Biological Therapy for Osteoarthritis, Efficacy and Safety: Focus on Monoclonal Antibodies against Nerve Growth Factor and Fibroblast Growth Factor-18

Lina Zaripova*, Mishi Pallav, Damira Tazhibaeva, Natalya Kabdualieva, Zhaina Aitbayeva, Gulshakhar Beglarova, Lazzat Yermentayeva, Karlygash Niyazbekova

1Department of Pathological Physiology named after V.G. Korpachev, Astana Medical University, Astana, Kazakhstan; 2Faculty of General Medicine, Astana Medical University, Astana, Kazakhstan

Abstract

Osteoarthritis (OA) is the most common chronic progressive musculoskeletal disease, affected cartilage, and surrounded tissues: Subchondral bones, ligaments, and meniscus. Current OA treatment based on non-steroidal anti-inflammatory drugs, acetaminophen (paracetamol), opioids, and intra-articular corticosteroid injections do not prevent the progression of the disease. Understanding of the pathogenesis of OA with continued structural damage accompanied by chronic pain led to appearance of monoclonal antibodies to fibroblast growth factor-18 (FGF-18) and anti-nerve growth factor (NGF). This review provides an overview of biological therapy with FGF-18 and anti-NGF for OA. Search process was conducted in PubMed and Google Scholar for the following terms: “FGF-18” or “anti-NGF” and “OA,” “monoclonal antibody” and “OA.” Results of the analysis of clinical trials revealed that therapy targeting NGF resulted in significant analgesic effect and functional improvement of joints in OA; however, it was associated with considerable increase in adverse events. The monoclonal antibody to FGF-18 demonstrated the structure-modifying effects on cartilage with decrease the cartilage loss and improvement of cartilage thickness. However, further clinical longitudinal studies characterized the risk-benefit are needed to establish safety and efficacy of these medications.

Introduction

Osteoarthritis (OA) is known as the most common chronic progressive joint disorder affected more than 300 million people globally and represented a formidable public health challenge [1], [2]. OA significantly influence on patients’ activities associated with pain, disability, and quality of life. The Global OA Patient Perception Survey revealed that more than 80% of patients demonstrated pain/tenderness, about 90% of individuals-limitations to physical activities and 50% pointed on a decrease of their quality of life because of OA [2].

According to the evidence-based recommendations, the central core treatments for OA are patients’ education, exercising, and weight loss [3]. Pain control treatment includes acetaminophen (paracetamol), nonsteroidal anti-inflammatory drugs (NSAIDs) and/or opioids, as well as intra-articular corticosteroid injections [4]. Comorbidity in OA is usually associated with cardiovascular and metabolic diseases, and the treatment with NSAID/ coxib (COX-2 selective inhibitor) might enhance the possibility of cardiovascular catastrophes [5].

Opioids are recommended from many guidelines in cases where paracetamol and topical agents are ineffective, though some authors found that opioids in the large joint OA have no additional benefit over non-opioid analgesics [6].

There is considerable interest in the possibility of the biochemical and structural modification of cartilage by glucosamine, chondroitin, diacerein, risedronate, strontium, and hyaluronic. A reduction in radiographic joint space loss was reported for glucosamine sulfate at the dose of 1500 mg/day and chondroitin sulfate at the dose of 800 mg/day, though the difference in comparison with placebo was small [7]. Some guidelines recommended symptomatic chondroitin and glucosamine sulfate as slow-acting drugs for OA, suggested that they may be effective and reduce functional impairment [3]. Thus, chondroitin is recommended by EULAR for managing pain and functional limitation for hand OA [8]. However,
data obtained from different research and systematic reviews on these products are heterogeneous and their prescription quite disputed.

Nowadays, despite of the huge amount of data on the issue of OA there is still no effective treatment that slows down the progression of the disease. The first international survey of OA revealed that about 40% of OA patients were not satisfied with their current OA treatment, which highlighted the need of search for further therapeutical approaches [2].

Monoclonal antibodies represent one of the new therapeutic approaches to the treatment of rheumatic diseases and suggested to be a promising method for the therapy of OA. First studies of monoclonal antibodies in OA were performed with anti-TNFα agents (adalimumab and etanercept) and anti-IL1 (anakinra and canakinumab). These drugs are successfully used as disease-modifying drugs for other types of inflammatory arthritis, such as rheumatoid, psoriatic, and enthesitis-related arthritis [9]. However, not anti-TNFα (adalimumab), or IL1 targeting drug (lutikizumab) significantly decrease pain, synovitis, or imaging outcomes in individuals with OA [10], [11].

Nerve growth factor (NGF) has been recognized as one of the major mediators of pain released by injured tissue and found elevated in the synovial fluid of OA patients [12]. It is characterized by potential pain modulation through binding to tropomyosin receptor kinase A (TrkA) and p75 on nociceptive neurons, cartilage, and bone [13]. Understanding the role of NGF in nociceptor sensitization led to the development of monoclonal antibodies against NGF that might be successfully used in chronic OA pain [14].

The place of disease-modifying OA drugs is still vacant; however, up-to-date research shows the potential of monoclonal antibodies that might target structural disease progression. Thus, fibroblast growth factor (FGF)-18 demonstrated a potential anabolic effect for cartilage by stimulation of hyaline extracellular matrix synthesis, chondrocyte proliferation and cartilage repair in an injury-induced OA [15]. FGF-18 was shown to maintain chondrocyte phenotype, up-regulate the expression of chondrocyte typical markers and improve the type II: I collagen ratio on different chondrocyte culture systems [16].

Objective

The objectives of the study were to evaluate the safety and efficacy of anti-NGF and FGF-18 in OA patients.

Methods

In the current study, the search process was conducted in PubMed and Google Scholar for English-language articles using the following keywords: “FGF-18” or “anti-NGF” and “OA,” “monoclonal antibody” plus “OA.” All articles in the format of Clinical Study, Clinical Trial Protocol, Clinical Trial, and Multicenter Study were reviewed. Papers published over the past 20 years till April 1, 2022, were included in the study. Outcomes were assessed using clinical scores, including the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Pain and Physical Function score, and Patient’s Global Assessment of OA (PGA-OA) which is an assessment of individuals perception of their status [17]. Safety assessment included different adverse events (AEs) recorded on medications or even after the treatment. The structural progression, cartilage repair and total femorotibial joint cartilage thickness were assessed using magnetic resonance imaging (MRI). Rapidly progressive osteoarthritis (RPOA) was registered as an atrophic destructive OA, characterized by rapid joint space narrowing and progressive atrophic bone [18]. The results of selected articles are discussed below.

Results

Twenty-three publications were found using the search strategy above: 16 with anti-NGF monoclonal antibodies and seven with recombinant human FGF-18. After omitting repetitions, the analysis of 11 articles with anti-NGF monoclonal antibodies (tanezumab, fasinumab, and fulranumab) and 6 with recombinant human FGF-18 (sprifermin) treatment was performed. In total the physical function, pain and progression of OA were analyzed in 6672 patients. A description of clinical studies of anti-NGF and FGF-18 therapy in OA is shown in the Table 1.

Nine apart from 11 studies about anti-NGF monoclonal antibodies were dedicated to tanezumab which was the first humanized IgG2 monoclonal antibody against β-NGF that blocks the interaction of NGF with its receptors, TrkA and p75 [19]. The earliest clinical trial with 450 patients revealed that tanezumab significantly reduced pain (p < 0.001) compared to placebo in patients with advanced knee OA who did not have a satisfactory response to nonopioid analgesics or considered to be candidates for surgical treatment [20].

All phase II and III clinical trials (Table 1) demonstrated the efficacy of tanezumab due to statistically significant enhancements in WOMAC Pain and Physical Function score compared to placebo [14], [21], [22], [23], [24], [25], [26]. Similarly, statistically significantly more tanezumab-treated patients than placebo met the criteria for outcome measures for rheumatology committee and osteoarthritis research society international standing committee for...
Table 1: Clinical experience of anti-nerve growth factor and fibroblast growth factor-18 monoclonal antibodies in therapy of osteoarthritis

<table>
<thead>
<tr>
<th>Name of the target molecule</th>
<th>Name of the drug</th>
<th>Patients (n) and dose</th>
<th>Outcomes</th>
<th>Authors, year, reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGF</td>
<td>Tanezumab</td>
<td>n = 283 for 2.5 mg, n = 284 for 5 mg; n = 282 for placebo</td>
<td>Decrease in WOMAC pain, WOMAC physical function and PGA-OA at 24 weeks significant from baseline for tanezumab 5 mg compared with placebo</td>
<td>Benoist et al. 2020 [22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 282 for placebo</td>
<td>An improvement in WOMAC pain and physical function, but not PGA-OA for tanezumab 2.5 mg group</td>
<td>Dakin et al. 2019 [13]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 567 (tanezumab 2.5 mg or 5 mg)</td>
<td>Significant reduction in loss of total and lateral femorotibial cartilage thickness and paresthesia (2.5%), hyperesthesia, peripheral neuropathy, and sensory disturbance (0.4%)</td>
<td>Gow et al. 2015 [23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 48 (1:1 to receive AMG 403, 1, 3, 10, or 30 mg intravenously; or 10 or 30 mg subcutaneously)</td>
<td>AMG 403 appeared to be well-tolerated after single and multiple doses, except for hypoaesthesia, pain, and paraesthesia (mild to moderate severity)</td>
<td>Karsdal et al. 2019 [35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 83 (tanezumab 10, 25, 50, 100, 200 µg/kg, or placebo)</td>
<td>Improvement of daily index joint pain within the first week with tanezumab 2.5 mg compared with placebo</td>
<td>Karsdal et al. 2019 [35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 849 (tanezumab 2.5 mg or 5 mg every 8 weeks)</td>
<td>Tanezumab-treated patients achieved treatment response criteria (≥30%, ≥50%, or ≥70% reduction in WOMAC Pain or OMERACT-OARSI response)</td>
<td>Schnitzer et al. 2020 [24]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 470 (dose of 10, 25, 50, 100, or 200 µg in patients weighing 50 kg and over)</td>
<td>Total joint replacements were similarly distributed across all three treatment groups (6.7%–7.8%). Tanezumab-treated patients experienced more paraesthesia (5%) and hypoaesthesia (both doses) than placebo</td>
<td>Lane et al. 2010 [20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 450 (dose of 10, 25, 50, 100, or 200 µg per kilogram of body weight on days 1 and 56)</td>
<td>Improvement of the rate of response according to OMERACT-OARSI Standing Committee for Clinical Trials Response Criteria Initiative OMERACT-OARSI ranged from 74% to 93% with tanezumab treatment, as compared with 44% with placebo (p &lt; 0.001)</td>
<td>Lane et al. 2021 [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 587 (tanezumab 2.5 mg or 5 mg every 8 weeks)</td>
<td>Significant improvement in WOMAC pain and physical function and PGA scores (p &lt; 0.001)</td>
<td>Lane et al. 2021 [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 621 (3 intravenous doses of tanezumab 2.5 mg, 5 mg, or 10 mg 32 weeks or placebo)</td>
<td>More tanezumab-treated patients achieved treatment response criteria (≥30%, ≥50%, or ≥70% reduction in WOMAC pain or OMERACT-OARSI response) efficacy was generally maintained throughout the 16 weeks treatment period</td>
<td>Lane et al. 2021 [25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 401 (1 mg every 4 weeks, 3 mg every 4 weeks, 6 mg every 8 weeks, or 10 mg every 8 weeks)</td>
<td>Improvement of the patients' global assessment measure (mean increases in score of 29 to 47 with various doses of tanezumab, as compared with 15% with placebo; p ≤ 0.001)</td>
<td>Lane et al. 2010 [20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 281 (tanezumab 50 µg/kg on days 1 and 8 weeks intervals (up to a total of eight infusions)</td>
<td>Improvement of the incidence of AEs equal 7.5% and include: Abnormal peripheral sensation; hypoaesthesia (3.2%), paraesthesia (2.5%), hyperesthesia, peripheral neuropathy, and sensory disturbances (0.4%)</td>
<td>Sanga et al. 2017 [17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 192, doses of 10 µg, 30 µg, and 100 µg</td>
<td>Improvement of WOMAC pain and physical function and PGA scores for 1mg every 4 weeks, 3 mg every 4 weeks, 10 mg every 8 weeks compared to placebo</td>
<td>Schnitzer et al. 2021 [27]</td>
</tr>
</tbody>
</table>

(Contd...)
clinical trials response criteria initiative (OMERACT-OARSI) response (p < 0.001) [20, 24, 25]. Among patients with moderate to severe OA of the knee or hip and inadequate response to standard alagesics, tanezumab resulted in improvements in PGA-OA in comparison with placebo, although the improvements were modest and not always statistically significant. Berenbaum et al. reported the improvement of PGA-OA by a mean (±standard error) of −0.19 ± 0.07 (p = 0.0051) for patients treated with tanezumab 5 mg but non-significant for the dose of 2.5 mg compared with placebo [22]. Overall knee pain and subject global assessment (SGA) of patients received tanezumab improved by a mean (±standard error) of −12.8 (±1.78) and 8.0 (±1.66) from baseline [27].

In addition to the clinical effect, it was shown no anti-drug antibody development that may affect the effect of anti-NGF medications [23].

The efficacy and safety of recombinant human FGF-18 for the treatment of OA were evaluated in 6 articles (Table 1). Spifermin demonstrated dose-dependent reductions in loss of total and lateral femorotibial cartilage thickness, joint space width narrowing in the lateral femorotibial compartment in phase II double-blind, placebo-controlled extension studies [28, 29]. Significant reduce of cartilage loss was shown for sprifermin 100 µg treatment every 6 months versus placebo (95% CI difference: 334 µm (114–554) [29]. Results of multicenter, randomized, double-blind, placebo-controlled, dose-finding, and phase II trial FORWARD study showed statistically significant dose-dependent modification of cartilage thickness of femorotibial joint change compared to placebo at year 2, with higher doses of sprifermin exhibiting less cartilage damage over time [30]. Less worsening of cartilage damage in patients treated with sprifermin was also confirmed by Roemer et al. [31]. Moreover, Eckstein et al. found the significant increase in the total cartilage thickening sum score in the 100-µg sprifermin group compared with placebo group was 237 µm (95% CI 34–440), p = 0.028 [20].

Of note, Eckstein et al. found the significant increase in the total cartilage thickening sum score in the 100-µg sprifermin group compared with placebo group was 237 µm (95% CI 34–440), p = 0.028. For bone marrow lesions, more improvement was observed from 6 to 12 months for whole knee analyses in sprifermin group (0.14, 95% CI −0.48–0.19 vs. placebo 0.44, 95% CI −0.15–1.04, p = 0.042) but no significant effects were seen in synovitis, menisci and osteophytes [22].

The most frequent AE was arthralgia (placebo: n = 46 (43.0%); 100 µg of sprifermin administered every 6 months: n = 50 (45.0%); 30 µg of sprifermin every 6 months: n = 40 (36.0%; and 30 µg of sprifermin every 12 months: n = 48 (44.0%))

Table 1: (Continued)

<table>
<thead>
<tr>
<th>Name of the target molecule</th>
<th>Name of the drug</th>
<th>Patients (n) and dose</th>
<th>Outcomes</th>
<th>Authors, year, reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant human FGF 18</td>
<td>Sprifermin</td>
<td>n = 75 sprifermin 100 µg (n = 57), placebo (n = 18)</td>
<td>Less worsening of cartilage damage was observed from baseline to 12 months in the patello-femoral joints (0.02, 95% CI −0.04–0.08 vs. placebo 0.22, 95% CI −0.05–0.49; p = 0.046)</td>
<td>Eckstein et al. 2016 [31]</td>
</tr>
<tr>
<td>Recombinant human FGF 18</td>
<td>Sprifermin</td>
<td>n = 168; Sprifermin 10, 30, or 100 µg</td>
<td>Spifermin significantly increases cartilage thickness: The mean difference in the total cartilage thickening sum score between the 100 µg sprifermin group and the placebo group was 331 µm (95% CI 24–685) (p ≤ 0.03)</td>
<td>Eckstein et al. 2015 [32]</td>
</tr>
<tr>
<td>Recombinant human FGF 18</td>
<td>Sprifermin</td>
<td>n = 387 intra-articular injections of sprifermin 30 µg or 100 µg; n = 87 (placebo)</td>
<td>No effects over 24 months were observed on osteophytes, menisci, and synovits</td>
<td>Roemer et al. 2020 [33]</td>
</tr>
</tbody>
</table>


https://oamjms.eu/index.php/mjms/index
approach where the total number of sub-regions with improvement was subtracted from the total number of sub-regions with worsening [33].

In addition, it was known that sprifermin targets not only FGFR-3 in chondrocytes promoted chondrogenesis and cartilage matrix formation [28], but also FGFR-3 in non-cartilaginous tissues, such as meniscus [33]. However, no meaningful differences between sprifermin and placebo were revealed in worsening in meniscus damage, osteophytes or synovium changes over 24 months [33].

FORWARD research did not reveal statistically significant differences in mean absolute change in total WOMAC scores for 100 μg of sprifermin administered every 6 months or every 12 months, for 30 μg of sprifermin every 6 months or every 12 months, compared with placebo [30]. However, selecting a more homogenous patient subgroup with risk of OA progression and high WOMAC pain at baseline resulted in receiving clinically relevant improvement in WOMAC pain score in 100 μg sprifermin-treated patients at year 3 [34].

The AEs of FGF-18 or anti-NGF therapies were not life-threatening; however, they may affect the compliance and satisfaction of patients and clinicians. The proportion of individuals with AEs in anti-NGF treatment group was higher than in FGF-18 group. Mainly, anti-NGF therapies demonstrated disorders of peripheral sensation and development of arthropathies [13], [21], [22], [27]. According to the analysis of the clinical trials with anti-NGF monoclonal antibodies the following adverse conditions were reported (percentage of patients): Abnormal peripheral sensation such as hypoesthesia (7.43%), paraesthesia (9.15%), hyperesthesia (0.36%), peripheral neuropathy and sensory disturbance (0.35%); arthralgia (15%); back pain (15.06%), extremity pain (10%), headache (9.11%), upper respiratory tract infection (10.65%), diarrhea (11.95%), sinusitis or nasopharyngitis (10.13%, Figure 1).

The results obtained from clinical trials revealed that patients treated with tanezumab more frequently had RPOA: 1.4% (4/283) in group of patients received 2.5 mg tanezumab and 2.8% (8/284) of individuals in the tanezumab 5 mg group [22]. However, Brown et al. (2013) reported that joint replacements had to perform in 1 patient received the 10 mg tanezumab, 2 in the 5 mg, 2 in the 2.5 mg, and 3 in the placebo group [14].

The differences in AEs between sprifermin and placebo were found insignificant; arthralgia became the most frequently reported event. Hochberg et al. reported 43.0% of arthralgia in placebo group, 41% in 100 μg of sprifermin administered every 6 months, 45% in 100 μg of sprifermin every 12 months, 36% in 30 μg of sprifermin every 6 months, and 44% in 30 μg of sprifermin every 12 months [30]. Lohmander et al. also described arthralgia and joint swelling, as well as infections and infestations (naso-pharyngitis), and headache as the most common treatment-emergent AEs; however, differences were not significant [28].

Discussion

In recent years, substantial progress has been achieved in the biologic therapy for OA. The recombinant human macrophage colony-stimulating factor (M-CSF) was investigated as an anabolic intra-articular OA medication. The phase II clinical trials showed the differences in longitudinal cartilage thickness change in the group of patients received sprifermin versus placebo [30], [31], [32], [33]. The significant dose-dependent reductions in loss of total and lateral femorotibial cartilage thickness were found in individuals received sprifermin [28], [29]. Moreover, sprifermin showed a positive effect on cartilage morphology on a global knee cartilage score; it does not only reduce cartilage loss but also increase cartilage thickness [29]. In terms of cartilage repair, five apart from six studies reported improvement in total femorotibial joint cartilage thickness by MRI. No clinically significant AEs were registered for FGF-18. Thus, according to the results of up-to-date studies, sprifermin may be considered as an effective therapy for knee OA with acceptable safety profile. Nevertheless, the disease-modifying drug for OA is supposed to bring structural improvements as well as symptomatic benefit in pain or function. According to the results obtained so far, clinical trials failed to demonstrate significant improvements in both outcomes. After treatment with FGF-18 (sprifermin) there was no significant improvement in WOMAC scores, however, this might be because of heterogeneous cohort of OA patients participating in the research. Subtracted the group of patients with high WOMAC scores and low joint space width at the beginning might demonstrate better results and finally reveal the improvement in WOMAC scores [34]. Further investigations with clear
selection of a homogeneous subgroup of OA defined in accordance with both joint-space width and pain score are needed [34].

Another group of biologic agents, NGF-inhibitors were suggested as an attractive tool for pain relief. Tanezumab, fasinumab and fulranumab have been investigated in OA and have shown promising results in terms of joints pain and function measured by WOMAC pain and physical function score, PGA-OA, OMERACT-OARSI criteria, and SGA [14], [21], [22], [23], [24], [25], [26], [27]. Apart from NGF inhibitors tanezumab has been the most widely studied. The high prevalence of progressively worsening OA among patients received anti-NGF drugs were registered in the phase II and phase III clinical trials. RPOA with radiographic evidence of bone necrosis was developed in 16 patients received tanezumab for OA followed by total joint replacements [20]. In June 2010, US Food and Drug Administration (FDA) put the tanezumab clinical program for OA on clinical hold until comprehensive information can be obtained to find the causality of these events. Following studies found no evidence that tanezumab was associated with the risk of osteonecrosis, but revealed the increased risk of RPOA associated with high dose of tanezumab in a combination with NSAIDs [18], [35], [36]. In June 2017, tanezumab received Fast Track designation by FDA for the treatment of OA. However, future longer-term safety studies will be necessary to provide more data to further characterize the risk-benefit of tanezumab in patients with OA.

There are some limitations to this research. The majority of OA trials, especially with FGF-18, have focused on knee OA, while OA as multarticicular disease affects different joints. The risk factors for development, progression, and prognosis of OA of the other joints might be different. Some data suggested that the same treatment may have a bigger effect size at the knee than at the hip, and that even the placebo effect in OA may vary between sites, being greatest at the hand, lower at the knee and least at the hip [37].

Another limitation of this analysis is that it included data taken over different time period. OA is known as a slowly progressed disease, and so longer study duration might be needed to identify the efficacy of anti-NGF and FGF-18 agents. Biological nature of tanezumab, fasinumab, fulranumab, and sprifermin also needed a comprehensive longitudinal safety research.

Conclusion

In conclusion, the analysis of clinical trials revealed that anti-NGF therapy palliates pain, enhances joint function and might be considered as an effective option for pain relief and functional improvement in OA non-responsive to conventional analgesics. Nonetheless, the risk of adverse effects is obviously high and may significantly limit the prescription. Another promising monoclonal antibody sprifermin (FGF-18) demonstrated the ability to decrease the cartilage loss and improve cartilage thickness with insignificant side events. This structure-modifying effects on cartilage made the basis for considering sprifermin as promising disease-modifying OA drug. However, further clinical longitudinal studies characterized the risk-benefit are needed to establish these medications safety and efficacy.

References

PMid:34695571

PMid:33160349

PMid:33116279

PMid:31908149

PMid:28487435

PMid:29509867

PMid:21826146

Zaripova et al. Biological therapy for osteoarthritis: efficacy and safety: focus on monoclonal antibodies against Nerve Growth Factor and Fibroblast Growth Factor-18

PMid:30154087


PMid:30653843


PMid:15366873


PMid:31207169


PMid:23553790


PMid:15896984


PMid:28823647


PMid:27748055


PMid:26554876


PMid:18505735


PMid:20942668


PMid:24653755


PMid:32234715


PMid:26449617


PMid:32252976


PMid:33728717


PMid:32264437


PMid:28696132


PMid:24740822


PMid:32098758


