immunohistochemical results showed a statistically significant increase of FGFR2 in epithelial dysplasias and squamous cell carcinomas compared with normal oral epithelium, and FGFR3 expression was statistically significantly increased in dysplastic and carcinomatous tissues compared with normal oral epithelium. While staining for FGF1 is decreased or lost in the development of epithelial dysplasia and carcinoma, FGF2 also showed increased intensity in squamous cell carcinomas<sup>48</sup>. FGFR3 is downregulated in CRC cells, while FGFR1 is overexpressed in CRC cells. Interestingly, by introducing of FGFR1 siRNA to disrupt the expression of FGFR1 in cells, the expression of FGFR3 was effectively increased as well as the tumor suppressive activities. These results showed that the reciprocal relationship between FGFR1 and FGFR3 in colorectal tissue may act as an important role in the progression of tumor formation<sup>49</sup>.

## FGF/FGFR signaling pathway

The binding of FGFs to FGFRs (aided by HSPGs) induces receptor dimerization, which triggers the activation of the FGFRs. The activation of FGFRs brings the intracellular kinases into close proximity, enabling them to transphosphorylate each other. This sets in motion a cascade of downstream signals, finally affecting mitogenesis and differentiation<sup>50,51</sup> (Figure 1). Several intracellular proteins have been implicated in promoting FGF-mediated signaling, such as phospholipase C $\gamma$  (PLC $\gamma$ ), fibroblast growth factor receptor substrate 2 (FRS2), src homology 2 domain-containing transforming protein B (Shb), Src kinase, ribosomal S6 protein kinase (RSK), signal transducers and activators of transcription (STATs), and CT10 regulator of kinase (Crk)<sup>6,52,40</sup>. Specifically, the adaptor growth-factor-receptor-bound protein 2 (GRB2) triggers the Ras/MAPK pathway and PI3K/Akt intracellular signaling cascades by binding to phosphorylated FRS2<sup>52</sup>. Rab5 small GTPase, a binding partner of activated FGFRs, is involved in maintaining the RAS-MAPK signaling but not PI3K-AKT signaling<sup>53</sup>.

The FGFR signaling pathway plays important biological roles in multiple processes include pro-survival signals as well as anti-apoptotic signals and stimulation of cell proliferation



**Figure 1** FGF/FGFR signaling pathways.

and cell migration<sup>6</sup>. There are two kinds of regulators that control the signaling output from activated FGFRs: one includes the negative regulators, such as Sprouty proteins, mitogen-activated protein kinase phosphatase 3 (MKP3) and the similar expression to the FGF (SEF)<sup>54-57</sup>; and the other includes the positive regulators, such as fibronectin-leucine-rich transmembrane protein (FLRT1), FLRT2 and FLRT3<sup>58,59</sup>. Sprouty proteins can decouple the downstream signaling of FGF by binding to GRB2<sup>54</sup>. Sprouty related enabled/vasodilator-stimulated phosphoprotein homology1 domain-containing protein (SPRED2), a Sprouty-related protein, was also shown to attenuate FGF signaling by redirecting FGFRs to lysosomes by interacting with the late endosomal protein neighbor of breast cancer early-onset 1 (NBR1)<sup>57</sup>.

## The role and clinical significance of FGFR signaling pathway in sarcomas

### Osteosarcoma

Osteosarcoma (OS), the most common primary bone tumor, is a genetically complex disease with a high incidence of pulmonary metastasis and poor prognosis, which mainly affects children and adolescents<sup>60</sup>. The amplification of several *FGFR* genes has been observed in OS, such as *FGFR1*, *FGFR2* and *FGFR3*<sup>61</sup>. However, a study on the expression of cell surface receptors revealed low expression of FGFR-2 and FGFR-3 across ten OS cell lines (OS229, OS232, OS231, OS238, OS242, OS252, OS290, OS293, OS308, and OS311)<sup>62</sup>. In addition, amplification of *FGFR-1* is disproportionately observed in the rare histological variants of OS. The amplification of *FGFR-1* was observed in 18.5% of patients who showed a poor response to chemotherapy, and no *FGFR-1* gene amplification was detected in the patients who responded well to therapy<sup>63</sup>. High expression levels of APE1, FGF2 and FGFR3 have been found in OS and are significantly related with poor prognosis. High expression of FGF2 and its receptor 3 FGFR3 was an adverse prognostic factor. We found that APE1 can promote angiogenesis by upregulating FGF2 and FGFR3 *in vitro* and *in vivo*<sup>64</sup>. Thus, the use of FGFR kinase inhibitors may be a promising treatment for patients with OS.

### Chondrosarcoma

Chondrosarcoma, the second most common primary malignancy of the bone, is difficult to treat. Wide excision is the only treatment owing to its resistance to both

chemotherapy and radiation<sup>65</sup>. FGFs and FGFRs are thought to be negative regulators of chondrocytic growth, such as achondroplasia and related chondrodysplasias, which are caused by constitutively active mutations in FGFR3<sup>66</sup>. Sahni et al.<sup>67</sup> reported that FGF inhibited chondrocytic growth through activating the STAT1 pathway, in which p21 might also play a role in inhibiting chondrocytic proliferation<sup>67</sup>. In rat chondrosarcoma cells, FGF2 arrested the cell cycle at G<sub>1</sub> phase and inhibited proliferation, which seemed to be mediated at least partially through p21 induction, the inactivation of cyclin E-Cdk2 and activation of pRb<sup>68</sup>. In addition, immunohistochemistry revealed high expression of FGFR3 and aberrant cellular localization of heparan sulfate proteoglycans in 42 dedifferentiated, 23 clear cell, and 23 mesenchymal chondrosarcoma tissues of human<sup>69</sup>. Taken together, FGF and FGFR may be a potential therapeutic target in chondrosarcoma.

### Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is a malignancy with features of skeletal muscle and the most frequent soft tissue sarcoma in children. There are two main kinds of variants of RMS: embryonal rhabdomyosarcoma (eRMS) and alveolar rhabdomyosarcoma (aRMS)<sup>70</sup>. Recently, several FGFR4 tyrosine kinase domain mutations were found in 7.5% primary human RMS tumors, and the mutants K535 and E550 showed autophosphorylation of the receptor. In RMS tumors, overexpression of FGFR4 was associated with advanced stage cancer and poor survival, while FGFR4 knockdown in a human RMS cell line reduced tumor growth<sup>71</sup>. FGFR4 protein is expressed in the two main variants of RMS (eRMS and aRMS), but protein expression is higher in aRMS. FGFR4 loss-of-function reduced cell proliferation in both subtypes *in vitro*. Interestingly, FGFR4 was necessary and sufficient for expression of the anti-apoptotic protein B-cell CLL/lymphoma 2-like 1 (BCL2L1) in aRMS; this was not observed in eRMS, indicating that FGFR4 may play dichotomous roles in RMS subtypes<sup>72</sup>. The fusion gene *FGFR1-FOXO1* has also been verified in RMS<sup>35</sup>. Inhibiting FGFR signaling might represent an important strategy to enhance the efficacy of current RMS treatments. FGFR4 signaling rescues only subgroups of alveolar RMS cells from apoptosis induced by compounds targeting the IGF1R-PI3K-mTOR pathway, and FGFR4-activated signaling is involved in the different behaviors of the phenotypes<sup>73</sup>. Together, these data suggest that FGFR4 may act as an oncogene in RMS, and these findings support the potential therapeutic targeting of FGFR4 in RMS.

## Liposarcomas

The fibroblast growth factor receptor substrate 2 (FRS2) is an adaptor protein that plays a critical role in FGFR signaling. Several studies showed the amplification of *FRS2* gene as well as the overexpression of FRS2 protein in liposarcoma, and the FGFR/FRS2 signaling was activated in about 75% of FRS2-positive high-grade liposarcomas. It was also observed in three high-grade liposarcoma cell lines: FU-DDLS-1, LiSa-2, and SW872<sup>74,75</sup>. Importantly, use of the FGFR selective inhibitor NVP-BGJ-398 can significantly inhibit the growth of two cell lines and suppressed the FGFR signal transduction. These findings indicate that FRS2 may serve as a potential therapeutic target in liposarcomas<sup>75</sup>. Comparative expression analyses using whole-genome microarrays were conducted in myxoid liposarcomas and fat tissue samples, and the results showed that FGFR2 was overexpressed and were validated by qPCR, immunohistochemistry and Western blot analysis in primary tumor samples. *In vitro*, inhibition of FGFR showed effects on proliferation and cell migration and induced apoptosis, which indicated that the FGFR might be a therapy target for myxoid liposarcoma<sup>76</sup>.

## Synovial sarcoma

Synovial sarcoma is a soft tissue malignancy that typically arises in young adults. The growth regulatory mechanisms are unknown<sup>2</sup>. A recent study shows that multiple FGF genes including *FGF2*, *FGF8*, *FGF9*, *FGF11* and *FGF18* were expressed in synovial sarcoma cell lines, and FGF8 showed growth stimulatory effects in all synovial sarcoma cell lines<sup>77</sup>. The growth stimulatory effect of FGF were transmitted through ERK1/2, and FGF signals in synovial sarcoma induced the phosphorylation of ERK1/2. While FGFR inhibitors induced significant growth inhibition *in vitro* and *in vivo* was only associated with a downregulation of phosphorylated ERK1/2 and an ERK kinase inhibitor showed growth inhibitory effects for synovial sarcoma<sup>77</sup>. Hence, FGF signals have an important role in the growth of synovial sarcoma, and inhibitory molecules will be of potential use for molecular target therapy in synovial sarcoma.

## Other sarcomas

Girnit et al.<sup>78</sup> showed that the bFGF pathway may be important for the maintenance of a malignant phenotype of Ewing's sarcoma cells through upregulating the EWS/FLI-1 protein. Another study found that bFGF-induced cell death was associated with upregulation of p21 and p53,

downregulation of PCNA and cyclin A and a decrease in active pRb1, consistent with accumulation of cells in G<sub>1</sub>. These data demonstrate that the bFGF pathway may be a therapeutic target in Ewing's sarcoma<sup>79</sup>. Overexpression of FGFR3, FGF2 and FGFR4 has been identified in the epidermal regions of dermatofibroma while the expression of FGF1 and FGF9 has not been found in dermatofibroma<sup>80</sup>. However, more studies are required. FGF2, alone or co-expressed with platelet-derived growth factor-B (PDGF-B) and vascular endothelial growth factor receptor-3 (VEGFR-3), involved in angiogenesis, is a significant independent negative prognosticator in widely tumor resected non-gastrointestinal stromal tumor soft-tissue sarcomas<sup>81</sup>.

## FGFR inhibitors and their role in sarcomas

Small-molecule tyrosine kinase inhibitors targeting the ATP-binding site of the intracellular tyrosine kinase domain in a number of different receptor tyrosine kinases (RTKs) have been successfully used for therapy for cancers such as NSCLC and breast cancer<sup>82,83</sup>. However, most of these inhibitors show broad specificity and target not only FGFRs, but also VEGFRs and/or PDGFRs, as they share structural similarities and have similar kinase domains. Several clinical trials targeting FGFRs have also been conducted in cancer. A phase 1 clinical trial of BAY1187982, an anti-FGFR2 antibody, in subjects with advanced solid tumors known to express FGFR2 has been conducted (<http://ClinicalTrials.gov>, NCT02368951). BGJ398 will be tested in phase 2 clinical trials in patients with advanced cholangiocarcinoma with FGFR2 gene fusions or other FGFR genetic alterations (<http://ClinicalTrials.gov>, NCT02150967). Lenvatinib, another example, will be tested in phase 1/2 clinical trials in children and adolescents with refractory or relapsed solid malignancies (<http://ClinicalTrials.gov>, NCT02432274). Other tyrosine kinase inhibitors such as BIBF1120, TKI258, nintedanib and ponatinib are also in phase 1 and/or 2 clinical trials (<http://ClinicalTrials.gov>)<sup>84-87</sup>.

Aberrations in FGFR signaling are involved in the pathophysiology of several malignancies and disorders. FGFR inhibitors such as small-molecule FGFR inhibitors could be of potential use for targeted therapy in sarcomas. A recent study showed that xenogenic mesenchymal stem cells (MSCs) can selectively migrate to the tumor site and may be under the guidance of FGF2/FGFR pathways, while no MSCs were found in other organs in a fibrosarcoma-bearing C<sub>3</sub>H/HeN mice model. In addition, nitric oxide synthase (iNOS) protein that was delivered by genetically modified

iNOS-MSC showed a significant anti-tumor effect both *in vitro* and *in vivo*<sup>88</sup>. Regorafenib (BAY 73-4506, Stivarga®) is an oral diphenylurea multi-kinase inhibitor that targets VEGFR1-3, TIE2, PDGFR-beta, and FGFR as well as KIT, RET, and RAF. Currently, a multinational, randomized, placebo controlled phase 2 trial of regorafenib in metastatic soft tissue sarcoma (REGOSARC, NCT01900743) has been initiated. The study recruited patients with metastatic soft tissue sarcoma (STS) that received at least doxorubicin (or another anthracyclin) as a previous treatment. No results are currently available<sup>89</sup>. FGFR4 is a tractable therapeutic target for patients with RMS. In a recent study, ponatinib (AP24534) was determined as the most potent FGFR4 inhibitor because it was shown to inhibit the growth of RMS cells expressing wild-type or mutated FGFR4 by increasing apoptosis. Similar results were observed in a RMS mouse model expressing mutated FGFR4. Therefore, it suggests that ponatinib might be a potentially effective therapeutic agent for RMS tumors<sup>90</sup>.

## Conclusions

The FGF/FGFR family has been linked to mechanisms underlying tumor progression. Several intracellular proteins such as PLC $\gamma$ , FRS2, Shb, Src kinase, RSK, STATs and Crk, as well as several regulators including Sprouty proteins, SPRED2, MKP3, SEF and FLRT1-3, play important roles in FGFR signaling pathways. The genetic alterations of FGF/FGFR are involved in sarcomas such as OS, chondrosarcoma, RMS, liposarcomas, and synovial sarcoma. In addition, several drugs have been researched in sarcoma such as regorafenib and ponatinib. Recent FGF/FGFR signaling pathway research suggests that the FGFR inhibitor combination with surgery, radiation and chemotherapy might enhance the therapy responses for sarcoma patients.

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## Conflict of interest statement

No potential conflicts of interest are disclosed.

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