

# Long-term Effect of Injection Treatment for Osteoarthritis in the Knee by Orthokin Autologous Conditioned Serum

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## Abstract

**Background.** Orthokin is an intra-articular autologous conditioned serum (ACS). Its use might have a beneficial biological effect on pain and function of osteoarthritis in the knee. However, earlier studies lack any consensus on its clinical application and disease modifying effect. **Objective.** The aim of this study was to investigate the long-term effect of Orthokin injection treatment on prevention of surgical treatment for end-stage knee osteoarthritis. **Study Design.** Prospective cohort study. **Methods.** Patients of the previously published Orthokin cohort were contacted to determine whether any intra-articular surgical intervention or osteotomy of the study knee had taken place during the past decade. A log-rank test was performed to evaluate the differences in the survival distribution for the 2 types of intervention: Orthokin versus placebo. **Results.** The survival distributions for the 2 interventions were not statistically significantly different,  $\chi^2(1) = 2.069$ ,  $P = 0.150$ . After  $7.5 \pm 3.9$  years, 46.3% of the placebo and 40.3% of the Orthokin group had been treated surgically. **Conclusion.** The use of Orthokin in knee osteoarthritis patients did not result in a delay regarding surgical treatment. **Clinical Relevance.** The intra-articular use of Orthokin does not seem to prevent or delay surgical intervention at 10 years after treatment for end-stage knee osteoarthritis.

## Keywords

osteoarthritis, Orthokin, interleukin-1, disease-modifying osteoarthritis drugs, placebo

## Introduction

The lack of options for the treatment of osteoarthritis (OA) has raised the focus on research of drugs that stop the progression of OA and postpone the need for total joint replacement. These drugs are called disease-modifying osteoarthritis drugs (DMOADs).

Current treatment options such as nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors proved to reduce symptoms of OA.<sup>1,2</sup> However, such drugs do not slow the disease progression, while the patient is at risk for serious side effects, such as an increased risk of upper gastrointestinal bleeding.<sup>3</sup>

Synoviocytes, activated immune cells and chondrocytes secrete cytokines and growth factors that play an important role in cartilage degeneration. DMOADs are believed to intervene with the pathways of these cytokines. Thereby influencing disease progression, decreasing disease symptoms, and improving quality of life.<sup>4,5</sup> Interleukin-1 $\beta$  is such a pro-inflammatory cytokine, suspected to play a prominent role in the pathophysiology of OA.<sup>6</sup> It stimulates matrix metalloproteinases and prostaglandin production, both of which have a negative effect on the cartilage matrix integrity.<sup>7</sup>

Orthokin (Orthogen, Dusseldorf, Germany) is a product designed to stimulate the synthesis of the IL-1 receptor antagonist (IL-1ra) and anti-inflammatory cytokines such as IL-4, IL-10 and IL-13. The product is produced of whole blood, incubated with CrSO<sub>4</sub>-coated glass beads.<sup>8</sup> The potentially beneficial effect of Orthokin and other DMOADs on symptoms and progression of OA has been investigated by certain studies in the past decade.<sup>9,10</sup> However, a follow-up longer than 1 or 2 years is lacking. This is remarkable, keeping in mind the long-lasting nature of this disease. Also, the overall goal of DMOADs is to postpone the need for surgical intervention in the long-term.

In 2004-2005, Auw Yang *et al.*<sup>9</sup> aimed to investigate the effect of intra-articular Orthokin compared to placebo (physiological saline) in reducing symptoms of OA.

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Injections with Orthokin or physiological saline were administered in the prospective double-blind placebo-controlled randomized multicenter trial, forming the Orthokin cohort.<sup>9</sup>

The study of Auw Yang *et al.*<sup>9</sup> demonstrated a biological response different from placebo. However, it was not clinically relevant and the use of Orthokin could not be recommended.<sup>9</sup> On the other hand, Orthokin improved clinical signs and symptoms of OA considerably in the study of Baltzer *et al.*<sup>10</sup> Both studies could not make conclusions on chondroprotective or disease modifying effects. Rutgers *et al.*<sup>11</sup> evaluated these effects *in vitro*. However, they found no effect compared with saline admission.<sup>11</sup> In short, there is no clear agreement on the biological and clinical effects of Orthokin.

The aim of this current study was to investigate the long-term effect of Orthokin injection treatment on prevention of surgical treatment for end-stage knee OA.

## Methods

All patients from the original cohort were approached for inclusion. Exclusion criteria were an unknown side of injection and a missing date of first injection with an event in 2004 or 2005. The latter criterion was chosen as it gave the possibility of admission after surgery. The date of the first surgical treatment was taken into account if the patient had received bilateral intra-articular injections. If the month of the surgical event was unknown, the month January of that year was chosen standardly.

This long-term follow-up has been approved by the Medical Ethics Committee of the University Medical Center in Utrecht (UMCU, reference number 15/101). Patients were approached by phone or letter and provided informed consent. For nonresponders the electronic health reports (EHR) were evaluated.

Patients were requested to provide information regarding the side and date of the knee injection. This was checked by the data from the initial Orthokin study. To assess the primary outcome, the type and date of any surgical treatment was collected for the study knee. If no surgical treatment had taken place, they were asked for progression of symptoms in both the study knee and the contralateral knee. Also, NSAID usage for knee symptoms in the past years and patients' awareness of the received regimen (Orthokin/placebo) were evaluated.

The results were analyzed using SPSS Statistics software package version 23.0 with the Kaplan-Meier method to estimate the probability of knee surgery (event) versus no surgery (censoring). An event was any intra-articular surgical intervention or osteotomy of the concerning knee, past given time points. For a valid inclusion in the Kaplan-Meier method the assumptions were checked. The Orthokin and placebo group were compared using the log-rank test. A *P*

value  $<0.05$  was considered statistically significant. Right-censoring means a patient had received no surgical treatment until a certain date, yet got lost to follow-up from that point. On the other hand, if a patient had had a surgically treated study knee on a time point without the exact date of surgery, there would be a case of left-censoring.

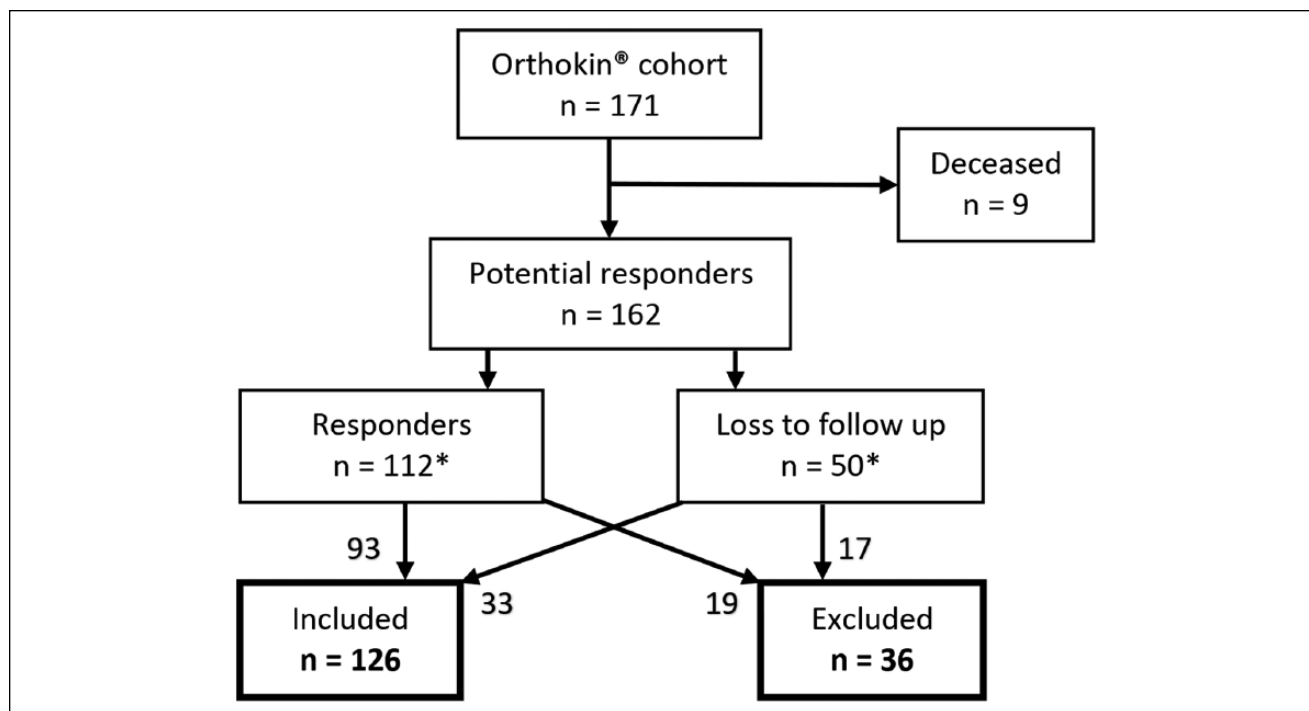
## Results

All assumptions for the Kaplan-Meier method were met. Left-censoring was avoided and there was an absence of secular trends influencing the chance of surgery. Besides, the reason why cases were censored was not because they were at greater risk of the event occurring. This gives an independence of (administrative) censoring. Finally, 13 patients from the placebo and 6 from the Orthokin group with data from the EHR had right-censored information before the end date of the study. However, there were no founded reasons to believe in other prospects causing this difference in amount and pattern of censorship. 3 Orthokin and 6 placebo patients had missing data regarding the date of first admission due to random errors in reporting. These patients got the mean date of admission of their treatment group. February 2016 was selected as the end date for patients without any surgical treatment, as all data was collected by then.

The flowchart in **Figure 1** describes the process of enrollment in which finally 126 patients were included. Thirty-six patients were excluded for several reasons such as unclear responses in surgery status and study treatment cross-over. On the other hand, 33 of the lost to follow-up group got included by means of useable data in the electronic health report.

Baseline values in **Table 1** of both treatment groups were comparable using Pearson's chi-square test for included variables. However, significantly more data was obtained from the EHR in the placebo group. For age we could assume equality of variances with Levene's test ( $P = 0.675$ ) and found comparable groups with the independent *T* test. As **Table 2** illustrates, total knee arthroplasty was the event with highest incidence in both groups. Overall, the types of events were not statistical significantly different between the groups ( $P = 0.100$ ).

**Figure 2** shows that the Orthokin group showed better survival after  $7.5 \pm 3.9$  years of follow-up. In total, 54 events occurred with an estimated mean time of 102.66 months (standard error [SE] = 4.56, 95% confidence interval [CI] = 93.73-111.60) since the initial Orthokin study inclusion. The 29 events in the Orthokin group had an estimated mean time of 109.27 months (SE = 5.52, 95%CI = 98.45-120.09), while the 25 events in the placebo group had an estimated mean time of 93.76 months (SE = 7.56, 95% CI = 78.94-108.58). At the end of this follow-up, 46.3% of the placebo and 40.3% of the Orthokin group had been



**Figure 1.** Flowchart of enrollment. The figure describes the process of inclusion for the long-term follow-up of the Orthokin cohort. A total of 126 patients were included out of 162 potential responders. Seventeen of the 112 responders were excluded as they received Orthokin later on, 2 others gave unclear responses in surgery status. Likewise, one of the loss to follow-up patients was excluded as Orthokin was admitted later on and 16 had no useable data in the electronic health report.

**Table 1.** Baseline Values.<sup>a</sup>

Treatment		Placebo (n = 54)		Orthokin (n = 72)		Comparison	
		n	%	n	%		P
Gender	Female	25	46.3	25	34.7	$\chi^2(1) = 1.727$	0.189
	Male	29	53.7	47	65.3		
Age	Mean	63 y, 8 mo		62 y, 11 mo		$t(124) = 0.460$	0.647
Time from injection	Mean	11 y, 2 mo		11 y, 3 mo			
NSAID usage	Not used	21	63.6	40	72.7	$\chi^2(1) = 0.801$	0.371
	Used	12	36.4	15	27.3		
Awareness of treatment	Unaware	3	9.4	14	24.1	$\chi^2(2) = 3.845$	0.146
	Aware	28	87.5	40	69.0		
	Incorrect	1	3.1	4	6.9		
Follow-up method	EHR	22	40.7	11	15.3	$\chi^2(1) = 10.349$	0.001
	Response	32	59.3	61	84.7		
Contralateral knee	Asymptomatic	11	34.4	32	55.2	$\chi^2(1) = 3.575$	0.059
	Symptomatic	21	65.6	26	44.8		

EHR = electronic health report; NSAID = nonsteroidal anti-inflammatory drug.

<sup>a</sup>The table compares the baseline values of both treatment groups.

treated surgically. A log-rank test showed that the survival distributions for the 2 interventions were not statistically significantly different,  $\chi^2(1) = 2.069$ ,  $P = 0.150$ .

The following covariates had no statistically significant outcomes using Cox regression: placebo or Orthokin

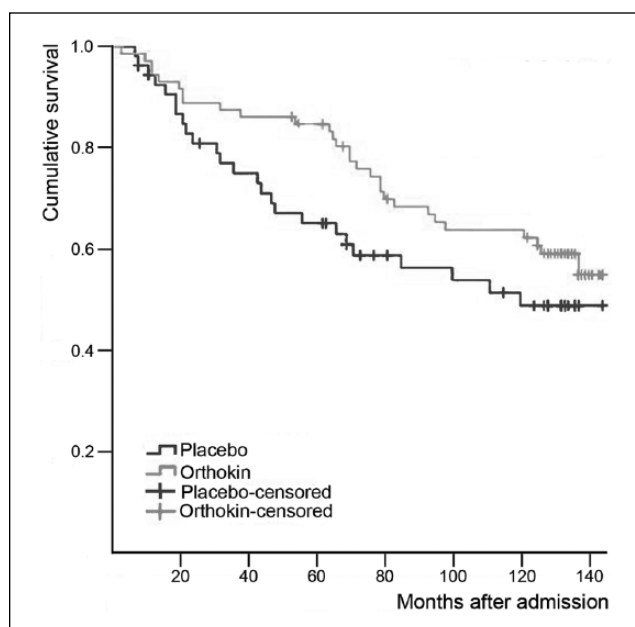
$B^2(1) = 0.791$ ,  $P = 0.527$ ; gender  $B^2(1) = 0.707$ ,  $P = 0.376$ ; contralateral knee symptomatology  $B^2(1) = 1.155$ ,  $P = 0.691$ ; and age  $B^2(1) = 1.023$ ,  $P = 0.307$ . The Kaplan-Meier analysis showed similar outcomes. However, NSAID users had a significantly higher risk of an event  $B^2(1) = 3.390$ ,

**Table 2.** Types of Events.<sup>a</sup>

Type of Surgery	Treatment			
	Placebo		Orthokin	
	n	%	n	%
No surgery	29	53.7	43	59.7
TKR	11	20.4	21	29.2
PKR	4	7.4	1	1.4
Osteotomy	3	5.6	4	5.6
Arthroscopy	4	7.4	0	0.0
Unknown type surgery	2	3.7	3	4.2
Distraction	0	0.0	0	0.0
Microfracturing	0	0.0	0	0.0
Arthrotomy	1	1.9	0	0.0

PKR = partial knee replacement; TKR, total knee replacement.

<sup>a</sup>The table shows that TKR was the most common type of event.



**Figure 2.** Survival distribution for the Orthokin and placebo treatment groups. At the very beginning of the follow-up, there is a crossing of survival distributions. This might have decreased the power of the log-rank test to some extent. The difference between the intervention groups had increased gradually, but decreased at the curves' last cumulative survival proportion.

$P < 0.001$ . This was also the case with the Kaplan-Meier analysis  $\chi^2(1) = 9.077$ ,  $P = 0.003$  using the log-rank test.

## Discussion

This study aimed to determine the long-term effect of Orthokin injection treatment on prevention of surgical treatment for end-stage knee OA. It showed for the first time

that the clinical use of Orthokin has no delaying or preventive effect, compared with placebo. This finding suggests no clinically relevant disease modifying effect of the treatment.

The effect on symptom relief is controversial in literature. Auw Yang *et al.*<sup>9</sup> formed the current cohort and found statistically significant improvement of Knee injury and Osteoarthritis Outcome Score (KOOS) symptom and sport parameters. However, their aimed clinical improvement was not achieved in the 12-month follow-up and the use of Orthokin could not be recommended.<sup>9</sup> On the other side, Baltzer *et al.*<sup>10</sup> performed a controlled clinical trial with an observer-blinded follow-up of 104 weeks after treatment. Orthokin gave statistically and clinically significant improvement in Patient Reported Outcome Measures (PROMs), compared to saline and hyaluronic acid.<sup>10</sup> These 2 studies had partially different inclusion criteria, statistical methods, outcome instruments and follow-up durations. Rutgers *et al.*<sup>11</sup> investigated the in vitro effects of Orthokin on cartilage proteoglycan metabolism, and cytokine production. The aim was to evaluate possible disease-modifying and chondroprotective aspects. They showed no difference between Orthokin and saline admission.<sup>11</sup> All in all, no clear consensus on the biological and clinical effect emerged from these studies.

The etiology and pathology of OA are poorly understood. Pro-inflammatory cytokines such as interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF) are known mediators in this process. This makes them possible therapeutic targets. However, little research has been conducted on the beneficial effects of blocking these mediators. Also, several other cytokines have been proven to play a role in the development of OA.<sup>12</sup> The choice for an IL-1 $\beta$  antagonist is rational as it plays a prominent role in the pathogenesis of OA. Patients with OA have a higher level of IL-1 $\beta$  in the synovial fluid and other compartments of their knees.<sup>13</sup> However, to our knowledge, no study has examined cytokine levels as a predictor of total knee replacement.

Intra-articular injections such as corticosteroids, hyaluronic acid, and autologous conditioned serum (ACS) are considered the final pharmacological option before arthroplasty. In particular, ACS has the potential to be a better option than established pharmacological treatments and surgery.<sup>14,15</sup> However, till now, no DMOAD has convincingly changed the structural progression of OA, such as cartilage loss and joint space narrowing. Therapies have only had symptomatic effects to some extent.<sup>13,16</sup> The complexity of the multiple cytokines involved, calls for a more sophisticated approach in therapeutic strategies. More research should be conducted on missing links.<sup>13</sup> However, the presence of inflammatory cytokines may be an irreversible point of a disturbed cytokine homeostasis. This indicates the need for focus on treatment of early stage OA before the onset of irreversible joint failure. Important for

this purpose are the identification of clinical risk factors and sensitive diagnostic modalities such as highly sensitive magnetic resonance imaging and serum biomarkers.<sup>13,16,17</sup> Also, the need for personalized patient care and patient specific molecular profiles is essential for choosing particular treatment strategies.<sup>14,18</sup> Furthermore, De Windt *et al.*<sup>19</sup> recently showed that mesenchymal stem cells (MSCs) originating from adult bone marrow may be safe and promising for chondrogenesis and cartilage regeneration. MSCs may secrete paracrine factors with tissue repair as a result. Also, MSCs would have an anti-inflammatory and immunomodulatory effect.<sup>19</sup> Intra-articular use of mesenchymal stem cells needs more research before definite conclusions and clinical translation can be made.<sup>14,15</sup> Then again, it may even be naive to think of one universal treatment given the complexity of the pathogenesis of OA.<sup>17,20</sup>

At the initial trial, Orthokin treatment was the last resort for patient who would otherwise have received knee arthroplasty. Noteworthy is the relatively large part of the cohort without an event almost a decade after admission in both groups (events in 46.3% of the placebo and 40.3% of the Orthokin group). Of importance is the KOOS score of the study of Auw Yang *et al.*<sup>9</sup> which was considerably lower than that of the same age population from the study of Paradowski *et al.*<sup>21</sup> Also, in comparison with hyaluronic acid studies of Waddell and Bricker<sup>22</sup> and Altman *et al.*<sup>23</sup> the relative number of patients without surgical treatment is high. This may be due to a selection bias in the initial trial. Patients might have been included without sufficient indication for joint arthroplasty. On the other hand, it may also be explained by a prolonged placebo effect and altered patient behavior.<sup>24</sup>

Limitations of this study are as follows. First, we did not take any use of nonsurgical treatment of knee OA into account. The use of hyaluronic acid, corticosteroids, or physiotherapy might have been a confounder, especially in placebo patients aware of their treatment. Second, 33 patients were included based on information from the electronic health report. This information did not contribute to the baseline data of patient awareness and NSAID usage. Nevertheless, NSAID users had a significantly higher chance of surgical treatment. A possible explanation is the confounding that patients in need of analgesics experience more pain. This could lead to an earlier desire for surgical treatment.

In conclusion, the use of Orthokin for knee OA did not result in a delay regarding surgical treatment for OA, compared with placebo. The findings suggest no clinically relevant disease-modifying effect of the treatment, around a decade after injections were given.

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### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Ethical Approval

This long-term follow-up has been approved by the medical ethics comity of the University Medical Center in Utrecht (UMCU, reference number 15/101). Patients were approached by phone or letter and provided informed consent. For nonresponders the electronic health reports (EHR) were evaluated.

### Informed Consent

Informed consent was obtained from all subjects before the study by phone or letter.

### Trial Registration

Not applicable.

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