has been postulated that RAR antagonists may prevent or reverse retinoid-mediated cartilage destruction and a RAR pan antagonist was previously shown to improve clinical scores in the collagen-induced arthritis (CIA) model, albeit with unacceptable adverse effects on testes. We have postulated that the primary beneficial joint effects of RAR antagonists are associated with RARgamma, while the adverse effects on testes are associated with RARalpha. Thus, we have identified a highly selective RARgamma antagonist (LY2813631) to test this hypothesis.

Methods: The RAR antagonist LY2813631 demonstrated in vitro selective affinity for RARgamma in a SPA-based binding assay using full length RAR alpha, beta and gamma protein and the synthetic pan agonist 3H-TTNPB. Functional antagonism and selectivity was demonstrated using a GAL4- RAR alpha, beta and gamma and Gal4 response element/Luciferase constructs, co-transfected into HEK 293 cells. Functional activity in chondrocytes was demonstrated using agonist TTNPB and primary bovine chondrocytes, looking at the ability of LY2813631 to regulate OA-relevant genes, such as MMP13, ADAMTS-5 and Type 2 Collagen. In vivo studies utilized Lewis rats to show reversal of RAR gamma agonist induced changes in OA relevant genes in the articular cartilage and reduction in OA-related neoepitopes in the synovial fluid. The collagen induced arthritis (CIA) model was used to determine joint efficacy in rats.

Results: In vitro, LY2813631 binds RARgamma (Ki = 0.74 nM) with significantly higher affinity than RARalpha (Ki = 400 nM) and RAR beta (Ki = 25 nM) and shows selective functional antagonism at RAR gamma (Kb = 42 nM) in HEK 293 cells, compared to RARalpha (Kb = 2010 nM) and RAR beta (Kb = 359 nM). Additionally, LY2813631 normalized RAR agonist-induced increases in the catabolic enzyme ADAMTS-5 in primary bovine chondrocytes. In vivo, a selective RARgamma agonist was shown to increase mRNA levels of ADAMTS-5 and decrease mRNA levels of type 2 collagen in the articular cartilage of rats after 3 days of oral dosing. The animals also showed a 5-fold increase in the type 2 collagen neoepitopes 9A4 and CTX-II in synovial fluid. These effects could be blocked by co-dosing RARgamma antagonist LY2813631, demonstrating specific RARgamma-mediated target engagement in the joint space. LY2813631 also improved ankle and knee histopathology scores in the rat CIA model at doses that did not cause testicular degeneration.

Conclusions: Our findings support a role for endogenous retinoids in the destruction of articular cartilage in OA and suggest that these effects are primarily mediated through RARgamma signaling. We have also shown that the beneficial effects of RARgamma antagonist LY2813631 can be demonstrated without concomitant adverse effects on the testes, supporting RARgamma as a potentially safe target for disease modification in arthritic diseases.

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IDENTIFICATION OF ENHANCERS OF BMP SIGNALING PATHWAY WITH CARTILAGE ANABOLIC PROPERTIES

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Purpose: Modulation of the BMP pathway remains an attractive target for development of novel anabolic treatments for osteoarthritis. We are aiming to identify chondro-specific small molecule enhancers of the BMP pathway. The ideal compound promotes the anabolic potential of the chondrocytes without pushing the cells into hypertrophy and does not induce/enhance TGFb-driven tumor formation/metastasis. We used a "chemical biology approach" whereby 25K compounds were screened in a BMP reporter cell-line for their enhancing activity. Next, we applied "phenotypic filter assays" to identify compounds with the desired profile. Like this, we take full advantage of the diversity available in the chemical library and maximize our chances to come up with innovative modulators of BMP pathway possessing tissue selective capacities and no/minor side effects.

Methods: 25K compounds were screened in a BMP reporter cell line for their BMP enhancing potential at 10μM (%PAC > 68%). Cytotoxic and general enhancers have been removed (NFkB counterscreen). Commercially available hit-analogues were ordered, evaluated in the BMP reporter (hit explosion) and in primary human adult articular chondrocytes in alginate bead culture system at 10μM to select promising series. The effect on aggrecan production was evaluated in the interterritorial matrix of the beads using a commercially available ELISA and gene expression profile was studied on the cell pellet using qRT-PCR (COL2, ACAN, SOX9, MIA, COL1, BGLAP). Medicinal chemistry efforts produced > 300 derivatives of promising series. Potency was evaluated as previously described and used to identify SAR of the series. In addition, tissue selectivity (osteogenic differentiation assay, TGFb-driven tumor formation in lung fibroblasts and breast cancer cell invasion model) and pathway specificity (TGFb reporter/NFkB (TNF) reporter) were performed to eliminate series/compounds showing unselective/aspecific side effects.

Results: From the 25K compounds, hits belonging to 12 chemical series were identified showing > 68% enhancement in the BMP reporter, showing no toxicity in hepG2 cells nor general transcriptional activation. Hit explosion confirmed the initial results and provided basic SAR. Using primary human chondrocytes the most promising series showed a concentration-dependent increase of aggrecan production; COL2, ACAN and MIA upregulation; no effect on hypertrophy-associated genes. The selected series are pathway specific (no TGFb enhancement - no TNF enhancement), tissue selective (no enhancement of osteogenic differentiation) and enhance/induce no TGFb-driven tumor formation/metastasis. Following hit to lead optimization, SAR has been refined and potency (EC50) already improved to sub-micromolar range.

Conclusions: Using this chemical biological approach we identified novel series showing pro-anabolic properties in the articular cartilage, tissue selectivity and pathway specificity, with no to minimal side effects (toxicity, TGFb-driven tumor formation/metastasis) and a clear structure activity relationship. Further in vitro and in vivo activity testing is ongoing and results are expected soon.

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ADJUDICATION PROCESS FOR JOINT EVENTS OF INTEREST IN FULRANUMAB CLINICAL TRIAL PROGRAM

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Purpose: Fulranumab, a novel human anti-nerve growth factor monoclonal antibody, has demonstrated efficacy compared with placebo in improving chronic pain and functional impairment in patients with knee or hip osteoarthritis (OA). Unexpected joint replacements and adverse events suspicious of rapidly progressive OA (RPOA) or osteonecrosis (ON), however, led the US Food and Drug Administration to place all ongoing fulranumab studies on clinical hold in December 2010. Accordingly, an independent Adjudication Committee (AC) was established by the sponsor to develop definitions of ON and RPOA consistent with the literature and the adjudicators' experience; review suspicious cases and render diagnoses based on blinded X-ray and clinical data; and attribute causality based on clinical data.

Methods: Adjudicators were selected for their expertise in ON, RPOA, rheumatology, orthopedic surgery, or radiology. For each case of joint replacement and adverse event possibly related to joint destruction, the AC agreed by majority vote on one of 5 predefined diagnoses: ON, RPOA, RPOA with a component of, or advancing to, ON (RPOA+ON), normal progression of OA (NPOA), or insufficient information (II). During case review, adjudicators were blinded to the patient's assignment to study drug and to previous radiologic diagnoses. Adjudicators assessed the case diagnoses based on structural evidence from available joint radiographs and clinical data. Case attributions were based on clinical evidence with the attributes of "definitely related", "probably related", "possibly related", and "not related".

Results: The adjudicators adopted the following definitions:

ON

Radiologic evidence:

- X-Ray: diagnosis of ON (based on: Cystic and sclerotic changes; collapse of the femoral head, femoral condyle, or tibial plateau;

change in the contour of the femoral head, femoral/tibial condyle, or tibial plateau, crescent sign)
- MRI: diagnosis of ON (based on: well-defined margin surrounding a focus of fat-like or fluid-like or low signal +/- surrounding edema, change in contour of the femoral head, femoral/tibial condyle, or tibial plateau)

Histopathologic evidence (if available):

- Evidence of ON at surgery histopathologic/gross specimen report. Histopathology must have corresponded to radiographic evidence.

RPOA
Radiologic evidence:

- Required availability of baseline comparison film(s)
- Initial radiograph: OA Kellgren-Lawrence (K-L) grade of 0 to 3 in the replaced joint
- Follow-up radiograph:
  - Focal joint space narrowing $\geq$ 50% or $\geq$ 2 mm per year
  - Flattening of the femoral head
  - Flattening of the femoral/tibial condyle and/or tibial plateau

Histopathologic evidence (if available):

- Histopathology consistent with totality of clinical and radiographic findings
- If ON present, evidence of focal ON only

RPOA sub-categorization

- Type I: Focal joint space narrowing $>$ 50% or $>$ 2 mm per year
- Type II: Rapid and aggressive destruction of bone, with or without subluxation of the joint

One hundred nine joints (94 patients, including 62 knees, 43 hips, 3 shoulders, and 1 lumbar spine) were adjudicated with the following results: 72 NPOA, 18 RPOA, 14 II, 0 ON, and 0 RPOA+ON. Five cases were considered not appropriate for review because of revisions of joint replacements or repair of lumbar device failure. Among the 18 RPOA, 7 (4 knees and 3 hips) were Type I and 11 (4 knees and 7 hips) were Type II. The adjudicators agreed unanimously on 81% of the cases. All 18 cases that had an original radiologic diagnosis of ON in the Clinical Centers were adjudicated by the AC as 9 RPOA, 8 NPOA, and 1 II. All 72 NPOA cases and 1 RPOA were assessed as "not related" to study drug, and 17 RPOA cases were assessed as "possibly related".
Conclusions: RPOA may occur in association with fulranumab use and is a safety signal. Among 109 cases reviewed, 18 were adjudicated as RPOA, all of which occurred in fulranumab-treated patients. ON was not identified as a safety signal in the fulranumab studies.

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A SELECTIVE RAR GAMMA ANTAGONIST RELIEVES OSTEOARTHRITIS LIKE KNEE PAIN IN THE RODENT

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Purpose: Osteoarthritis (OA) is a chronic debilitating joint disease with resulting pain and cartilage degradation in the affected joint. It is known that levels of retinoids are increased in both plasma and synovial fluid of patients with OA. We therefore hypothesize that retinoic acid receptor gamma (RARγ) antagonists may be beneficial in the treatment of OA pain. As antagonism of the RARα receptor has been linked to testicular toxicity we chose to target RARγ specific antagonism as there was no reported toxicity associated with this receptor. We confirmed that an RARγ agonist can induce knee pain when given intra-articularly. Also a selective RARγ agonist when co-treated reversed the pain efficacy of the selective antagonist, indicating the critical role for RARγ in mediating pain efficacy. Accordingly, we identified a highly selective RARγ antagonist (LY2813631) and tested its affect on pain in three models of OA- like knee pain, the rat monoiodoacetate (MIA) model, the nonsteroidal anti-inflammatory drug (NSAID) non-responding MIA model and the rat meniscal tear (MT) model.
Methods: In the rat MIA model injection of MIA (0.3mg) into the right knee joint produces an acute inflammatory insult which develops into chronic degeneration of the joint tissues. In contrast, in the MT model rapidly progressing degenerative changes in the joint, including cartilage fibrillation and erosion and osteophyte formation occur when the medial collateral ligament and meniscus of the right knee are transected. The pain resulting from the joint injury in both models can be measured via differential weight bearing of the hind legs using an incapacitance tester. To test whether RARγ antagonist (LY2813631) could reduce pain in these models compound was dosed after pain induction and differential weight bearing measured. This was done as a dose response in the standard MIA model 12 days post MIA injection. In the MT model LY2813631 was assessed for pain efficacy at a single dose 13 and 17 days post MT surgery. LY2813631 was also tested in a version of the MIA model that does not show pain relief with non steroidal anti-inflammatory drugs (NSAIDs) at 43 days post MIA (1mg) injection.
Results: **MIA model-** At 12 days post MIA injection RARγ antagonist (LY2813631) significantly inhibited pain at doses of 0.3, 1, 3 and 10mg/kg test (*$p<0.05$ table 1)

Table 1
MIA Pain Measurements (Saline-MIA) (g)

Treatment	Day9
Vehicle	21.94±0.55
0.1mg/kg LY2813631	21.14±0.72
0.3mg/kg LY2813631	18.29±0.25*
1mg/kg LY2813631	16.87±0.75*
3mg/kg LY2813631	13.78±0.69*
0mg/kg LY2813631	12.01±0.51*

Mean±SEM n=5

NSAID non-responding MIA model- At 43 day post MIA injection RARγ antagonist (LY2813631) significantly inhibited pain in the NSAID non-responding MIA model (*$p<0.05$ table 2). As expected the NSAID diclofenac did not show efficacy in this model.

Table 2
NSAID non-responding MIA Pain Measurements (Saline-MIA) (g)

Treatment	Day 43
Vehicle	52.08±1.14
5mg/kg diclofenac (NSAID)	52±1.17
1mg/kg LY2813631	45.64±0.88*
3mg/kg LY2813631	44.64±1.57*

Mean±SEM n=6

MT model- At 13 and 17 days post MIA injection RARγ antagonist (LY2813631) significantly inhibited pain (*$p<0.05$ table 3)

Table 3
MT Pain Measurements (Contra-lateral non surgical-surgical)(g)

Treatment	13 days	17 days
Vehicle	48.69±0.85	47.4±0.9
10mg/kg LY2813631	43.76±0.76*	38.14±0.94*

Mean±SEM n=6

Conclusions: RARγ antagonist (LY2813631) was effective at reducing in three different models of osteoarthritic like knee pain: the MIA model, the NSAID non-responding MIA model, and the MT model. Therefore such a selective agent could provide therapeutic benefit when tested in clinical studies without some of the obvious deleterious effects of a non specific retinoid acid receptor antagonist.