

Effect of Intraarticular Hyaluronan Injection on Vertical Ground Reaction Force and Progression of Osteoarthritis After Anterior Cruciate Ligament Transection

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ABSTRACT. Objective. To determine if intraarticular (IA) injection of hyaluronan (HA) into the canine knee after anterior cruciate ligament transection (ACLT) alters the progression of osteoarthritis (OA) and the perception of pain in this model.

Methods. OA was induced in 30 adult dogs of mixed breed by ACLT. The dogs were divided into 3 groups and given 5 weekly IA injections into the unstable knee during Weeks 1–5 and 13–17. The prophylactic treatment group received HA in the first series and saline during the second series. The therapeutic group received saline in the first series and HA in the second series. The control group received saline during both injection series. The progression of joint damage of OA was evaluated by arthroscopy 12 weeks after ACLT and by gross examination 32 weeks after ACLT. Histologic and biochemical changes of OA were evaluated. Loading of the unstable limb during gait was determined by force-plate analysis before surgery, after each series of injections, and the week before euthanasia.

Results. Arthroscopic examination 12 weeks after ACLT revealed OA changes in the cruciate-deficient knees. Chondropathy scores ranged from 0 to 8 (possible range 0–65). The mean chondropathy score was 2.5 ± 1.3 (mean \pm SD) for the controls, 2.5 ± 2.5 for the therapeutic group, and 2.1 ± 1.3 for the prophylactic group. At the termination of the experiment 32 weeks after ACLT, the gross chondropathy scores were 14.0 ± 5.2 for controls, 17.6 ± 6.8 for the therapeutic group, and 13.3 ± 5.0 for the prophylactic group. There were no significant differences among the means of the gross scores, the histologic scores, or biochemical composition of articular cartilage. The peak vertical ground reaction force (VGRF) generated by the unstable limb was reduced by ~60% after ACLT, and slowly increased to ~75% of the baseline value over the 32 weeks after ACLT. HA injection had no effect on the VGRF or on the pathologic changes of OA.

Conclusion. Intraarticular HA injection did not alter the progression of OA in the cruciate-deficient canine knee or alter the loading of the unstable limb. (J Rheumatol 2005;32:325–34)

Key Indexing Terms:

OSTEOARTHRITIS HYALURONAN ANTERIOR CRUCIATE LIGAMENT TRANSECTION

Intraarticular (IA) injection of hyaluronan (HA) for relief of joint pain in humans with knee osteoarthritis (OA) is growing in popularity. Proponents argue that HA injection relieves pain and slows the progression of joint damage^{1–3}. In a previous study we injected HA into the unstable knee of dogs weekly for 5 weeks, beginning the day after anterior

cruciate ligament transection (ACLT), and examined the joint 12 weeks after surgery (7 weeks after the last injection). The extent of joint damage in the unstable knee was comparable to that in OA knees injected with saline on the same schedule. However, the proteoglycan (PG) concentration of the articular cartilage from knees injected with HA was significantly lower than that from cartilage of the contralateral knee⁴. In contrast, the PG concentration of articular cartilage from OA knees injected with saline was significantly higher than that in the cartilage from the contralateral knees, as has been observed consistently in this model⁵.

Because the compressive stiffness of cartilage is dependent on the PG concentration⁶, accelerated progression of damage might be expected in OA joints in which the PG concentration of the matrix was reduced by IA injection of HA. A similar increase in PG concentration is seen in human OA, and is interpreted as a manifestation of repair activity

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by the chondrocytes⁷. The depletion of articular cartilage PG noted in these studies raised questions about the efficacy and the safety of intraarticular HA injection.

To address this concern, this study was designed to clarify the effects of the duration of knee instability and of the timing of the intervention on the composition of articular cartilage and progression of OA after HA injection. We tested the effects of prophylactic treatment with HA in one group of animals by initiating the injections immediately after surgery, and of therapeutic treatment in a second group of animals in which injections were initiated after pathological changes of OA were clearly established. We extended the duration of the study to 32 weeks to permit the development of severe changes of OA in the cruciate-deficient knees.

Finally, the vertical ground reaction force (VGRF) generated by the unstable limb was determined to assess the effect of intraarticular HA injection on limb loading after ACLT, with the premise that the extent to which the animal loads the unstable limb reflects the discomfort associated with the use of the limb.

MATERIALS AND METHODS

Experiment design. To determine the effect of intraarticular HA injection on the progression of OA and whether this treatment affected loading of the OA limb, 36 dogs were treated as illustrated in Figure 1. All animal procedures were approved by the Institutional Animal Care and Use Committee.

To confirm that the PG depletion seen in our previous studies was related to HA injection of the OA knee, 6 dogs were subjected to a sham surgical procedure and evaluated after 12 weeks (Figure 1A). Each dog was

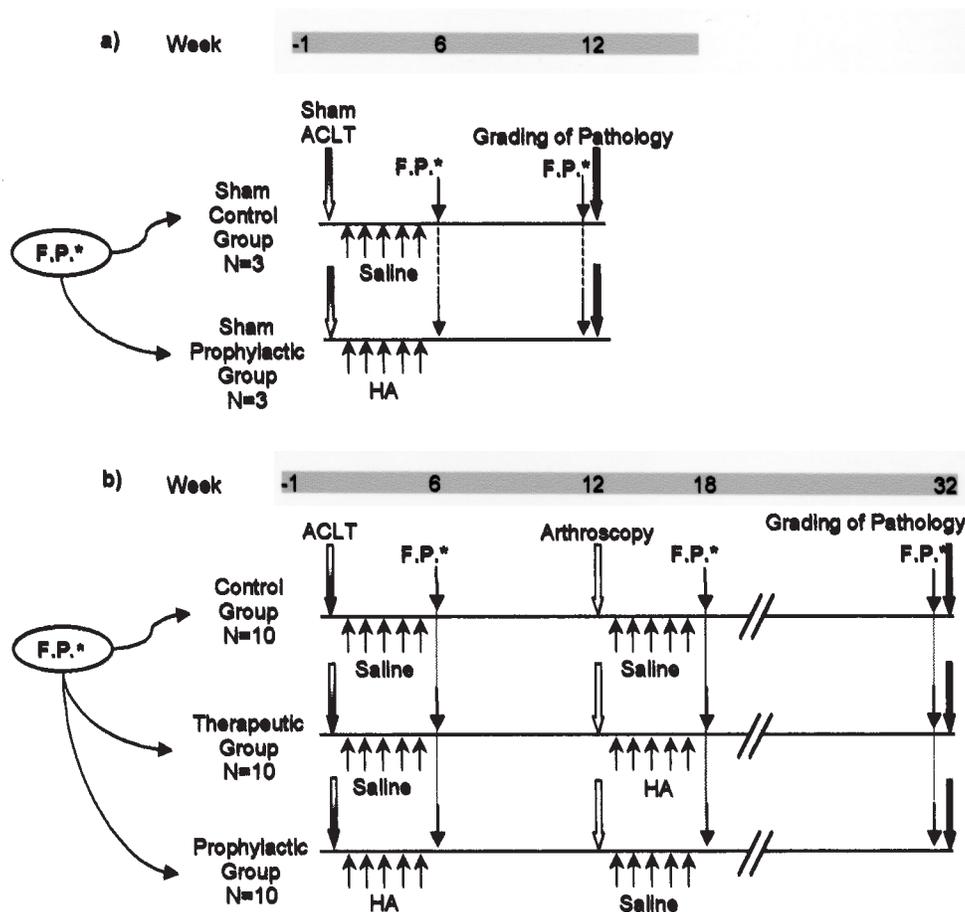


Figure 1. Experiment design. A. Sham groups. After force-plate analysis (F.P.) of VGRF confirmed normal limb loading, dogs were randomly assigned to treatment groups. Two groups of 3 dogs each underwent a sham ACLT procedure in which the ACL was exposed but not cut, and then the operated knees were injected with either saline (sham control group) or HA (prophylactic control group) weekly for 5 weeks, beginning the day after surgery. The VGRF generated by each limb was determined the week after the last injection and the week before euthanasia and joint analysis. The knee joints were graded for OA changes at necropsy, 12 weeks after surgery. B. ACLT groups. Three groups of 10 dogs each underwent ACLT, and then the operated knee of each dog received weekly IA injections from Weeks 1–5 and Weeks 13–17. The left knee of each dog in the control group was injected with saline during Weeks 1–5 and Weeks 13–17; in the therapeutic group, with saline during Weeks 1–5 and with HA from Weeks 13–17; and in the prophylactic group, with HA from Weeks 1–5 and with saline from Weeks 13–17. The dogs were examined by arthroscopy 12 weeks after surgery and by force-plate analysis the week following each series of injections and 32 weeks after surgery, before euthanasia and grading of OA pathology.

examined radiographically to confirm that the growth plate was closed and to exclude preexisting joint pathology. As indicated in Figure 1A, each dog was then evaluated by force-plate analysis to verify that the limbs were normally loaded during gait. After the initial gait analysis, the dogs were randomly assigned to 2 groups of 3 dogs each and the ACL of the left knee of each dog was exposed but not cut. The dogs then received a series of 5 weekly IA injections of either 1 ml saline (the sham control group) or 1 ml saline containing HA (10 mg/ml sodium hyaluronate, MW 0.5–7.3 × 10⁶ Da; Hyalgan®, lot no. 064600; Fidia, Abano Terme, Italy; the sham prophylactic group). Both knees of each dog were evaluated at necropsy, 12 weeks after surgery.

An additional 30 dogs were randomly assigned to 3 groups of 10 dogs each after gait analysis. The ACL of the left knee was transected, and the dogs were treated as shown in Figure 1B. All dogs were examined by arthroscopy at Week 12 after surgery, and were analyzed in the gait laboratory during Weeks 6, 18, and 32. The operated knees of the dogs in the control group were injected with saline during Weeks 1–5 and Weeks 13–17; the dogs in the therapeutic group, with saline during Weeks 1–5 and with HA during Weeks 13–17; the dogs in the prophylactic group, with HA during Weeks 1–5 and with saline during Weeks 13–17.

Induction of OA. Adult male dogs (20–25 kg) of mixed breed were randomly divided into 3 groups of 10 dogs each, and the ACL of the left knee of each dog was transected as described⁵. The dogs were anesthetized, the joint capsule was entered from the medial aspect, and the ligament was exposed and transected with a curved scalpel. An additional 6 dogs were subjected to a sham surgical procedure in which the ligament was exposed but was not transected⁶.

The dogs were kept in 0.85 × 2.1 m runs for the first 5 weeks after surgery, while they received weekly IA injections, and were then kept in indoor/outdoor runs (1.3 × 2.1 m indoors, 1.3 × 5 m outdoors) during Weeks 6–12. The sham-operated dogs were killed after 12 weeks, and the knees were evaluated for pathologic changes. The dogs that had undergone ACLT were returned to the indoor runs during Weeks 13–17 for a second series of injections, and were returned to the indoor/outdoor runs during Week 18, where they remained for the final 14 weeks of the experiment. While in the indoor/outdoor runs, the dogs were placed in a fenced 30 m × 50 m area for 30 min each weekday and encouraged to run and use the operated limb. In the indoor runs, the dogs were allowed 30 min/weekday of free activity outside the runs.

HA injections. For the injection procedure, after the dog was sedated with sodium pentathol, a 21 gauge needle was inserted into the joint space through the lateral aspect of the knee and as much fluid as possible was withdrawn. One milliliter HA or an equal volume of sterile saline was injected into the joint through the same needle. The dogs were monitored in the recovery room while they recovered from anesthesia and were returned to their runs the following day.

Gait analysis. To determine if IA injection of HA into canine knees following ACLT changed the extent to which the animal loaded the unstable knee, the dogs were walked over a specially constructed runway with an AMTI force platform mounted flush with the surface^{9,10}. The average speed was ~1.8 m/s, a speed at which the dogs broke into a trot. The trials were videotaped and the forces generated when the forelimb and ipsilateral hind limb struck the force-plate were recorded. Ten records for each pair of limbs, in which both limbs struck the force-plate cleanly and in which a distinct peak for each limb was apparent, were saved for analysis. The peak VGRF generated by the operated and contralateral unoperated limbs was measured before surgery; 6 weeks after ACLT, following the first series of injections; 18 weeks after ACLT, following the second series of injections; and 32 weeks after ACLT, shortly before euthanasia (Figure 1). The sham-operated animals were studied at baseline; after the injection series, 6 weeks after surgery; and at the end of the study, 12 weeks after surgery.

Arthroscopic observations. The severity of pathology in the operated knee of each dog that underwent ACLT was evaluated using a 2.7 mm glass lens arthroscope (Smith-Nephew Dyonics, Memphis, TN, USA) at Week 12,

immediately prior to the second series of injections^{5,11}. The dogs were anesthetized and the skin of the operated knee was shaved and scrubbed with 1% aqueous Zephiran. Synovial fluid was aspirated with a 21 gauge needle, the joint was then distended with 20–30 cc of sterile saline, and then entered with the arthroscope. A probe was inserted into the joint as an internal dimensional standard. After evaluation of joint pathology, all fluid was drained from the joint and the arthroscope was removed. The small puncture wounds created by the procedure did not require suturing. The duration of the examination was about 40 min.

Inspection of the femoral cartilage during arthroscopy was performed systematically to identify the location, size, and severity of cartilage ulcers. The entire examination, including views of the medial and lateral condyles in flexion and extension, the intercondylar notch, and the trochlea, was recorded on videotape. The degree of marginal osteophytosis on the femoral trochlea and appearance of the synovial vasculature were noted.

The defects were assigned a severity grade based on their depth: 1 = superficial fissure/abrasion; 2 = partial-thickness erosion/fibrillation; 3 = full-thickness erosion/defect. A chondropathy score was calculated for each lesion by multiplying its weighted severity factor (grade 1 = 0.14, grade 2 = 0.34, grade 3 = 0.65) by the area of the lesion, expressed as a percentage of the total femoral condyle surface area^{5,11,12}. The surface area of each femoral condyle was estimated post mortem by morphometric analysis of a photograph of the distal femur, as described below. The scores for all cartilage defects were summed to provide an arthroscopic chondropathy score for the femur (possible range = 0 to 65)⁵.

Cartilage changes of OA. At necropsy, both knees of each dog were evaluated. The femur and tibia were disarticulated and the articular cartilage surfaces and synovium were photographed. The joints were examined for gross evidence of OA changes, including the size, location, and severity of cartilage defects^{5,12,13}. The lesion area was determined as a percentage of the total condylar surface area, which was measured on a digitized photograph that included an internal dimensional standard. Osteophytic cartilage was excluded from this measurement, but no adjustment for the curvature of the surface was attempted. A severity score was calculated for each lesion, using the scoring system described above for the arthroscopic grading of lesions. A similar score was developed for the tibial surface. Gross chondropathy scores for the femur and tibia of each joint were calculated by summing the individual lesion scores.

Osteophytes. Osteophytes along the margins of the trochlear ridges, within the intercondylar notch, on the patella, and on the tibial plateau were counted and the length and width of the largest osteophyte in each location were recorded. The severity of osteophytosis at the margin of the femoral articular cartilage was graded using a scale from 0 to 4, where 0 = no osteophyte, 1 = mild osteophytosis (thickening of the trochlear margin); 2 = moderate osteophytosis (≤ 6 distinct osteophytes); 3 = marked osteophytosis (> 6 large osteophytes, covering much of the femoral margin); and 4 = extensive (large osteophytes covering the entire femoral margin).

For osteophyte scoring for the tibial plateaus, we used a similar scale, where 0 = no osteophytes; 1 = slight remodeling of the plateau margin; 2 = 20% of the margin covered by osteophytes; 3 = 50% of the margin covered by osteophytes; and 4 = 100% of the margin covered with osteophytes and remodeled.

Osteophytes within the intercondylar notch were graded for the lateral and medial margins of the notch according to the percentage of the notch margin involved by the osteophyte, where 0 = none; 1 = 25% of the area; 2 = 50% of the area; 3 = 75% of the area; and 4 = 100% of the area.

Patellar osteophytes were graded on the scale: 0 = no osteophytes; 1 = only 1 or 2 small osteophytes (< 10 mm²); 2 = > 3 small osteophytes and/or osteophytes from 10–20 mm² area; 3 = 1 or 2 large osteophytes; 4 = ≥ 3 large osteophytes (> 100 mm²).

Cartilage histology. A full-thickness block of cartilage roughly 5 mm wide, extending through the subchondral bone, was removed from the anterior portion of the medial femoral condyle (Figure 2) with a bone saw and was fixed for histologic examination. The block extended from the proximal

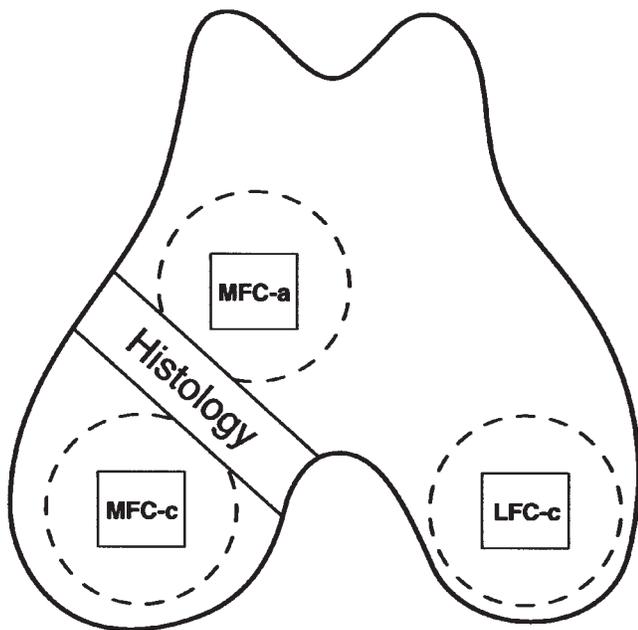


Figure 2. Sites of sample collection on the canine femoral condyles (left femur). A sample of cartilage (represented by squares) was taken from the central weight-bearing region of the medial femoral condyle (MFC-c), the central weight-bearing region of the lateral femoral condyle (LFC-c), and from a site on the anterior surface of the femoral condyle proximal to the intercondylar notch and near the base of the trochlear ridge (MFC-a), for determination of the biochemical composition of articular cartilage. Cartilage slices from the area around each of these samples (broken circle) were cultured with radioactive sulfate to determine net PG synthesis. A wedge of tissue extending from the proximal end of the intercondylar notch to the proximal margin of the condyle, including the cartilage and subchondral bone, was taken for histology.

margin of the intercondylar notch to the outer margin of the condyle. Sections (5 μ m) were cut and stained with Safranin-O/fast green. They were graded for pathologic changes of OA, using the criteria described by Mankin, *et al*¹⁴, by 2 observers (GNS, SLM), who were unaware of the treatment group assignment. When scores of the 2 readers differed by more than 2 points, the readers examined the slides together and assigned a consensus score to the sample. If the difference was 1 point, the scores were averaged.

Histology of synovium. Samples of synovial tissue from the area adjacent to the anterior horn of the medial meniscus were fixed in formalin, embedded in paraffin blocks, sectioned (4 μ m thick), and stained with hematoxylin and eosin (H&E). Sections were graded for mononuclear cell infiltration on a scale of 0–4, where 0 = no mononuclear cells; 1 = a few scattered mononuclear cells; 2 = 5–15 mononuclear cells per high power field; 3 = diffuse mononuclear cell infiltration or 1–2 dense mononuclear cell aggregates; and 4 = > 2 dense mononuclear cell aggregates per section¹⁵. The synovial slides were scored by 2 observers (GNS, SLM) who were unaware of the treatment group to which each animal had been assigned.

Biochemical composition of articular cartilage. The water and PG content of the articular cartilage were determined using full-thickness samples taken from the central weight-bearing area of the medial femoral condyle (mfc-c), the lateral femoral condyle (lfc-c), and the anterior face of the medial femoral condyle, near the base of the medial trochlea of the knee (mfc-a) (Figure 2). The samples were weighed, extracted with acetone, dried to a constant weight, and incubated overnight at 56°C with pronase (1 mg Pronase B in 1 ml 0.05 M Tris buffer, pH 7.5). The glycosaminoglycans in the pronase digest were precipitated with acridine orange and converted

to their water-soluble sodium salts by treatment with Bio-Rad Cation Exchange Resin AG50W-X8, then the uronic acid content was determined by the carbazole reaction¹⁶.

Incorporation of radiolabeled sulfate into cartilage PG. After the samples were taken for determination of cartilage composition, cartilage slices removed from the margins of each sample site (indicated by broken circles in Figure 2) were cultured with ³⁵SO₄⁻² to determine net PG synthesis, as described⁵. Briefly, thin slices (< 0.5 mm) of cartilage were incubated for 20 h in Ham's F12 medium supplemented with 10% newborn calf serum and antibiotics. The medium was replaced with medium containing 20 μ Ci/ml Na₂³⁵SO₄, and incubation was continued for an additional 4 h. Total nondialyzable radioactivity from the spent medium was added to that from the pronase digest of the tissue slices, and the net incorporation of radioactive sulfate was calculated as cpm/mg wet weight. The results are reported as the ratio of the radioactivity incorporated by the cartilage sample from the OA knee (cpm/mg wet weight) to that incorporated by the cartilage from the corresponding site in the contralateral knee.

Statistics. The arthroscopy scores and chondropathy scores and the data for uronic acid concentration, water content, net incorporation of radioactive sulfate into cartilage samples, and VGRF were evaluated by ANOVA, and the means were compared by Student-Newman-Keuls test. The correlation between chondropathy score and VGRF was evaluated by linear regression analysis. The nonparametric measures — cartilage histology scores and synovitis scores — were evaluated by Kruskal-Wallis test.

RESULTS

Sham-operated animals. Very slight cartilage damage on the femoral condyles and the tibial plateaus was noted at necropsy in the 6 sham-operated animals when they were evaluated 12 weeks after surgery. The gross chondropathy scores for these dogs ranged from 0 to 2.1, with a mean score \pm SD of 0.44 \pm 0.81 and a median score of 0.14. The score distribution was skewed greatly by a dog from the saline-injected group with large, grade 1 lesions on both condyles of the sham-operated knee. However, the contralateral, unoperated knee of this dog had a similar lesion, suggesting that this defect was not related to the sham surgery or injection of saline. There were no osteophytes or evidence of synovial pathology in any of the sham-operated knees, whether they were injected with HA or with saline. There was no statistically significant difference between the cartilage proteoglycan concentration in the right and left limbs of the sham-operated dogs. The VGRF generated by the left hind limb 6 and 12 weeks after surgery was comparable to the baseline value, indicating that surgery and IA injection had no persistent effect on loading of the limb.

Pathology 12 weeks after ACLT. Arthroscopic examination of the unstable knees 12 weeks after ACLT revealed chondropathy, regardless of treatment group assignment, on the articular surface of the femoral condyles in 29 of 30 dogs. The single dog with no pathology on the femoral condyles exhibited significant defects on the tibial plateaus, damage to the lateral meniscus, and large osteophytes. The arthroscopic chondropathy score from the prophylactic HA group (2.1 \pm 1.3, mean \pm SD) indicates that HA treatment did not change the early progression of OA, in comparison with the control group (2.5 \pm 1.3) or with the therapeutic HA treatment group (2.5 \pm 2.5) (Figure 3).

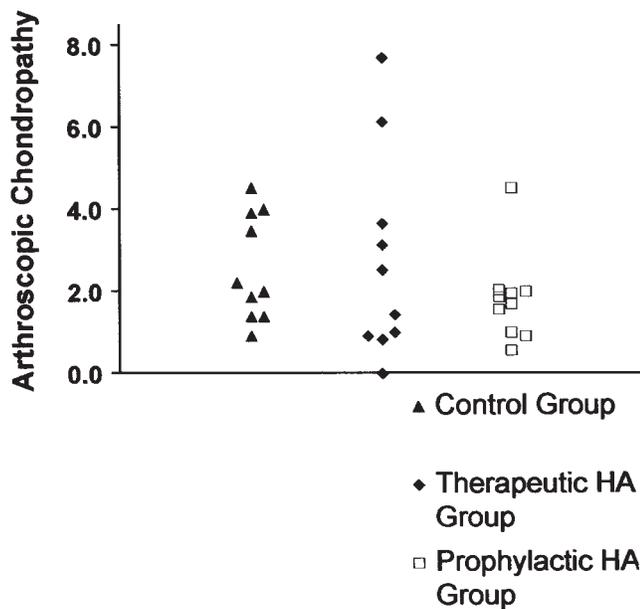


Figure 3. The unstable knee was examined by arthroscopy 12 weeks after ACLT to assess the progression of OA changes. The size of articular cartilage lesions on the femoral condyle was estimated, using a probe inserted into the joint as a size standard, and the lesions were graded on a 4-point scale, where 0 = normal surface, 1 = surface roughening, 2 = significant pitting and fibrillation, and 3 = lesions that reach the subchondral bone. The surface area of the lesions was estimated as a percentage of the total surface area of the condyles determined directly at necropsy 32 weeks after ACLT (and 20 weeks after arthroscopy). The score was calculated as described⁵.

Osteophytes were prominent along the lateral and medial margins of the trochlea, and the synovium exhibited vascular engorgement and displayed prominent villi in all OA knees 12 weeks after ACLT. However, neither the synovial reaction nor the presence and location of osteophytes were related to the treatment group.

Pathology 32 weeks after ACLT. Gross examination of the knees at necropsy, 32 weeks after ACLT, revealed significant changes of OA in all treatment groups. However, the mean scores for cartilage damage on the femoral condyles of dogs in the prophylactic HA group (12.9 ± 5.1 , $n = 10$), therapeutic HA group (17.3 ± 6.9 , $n = 10$), and control group (14.4 ± 4.9 , $n = 10$) did not differ significantly (Figure 4).

Similarly, the chondropathy scores for the tibial plateaus of dogs in the prophylactic HA group (13.7 ± 5.1), therapeutic HA group (18.5 ± 5.0), and control group (18.9 ± 5.8) did not differ significantly.

Osteophytosis was observed in every OA knee (Table 1). The treatment groups did not differ with respect to the mean severity of osteophytes.

Histology of articular cartilage. Cartilage from the unstable knee 32 weeks after ACLT exhibited significant histologic changes of OA, including surface disruption ranging from mild surface defects to clefts extending into the radial zone. Cellular changes were present in most samples, ranging

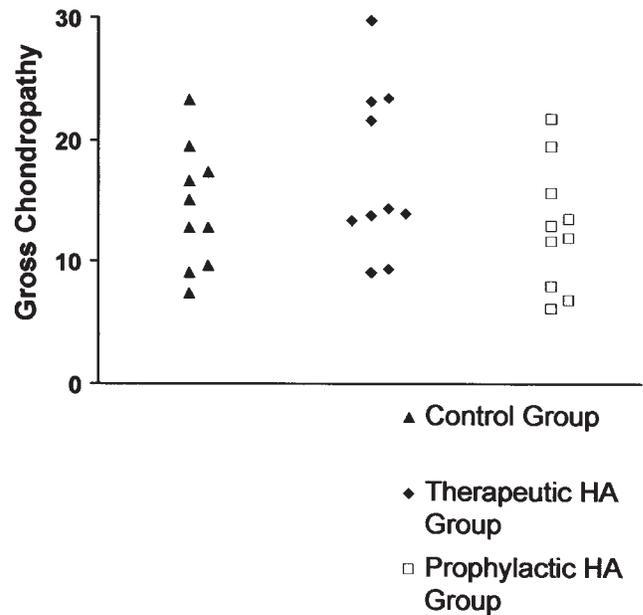


Figure 4. Chondropathy scores for femoral condyles of dogs 32 weeks after ACLT. Femoral condyle lesions were measured at necropsy and grades were assigned to each lesion, using the scale used at arthroscopy. The relative area of each lesion was calculated as a percentage of the total surface area of the condyle determined from a digitized photograph, and a chondropathy score was calculated by multiplying the relative lesion area by a factor of 0.14 for grade 1 lesions, 0.34 for grade 2 lesions, and 0.65 for grade 3 lesions^{5,13}.

from mild hypercellularity to regions of hypocellularity. Cloning was common and in some cases very large brood capsules were present. Focal reduction of Safranin-O staining occurred in some samples. However, the mean scores for the 3 groups revealed no differences in treatment effects (Table 2).

Biochemical composition. Cartilage samples from the lateral condyles and from the sampling site on the anterior aspect of the medial condyle (Figure 2, Table 3) consistently exhibited a higher PG concentration than the corresponding sites from the contralateral knee (Table 3). In contrast, the PG concentration in cartilage from the central weight-bearing region of the medial femoral condyle of the knees of dogs in the therapeutic HA group was no higher in the operated knee than in the contralateral knee. For cartilage from this region, the ratio of the PG concentration in cartilage from the OA knee to that in cartilage from the contralateral knee was significantly lower for the dogs in this therapeutic HA group than for either the control dogs or the prophylactic HA dogs.

In all treatment groups, the water content of the cartilage of unstable limbs was increased 4%–7% relative to that of the contralateral limb. There were no significant differences among the 3 treatment groups.

PG synthesis. No difference in net sulfate incorporation between cartilage from treated and untreated knees was detected (Table 4). Even those cartilage samples from the

Table 1. Distribution and severity of osteophytes in the OA knees of dogs 32 weeks after ACLT.

Treatment Group	Femoral Condyle*		Tibial Plateau**		Intercondylar Notch [†]		Patella ^{††}
	Medial	Lateral	Medial	Lateral	Medial	Lateral	
Control	2.9 ± 0.7	3.1 ± 0.7	3.6 ± 0.5	3.0 ± 0.8	1.4 ± 1.5	3.4 ± 0.8	2.6 ± 0.9
Therapeutic HA	2.9 ± 0.9	3.3 ± 0.8	3.7 ± 0.5	3.3 ± 0.6	2.0 ± 1.7	3.8 ± 0.4	3.0 ± 0.9
Prophylactic HA	2.9 ± 1.3	3.1 ± 0.9	3.6 ± 1.0	3.0 ± 1.1	1.4 ± 1.4	3.4 ± 1.2	2.6 ± 1.0

* Osteophyte scoring for the femoral condyles, 0 = no osteophytes; 1 = mild osteophytosis (thickening of the trochlear margin); 2 = moderate osteophytosis (≤ 6 distinct osteophytes); 3 = marked osteophytosis (> 6 large osteophytes, covering much of the femoral margin); 4 = extensive osteophytosis (large osteophytes covering the entire femoral margin). ** Osteophyte scoring for the tibial plateaus, 0 = none; 1 = slight remodeling of the plateau margin; 2 = 20% of the margin covered by osteophytes; 3 = 50% of the margin covered with osteophytes; 4 = 100% of the margin covered with osteophytes and remodeled. [†] Notch osteophytes were graded for the lateral and medial margins of the notch according to the percentage of the notch margin involved by the osteophyte, where 0 = none; 1 = 25% of the area; 2 = 50% of the area; 3 = 75% of the area; 4 = 100% of the area. ^{††} Patella osteophytes were graded: 0 = no osteophytes; 1 = only 1 or 2 small osteophytes (< 10 mm²); 2 = more than 3 small osteophytes and/or osteophytes 10–20 mm² area; 3 = 1 or 2 large osteophytes; 4 = 3 or more large osteophytes (> 100 mm²).

Table 2. Histologic changes of OA 32 weeks after ACLT.

Treatment Group	Cartilage Histology*, Mean ± SD		Synovitis**, Mean ± SD	
	OA Knee	Contralateral	OA Knee	Contralateral
		Knee		Knee
Control	4.7 ± 1.6	0.0	2.4 ± 1.4	0.7 ± 0.7
Therapeutic HA	4.4 ± 1.7	0.1	3.2 ± 1.2	0.3 ± 0.5
Prophylactic HA	4.7 ± 1.9	0.0	2.5 ± 1.4	0.3 ± 0.7

* Cartilage histology graded¹⁴ on a 15-point scale. ** Synovitis estimated on a 5-point scale, where 0 = no mononuclear infiltrate; 1 = occasional mononuclear cells, 2 = 5–15 mononuclear cells/high power field; 3 = diffuse mononuclear cell infiltration or 1–2 dense mononuclear cell aggregates/section; 4 = more than 3 dense mononuclear cell aggregates/section¹⁵.

Table 3. Uronic acid (UA) concentration of cartilage samples from canine knees 32 weeks after ACLT.

Treatment Group	Sample*	UA (µg/mg dry weight), mean ± SD		Ratio OA Knee/Control Knee
		Control Knee	OA Knee	
Control	MFC-c	4.7 ± 0.5	5.6 ± 0.6	1.19
Therapeutic HA	MFC-c	5.2 ± 0.3	5.3 ± 0.4	1.03**
Prophylactic HA	MFC-c	4.5 ± 0.6	5.8 ± 0.6	1.24
Control	LFC-c	4.9 ± 0.5	5.6 ± 0.6	1.19
Therapeutic HA	LFC-c	5.1 ± 0.3	5.9 ± 0.4	1.15
Prophylactic HA	LFC-c	4.8 ± 0.6	5.8 ± 0.6	1.24
Control	MFC-a	3.1 ± 0.9	5.0 ± 0.7	1.59
Therapeutic HA	MFC-a	2.8 ± 0.6	5.0 ± 0.7	1.78
Prophylactic HA	MFC-a	3.0 ± 0.5	4.9 ± 0.9	1.63

* Values were obtained for 3 full-thickness cartilage samples per knee, taken from the central weight-bearing area of the medial femoral condyle (MFC-c), the central weight-bearing area of the lateral femoral condyle (LFC-c), and the anterior face of the medial femoral condyle (MFC-a), a site where lesions frequently develop in this model. ** Different from control and prophylactic HA treatment groups by ANOVA and Student-Newman-Keuls test, p = 0.05.

anterior face of the medial condyle, which had almost twice the PG content of the corresponding site on the contralateral knee (Table 4), did not exhibit elevated synthesis of PG in the samples taken 32 weeks after ACLT.

Synovitis. Mononuclear cell infiltration of the synovium was observed in more than 90% of the OA knees (Table 2). Notably, a synovitis score of 4 (indicating ≥ 3 dense aggre-

gates of mononuclear cells) was found in 50% of the samples from knees of dogs in the therapeutic HA group, and in 30% of those from the dogs in the prophylactic HA group, but not in any sample from the control group. Nevertheless, no statistically significant difference was detected in the severity of synovitis among the 3 treatment groups.

Effect of intraarticular HA injection on loading of the unsta-

Table 4. Incorporation of radiolabelled sulfate into glycosaminoglycans by articular cartilage slices from canine knees 32 weeks after ACLT*. Data are mean \pm SD.

Treatment Group	MFC-c	Cartilage Sample Site**	
		LFC-c	MFC-a
Control	0.97 \pm 0.12	0.87 \pm 0.17	0.97 \pm 0.28
Therapeutic HA	1.03 \pm 0.35	0.82 \pm 0.15	0.98 \pm 0.21
Prophylactic HA	0.85 \pm 0.18	0.87 \pm 0.20	0.92 \pm 0.14

* Ratio of net sulfate incorporation (cpm/mg wet weight of cartilage) by cartilage slices from the OA knee/contralateral knee (mean \pm SD). ** Sample sites are indicated in Figure 2, where MFC-c = medial femoral condyle, central weight-bearing area, LFC-c = lateral femoral condyle, central weight-bearing area, and MFC-a = medial femoral condyle, anterior surface.

ble limb. During a trot, the forelimb and the ipsilateral hind limb of a dog generate 2 distinct peaks on a plot of VGRF versus time (Figure 5). In the 30 dogs in this study the peak VGRF generated by the hind limbs prior to ACLT averaged about 67% of body weight (mean \pm SD for the right hind limb, 66.6% \pm 5.8%; for the left hind limb, 67.6% \pm 4.7%; Figure 6). The forelimbs generated a VGRF of ~118% of body weight (right 118.1% \pm 8.7%; left 118.3% \pm 8.1%). Sham ACLT did not alter the VGRF generated by the operated limb of the saline- or HA-injected dogs 6 or 12 weeks after surgery, indicating that neither surgery nor intraarticular HA injection had an effect on the loading of the normal, stable canine knee.

When the dogs were evaluated 6 weeks after ACLT after injection of either saline (the control group and the therapeutic group) or HA (the prophylactic group), the VGRF

generated by the unstable left hind limb was reduced to 26.7% \pm 5.8% of static body weight (39.5% \pm 9% of the baseline value; Figure 6). The right hind limb and right forelimb generated slightly greater VGRF than they did before ACLT, although the differences were not statistically significant and were not apparent 6 weeks after ACLT.

No significant differences existed in the mean VGRF generated by the unstable limb of the saline-injected control group (41% \pm 7%), the therapeutic group (36% \pm 9%), or the prophylactic group (42% \pm 10%; Figure 7). After the second series of injections, 18 weeks after ACLT, VGRF generated by the unstable hind limb increased, relative to the value at 6 weeks, in all 3 groups (control group 55% \pm 9%, therapeutic group 58% \pm 22%, prophylactic group 63% \pm 11%), but no significant difference between the mean VGRF of the 3 treatment groups were observed.

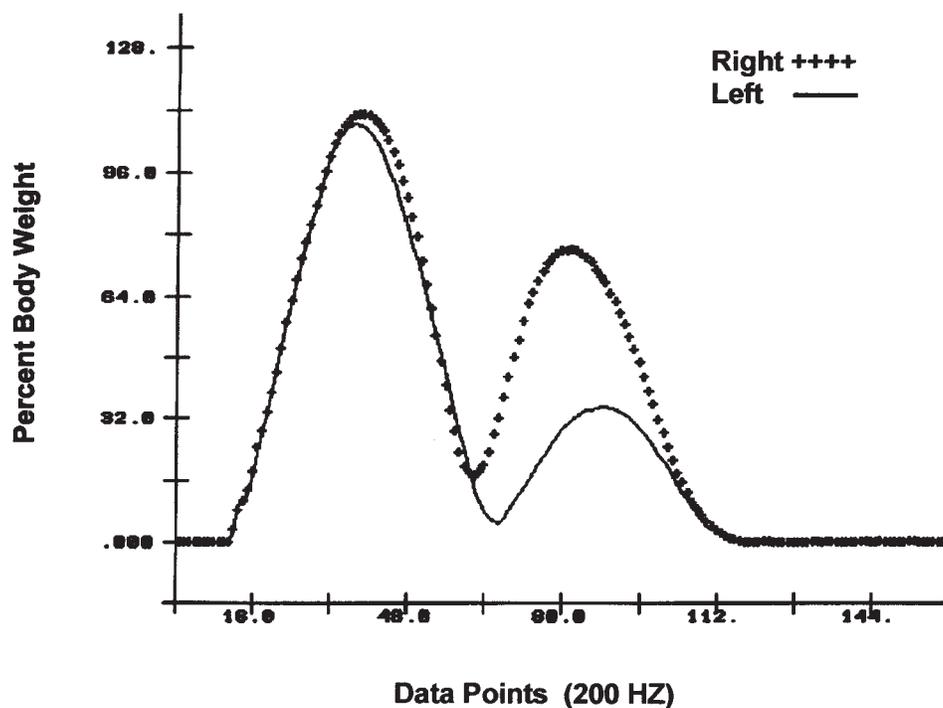


Figure 5. Typical record of VGRF generated by the forelimb and hind limb of a dog during a trot. The unstable (left) hind limb generates a lower VGRF than the contralateral stable hind limb.

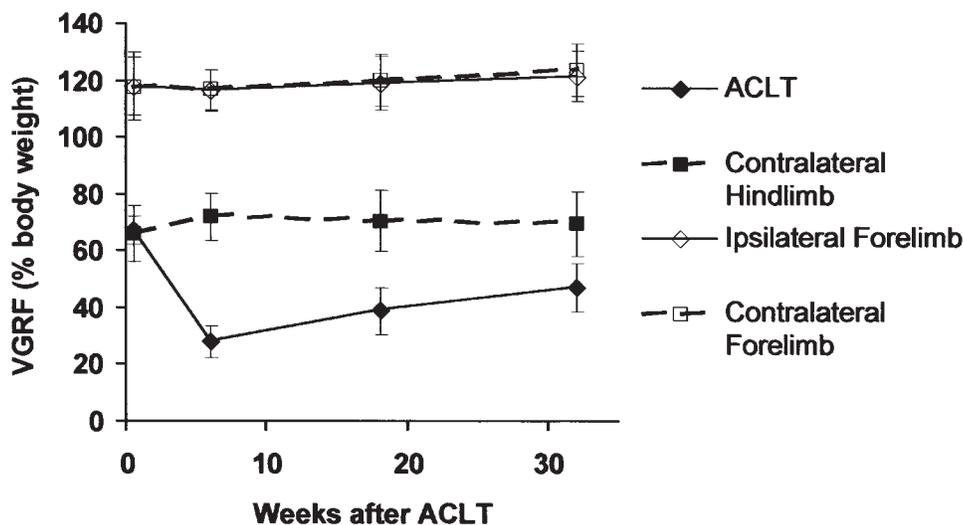


Figure 6. Effect of left ACLT on VGRF. The distribution of VGRF during a trot is altered after ACLT. Six weeks after surgery, the VGRF generated by the unstable limb (left hind limb) is reduced to about 40% of the baseline value. Loading of the left hind limb then increases with time and reaches approximately 70% of the baseline value 32 weeks after ACLT. The other 3 limbs generate slightly higher VGRF after the surgery, but the increases, relative to the baseline values, are not statistically significant.

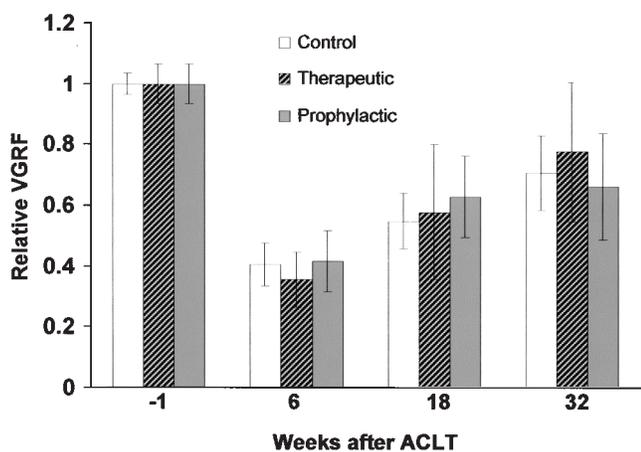


Figure 7. Relative VGRF generated by unstable limb after ACLT and IA injection of HA. IA injection of HA did not affect the relative VGRF (VGRF/baseline VGRF) generated by the unstable limb.

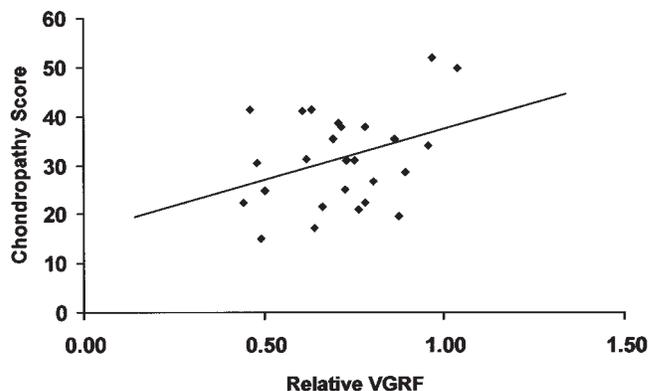


Figure 8. Relationship between VGRF generated by the unstable limb shortly prior to euthanasia and the extent of cartilage damage in the OA knee at euthanasia ($r = 0.35$, $p = 0.04$). The extent of joint damage increases with increased loading on the unstable knee.

Finally, 32 weeks after ACLT in all 3 treatment groups, the VGRF generated by the unstable knee was increased, relative to the value at 18 weeks, although the differences between groups were not significant (Figure 7).

Relationship between VGRF and chondropathy. All the cruciate-deficient knees subjected to ACLT developed OA after 32 weeks. The severity of OA, based on post mortem gross analysis of the damage to the tibia and femoral condyles, was correlated with the VGRF measured shortly before euthanasia ($r = 0.35$, $p = 0.04$; Figure 8).

DISCUSSION

In this study and in previous study from our laboratory⁴, intraarticular injection of HA did not affect the morphologic progression of OA. Although we have not found evidence that HA alters the rate of OA progression in the cruciate-deficient canine knee, others, working with different models and using a variety of outcome measures, have reported both positive and negative effects after IA injection of various HA preparations³. The discrepancies may be attributed to fundamental differences in the animal models employed in

these studies, such as differences in gait, and the properties of HA in normal joint fluid among species. HA injection in an ovine partial medial meniscectomy model of OA resulted in an increase in osteophytosis and slight increase in loading, with no effect on the progression of cartilage damage^{17,18}. Intraarticular HA injection into the rabbit knee after either ACLT or meniscectomy has been reported to have a positive effect on several parameters of OA progression, including the slowing of progression of structural damage and reduction in expression of matrix metalloproteinase (MMP-3) and interleukin 1 β (IL-1 β)¹⁹⁻²². However, in rabbits with less severe OA, IA injection of HA was reported to result in an increase of MMP-3 expression²⁰. A similar response was seen in bovine cartilage that had been depleted of PG by treatment with fibronectin fragments *in vitro*²³. Inclusion of HA in the medium resulted in an increase in MMP-3 expression and loss of PG during the first week in culture, but cartilage slices incubated in the presence of HA for 28 days restored the PG concentration to near-pretreatment levels. The authors suggested that HA treatment improved PG recovery by accelerating early PG loss and triggering regenerative pathways²³.

In our previous study, injection of HA into the knee for the 5 weeks immediately after ACLT resulted in 10%–30% depletion of cartilage PG 7 weeks later, i.e., 12 weeks after ACLT⁴. Because the compressive stiffness of articular cartilage is directly related to its PG concentration⁶, this result suggested that cartilage might become softer and therefore more susceptible to damage in OA joints injected with HA. This raised the concern that prophylactic injection of HA into the joint might accelerate the progression of OA if PG depletion persisted over an extended duration of joint instability. However, when we extended the duration of study to 32 weeks after ACLT, no acceleration of progression was noted. In addition, in our current study, loss of PG from the articular cartilage was not apparent at Week 32, i.e., 27 weeks (the prophylactic group) or 15 weeks (the therapeutic group) after the last HA injection.

One possible explanation for the absence of PG depletion in this study when we noted PG depletion in our previous study is that the chondrocytes resynthesized PG after the period of HA injection. The results from the dogs in the therapeutic group, which were injected with HA between Weeks 13 and 17 (Table 3), suggest this might be the case, since the PG concentration in articular cartilage of the weight-bearing area of the medial femoral condyles from those knees — which was analyzed 15 weeks after the last HA injection — was comparable to that in the contralateral knee cartilage. In contrast, the prophylactic group, which was analyzed 27 weeks after the last HA injection, and the control group, which was not injected with HA, exhibited a higher PG concentration in cartilage from the OA knee than in that from the contralateral knee, consistent with the phenomenon of hypertrophic repair in OA⁷.

Alternatively, PG depletion may not have occurred in this study because we used a different HA preparation than that used in the initial study, after the initial preparation was not approved for human use by the US Food and Drug Administration. The molecular weight of the HA in the present study ($0.5\text{--}0.73 \times 10^6$ Da) was only about half that used in our earlier study (1.5×10^6 Da). However, in a preliminary study of 2 dogs analyzed 12 weeks after ACLT, IA injection of Hyalgan[®], the HA preparation employed in this study, reduced the PG concentration in cartilage from the medial femoral condyle of the unstable knee by $18\% \pm 3\%$ relative to that of cartilage of the contralateral knee⁸. In contrast, we found previously that cartilage from saline-injected OA knees exhibited a 15%–30% increase in PG concentration, an indication of hypertrophic repair⁴.

In the present study, intraarticular HA injection did not slow OA progression as measured by morphologic changes to cartilage, synovium, or meniscus, or osteophytosis and had no effect on histopathologic changes of cartilage or synovium. Clearly, if intraarticular HA injection has an effect on the morphologic progression of cartilage damage in experimental canine OA, it is not robust, based on these results and our previous studies in the cruciate-deficient canine. We have not observed either slowing of OA progression or improvement in concentration or molecular weight of synovial fluid HA^{4,24}.

HA injection also had no apparent effect on the loading of the unstable limb in the present study. If the extent to which the animal loads the limb is a reflection of the pain associated with load-bearing, these data suggest that HA has little effect on the perception of pain in this animal model. Finally, the data suggest a correlation between the progression of OA pathology and the extent of loading in this model.

The primary effect demonstrated in clinical trials of intraarticular HA is reduction of joint pain. In studies of humans with medial compartment knee OA, a decrease in joint pain upon treatment with an analgesic/nonsteroidal antiinflammatory drug (NSAID) was accompanied by an increase in the adductor moment and net quadriceps moment, substantially increasing the load on the damaged compartment; cessation of analgesic/NSAID therapy was followed by an increase in knee pain and reduction in forces acting on the medial compartment²⁵. Studies of gait in the ovine meniscectomy model suggest that intraarticular HA injection increases loading of the affected joint, an effect that was associated with increased osteophytosis, reduced PG concentration, and reduced PG synthesis in the OA cartilage^{19,20}. These observations and our finding that the severity of joint pathology is correlated with loading of the OA knee suggest that relief of joint pain without concomitant inhibition of the pathologic changes of OA may accelerate disease progression.

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