

Therapeutic Effects of Ribunucleinate (Ribonucleotides) in Immuno-Inflammatory and Arthritic Diseases

G. Stommel, S. Schuehlein, K.-H. Schuehlein and K.D. Rainsford

Abstract Ribonucleic acids from different organs and from yeast have been used for the treatment of chronic and degenerative diseases in the context of naturopathic medicine in the last 60 years. This chapter provides general information about ribonucleinates as therapeutic agents. Past and present pharmacological and clinical investigations are discussed in the field of the central nervous system, sensory organs, cancer and degenerative diseases of joints and vertebra.

Keywords Osteoarthritis · Ribonucleotides · Cartilage · Bone · Synovia · Proteoglycans · Micro-RNA · Silencing RNA

1 Introduction

It is now well established that diseases affecting the nervous, immune and articular systems are among the most serious and debilitating conditions affecting human populations worldwide. The impact of these chronic conditions both on afflicted patients, the economies and health care systems is by far the most demanding of all human conditions. At present, the prevention and treatment of these conditions are based on a wide range of therapies and surgical procedures all of which are costly and in some cases only partially alleviate or ameliorate the various conditions. The possibility that a single group of therapeutic substances might be effective in treating such a wide range of conditions has some appeal. The reality is that this may not always prove to be the case in the long term.

G. Stommel (✉) · S. Schuehlein · K.-H. Schuehlein
Dyckerhoff Pharma GmbH & Co. KG, Robert-Perthel-Straße 49,
50739 Cologne, Germany
e-mail: g.stommel@dyckerhoff-pharma.de

K.D. Rainsford
Biomedical Research Centre, Sheffield Hallam University,
Howard Street, Sheffield S1 1WB, UK

The concept that ribonucleic acids from different organs might regulate the regeneration of these organs was developed over 60 years ago by Professor Dr. Hanns Dyckerhoff and further commercialised by a company he formed (Dyckerhoff Pharma GmbH & Co. KG, Köln, Germany) (Becker et al. 1995). In essence, the concept he formulated was that ribonucleic acids (RNAs) are the key regulator of regeneration which is necessary to maintain healthy tissue and prevent disease (Becker et al. 1995). The thesis about RNA involvement in regeneration is that after the age of 40 the body lacks sufficient biologically active RNA. The RNA derived from specific organs is used therapeutically to enhance the production of proteins in the organs which should benefit from the treatment.

1.1 Development of RNA Regenerative Therapy

Decades ago, ribonucleic acid was expected to have modulating effects in the cellular metabolism especially the protein biosynthesis with high potential in regenerating cellular metabolism in degenerating tissues. RNA from different sources was tested in biological studies about the influence on the synthesis of RNA (Kanehisa et al. 1977; Grabowska et al. 1981; Liu et al. 1981; Novakova et al. 1979), the synthesis of DNA (Beljanski and Plawecki 1979; Plawecki and Belianski 1981; Lodemann et al. 1989) and the protein biosynthesis (Amos and Moore 1963; Malpoix 1964, 1967; Rollins et al. 1966; Bogdanovsky et al. 1973; Kelly et al. 1983).

1.1.1 Pharmacodynamics

Early research was done in the field of RNA effects in the brain showing improved brain function and learning tested in different animal species (Hydén and Pigon 1960; Hydén and Egyhazi 1963; Cook and Davidson 1963; Solyom et al. 1967; Guyette et al. 1980; Rosenzweig 1984; Davis and Squire 1984; Högger 1999).

A variety of modulating effects of RNA on the immune response were shown including interferon induction (Aksenov et al. 1970; Taborsky and Dolnik 1977; Wacker et al. 1981; Lacour et al. 1984; Sula and Nouza 1984; Lodemann et al. 1989), virus inhibition (Gifford 1965; Stebbing et al. 1977; Stebbing and Lindley 1980; Zemskov 1977; Iliescu et al. 1983; Repanovici et al. 1983; Nosik et al. 1984; Ignat'ev et al. 1988), stimulation of macrophages and propagation of plasma cells (Merritt and Johnson 1965; Engibarman 1977; Stebbing et al. 1980; Zemskov 1980; Razvorotnev et al. 1987; Ikeda et al. 1994), increase in colony stimulating cells of the hematopoietic system (Semina et al. 1976), increase in host versus graft immune-tolerance (Ashley et al. 1960; Jolley et al. 1961; Largiadèr et al. 1968; Groth et al. 1968), anti-Inflammatory effects (Davis et al. 1981, 1985) and the inhibition of cancer (DeCarvalho and Rand 1961; Esposito 1964; Matienko et al. 1971; Demin 1973; Beljaew et al. 1974).

Hormone-like effects of RNA preparations have been tested for RNA extracts including those from adrenal glands and testis (Vilee 1967), thyroid gland and liver (Mu 1973), uteri, kidney, lungs, skeletal muscle, thymus and liver (Mansour 1968;

Fencel and Vilee 1971) and from seminal vesicle, ovary, prostate and liver (Fujii and Vilee 1969; Niu et al. 1973). In these studies, RNA preparations from different tissues showed organ-specific effects.

Regenerative effects were found in the healing of bones and wounds (Williamson and Guschlbauer 1961a, b, 1963; Belous and Pankow 1966, 1969; Babiichuk et al. 1969; Klyuewa et al. 1977; Semochkin et al. 1999, 2001; Bekman et al. 2001), in the regeneration of nerves (Batkin 1966; Razumova 1970; Vichikova 1982) and of heart, liver, pancreas and bone marrow (Wool et al. 1968; Chernukh et al. 1970, 1971; Breslavskii et al. 1978; Skuba and Levkova 1980; Beljanski et al. 1983). RNA extracts induce regeneration after radiation damages (Sugahara et al. 1966; Ebel et al. 1969; Vladimirov et al. 1985). There is an indication for RNA eliciting differentiation in embryonic myocardium cells (Deshpande et al. 1977; Deshpande and Siddiqui 1978; McLean et al. 1977).

1.1.2 Pharmacokinetics

Cellular uptake of oligonucleotides has been shown to occur by receptor-mediated endocytosis and by unspecific non-receptor-mediated mechanism (Bennet et al. 1988, 1991; Yakubov et al. 1989; Loke et al. 1989; Rieber et al. 1989; de Smidt et al. 1991; Barry et al. 1993; Geselowitz and Neckers 1992; Iversen et al. 1992; Krieg et al. 1991, 1993; Vlassov et al. 1993; Saijo et al. 1994; Wu-Pong et al. 1994; Zamecnik et al. 1994; Giles et al. 1995). Oligonucleotides can be found in the nucleus and in the cytoplasm of incubated cells (Yakubov et al. 1989; Chin et al. 1990; Hawley and Gibson 1992; Barry et al. 1993; Gao et al. 1993; Wu-Pong et al. 1994; Giles et al. 1995). After intravenous, intraperitoneal, intradermal, oral or mucosal administration of oligonucleotides, these are distributed throughout the body (Bazanov et al. 1991; Vlassov et al. 1993; Cossum et al. 1994; Crooke et al. 1994; Sands et al. 1994; Saijo et al. 1994; Agrawal et al. 1995; Zhang et al. 1995). Oligonucleotides are metabolised in cells and serum and excreted mainly in the urine (Crooke et al. 1994; Galbraith et al. 1994; Agrawal et al. 1995). Toxicological studies showed no evidence of acute or chronic toxic effect, teratogenic effects or mutagenicity (Goossens and Gastpar 1960; Caujolle 1966; Lapik and Matienko 1970; Bormann and Reyher-Pauly 1972; Leuschner 1974a, b, c, d, 1975, 1984, 1988). No cancerogenic effect of oligonucleotides could be found in RNA extracts from healthy tissues (Niu et al. 1961; Esposito 1964; Demin 1973; Beljaew et al. 1974; Svirnovskii et al. 1974; McLean et al. 1977).

1.2 Modern Concepts of RNA Regenerative Therapies

1.2.1 Micro-RNA/Silencing RNA

Current research on micro-RNA/silencing RNA shows that there is highly specific effects of these small RNA chains in cardiovascular disease (Kataoka and Wang 2014), disorders of the immune system and inflammatory diseases

(Tomakova et al. 2011) and osteoarthritis (Stanczyk et al. 2008, 2011; Miyaki et al. 2009; Nakamachi et al. 2009; Tardif et al. 2009; Abouheif et al. 2010; Niimoto et al. 2010; Kawano and Nakamachi 2011; Kurowska-Stolarska et al. 2011; Li et al. 2011, 2012a, b; Nakasa et al. 2011; Tew et al. 2011; Yu et al. 2011; Dai et al. 2012; Díaz-Prado et al. 2012; Dong et al. 2012; Goldring and Marcu 2012; Liang et al. 2012; Martinez-Sanchez et al. 2012; Steck et al. 2012; Swingle et al. 2012; Ukai et al. 2012; Yamasaki et al. 2012; Akhtar and Haqqi 2012; Le et al. 2013; Matsukawa et al. 2013; Song et al. 2013; Trenkmann et al. 2013; Wang et al. 2013). Synthetic micro-RNA for medicinal use is of rapidly expanding relevance (Gibson 2014).

1.2.2 Natural RNA Extracts Containing Micro-RNA

RNA regenerating therapy is based on pharmacological data described above. This may be related to the actions of the components of Regeneresen[®] comprising the RNA extracts from different organs and from yeasts. Recent analysis of Regeneresen[®] RNA extracts shows the complex composition of these natural organ extracts. Thus, the chain length of nucleotides (nt) has been found to be below 500 nt when extracts were tested using an Agilent Bioanalyzer (Fig. 1). This analysis shows that the majority of small RNA chains comprise transfer-RNA or smaller RNA types. Micro-RNA was analysed by RNA hybridisation in Regeneresen[®] RNA extracts which showed the presence of more than 100 micro-RNA types compared with those found in known sequences from different mammalian species

Fig. 1 BioanalyzerData (Exiqon, Denmark) of organ extracts from placenta (average of 4 batches), synovia (average of 3 batches) and from yeast extract (average of 2 batches). These extracts are the active ingredients of Regeneresen[®]. Standard nucleotides (nt) with chain length from 25 to 6000 nt were used as markers

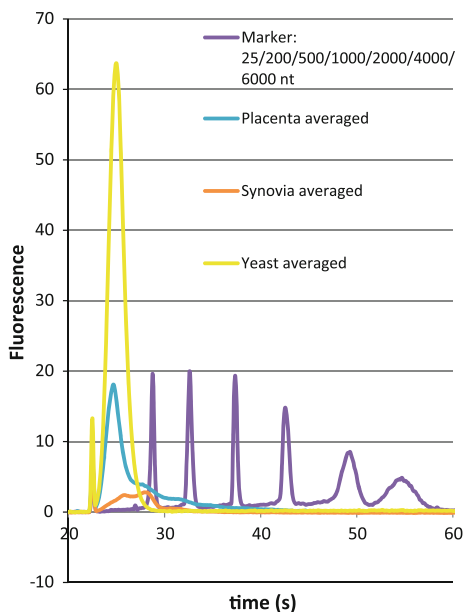
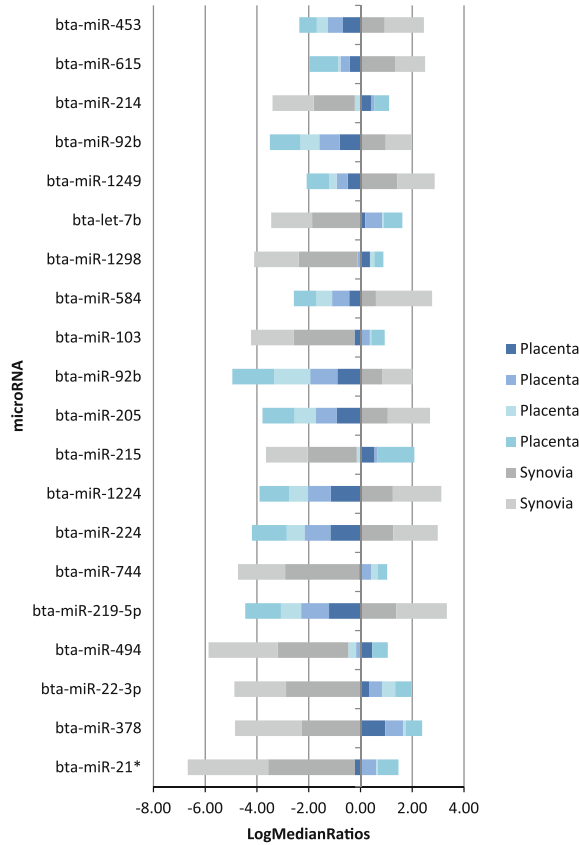


Fig. 2 Micro-RNA hybridisation analysis (miRCURY™ LNA Array, Exiqon, Denmark) of RNA extracts from synovia (2 batches) and from placenta (4 batches). 20 micro-RNAs have been sorted on basis of the standard deviation values with the highest differential expression on top. The numbers are log2(Hy3/Hy5) ratios



shown in standard databases. These hybridisation assays showed differences in the spectrum of micro-RNA for different organ RNA extracts (Fig. 2). Micro-RNA might contribute to organ-specific effects of natural RNA extracts found in pre-clinical tests.

1.3 Clinical Relevance of RNA Regenerative Therapies

There is evidence that RNA extracts of different sources and even synthetic “non-sense” RNA are of clinical relevance as well. RNA extracts from yeast or liver enhanced the recovery from hepatitis and fatty liver with long-term benefits (Levina et al. 1975; Frolov and Razenkova 1980; Rychnev et al. 1982; Hou et al. 1988). In patients with diabetes mellitus, the clinical chemistry of parameters of the pentose phosphate cycle including the activity of ATP-ase, 3-nucleotidase and transketolase increased, while the purine nucleotide concentrations were improved (Karabun and

Yefimov 1975). In patients with *Tapetoretinal dystrophia* or *Retinitis pigmentosa*, treatment with yeast RNA improved the visual field and acuity as well as the dark adaptation (Fuks et al. 1969, 1971; Shershevskaya et al. 1971; Shershevskaya and Levina 1978; Trutneva et al. 1972). Yeast RNA extract reduced dyspnoea and asthma in chronic obstructive lung disease (Zemskov et al. 1979; Silvestrov et al. 1981). Improved wound healing has been found after surgical intervention in *Otitis media epitympanic* after treatment with homologous bone RNA (Filatov et al. 1977). Phage double-stranded RNA accelerated the recovery of Herpes simplex infections and improved symptoms of *Herpes genitalis* or other virus-related dysplasia (Borecky et al. 1978; Gasparyan et al. 1991; Cheknev et al. 1994). Adjuvant treatment with synthetic “non-sense” polyadenylic–polyuridylic acid increased tumour-free intervals and the lifespan of patients with breast cancer in 8 years of observation (Lacour et al. 1980, 1984, 1988). A synthetic “non-sense” dsRNA Poly (I)-poly(CU) improved symptoms of lethargy and fatigue in patients with Chronic Fatigue Syndrome (Strayer et al. 1994). In patients with age-related dementia or memory loss due to other diseases of the central nervous system, yeast RNA improved memory and vigilance (Cameron and Solyom 1961; Cameron et al. 1963; Kral et al. 1967). Clinical experience and trials with Regeneresen® RNA Extracts are detailed below.

2 Regeneresen®

2.1 General Properties

Regeneresen® is a trade name for sodium salt-ribonucleic acid extracts (RNA) preparations from about 50 bovine organs and from yeast as mixtures of specific amounts of organ RNA and yeast RNA. One type of this is RN 13 Regeneresen which comprises a specific mixture of RNA components from adrenal cortex, cerebral cortex, heart, hypothalamus, kidney, liver, ovary, pituitary gland, placenta, spleen, testes, thalamus, vessel wall and yeast. RN13 has been employed for treating patients with geriatric conditions, age-related endocrine involution, general manifestations of ageing, immune deficiencies and for improving muscular strength. Another type of Regeneresen, known as AU4 Regeneresen comprises RNA derived from the auditory system, has been used in patients with presbycusis, degenerative diseases or toxic injury to the internal ear, sudden deafness and tinnitus. Osteochondrin® is a mixture from connective tissues as described in the subsequent section.

Regeneresen®/AU 4/Osteochondrin® is administered by intramuscular injection, intravenous infusion, intraperitoneally, orally, or applied directly to the mucous membranes or the skin. While intramuscular injection has been the standard method of application over decades, topical application has been proven effective to some extent (Vlassov et al. 1993).

The dosage of Regeneresen[®] is 6 mg RNA per 5 ml ampoule with recommended daily injection of 2 ampoules. The weekly dosage is 4–12 ampoules with a total of 12–18 ampoules per treatment. There is also some clinical experience with intravenous infusion (Westphal 1997) as well. Since uric acid is a metabolite of RNA and phenylalanine was used as excipient patients with manifest gout, phenylketonuria could not be treated with Regeneresen[®]. Hypersensitivity reactions manifest in the form of itching, and exanthema occurs infrequently at a rate of less than 1:10,000 applications. In these very rare cases, the treatment was discontinued.

2.2 Clinical Observations

Clinical studies with Regeneresen[®] have been conducted in patients with diseases of the central nervous system, sensory organs, cerebral insufficiency and cancer.

Diseases of the auditory system have been the subject of several studies with adjuvant treatment with Regeneresen[®] compared to standard therapy alone or with placebo.

A randomised study with standard therapy and Regeneresen[®] containing RNA derived from auditory system (AU4 Regeneresen[®]), vessel wall, placenta and yeast compared with standard treatment alone (low-molecular dextran; naftidrofurylhydrogenoxalate; Vitamin B; saluretic medication) was performed in 50 patients in 4 study groups with Ménière's disease (2×10 patients), sudden deafness (onset recently 2×5 and onset formerly 2×5 patients) and old acoustic trauma (2×5 patients). Of these, 25 patients received standard therapy alone and 25 additional Regeneresen (120 mg RNA) injections over 3 weeks (Pilgramm and Schumann 1985). Relative changes in hearing derived from audiometric data averaged from 0.25/0.5/1/2/3/4/5/6 to 8 kHz showed no significant differences between standard treatment and adjuvant Regeneresen treatment. Dizziness was reported by 20 patients in the group with Ménière's disease before treatment. After treatment with standard therapy, 6 patients reported improvement of dizziness and 5 patients in the Regeneresen group. A complete relief of Tinnitus was found in the Ménière's disease group (standard treatment 33 % vs. Regeneresen 62 %), in the group with sudden deafness with recent onset (standard treatment 60 % vs. Regeneresen 100 %), in the group with sudden deafness and formerly onset (standard treatment 40 % vs. Regeneresen 80 %) and in the group with old acoustic trauma (standard treatment 0 % vs. Regeneresen 20 %) showing a benefit due to adjuvant Regeneresen treatment in all study groups. A total of 3 patients showed mild inflammation at the injection site of Regeneresen, and one patient interrupted the treatment after breaking out into sweat and sensation of heat.

AU 4 Regeneresen[®] containing RNA from the auditory system and yeast (36 mg RNA) was compared with placebo (glucose) in a controlled study with 20 patients after acoustic trauma (Pilgramm and Schumann 1986). All patients received standard treatment with dextran40. Hearing loss was evaluated by audiometry, and tinnitus shown a proportion of patients with full relief of symptoms. Hearing gain

was 24.6 % after 10 days and 25.2 % after 42 days in the Regeneresen[®] group and 24.1 % after 10 days and 24.2 % after 42 days in the placebo group. Tinnitus revealed in 60 % of patients after 10 days and in 70 % of patients after 42 days in the Regeneresen[®] group and in 70 % after 10 days and 60 % after 42 days in the placebo group. These changes were not significantly different between placebo and Regeneresen[®] group. The treatment was well tolerated.

RNAs derived from auditory system (AU4 Regeneresen[®]), vessel wall placenta and yeast were tested in a randomised double-blinded study with 2×20 patients with tinnitus after acoustic trauma or sudden deafness (Gottwik 1989). All patients received standard therapy with pentoxifylline and dextran in addition to Regeneresen[®] (120 mg RNA) or placebo (containing the excipients from Regeneresen[®] and low-dose riboflavine). Placebo and verum groups were divided each into 2 subgroups with recent onset of tinnitus or longer existing tinnitus. Hearing loss was evaluated by audiometry and rated (0 = no symptom; $1 < -20$ dB, $2 < -40$ dB, $3 < -50$ dB). Tinnitus was rated by patients (full relief, improved, unchanged, worse). Improvement in tinnitus was seen after 3 weeks of treatment with Regeneresen[®] (Regeneresen: 50 % of patients improved, placebo 40 % of patients improved). Hearing gain (2 before treatment, 1.3 after 3 weeks; $p < 0.05$) was seen in the subgroup with recent onset of tinnitus (mean 0, 81 years). No significant hearing gain was seen in other subgroups. The treatment was well tolerated.

A placebo-controlled double-blinded study with RN 13 Regeneresen[®] was executed in 60 patients with cerebral insufficiency mainly due to degeneration (Held et al. 1989). RN 13 (RNA from adrenal cortex, cerebral cortex, heart, hypothalamus, kidney, liver, ovary, pituitary gland, placenta, spleen, testes, thalamus, vessel wall and yeast) and placebo (low-dose Vitamin B2) were injected intramuscular over 3 weeks. The dosage of RN 13 was 3×12 mg per week. There was no significant difference in effects of RN 13 (SCAG: -6.1 %) compared to placebo (SCAG: -5.2 %) in the Clinical Assessment Geriatric Scale (SCAG) as well as in the tolerance of the treatments. Mild local inflammation at the injection site was seen in 6 of 30 patients in the placebo group and in 7 of 30 patients in the RN 13 group.

However, a subgroup of 24 patients (14 RN 13, 10 placebo) with severe clinical signs (SCAG at least 75) showed significant effects for RN 13 in SCAG (RN 13: -10.9 %, placebo: -3.5 %; $p < 0.039$) after 3 weeks. There was some evidence of effects after week 1 (RN 13: -8.1 %, placebo: -2.2 %; $p < 0.055$), but this tapered off after injections ceased; after week 5 (RN 13: -11.0 %, placebo: -4.3 %; $p < 0.093$) and week 8 (RN 13: -10.4 %, placebo: -6.6 %; $p < 0.68$), a trend to better results with RN 13 was found.

A total of 13 patients with Parkinson's disease were treated with 132 mg RNA from cerebral tissues as adjuvant treatment over a period of 12 days (Fornadi 1993). In this pilot study, all patients received L-Dopa and at least one further medication for this disease and physical therapy. Mini-Mental-State Scale, ZUNG Depression Scale and CURS (Columbia University Rating Scale) were evaluated.

After 5 weeks, the CURS rating was reduced from 19.3 to 11.4. The other parameters were unchanged. The treatment was well tolerated.

The adjuvant treatment with RN 13 Regeneresen and RNA from bone marrow was tested in an open-controlled randomised study with 45 patients with breast cancer (Tchaika et al. 1999). All patients underwent surgery and chemotherapy (cyclophosphamide, methotrexate, fluorouracil; 4 cycles with 3 months time interrupt). Regeneresen (72 mg RNA) was tested versus standard adjuvant treatment (Hämodes, Aerosyl, Cerucal, Navoban). Patients were divided into three groups with 15 patients each receiving standard, adjuvant treatment, RN 13 Regeneresen[®] and Regeneresen[®] bone marrow. Primary endpoints were leukocyte and thrombocyte count. In patients treated with Regeneresen[®], bone marrow leukocytes recovered in mean from 3.3 Gpt/l before to 4.7 Gpt/l after 10 days of treatment. Thrombocytes recovered in mean from 145.4 Gpt/l before to 220.7 Gpt/l after treatment. In patients treated with RNA, 13 leukocytes recovered in mean from 3.1 Gpt/l before to 4.7 Gpt/l after 10 days of treatment. Thrombocytes recovered in mean from 168.1 Gpt/l before to 233.4 Gpt/l after treatment. In patients treated with standard therapy, leukocytes recovered in mean from 3.1 Gpt/l before to 4.2 Gpt/l after 10 days of treatment. Thrombocytes recovered in mean from 159.8 Gpt/l before to 202.4 Gpt/l after treatment differences were not statistically significant. One patient treated with Regeneresen bone marrow showed allergic exanthema after one injection. The consecutive treatment was well tolerated, and the patient finished the treatment accordingly.

3 Osteochondrin[®]

3.1 General Properties

Osteochondrin[®] is a trade name for a special mixture of Regeneresen[®] containing sodium salt-ribonucleic acid extracts (RNA) preparations from cartilage, intervertebral disc, synovia, placenta and yeast. It has been used for treatment of patients with osteochondrosis, osteoporosis, osteoarthritis, spondylosis and brachialgia.

3.1.1 Current Therapies for Osteoarthritis

Osteoarthritis (OA) affects over 90 % of the western population with increase in frequency and severity with age (Buchanan et al. 2003). Recent studies have given much information on the type and pattern of inflammatory changes that accompany this disease (Pelletier et al. 2001). The treatment of OA is conventionally symptomatic and relies on drugs and/or physical therapies to relieve the symptoms of pain, swelling, stiffness and immobility (Altmann 1991; Buchanan and Kean 2002a). Joint pain is the most significant clinical parameter for the patient

(Buchanan and Kean 2002a), but the precise origins of this are not clear (Creamer et al. 1998).

There are no therapies currently available that can either arrest or reverse the disease (Wieland et al. 2005) although there are some new developments which are encouraging (Wu and Kalunian 2005). In severe OA, surgery is the only realistic option where there is unremitting pain, immobility or instability of the affected joint (Brandt and Flusser 1991; Buchanan and Kean 2002a, b, c; Buchanan et al. 2003). While considerable advances have been made in surgical techniques since the development of the “Charnley” prosthetic hip nearly half a century ago and with advances in biomaterials, conventional surgery is still a procedure of last resort, which has considerable costs, some risks of failure and in some cases limited benefit within the scope of the lifespan of the individual (Brandt and Flusser 1991; Buchanan and Kean 2002a, b, c; Buchanan et al. 2003). Stem cell therapy, while attractive as a means of reversing joint damage, is still only experimental (Baker and Ferguson 2005). Most patients with OA will rely on therapy (self- or doctor-prescribed) with drugs comprising non-steroidal anti-inflammatory drugs (NSAIDs), non-narcotics (e.g. paracetamol, dipyrrone), narcotics and a range of herbal or natural products to relieve symptoms of pain and joint inflammation (Buchanan and Kean 2002a). While having some benefit, these natural therapies have variable responses and the risks of developing adverse reactions, despite relative safety, are sometimes limitations to their applications. More significant is that despite claims for “chondro”- or cartilage protection with some NSAIDs (Rainsford 1996, 1999), and these claims are clinically unproven. With a few NSAIDs (e.g. aspirin, indomethacin), there is evidence that they may even accelerate cartilage destruction either as a consequence of over-use from analgesia or biochemical effects (e.g. impaired connective tissue metabolism).

3.1.2 Natural Products and Derivatives

In the past decades, much interest has been shown in therapy with oral glucosamine sulphate and/or chondroitin sulphate (Pavelka et al. 2002; Brenner et al. 2004; Bruyere et al. 2004; Reginster et al. 2001; Richy et al. 2003), and intra-articular hyaluronic acid (Hyal[®], hyaluronan) (Leopold et al. 2003; Caborn et al. 2004; Kotevoglu et al. 2006; Neustadt et al. 2005; Ozturk et al. 2006; Raynauld et al. 2002, 2005) and intramuscular injection of galactosamino–glycuronylglycan sulphate (Rovetta 1991; Chevallard et al. 1993; Baker and Ferguson 2005; Moskowitz and Hooper 2005; Goldberg and Buckwalter 2005) as treatments for control of joint destruction as well as achieving relief of pain and inflammation. The biochemical rationale for the case of glucosamine/chondroitin sulphate, while not proven, rests on claims for inhibiting pro-inflammatory cytokine-mediated cartilage destruction as well as stimulating proteoglycan synthesis (by mechanisms that are not clear but could depend on provision of substrates). There are claims for analgesic and anti-inflammatory efficacies, and these effects are mild to moderate. There is also little or no substantial clinical benefit proven with chondroitin/glucosamine sulphate

preparations in *reversing joint damage per se*, although there may be some protection of cartilage. Similar conclusions can be drawn with the use of hyaluronic acid preparations (Buchanan and Kean 2002a; Brandt and Mazzuca 2005). Here, the utility and applications, like those of intra-articular corticosteroids (Bellamy et al. 2005a, b; Raynauld et al. 2005), are limited because of the need for injection into joints with associated risks of operative injury or side effects from the therapy.

Antioxidants have been proposed as another means for preventing or controlling cartilage destruction in OA. For some preparations, there is evidence for these to inhibit pro-inflammatory cytokine-mediated connective tissue degradation, but there is little clinical evidence that this is prevented with these agents Baker and Ferguson 2005; Moskowitz and Hooper 2005).

The central issue with the applications of all these agents is that there is no proven reversal of damage to both bone (notably subchondral bone) as well as cartilage in the joints of patients with OA. Here, some natural product extracts derived from connective tissues (e.g. Rumalon[®], a cartilage-bone marrow-placental extract; Katona 1987; Pavelka et al. 2000) or polysulphated derivatives of glucosamine (e.g. Arteperon[®]; Arck 1982; Ghosh et al. 1992; Pavelka et al. 2000) have been tried, based on some experimental evidence for their effects in “controlling” joint destruction in animal models of joint injury. Concerns about unspecific immune reactions with Rumalon[®] (Ghosh et al. 1992) and liver toxicity from Arteperon[®] lead to their withdrawal or reduced interest (Rainsford 1996). Sterile abscesses in joints, and other adverse reactions have been reported in patients who have received these preparations (Schadelin et al. 1981; Berg et al. 1992) raising issues about the safety of these glycosaminoglycan products.

3.1.3 Osteochondrin

Osteochondrin is unique natural product derived from connective tissue sources but it is different from the above mentioned (which are glycoproteins or glycosaminoglycans; GAGs), where it is a ribonucleic acid/ribonucleotide extract product (RNP) derived from connective tissues and yeast (Schroeder et al. 1989; von Sulecki 1990; Rainsford 1996). The rationale for this (as well as a range of RNP's (including Regeneresen[®]) is that these stimulate regenerative processes, e.g. in bone and cartilage in an attempt to reverse or control the joint degenerative processes. There is evidence to support accelerated repair of experimentally induced fracture injury (Babayan et al. 1979; Bethge et al. 1979; Lodemann et al. 1989) and stimulation of joint GAGs and collagen production in regenerating bone of rats (Babiichuk et al. 1969) and other healing processes (Belous 1971). Osteochondrin/Regeneresen[®] products have been employed as medicinal products in some European and some other countries for several decades for relieving degenerative conditions in joints, the central nervous system (CNS) and other organs (Lodemann et al. 1989; Schroeder et al. 1989; von Sulecki 1990).

The basis for the actions of Osteochondrin in OA may be that it has combined actions on immuno-inflammatory reactions and connective tissue metabolism.

OA is accompanied by a variety of local immunological reactions with well-defined T-cell responses (Schlaak et al. 1995; Liossis and Tsokos 1998; Nakamura et al. 1999; Sakkas and Platsoucas 2002; Sakkas et al. 2004; Sturmer et al. 2004). Some of these immunological reactions are due to breakdown products of collagen, proteoglycan and bone connective tissues that are degraded in the osteoarthritic process (Liossis and Tsokas 1998; Schroeder et al. 1989).

Part of their actions may have an immunological basis since RNPs have been shown to act as immune stimulants (Wacker and Eichler 1981; Lacour et al. 1984; Zemskov et al. 1984; Berg et al. 1992; Ikenda et al. 1994) possibly in part by regulating T-cell functions (Rudolf et al. 1984; Kulkarni et al. 1986; Bekman et al. 2001) so controlling chronic inflammatory conditions (Vladimirov et al. 1985; Beljanski 1991; Burneister and Rainsford 1991) and the immune deficiencies from infection (Rudoff et al. 1984; Kulkarni et al. 1986). How these T-cell-mediated actions of RNPs affect degenerative processes in such diverse organs as the CNS and arthritic joints is not known. However, there is evidence from a wide range of immunological models for RNPs influencing abnormal immune functions and for the manipulation of T-cell functions to achieve reversal or control of chronic inflammatory-degenerative conditions.

Another action of RNPs may arise from the roles that (ribo)-nucleotides and RNAs have in regulating cell growth (Semochkin et al. 1999, 2001). Ribonucleotides and RNAs stimulate a number of metabolic reactions including mitochondrial oxidative metabolism (Germaniuk and Minchenko 1972, 1982; Germaniuk and Goidoash 1976; Germaniuk et al. 1976; Minchenko and Germaniuk 1976), and this may lead to increased metabolic reactions that lead to repair in connective tissues (Lodemann et al. 1989). Hitherto, difficulties that were envisaged in uptake of RNPs into cells so that they can stimulate growth processes have been overcome in well-characterised models. Moreover, the recent discovery of inhibiting RNAs (iRNA) that block specific translation of RNAs coding for proteins that in some cases may include pro-inflammatory cytokines, metalloproteinases and various inflammatory mediators (e.g. PLA₂, COX-2) raises the tantalising prospect that some, albeit unspecific, iRNA-like activity may be present in RNPs to act as inhibitors of mRNAs coding for those cytokines and metalloproteinase enzymes (e.g. Jiang et al. 2005; Fukuyama et al. 2005) some of which are known to be central to the joint destructive processes in OA. Furthermore, ribonucleate sodium (Osteochondrin S) has been shown to inhibit cytokine-induced degradation of bones and cartilage (Rainsford et al. 2008) and modifies osteoclast activity reducing bone resorption in vitro (Cantley et al. 2010a, b). Osteochondrin® and its components showed a concentration-dependent inhibition of human osteoclast activity (Cantley et al. 2013). This inhibition by the RNA components of Osteochondrin® of the resorptive ability of osteoclasts is likely to occur at a late stage during osteoclast formation, downstream from the sites of action of NFATc1. Overall, the findings show that Osteochondrin S inhibition of osteoclast activity may be responsible for its beneficial effects on diseases of the bones such as osteoarthritis (Cantley et al. 2013).

Although these putative actions of RNPs have not yet been proven to have effects in control of regenerative or degenerative cell processes, let alone those

involved in joint destruction in OA, the need for an agent to attack these joint degenerative processes which is based on a non-protein, RNP product with some evidence for its clinical utility makes clinical studies with Osteochondrin[®], a unique and novel strategy for treating OA.

3.2 Clinical Observations

Clinical studies have been conducted with Osteochondrin in patients with a variety of musculo-skeletal or arthritis condition. Among the most significant observations are significant improvements in osteoarthritis, lumbago and ischialgia.

A double-blinded randomised study with 57 patients with osteoarthritis of the ankle, knee or patella treated with Osteochondrin[®] S (27 patients, 120 mg RNA) or placebo (30 patients, low-dose vitamin B2) was conducted. All patients received standard physical therapy. Pain, stiffness and general function parameters were evaluated after 4, 8 and 12 weeks (Schröder et al. 1989). Osteochondrin S and placebo groups showed significant improvements of almost all parameters at week 12 compared to values before the treatment, and only the duration of pain was not significantly changed in the placebo group. Matched pair analysis with 18 patients each from placebo and verum groups could be applied considering age, severity and localisation of osteoarthritis showing superiority of verum in 61 %, equal results in 11 % and superiority of placebo in 28 % of the matched pairs suggesting that patients might benefit from an adjuvant treatment with Osteochondrin S. The treatment was well tolerated in both groups.

A total of 118 Patients with Lumbago or Ischialgia were included into a controlled study with Osteochondrin S (120 mg RNA) in two groups comparing efficacy and safety of paravertebral injections (58 patients) compared to intragluteal injections of Osteochondrin S (60 patients). Examination of the patients and injection of the patients were executed by two doctors independently achieving a blinding of the study. Superiority of paravertebral injection should be tested compared to intragluteal injection. Primary endpoints were the sum of the scores of pain, paraesthesia in the legs and reflexes after 2 weeks (118 patients) and after 3 months (100 patients). All patients received a standard physical therapy. The sum of the scores was $7.45 + 1.77$ (mean + SD) before intragluteal injection and $7.64 + 1.97$ (mean + SD) before paravertebral injection. The sum of scores (mean + SD) was reduced after 2 weeks by $1.90 + 1.79$ with intragluteal injection and by $1.60 + 1.72$ with paravertebral injection. After 3 months, the reduction was $2.66 + 1.93$ (intragluteal) and $2.74 + 2.13$ (paravertebral). Both groups were not significantly different. The treatment was well tolerated. Painful injections were reported in 28.3 % of the intramuscular treatments, and 47.5 % of paravertebral injection which was significantly more. Two patients finished the study after the first paravertebral injection one after a heat sensation in the back and the other after pain at the injection site with nausea and insomnia. These data suggest that both routes of administration might be effective but the paravertebral route causes more

adverse effects so the intragluteal injection might be recommended for Osteochondrin S as the standard application.

3.2.1 Multi-centre Clinical Trial

To establish the efficacy of Osteochondrin in patients, a randomised, parallel-group, multi-centre study was undertaken to investigate the effects of this RNP preparation in controlling pain and joint swelling in osteoarthritis of the knee

There were initially 20 centres recruited in this multi-centre study (under Chefarzt Dr. Med. Wolfgang Bolten, Aertzlicher Direktor, Klaus Miehke Klinik, Wiesbaden as Principal Investigator). At the same time, there was a study at a single centre in Moscow. The study was designed and managed by the CRO, the Institut fuer Angewandte Statistik GmbH (IAS) (Bielefeld) under the Trial Manager, Dr. rer. Nat. Jörg Schnitker. Dipl. Math.

A total of 168 patients were enrolled in the 20 study centres in Germany and a further 48 at a centre of Moscow; the latter centre is excluded from further investigation in this report because of some protocol violations and inhomogeneity. Thus, the main report considered here is of the 168 patients initially enrolled in Germany. To these were applied standardised and appropriate Inclusion and Exclusion Criteria. Two patients withdrew consent, and thus 166 patients were randomised and considered for safety evaluation. After exclusion of two centres with substantial errors in records and other unacceptable deviations and patients with early termination without relation to efficacy, 145 patients constituted the Full Analysis Set (FAS). Subsequently, there were 12 major protocol deviations in the Osteochondrin Group and 13 in the placebo group treatment, and there were 60 patients in each group that comprised the Valid Case (VC) Set (Table 1).

The loss of patients due to deviations and violations was acceptable considering the circumstances in the trial which have been fully accounted for in the Report. From the statistical viewpoint, the number of patients in each of the treatment

Table 1 Demographics of patients enrolled for randomised treatment allocations

Patients	OST	PLA	All patients
Screening			168
Patients withdrew consent			2
Randomisation			166
Safety Analysis	84 (100 %)	82 (100 %)	166 (100 %)
Early termination not related to efficacy	4 (4.8 %)	1 (1.2 %)	5 (3.0 %)
Exclusion of centre No. 17 ^a	4 (4.8 %)	4 (4.9 %)	8 (4.8 %)
Exclusion of centre No. 21 ^a	4 (4.8 %)	4 (4.9 %)	8 (4.8 %)
Full Analysis Set (FAS)	72 (85.7 %)	73 (89.0 %)	145 (87.3 %)
Major protocol violations (with FAS)	12 (14.3 %)	13 (15.9 %)	25 (15.1 %)
Valid Case Set (FAS)	60 (71.4 %)	60 (73.2 %)	120 (73.2 %)

^aThese centres were excluded because of major protocol violations

groups of the FAS and VCS, respectively, is well balanced so that type II errors relating to loss of power (DiGiovanni and Hayes 2001) would be unlikely.

Measuring the Progression of OA and Responses to Therapy

OA (Osteoarthritis) is a complex disease which has variable clinical progression and outcomes (Altman 1991; Buchanan and Kean 2002b, c). In OA of the knee, long-term clinical and radiological studies have shown that although most deteriorate that a small proportion will actually improve and some will remain the same (Massardo et al. 1989). This variability in outcome presents considerable challenges to the investigator for measuring changes that occur in the knee and other joints in response to therapies (Dworkin et al. 2014). While routine X-ray and magnetic resonance imaging (MRI) may give a visual representation of changes in joints, application of these techniques to reliably quantify changes in bone and cartilage in OA has sometimes not been performed with standardised and fully validated techniques so that the assessment of changes and progression of joint injury or pathology may be highly variable (Buchanan and Kean 2002c). For the patients, the most significant clinical symptoms are pain and impairment of joint function (Buchanan and Kean 2002c). In assessing the treatment of OA, it will, therefore, be these parameters that will have greatest clinical significance (Buchanan and Kean 2002c). Quantifying changes in these clinical parameters as well as quality of life (QoL) assessments under standardised and validated procedures (Chassany et al. 2002) are the key components of assessing clinically relevant changes during therapy. In the present study, a standard clinometric approach was employed employing a health status instrument and assessment of QoL and global health status to determine the responses to Osteochondrin therapy.

Use of the WOMAC Instrument

In this study, the Western Ontario and McMaster Universities OsteoArthritis (WOMAC) Index was employed as the primary instrument for determining health status of the study patients.

WOMAC has been widely used as a health status instrument to determine outcomes from therapy with a wide variety of pharmacological agents, physical treatments and procedures used to treat osteoarthritis including that in the knee (Bellamy 2005; Lequesne and Maheu 2003; Salaffi et al. 2003, 2005).

Linguistic Forms of WOMAC and Applications

WOMAC has been evaluated in a number of different language forms, notably in German (Lesquesne 1994; Stucki et al. 1996; Kirschner et al. 2003). In the present study, the CRO has stated that the German version of WOMAC published by Lesquesne (1994) was employed.

Main Outcomes

Patients received treatments with intramuscular injection of 20 ampoules of Osteochondrin S with a dosage of 3×2 ampoules per week or placebo ampoules containing the excipients of Osteochondrin S and low-dose riboflavin (Fig. 3). Three subsequent treatment periods were conducted starting from 12th week after the onset of the previous cycle. Overall, the Primary Endpoint of the study in which the WOMAC total index showed response ($\geq 20\%$ reduction of the baseline values) was achieved. This was shown in the form of statistically significant differences between treatment and control in the total index. Along with this were improvements in Osteochondrin c.f. placebo in the Pain, Stiffness and Physical Function Scales of WOMAC; these being Secondary Endpoints in the Valid Case Set using χ^2 test and logistic regression analysis and in the Full Analysis set using logistic regression following completion to series 2 and 3 of treatments (Rainsford et al. 2004; Stommel et al. 2008). In essence, this means that patients who received Osteochondrin showed significant improvement in total index, pain, stiffness and physical function over placebo.

Examination of the time-dependent changes reveals, overall, qualitative in the Valid Case (VC) population and there were, overall, striking improvements in the total WOMAC Index as well as the individual scales in both Osteochondrin and placebo groups (Fig. 4). The overall trend in the total WOMAC Index and all these component scales was a progressive reduction of about one-half in the VAS scores from the starting baseline (V1) over the three periods (V3, V5 and V7) of the VAS scores. While there was a slight reduction in some of the VAS scores between the period measurements at V5 (post series 2) and V7 (post series 3), the most striking

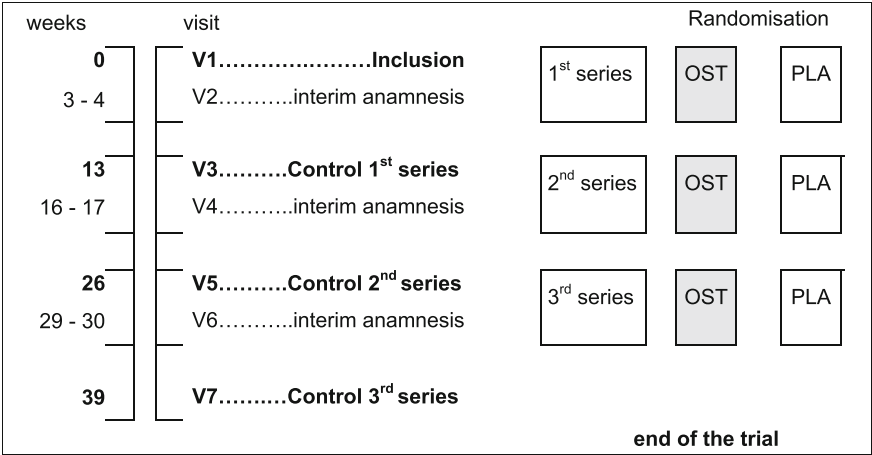


Fig. 3 Treatment and observation assessment. After randomisation patients were treated with 20 ampoules Osteochondrin S (OST) or Placebo (PLA) in three subsequent series with control of the parameters before treatment (V1), and 12 weeks after onset of each series of treatment (V3, V5, V7)

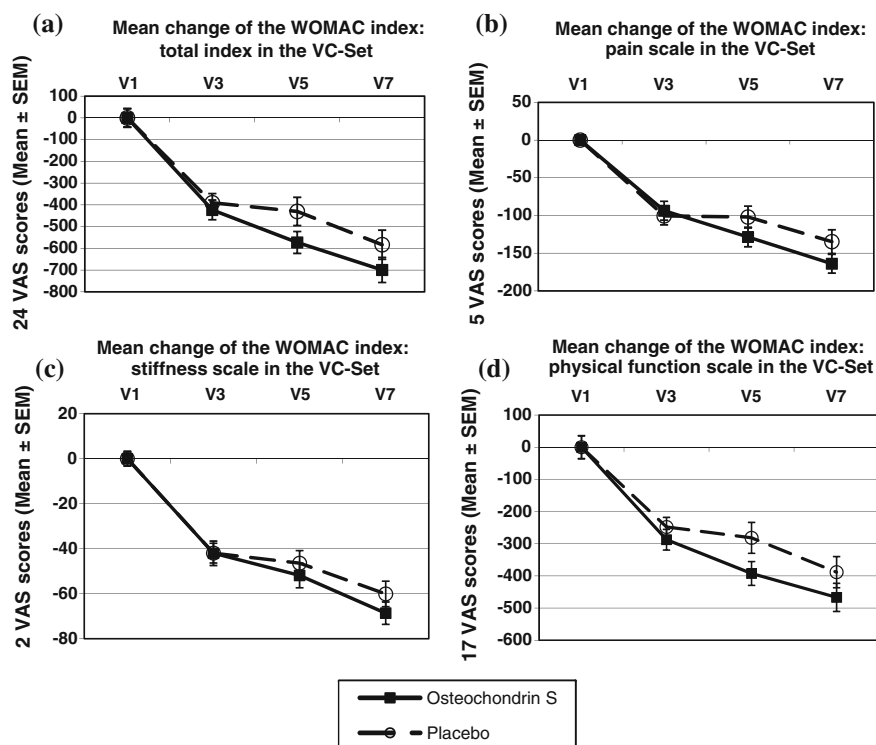


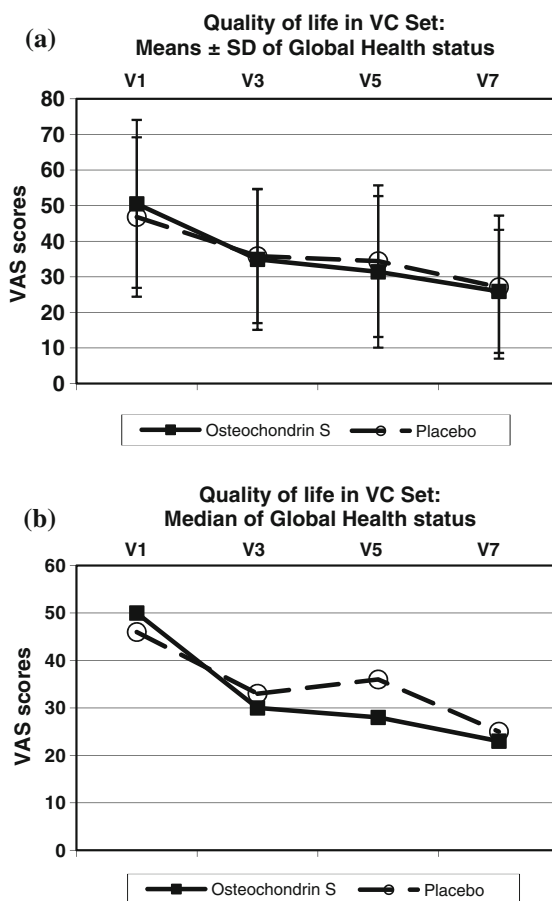
Fig. 4 Mean change of the WOMAC parameters (absolute values) in the Valid Case Set at study onset (V1) and in the course of treatment after the three series of treatment (V3, V5, V7). Differences in placebo and Osteochondrin groups are more obvious after shifting V1 mean values to 0. **a** WOMAC total index; V1 absolute mean values: 1332 in Osteochondrin S group and 1247 in placebo group. **b** WOMAC pain scale, V1 absolute mean values: 284 in Osteochondrin S group and 264 in placebo group. **c** WOMAC stiffness scale, V1 absolute mean values: 121 in Osteochondrin S group and 115 in placebo group. **d** WOMAC physical function scale, V1 absolute mean values: 928 in Osteochondrin S group and 865 in placebo group

changes in physical function, pain and total index seem to be evident at V5 (post series 2) compared with those at the other time points. These data suggest that there was a substantial influence of both treatments in the clinical outcomes shown by WOMAC measurements from the study.

Similarly, overall trends of both groups are evident in the VC population in the global health status (Fig. 5), as well as the OA status (Fig. 6). Again, the trend is towards a reduction of about one-half in the VAS scores over the entire period from the baseline in these global clinical parameters, with some trends being slightly greater than others. There is clearly a positive placebo effect evident, and since the trends towards improvements are apparent overall, this tends to disguise differences that are apparent with Osteochondrin treatment over placebo making the latter appear proportionately less.

Fig. 5 Quality of life VAS scores in the Valid Case Set in the course of treatment after the three series of treatment (V3, V5, V7) with Osteochondrin S and placebo. Scales from 0 (very good) to 100 (very bad).

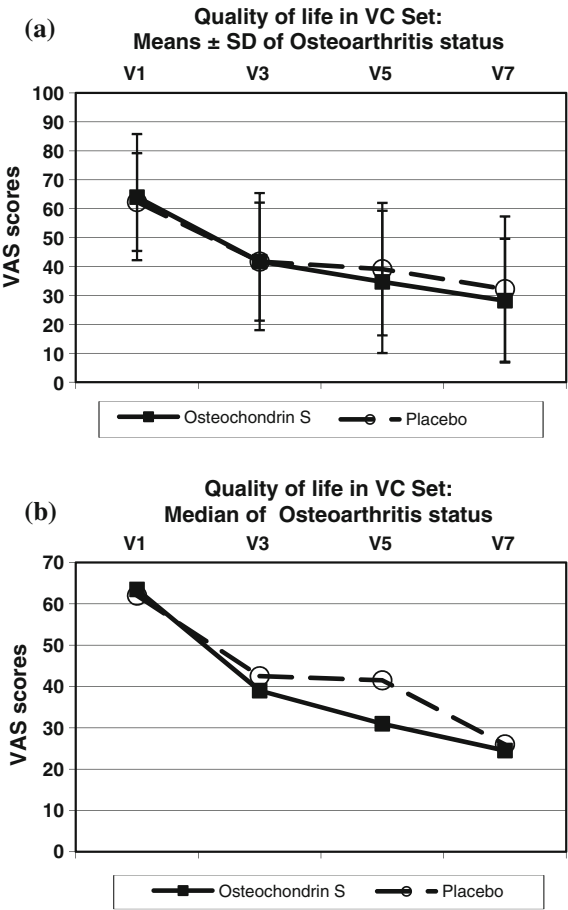
a Means \pm SEM of global health status. **b** Median values of global health status



Analgesic Consumption

As an indicator of pain status during the periods of the trial, the consumption of ibuprofen tablets (400 mg) did not reveal any differences in self-administration of this drug in the two treatments; whether the comparison of individual periods were considered (Table 2) or in the post-V7 period compared with V1 (1st injection) and there were no differences between the groups at baseline in both the VC or FAS groups. Overall, the consumption of ibuprofen was about 0.5–1.8 tablets per week which is relatively small and does not present a problem for developing gastro-intestinal or other side effects (Rainsford 1999). Although there was a trend to reduction in the intake of ibuprofen with both Osteochondrin and placebo groups, this difference did not achieve statistical significance in either the FAS or VC series populations. However, it was notable that patients in both groups were initially taking about 5 tablets of ibuprofen per week in both FAS and VC series so the relative reduction in rescue medication is small (since this was about 0.5–1.8 tablets/week).

Fig. 6 Quality of life VAS scores in the Valid Case Set in the course of treatment after the 3 series of treatment (V3, V5, V7) with Osteochondrin S and placebo. Scales from 0 (very good) to 100 (very bad).
a Means \pm SEM of Osteoarthritis status.
b Median values of Osteoarthritis status



Joint Parameters

The assessments of tenderness, mobility, circumference and swelling of knee joints were designated “Exploratory Target Criteria” in the measurements of efficacy in this study. These parameters are of considerable clinical significance and have advantages in being to some extent objective measures of joint inflammation and associated pain responsiveness to pressure application.

In both the FAS and VC series, the reduction in tenderness to application of pressure above the articular space of the affected knee (Table 3) was shown for the individually last series “post7”. This is a substantial reduction in pain responsiveness. Even though there were no statistically significant differences between the two treatments, this trend shows parallel with the WOMAC parameters of pain and physical function noted earlier. There was a reduction in the pain tenderness of contralateral knees in the FAS and VC series of both treatment groups as well but

Table 2 Rescue medication with Ibuprofen per week in the 1st, 2nd and 3rd series of treatment with Osteochondrin (OST) and placebo (PLA)

Rescue medication (FAS)	V1–V2		V2–V3		V3–V5		V5–V7		Post 7	
	OST	PLA	OST	PLA	OST	PLA	OST	PLA	OST	PLA
Mean	5.69	5.52	4.25	4.08	3.92	3.93	3.23	3.61	3.23	3.8
SD	7.25	6.16	4.88	4.70	5.55	5.02	5.46	5.13	5.36	5.45
Median	3.75	3.77	2.43	2.48	0.88	2.17	0.48	1.13	0.48	1.13

^a[FAS]

Rescue medication (VC)	V1–V2		V2–V3		V3–V5		V5–V7	
	OST	PLA	OST	PLA	OST	PLA	OST	PLA
Mean	4.99	5.39	4.11	3.75	3.99	3.58	3.04	3.37
SD	5.7	5.8	4.8	3.93	5.28	4.33	4.85	4.70
Median	3.75	3.96	2.43	2.51	1.12	2.15	0.64	1.16

^b[VC-Set]

^aFindings in the time periods V1–V2, V2–V3, V3–V5, V5–V7 and in the individually last period (post7) compared to the first injection phase in Full Analysis Set

^bFindings in the time periods V1–V2, V2–V3, V3–V5, V5–V7 in the Valid Case Set

again there were no differences between the treatment groups. The tenderness pain in the contralateral knees is about half that of the affected knees but is still quite pronounced. This is an interesting aspect and reflects the view that OA has systemic components.

The changes in tenderness pain had, to some extent, parallels with swelling of the knee joints (Table 4) which was reduced by about one-half, and the patients were symptom-free or improved over the period to the individually last series “post7” from baseline in about 60–70 % of individuals in both treatment groups. In the same way, the tenderness pain was symptom-free or improved in some 70–80 % of patients on both treatments (Table 3).

In values of the mobility of the affected joints, the values for the angles of stretch, bend and degree of mobility were improved in both treatment groups over the V1 to post V7 period by about 10 degree for the degree of mobility, and there were no changes in these parameters on the contralateral side (Table 5). Likewise, the values for the circumference of the knee joints of the affected side were reduced by about 10 mm in both treatment groups with a trend to get better results in the OST-group shown by the differences at the 1st, 2nd and 3rd series versus V1 at the affected side and contralaterally (Tables 6 and 7).

Overall, these parameters of joint inflammation and pain show that inflammatory pain is reduced in both treatment groups as well as indices of joint movement. There are no differences between the two treatment groups. The patient diary records show changes in pain in the knee by 40 % reduction in the OST-group and 30 % in the PLA-group for the last series compared to the first (Table 8), and this may relate to the changes in joint inflammatory/pain components for both treatments noted above with a clear trend to favour OST.

Table 3 Changes in tenderness on pressure from baseline to the individually last series 'post7' at

Changes from baseline	OST	PLA
Number of patients	72	73
Not affected	2	1
Symptom-free	24 (34.3 %)	23 (31.9 %)
Improved	32 (45.7 %)	31 (43.1 %)
Unchanged	11 (15.7 %)	15 (20.8 %)
Worse	3 (4.3 %)	3 (4.2 %)
^a $[\chi^2 \text{ test: } p = 0.891]$, affected side (FAS)		
Changes from baseline	OST	PLA
Number of patients	72	73
Not affected	37	31
Symptom-free	18 (51.4 %)	23 (54.8 %)
Improved	4 (11.4 %)	5 (11.9 %)
Unchanged	11 (31.4 %)	12 (28.6 %)
Worse	2 (5.7 %)	2 (4.8 %)
^b $[\chi^2 \text{ test: } p = 0.988]$, contralateral side (FAS)		
Changes from baseline	OST	PLA
Number of patients	60	60
Not affected	2	–
Symptom-free	19 (32.8 %)	20 (33.3 %)
Improved	29 (50.0 %)	24 (40.0 %)
Unchanged	8 (13.8 %)	14 (23.3 %)
Worse	2 (3.5 %)	2 (3.3 %)
^c $[\chi^2 \text{ test: } p = 0.552]$, affected side (VC)		
Changes from baseline	OST	PLA
Number of patients	60	60
Not affected	33	27
Symptom-free	14 (51.9 %)	18 (54.6 %)
Improved	2 (7.4 %)	5 (15.2 %)
Unchanged	9 (33.3 %)	8 (24.2 %)
Worse	2 (7.4 %)	2 (6.1 %)
^d $[\chi^2 \text{ test: } p = 0.739]$, contralateral side (VC)		
^a The affected side [FAS]		
^b The contralateral side [FAS]		
^c The affected side [VC]		
^d The contralateral side [VC]		

The measurements of walking time do not show any appreciable differences for both treatments, and there are no significant differences between the two treatments (Table 9).

The QoL Global Health State shows improvement in both groups from V1 to post 7 (Table 10) as well as the results in osteoarthritis status (Table 11) and global quality of life (Table 12) without significant differences between Osteochondrin and placebo.

The investigator's Global Assessment of Efficacy in relation to Clinical Global Impressions (CGI) showed an overall trend for improvement at the various periods

Table 4 Changes in swelling of the knee joint from baseline to the individually last series ‘post7’ at

Changes from baseline	OST	PLA
Number of patients	72	73
Not affected	13	5
Symptom-free	28 (47.5 %)	33 (48.5 %)
Improved	12 (20.3 %)	13 (19.1 %)
Unchanged	18 (30.5 %)	19 (27.9 %)
Worse	1 (1.7 %)	3 (4.4 %)
^a [χ^2 test: $p = 0.839$], (FAS)		
Changes from baseline	OST	PLA
Number of patients	60	60
Not affected	9	4
Symptom-free	25 (49.0 %)	29 (51.8 %)
Improved	12 (23.5 %)	10 (17.9 %)
Unchanged	14 (27.5 %)	16 (28.6 %)
Worse	–	1 (1.8 %)
^b [χ^2 test: $p = 0.710$], (VC)		

^aThe affected side [FAS]^bThe affected side [VC]

for both treatment groups (Table 13). When the values for CGI were correlated with changes in the WOMAC index in the FAS and VC groups that received Osteochondrin and placebo (Table 14), where there appeared to be no differences between the two treatments, there were overall improvements with both the two treatments. This is shown by the predominance of changes in improvements of the CGI of 1–3 (“very much improved” to “minimally improved”) with relative changes from baseline in the WOMAC index >20 % (which are outlined in double-lined boxes in Table 14). Outliers have been listed in the comments in Table 14. However, the underlying assessment CGI and the components of the WOMAC Index would not be expected to have identical clinical responsiveness.

The Patient’s Global Assessment of Efficacy (FAS and VC) showed a similar trend to that seen with the Investigator’s assessment but with a trend to better results in favour of OST after the 2nd series of treatment (Table 15).

Adverse Events and Safety

About half the patients who received the treatments experienced adverse events (Table 16), and it was assumed that the treatments were possibly related in five patients who received Osteochondrin (6.0 %) and 6 who had placebo (7.3 %). The events reported (WHO Terms) were all minor, and there was no clear pattern of their occurrence (Table 17). It might be argued that the reports of pruritus (1 case) and erythematous rash (1 case) (both types of events have been reported earlier and are described in the SPC) might have resulted from the therapy because of vague possibility of some unspecified immunological reactions to the injection.

Table 5 Mobility of the Knee Joint (stretch, bend and degree of mobility) in the 1st, 2nd and 3rd series of treatment

OST					
Stat. estimate	V1	V3	V5	V7	post7
N	72	72	69	68	72
Mean	1.3	2.6	2.8	3.7	3.3
SD	5.9	5.2	5.7	5.7	5.9
Median	0.0	0.0	0.0	0.0	0.0
PLA					
N	73	73	71	71	73
Mean	1.8	2.0	2.7	2.6	2.6
SD	5.5	5.3	5.8	5.5	5.5
Median	0.0	0.0	0.0	0.0	0.0
^a Stretching [°] of the affected knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	72	72	69	68	72
Mean	115.2	118.0	121.1	123.0	121.9
SD	15.8	16.8	15.4	15.4	16.5
Median	120.0	120.0	120.0	125.0	125.0
PLA					
N	73	73	71	71	73
Mean	112.8	117.0	119.5	121.3	120.7
SD	15.8	15.4	14.7	14.8	15.1
Median	120.0	120.0	120.0	120.0	120.0
^b Bending [°] of the affected knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	72	72	69	68	72
Mean	116.5	120.6	123.9	126.8	125.2
SD	18.6	19.1	17.2	17.0	18.7
Median	120.0	121.5	125.0	130.0	130.0
PLA					
N	73	73	71	71	73
Mean	114.6	119.0	122.2	123.9	123.3
SD	17.8	17.3	16.8	16.8	17.1
Median	115.0	120.0	125.0	130.0	129.0
^c Degree of mobility [°] at the affected knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	60	60	60	60	
Mean	1.9	3.0	2.7	3.5	

(continued)

Table 5 (continued)

OST					
Stat. estimate	V1	V3	V5	V7	
SD	5.7	5.0	5.8	5.5	
Median	0.0	0.0	0.0	0.0	
PLA					
N	60	60	60	60	
Mean	2.1	2.4	3.3	3.0	
SD	5.7	5.3	5.8	5.6	
Median	0.0	0.0	2.5	0.0	
^d Stretching [°] of the affected knee (VC)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	60	60	60	60	
Mean	114.5	117.9	120.3	121.8	
SD	14.3	15.2	14.9	15.5	
Median	117.5	120.0	120.0	125.0	
PLA					
N	60	60	60	60	
Mean	112.9	116.7	118.7	120.6	
SD	15.6	15.2	14.5	14.5	
Median	120.0	120.0	120.0	120.0	
^e Bending [°] of the affected knee (VC)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	60	60	60	60	
Mean	116.4	120.9	123.1	125.2	
SD	16.9	17.3	17.2	17.3	
Median	120.0	121.5	124.5	130.0	
PLA					
N	60	60	60	60	
Mean	115.0	119.1	122.0	123.6	
SD	17.2	16.6	16.7	16.5	
Median	120.0	120.0	122.5	129.5	
^f Degree of mobility [°] at the affected knee (VC)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	70	70	67	66	70
Mean	5.4	5.5	4.9	5.3	5.2
SD	5.1	5.0	5.3	5.1	5.1
Median	5.0	5.0	5.0	5.0	5.0

(continued)

Table 5 (continued)

OST					
Stat. estimate	V1	V3	V5	V7	post7
PLA					
N	72	72	70	70	72
Mean	5.1	4.5	4.7	4.7	4.6
SD	4.9	5.9	6.2	5.4	5.4
Median	5.0	5.0	5.0	5.0	5.0
^g Stretching [°] of the contralateral knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	70	70	67	66	70
Mean	131.1	131.5	129.9	133.0	133.2
SD	12.3	12.8	18.0	17.3	18.1
Median	130.0	132.5	135.0	132.5	130.0
PLA					
N	72	72	70	70	72
Mean	130.0	130.7	129.1	128.7	128.5
SD	10.8	10.4	16.3	17.8	17.6
Median	130.0	130.0	130.0	130.0	130.0
^h Bending [°] of the contralateral knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	70	70	67	66	70
Mean	136.5	137.0	134.8	138.3	138.4
SD	14.5	14.6	20.4	18.2	19.0
Median	140.0	140.0	140.0	140.0	140.0
PLA					
N	72	72	70	70	72
Mean	135.1	135.2	133.7	133.4	133.1
SD	13.2	13.2	18.1	18.8	18.6
Median	135.0	137.5	140.0	137.5	135.0
ⁱ Degree of mobility [°] at the contralateral knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	58	58	58	58	
Mean	5.9	5.7	5.0	5.4	
SD	5.2	5.1	5.4	5.2	
Median	9.0	9.0	5.0	7.0	
PLA					
N	59	59	59	59	
Mean	5.2	5.0	5.2	4.9	

(continued)

Table 5 (continued)

OST				
Stat. estimate	V1	V3	V5	V7
SD	4.6	5.5	5.6	5.2
Median	5.0	5.0	5.0	5.0

^jStretching [°] of the contralateral knee (VC)

OST				
Stat. estimate	V1	V3	V5	V7
N	58	58	58	58
Mean	130.7	131.6	129.8	131.0
SD	10.8	10.3	18.7	11.5
Median	130.0	132.5	132.5	130.0

PLA

N	59	59	59	59
Mean	130.7	131.3	128.5	128.3
SD	10.0	9.6	16.9	18.4
Median	130.0	130.0	130.0	130.0

^kBending [°] of the contralateral knee (VC)

OST				
Stat. estimate	V1	V3	V5	V7
N	58	58	58	58
Mean	136.6	137.3	134.8	136.4
SD	13.3	12.6	21.4	13.5
Median	140.0	140.0	140.0	140.0

PLA

N	59	59	59	59
Mean	135.9	136.3	133.7	133.2
SD	11.4	11.1	17.8	18.8
Median	140.0	140.0	140.0	135.0

^lDegree of mobility [°] at the contralateral knee (VC)

Findings at V1, V3, V5, V7 in VC-Set (VC) and additionally for the initially last series “post7” in Full Analysis Set (FAS)

^aStretching of the affected side (FAS)^bBending of the affected side (FAS)^cDegree of mobility at the affected side (FAS)^dStretching of the affected side (VC)^eBending of the affected side (VC)^fDegree of mobility at the affected side (VC)^gStretching of the contralateral side (FAS)^hBending of the contralateral side (FAS)ⁱDegree of mobility at the contralateral side (FAS)^jStretching of the contralateral side (VC)^kBending of the contralateral side (VC)^lDegree of mobility at the contralateral side (VC)

Table 6 Circumference of the Knee Joint (mm) in the 1st, 2nd and 3rd series of treatment

OST					
Stat. estimate	V1	V3	V5	V7	post7
N	71	71	68	67	71
Mean	422.8	418.4	414.7	413.0	411.8
SD	50.0	53.2	52.4	51.4	50.2
Median	410.0	400.0	400.0	400.0	400.0
PLA					
N	73	73	71	71	73
Mean	415.4	410.9	407.3	404.8	406.0
SD	40.6	38.4	37.9	39.3	39.5
Median	410.0	410.0	405.0	400.0	400.0
^a Circumference [mm] of the affected knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	59	59	59	59	
Mean	428.0	423.7	418.2	415.8	
SD	52.8	56.3	54.8	53.4	
Median	429.0	416.0	417.0	410.0	
PLA					
N	60	60	60	60	
Mean	414.2	410.3	407.9	404.8	
SD	42.1	40.2	39.8	40.7	
Median	410.0	407.5	405.0	400.0	
^b Circumference [mm] of the affected knee (VC)					
OST					
Stat. estimate	V1	V3	V5	V7	post7
N	67	67	64	63	67
Mean	410.9	409.8	409.6	409.8	407.6
SD	51.4	51.4	51.3	51.8	51.1
Median	400.0	400.0	408.0	400.0	400.0
PLA					
N	68	68	67	67	68
Mean	403.2	403.7	402.8	401.2	401.7
SD	42.2	40.8	40.5	38.8	38.7
Median	400.0	400.0	400.0	400.0	400.0
^c Circumference [mm] of the contralateral knee (FAS)					
OST					
Stat. estimate	V1	V3	V5	V7	
N	55	55	55	55	
Mean	416.3	415.3	413.3	412.6	

(continued)

Table 6 (continued)

OST				
Stat. estimate	V1	V3	V5	V7
SD	54.4	54.4	53.9	54.3
Median	420.0	415.0	410.0	410.0
PLA				
N	57	57	57	57
Mean	402.4	403.3	403.2	401.8
SD	43.6	42.2	42.2	40.8
Median	400.0	400.0	400.0	400.0

^dCircumference [mm] of the contralateral knee (VC)

Findings at V1, V3, V5, V7 in Valid Case Set (VC) and additionally for the initially last series “post7” in Full Analysis Set (FAS)

^aCircumference at the affected side (FAS)

^bCircumference at the affected side (VC)

^cCircumference at the contralateral side (FAS)

^dCircumference at the contralateral side (VC)

Table 7 Differences in the Circumference of the Knee Joint [mm] versus V1 in the 1st, 2nd and 3rd series of treatment in the Valid Case Set [VC]

Affected side								
V3 versus V1			V5 versus V1			V7 versus V1		
Stat. estimate	OST	PLA	Stat. estimate	OST	PLA	Stat. estimate	OST	PLA
<i>N</i>	59	60	<i>N</i>	59	60	<i>N</i>	59	60
min.	−40	−40	min.	−50	−50	min.	−60	−40
max.	40	60	max.	35	50	max.	30	50
median	0.0	−0.5	median	−10.0	−5.0	median	−10.0	−10.0
mean	−4.2	−3.8	mean	−9.8	−6.3	mean	−12.1	−9.4
standard dev.	12.5	14.2	standard dev.	14.7	13.9	standard dev.	17.2	14.9
t test: <i>p</i> = 0.4348			t test: <i>p</i> = 0.0938			t test: <i>p</i> = 0.1730		
U test: <i>p</i> = 0.6521			U test: <i>p</i> = 0.1149			U test: <i>p</i> = 0.3671		
Contralateral side								
<i>N</i>	55	57	<i>N</i>	55	57	<i>N</i>	55	57
min.	−25	−28	min.	−30	−37	min.	−55	−36
max.	15	40	max.	15	65	max.	40	65
median	0.0	0.0	median	0.0	0.0	median	0.0	0.0
mean	−1.0	0.8	mean	−3.0	0.8	mean	−3.7	−0.7
standard dev.	6.8	8.7	standard dev.	8.4	12.0	standard dev.	14.4	13.4
t test: <i>p</i> = 0.1135			t test: <i>p</i> = 0.0282			t test: <i>p</i> = 0.1302		
U test: <i>p</i> = 0.1243			U test: <i>p</i> = 0.0460			U test: <i>p</i> = 0.3924		

[one-sided p values]

Table 8 Patient’s diary in the 1st, 2nd and 3rd series of treatment for pain in the knee as an average of diary entries [0 = no, 1 = mild, 2 = severe]

OST					
Stat. estimate	V1–V2	V2–V3	V3–V5	V5–V7	post7
N	71	71	69	67	71
Mean	1.28	1.09	0.92	0.76	0.80
SD	0.36	0.43	0.49	0.47	0.49
Median	1.28	1.11	1.00	0.81	0.84
PLA					
N	71	71	70	69	71
Mean	1.23	1.05	0.95	0.85	0.87
SD	0.41	0.46	0.47	0.53	0.53
Median	1.22	1.06	1.00	0.99	1.00
^a (FAS)					
OST					
Stat. estimate	V1–V2	V2–V3	V3–V5	V5–V7	
N	59	59	59	59	
Mean	1.25	1.06	0.90	0.75	
SD	0.36	0.43	0.49	0.48	
Median	1.27	1.10	1.00	0.81	
PLA					
N	58	58	58	58	
Mean	1.24	1.06	0.96	0.87	
SD	0.34	0.41	0.44	0.52	
Median	1.22	1.06	1.00	0.98	
^b (VC)					
^a Findings in the time periods V1–V2, V2–V3, V3–V5, V5–V7 and in the individually last series in the FAS					
^b Findings in the time periods V1–V2, V2–V3, V3–V5, V5–V7 in the VC-Set					

Table 9 Walking time [s] for 15 m in the 1st, 2nd and 3rd seriesof treatment in the VC-Set

OST				
Stat. estimate	V1	V3	V5	V7
N	60	60	60	60
Mean	23.2	21.9	21.4	20.6
SD	11.6	12.0	11.7	11.1
Median	19.5	18.5	18.0	17.0
PLA				
N	58	58	58	58
Mean	22.0	20.8	20.0	19.2
SD	11.3	11.0	10.4	9.5
Median	19.0	18.0	17.0	17.0

Table 10 Changes from baseline to the individually last series 'post7' in global health status in FAS and VC-Set

FAS		
Stat. estimate	OST	PLA
Number of patients	71	72
Median	-19.0	-17.5
Mean	-21.7	-19.3
Standard deviation	23.5	26.4
t test: $p = 0.2838$ one-sided; U test: $p = 0.3292$ one-sided		
VC		
Number of patients	59	59
Median	-21.0	-21.0
Mean	-24.6	-19.8
Standard deviation	23.9	26.6
t test: $p = 0.1504$ one-sided; U test: $p = 0.2058$ one-sided		

Table 11 Changes from baseline to the individually last series 'post7' in osteoarthritis status in FAS and VC-Set

FAS		
Stat. estimate	OST	PLA
Number of patients	72	73
median	-32.5	-32.0
mean	-32.7	-30.4
standard deviation	27.2	27.0
t test: $p = 0.3019$ one-sided; U test: $p = 0.3154$ one-sided		
VC		
Number of patients	60	60
Median	-35.0	-32.5
Mean	-35.8	-30.1
Standard deviation	26.0	26.5
t test: $p = 0.1178$ one-sided; U test: $p = 0.1386$ one-sided		

There were no statistically significant differences between the two treatment groups in the overall incidence of adverse events and most were minor general disorders, principally in the gastro-intestinal (GI), musculo-skeletal and the respiratory systems (Table 18).

Influenza-like symptoms (6 cases) in three patients who received Osteochondrin 3 with placebo could be coincidental even though these symptoms sometimes appear as a result of leucocyte reactions to immunological agents. Since there were no indications of leucocyte changes, there being only three patients who received Osteochondrin and five on placebo who exhibited abnormal leucocyte counts (Table 19). There was no evidence of fever in any of the patients given either of the

Table 12 Changes from baseline to the individually last series ‘post7’ in quality of life in FAS and VC-Set

FAS		
Stat. estimate	OST	PLA
Number of patients	72	72
Median	−16.0	−15.0
Mean	−18.7	−16.5
Standard deviation	24.5	24.0
t test: $p = 0.2927$ one-sided; U test: $p = 0.3345$ one-sided		
VC		
Number of patients	60	59
median	−17.5	−14.0
mean	−20.2	−16.8
standard deviation	24.2	23.8
t test: $p = 0.2195$ one-sided; U test: $p = 0.2316$ one-sided		

Table 13 Investigator’s global assessment of efficacy as Responder rates (= incidences of improved physical status) from 1st, 2nd, 3rd series and the individually last series (post7) in FAS and from 1st, 2nd and 3rd series from VC-Set

FAS								
	V3		V5		V7		post7	
Stat. estimate	OST	PLA	OST	PLA	OST	PLA	OST	PLA
Responder rates	54/70 (77.1 %)	53/72 (73.6 %)	52/69 (75.4 %)	52/71 (73.2 %)	56/68 (82.4 %)	59/71 (83.1 %)	56/70 (80.0 %)	59/72 (81.9 %)
	$p = 0.625$		$p = 0.774$		$p = 0.907$		$p = 0.768$	
VC								
	V3		V5		V7			
Stat estimate	OST	PLA	OST	PLA	OST	PLA		
Responder rates	47/60 (78.3 %)	44/60 (73.3 %)	46/60 (76.7 %)	43/60 (71.7 %)	49/60 (81.7 %)	51/60 (85.0 %)		
	$p = 0.522$		$p = 0.532$		$p = 0.624$			

two treatments. Fever has been related to the use of polynucleotides designed for anti-viral therapy (Powanda et al. 1977).

Changes from baseline to endpoint of laboratory findings showed similar results for OST and PLA (Table 20). A difference of the p -value < 0.150 was obtained in HDL cholesterol ($p = 0.068$), Triglycerides ($p = 0.086$) and Total bilirubin ($p = 0.036$). These differences were due to different baseline values or within the normal range and clinically not significant.

Clinically relevant shifts (Table 21) occurred in two OST-treated patients (LDL, HDL and total cholesterol resp. triglycerides) and in one PLA-treated patient (γ -GT and alk. phosphatase). Laboratory findings were evaluated at V1 and at least at one visit after treatment in most cases at V7.

Table 14 Correlation between changes in WOMAC index and changes in patient's condition as compared to the start of therapy according to Clinical Global Impressions Item 2

OST								
Relative changes from baseline in WOMAC index	Changes in condition CGI Item 2							
	1	2	3	4	5	6	7	MV
≤-90 %	4	2	-	-	-	-	-	-
>-90 bis ≤-80 %	-	5	-	-	-	-	-	-
>-80 bis ≤-60 %	3	15	2	-	-	-	-	-
>-60 bis ≤-40 %	3	5	4	1	-	-	-	-
>-40 bis ≤-20 %	1	2	7	5	-	-	-	-
>-20 bis ≤+20 %	-	-	1	6	1	1	-	2
>+20 %	-	1	1	-	-	-	-	-

1 very much improved; 2 much improved; 3 minimally improved; 4 no change; 5 minimally worse; 6 much worse; 7 very much worse; MV missing value

Comments:

- 6 patients with improved WOMAC index ≥20 % were classified as unchanged or worse
- 1/9 patient with marginal changes in WOMAC index within <20 % was classified as improved

PLA

≤-90 %	5	5	1	-	-	-	-	-
>-90 bis ≤-80 %	-	4	-	-	-	-	-	-
>-80 bis ≤-60 %	6	11	1	-	-	-	-	-
>-60 bis ≤-40 %	-	9	1	-	-	-	-	-
>-40 bis ≤-20 %	-	2	4	1	1	-	-	-
>-20 bis ≤+20 %	-	3	7	7	2	1	-	1
>+20 %	-	-	-	-	1	-	-	-

1 very much improved; 2 much improved; 3 minimally improved; 4 no change; 5 minimally worse; 6 much worse; 7 very much worse; MV missing value

Comments:

- 2 patients with improved WOMAC index ≥20 % were classified as unchanged or worse
- 10/20 patients with marginal changes in WOMAC index within <20 % were classified as improved

^a(FAS)

OST								
Relative changes from baseline in WOMAC index	Changes in condition CGI Item 2							
	1	2	3	4	5	6	7	
≤-90 %	3	2	-	-	-	-	-	-
>-90 bis ≤-80 %	-	5	-	-	-	-	-	-
>-80 bis ≤-60 %	3	15	2	-	-	-	-	-
>-60 bis ≤-40 %	1	4	4	1	-	-	-	-
>-40 bis ≤-20 %	-	2	6	4	-	-	-	-

(continued)

Table 14 (continued)

OST							
Relative changes from baseline in WOMAC index	Changes in condition CGI Item 2						
	1	2	3	4	5	6	7
>−20 bis ≤+20 %	–	–	1	5	1	–	–
>+20 %	–	–	1	–	–	–	–
1 very much improved; 2 much improved; 3 minimally improved; 4 no change; 5 minimally worse; 6 much worse; 7 very much worse; MV missing value							
Comments:							
• 5 patients with improved WOMAC index ≥20 % were classified as unchanged							
• 1/7 patient with marginal changes in WOMAC index within <20 % was classified as improved							
PLA							
≤−90 %	3	4	1	–	–	–	–
>−90 bis ≤−80 %	–	4	–	–	–	–	–
>−80 bis ≤−60 %	4	10	1	–	–	–	–
>−60 bis ≤−40 %	–	8	1	–	–	–	–
>−40 bis ≤−20 %	–	2	4	–	1	–	–
>−20 bis ≤+20 %	–	3	6	5	2	1	–
>+20 %	–	–	–	–	–	–	–
1 very much improved; 2 much improved; 3 minimally improved; 4 no change; 5 minimally worse; 6 much worse; 7 very much worse; MV missing value							
Comments:							
• 1 patient with improved WOMAC index ≥20 % was classified as unchanged or worse							
• 9/17 patients with marginal changes in WOMAC index within <20 % were classified as improved							
^b (VC)							

^aIn the individually last series 'post7' FAS^bAfter series 3 in the VC-Set**Table 15** Patient's global assessment of efficacy as Responder rates (= incidences of improved physical status) from 1st, 2nd and 3rd series and the individually last series (post7) in FAS and from 1st, 2nd and 3rd series from VC-Set

FAS								
	V3		V5		V7		post7	
Stat. estimate	OST	PLA	OST	PLA	OST	PLA	OST	PLA
Responder rates	39/70 (55.7 %)	41/72 (56.9 %)	49/69 (71.0 %)	43/71 (60.6 %)	47/68 (69.1 %)	47/71 (66.2 %)	47/70 (67.1 %)	47/72 (65.3 %)
	$p = 0.883$		$p = 0.193$		$p = 0.713$		$p = 0.814$	
VC								
	V3		V5		V7			
Stat. estimate	OST	PLA	OST	PLA	OST	PLA		
Responder rates	35/60 (58.3 %)	35/60 (58.3 %)	43/60 (71.7 %)	35/60 (58.3 %)	42/60 (70.0 %)	40/60 (66.7 %)		
	$p = 1.000$		$p = 0.126$		$p = 0.695$			

Table 16 Adverse events

	Observed adverse events	
	OST	PLA
Number of patients	48 (57.1 %)	53 (64.6 %)
	X^2 test: $p = 0.323$	
	<i>At least related as possible</i>	
Number of patients	5 (6.0 %)	6 (7.3 %)

Table 17 Display of adverse events with at least possible relationship. Symptoms listed as WHO preferred terms from cases related to the study drug

OST	
WHO preferred terms	Action
Pruritus	No action taken
Circulatory failure	Study drug discontinued
Rash erythematous	Study drug discontinued
Sweating increased; Cramps legs	No action taken
Headache	No action taken
PLA	
Myalgia	Check for thrombosis
Arthralgia	Study drug discontinued
Paraesthesia	No action taken
Arthralgia; Hypoaesthesia; Fatigue and pain	No action taken
Dizziness; Fatigue	No action taken
Somnolence	Study drug discontinued

There do not appear to be any appreciable changes in the laboratory values over the treatment periods, and there appeared to be no differences in the occurrence of abnormal high values. There were some abnormal high values of cholesterol and LDL cholesterol in both treatment groups, but no trends were evident over the treatment periods. These high values like those of the liver enzymes probably reflect the conditions of the relatively older group of patients who have been recruited for the study.

The cardiovascular and other vital signs (Table 22) likewise reflect those expected the type of patients who were recruited in this study, and the comparison of changes from baseline to end of study did not differ between OST and PLA. Aside from some missing values, there were in mean no obvious abnormalities in vital signs that occurred during the study (Table 23) and presumably none could be related to the treatments unless they appeared as adverse events. The intake of concomitant medications, particularly cardiovascular, anti-thrombotic and GI agents, reflects that expected in the study population, while other drugs are of little

Table 18 Incidences of adverse events on the basis of SOC

SOC	OST [N = 84]		PLA [N = 82]		X ² test
	N	%	N	%	p-value
Skin and appendages disorders	9	10.7	6	7.3	0.445
Musculo-skeletal system disorders	9	10.7	15	18.3	0.165
Central and peripheral nervous system disord.	5	6.0	7	8.5	0.520
Vision disorders	2	2.4	2	2.4	0.981
Hearing and vestibular disorders	2	2.4	1	1.2	0.574
Psychiatric disorders	5	6.0	5	6.1	0.969
Gastro-intestinal system disorders	11	13.1	17	20.7	0.189
Liver and biliary system disorders	1	1.2	1	1.2	0.986
Metabolic and nutritional disorders	4	4.8	2	2.4	0.423
Endocrine disorders	–		1	1.2	0.310
Cardiovascular disorders, general	4	4.8	7	8.5	0.328
Myo-, endo-, pericardial and valve disorders	1	1.2	2	2.4	0.560
Heart rate and rhythm disorders	–		1	1.2	0.310
Vascular (extracardiac) disorders	3	3.6	2	2	0.670
Respiratory system disorders	7	8.3	12	1.6	0.202
Red blood cell disorders	–		1	1.2	0.310
White cell and reticuloendothelial system disorders	1	1.2	–		0.322
Platelet, bleeding and clotting disorders	2	2.4	–		0.160
Urinary system disorders	4	4.8	3	3.7	0.724
Reproductive disorders, male	1	1.2	–		0.322
Reproductive disorders, female	–		2	2.4	0.150
Neoplasm	–		1	1.2	0.310
Body as a whole—general disorders	20	23.8	19	23.2	0.923
Resistance mechanism disorders	6	7.1	7	8.5	0.738
Operations	–		1	1.2	0.310
Injuries	4	4.8	8	9.8	0.214

consequence for safety. No drug–disease or drug–drug interactions were noted, and no deaths were reported.

The assessments of local tolerability assessed by the patients and investigators (Table 24) showed that the injections were overall acceptable and well tolerated with no reports of “bad” tolerability. At study end, 78 of 79 patients treated with OST and 77 of 80 patients treated with PLA rated the tolerability ‘good’ or ‘very good’. There were four serious adverse events in the patients who received Osteochondrin and four who were in the placebo group (Table 25). It was considered that the relationship with the study group was excluded or rated unlikely in all these cases. The outcomes for most of these cases were either complete recovery after intervention or in the case of the arthritic conditions would be expected to be unresolved because of the progressive nature of the disease.

Table 19 Deviations of normal laboratory ranges

Parameter	OST		PLA	
	<i>N</i>	—+—	<i>N</i>	—+—
Leucocytes	66	1...2	70	1...4
Erythrocytes	69	6...2	66	6...3
Haematocrit	68	1...1	65	2...3
Haemoglobin	70	1...1	61	2...1
Platelets	68	1...2	74	— 2
Neutrophils	60	6...—	62	3...1
Basophils	60	—...3	60	2...1
Eosinophils	59	1...4	61	1...5
Lymphocytes	54	6...6	57	5...1
Monocytes	58	—...1	66	1...4
Others	69	—...—	67	—...2
Quick's time	66	—...1	67	1...1
PTT	58	— 2	53	—...—
Sodium	62	2...—	70	—...1
Potassium	65	—...1	72	2...2
Calcium	67	5...—	71	1...2
Phosphate	40	4...—	39	4...—
Glucose	58	1...4	57	3...4
Total cholesterol	28	— 8	26	— 5
LDL cholesterol	38	—...5	36	—...3
HDL cholesterol	36	4...1	32	2...2
Triglycerides	55	— 8	50	— 2
Creatinine	68	1...5	68	1...2
Uric acid	56	— 6	69	— 4
Urea	57	1...5	55	—...3
Blood-urea nitrogen	6	—...2	2	—...1
GOT (AST)	60	—...3	60	—...2
GPT (ALT)	64	— 7	68	— 2
γ-GT	54	—...5	62	—...4
Alk. phosphatase	67	—...2	68	1...5
Total bilirubin	67	—...3	68	— 5

Pre-post changes of the laboratory parameters are represented by means of shift tables. In case of normal pre-treatment values, changes to abnormal occurred in the following frequencies
N number of patients with normal pre-treatment values.
 — = abnormal low after treatment. + = abnormal high after treatment

It is worth noting that the study population had a considerable number of concomitant circulatory, endocrine, nutritional and metabolic diseases and immunity disorders (Table 26). The occurrence of other chronic conditions (e.g. malignancies, hyperthyroidism, GI and mental conditions) whether treated or untreated was relatively low and probably reflected the population at large. Since no drug–disease interactions were noted, it is unlikely that the presence of these conditions appreciably influenced the outcome of responses to the treatments.

Table 20 Laboratory findings from clinical chemistry and haematological screening

Parameter		OST			PLA			t test (p-value)
		N	X	SD	N	X	SD	
Leucocytes	[10 ⁹ /L]	74	0.32	2.36	75	-0.02	1.76	0.332
Erythrocytes	[10 ¹² /L]	74	0.01	0.40	75	-0.02	0.34	0.617
Haematocrit	[%]	74	-0.05	3.14	75	0.22	2.33	0.546
Haemoglobin	[g/dL]	74	-0.08	1.04	75	0.07	0.82	0.351
Platelets	[10 ⁹ /L]	74	-8.62	58.27	76	1.91	69.20	0.316
Neutrophils	[%]	70	-0.10	8.51	70	0.59	8.17	0.622
Basophils	[%]	70	0.16	1.31	70	-0.07	0.78	0.214
Eosinophils	[%]	70	-0.25	1.70	70	-0.00	2.55	0.503
Lymphocytes	[%]	70	0.11	7.14	70	-0.30	6.85	0.731
Monocytes	[%]	70	-0.14	3.07	70	-0.07	2.07	0.882
Others	[%]	70	0.19	1.27	70	-0.17	1.72	0.161
Quick's time	[%]	68	0.81	7.26	67	-0.81	6.95	0.190
PTT	[s]	61	-0.23	3.25	61	-0.54	5.23	0.698
Sodium	[mmol/L]	70	1.51	4.46	76	0.84	4.13	0.346
Potassium	[mmol/L]	71	-0.16	0.87	76	-0.20	0.67	0.762
Calcium	[mmol/L]	71	-0.03	0.15	73	0.01	0.30	0.314
Phosphate	[mg/L]	44	-0.11	0.98	45	-0.04	1.11	0.736
Glucose	[mg/dL]	72	-5.51	40.05	75	0.89	20.52	0.222
Total cholesterol	[mg/dL]	74	-5.91	36.03	75	-12.43	28.98	0.225
LDL cholesterol	[mg/dL]	55	-9.38	29.74	56	-7.50	20.56	0.699
HDL cholesterol	[mg/dL]	54	-0.62	10.39	54	3.40	12.19	0.068
Triglycerides	[mg/dL]	72	9.19	62.82	74	-7.47	53.21	0.086
Creatinine	[mg/dL]	74	0.00	0.14	76	0.00	0.10	0.986
Uric acid	[mg/dL]	73	-0.02	1.23	76	0.04	1.01	0.723
Urea	[mg/dL]	58	2.05	8.50	63	0.32	9.89	0.308
Blood-urea nitrogen	[mg/dL]	6	0.60	5.50	5	4.80	8.29	0.340
GOT (AST)	[U/L]	74	-0.10	3.22	72	0.85	5.29	0.193
GPT (ALT)	[U/L]	72	1.13	4.70	76	0.24	7.62	0.398
γ-GT	[U/L]	74	0.65	12.04	76	4.92	35.16	0.323
Alk. phosphatase	[U/L]	71	1.51	30.01	74	7.12	59.02	0.474
Total bilirubin	[mg/dL]	68	-0.04	0.19	69	0.03	0.20	0.036

Changes from baseline to endpoint in each individual laboratory parameter were compared between the trial group. N = number of patients

Table 21 Display of clinically significant abnormalities of laboratory findings (bold type: abnormal, in brackets: clinically significant)

Group	Parameter	V1	V3	V5	V7
OST	Total cholesterol [mg/dL]	264	–	–	(290)
	LDL cholesterol [mg/dL]	146	–	–	(160)
	HDL cholesterol [mg/dL]	42	–	–	(36)
OST	Total cholesterol [mg/dL]	(245)	–	–	260
	LDL cholesterol [mg/dL]	(160)	–	–	140
	HDL cholesterol [mg/dL]	(41)	–	–	40
OST	Glucose [mg/dL]	(185)	–	–	(196)
OST	Total cholesterol [mg/dL]	(343)	–	–	229
	LDL cholesterol [mg/dL]	(239)	–	–	120
OST	Glucose [mg/dL]	(297)	–	–	65
	Total cholesterol [mg/dL]	(258)	–	–	263
	LDL cholesterol [mg/dL]	(217)	–	–	159
	HDL cholesterol [mg/dL]	(32)	–	–	44
	Triglycerides [mg/dL]	(376)	–	–	233
	Uric acid [mg/dL]	(11.7)	–	–	6.6
	GOT (AST) [U/L]	(21)	–	–	15
	γ-GT [U/L]	(290)	–	–	223
OST	Triglycerides [mg/dL]	268	–	–	(461)
	γ-GT [U/L]	37	–	–	(68)
OST	Triglycerides [mg/dL]	(404)	–	–	(324)
OST	Neutrophils [%]	43.9	(71.2)	–	42.5
	Lymphocytes [%]	39.2	(22.3)	–	45.3
OST	Total cholesterol [mg/dL]	(250)	198	–	198
	LDL cholesterol [mg/dL]	(182)	138	–	117
OST	γ-GT [U/L]	(109)	81	–	–
OST	Glucose [mg/dL]	(130)	–	–	130
OST	Glucose [mg/dL]	(143)	118	–	101
OST	Glucose [mg/dL]	(292)	–	–	180
PLA	Total cholesterol [mg/dL]	(250)	–	–	(240)
	LDL cholesterol [mg/dL]	(160)	–	–	(155)
	HDL cholesterol [mg/dL]	(36)	–	–	(38)
	Triglycerides [mg/dL]	(205)	–	–	(196)
PLA	Glucose [mg/dL]	(166)	–	–	(176)
	Total cholesterol [mg/dL]	(339)	–	–	(350)
	LDL cholesterol [mg/dL]	(160)	–	–	(165)
	HDL cholesterol [mg/dL]	(32)	–	–	(30)
	Triglycerides [mg/dL]	(210)	–	–	(235)
PLA	Triglycerides [mg/dL]	(723)	322	505	688
	Uric acid [mg/dL]	(11.4)	9.3	7.2	10.9
	γ-GT [U/L]	(163)	72	171	241
PLA	HDL cholesterol [mg/dL]	(49)	–	–	51
PLA	Eosinophils [%]	2.1	(6.3)	–	3.4
	Lymphocytes [%]	30.8	(17.2)	–	32.5
	Total cholesterol [mg/dL]	(324)	179	–	219

(continued)

Table 21 (continued)

Group	Parameter	V1	V3	V5	V7
PLA	Total cholesterol [mg/dL]	(249)	(315)	–	221
	LDL cholesterol [mg/dL]	137	(189)	–	149
	Triglycerides [mg/dL]	104	(244)	–	53
PLA	Leucocytes [10 ⁹ /L]	8.7	(13.8)	–	5.1
PLA	Potassium [mmol/L]	4.7	4.4	(3)	3.7
PLA	Haemoglobin [g/dL]	(7.5)	–	–	–
PLA	Glucose [mg/dL]	(173)	–	–	80
PLA	γ-GT [U/L]	33	–	–	(313)
	Alk. phosphatase [U/L]	176	–	–	(579)
PLA	Glucose [mg/dL]	(120)	–	–	137
	Total cholesterol [mg/dL]	(323)	–	–	267
	LDL cholesterol [mg/dL]	(197)	–	–	176
	HDL cholesterol [mg/dL]	(39)	–	–	40
	Triglycerides [mg/dL]	(389)	–	–	233
PLA	Glucose [mg/dL]	(272)	–	–	–

13 patients in the OST-group and 13 patients in the PLA-group were concerned with at least one clinically significant abnormality (V1 and control value at V3, V5 or V7 present)

Table 22 Vital signs (Heart rate, HR; Systolic blood pressure, BPS; Diastolic blood pressure, BPD) in the course of study. Comparison between OST and PLA of changes from baseline to end of study in vital signs

Parameter	OST			PLA			t test (<i>p</i> -value)
	<i>N</i>	<i>X</i>	SD	<i>N</i>	<i>X</i>	SD	
HR [bpm]	75	0.20	6.24	74	1.20	8.27	0.405
BPS [mmHg]	75	–2.92	13.12	74	–3.01	14.80	0.968
BPD [mmHg]	75	–0.15	7.37	74	–0.84	6.89	0.555

Table 23 Vital signs (Heart rate, Systolic and Diastolic blood pressure) in the course of treatment

Heart rate after 5' in sitting position [bpm]: OST-group				
Stat. estimate	V1	V3	V5	V7
N	75	54	42	70
Mean	73.9	72.9	74.3	74.0
SD	8.7	8.0	8.4	7.1
Median	72.0	72.0	76.0	73.0
Heart rate after 5' in sitting position [bpm]: PLA-group				
N	74	48	46	70
Mean	73.2	73.9	75.5	74.5
SD	6.8	8.8	8.3	9.0
Median	72.0	72.0	76.0	73.0

(continued)

Table 23 (continued)

Heart rate after 5' in sitting position [bpm]: OST-group				
Stat. estimate	V1	V3	V5	V7
<i>Systolic blood pressure after 5' in sitting position [mmHg]: OST-group</i>				
N	75	55	42	70
Mean	140.7	138.0	138.1	138.2
SD	13.3	16.1	12.1	12.7
Median	140.0	140.0	140.0	140.0
<i>Systolic blood pressure after 5' in sitting position [mmHg]: PLA-group</i>				
N	74	49	46	71
Mean	142.2	140.3	138.7	139.4
SD	16.5	14.7	17.0	15.1
Median	143.5	140.0	140.0	140.0
<i>Diastolic blood pressure after 5' in sitting position [mmHg]: OST-group</i>				
N	75	55	42	70
Mean	82.8	81.9	82.1	82.7
SD	6.1	8.1	6.6	7.1
Median	80.0	80.0	80.0	80.0
<i>Diastolic blood pressure after 5' in sitting position [mmHg]: PLA-group</i>				
N	74	49	46	71
Mean	83.9	83.7	82.8	83.1
SD	7.7	6.2	7.3	6.8
Median	81.0	80.0	80.0	80.0

Table 24 Global assessment of tolerability

Patients assessment ($p = 0.674$)				
Judgement	OST		PLA	
	Frequency	(%)	Frequency	(%)
Very good	50	63.3	52	65.0
Good	28	35.4	25	31.3
Moderate	1	1.3	2	2.5
Bad	–	–	1	1.3
Investigators assessment ($p = 0.571$)				
Very good	52	65.8	56	70.0
Good	26	32.9	21	26.3
Moderate	1	1.3	2	2.5
Bad	–	–	1	1.3

High placebo responses as shown in this study were already known in osteoarthritis studies especially when the medication was applied by injection (Zhang et al. 2008; Doherty and Dieppe 2009; Zeidler 2011; Abhishek and Doherty 2013). Since this is an unsolved problem in clinical studies, the proved safety of Osteochondrin S might be of high significance.

Table 25 Serious adverse events

Treatment group	Item	Findings
OST	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a Follow-up	Arthrosis Acute exacerbation of coxarthrosis 73 years Female No AE persisting/still under treatment Completely recovered at follow-up dated 471 days after onset of the event
OST	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a	Haematemesis Gastric haemorrhage 62 years Male Unlikely Recovered completely
OST	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a	Diabetes mellitus Exacerbation of Diabetes mellitus 71 years Female No Recovered completely
OST	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a	Arthralgia Acute pain in the left knee 46 years Male Unlikely Recovered completely
PLA	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a	Fistula of the bladder Vesical fistula 74 years Female No Recovered completely
PLA	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a	Chest pain Unclear chest pain 74 years Female No Recovered completely
PLA	WHO preferred term AE Age of patient Gender Drug relationship Outcome ^a Follow-up	Arthrosis Joint effusion of the knee 63 years Female No AE persisting/still under treatment Knee joint endoprosthesis at both knees within 2 years after onset of the event

(continued)

Table 25 (continued)

Treatment group	Item	Findings
PLA	WHO preferred terms	Uterine carcinoma, Uterine disorder nos
	AE	Ca. in situ, descensus uteri vaginae
	Age of patient	71 years
	Gender	Female
	Drug relationship	No
	Outcome ^a	Recovered completely

^aOutcome at individual discontinuation of study

Table 26 Rate of concomitant diseases at study onset

ICD-9 Code	Diagnosis	Frequency (%)
ICD VII	Diseases of the circulatory system	54.8
ICD III	Endocrine, nutritional, and metabolic diseases and immunity disorders	35.5
ICD IX	Diseases of the digestive system	10.8
ICD XVI	Symptoms, signs, and Ill-defined conditions	10.2
ICD VI	Diseases of the nervous system and sense organs	9.6
ICD VIII	Diseases of the respiratory system	9.6
ICD X	Diseases of the genitourinary system	8.4
ICD V	Mental disorders	3.6
ICD XIII	Diseases of the musculo-skeletal system and connective tissue	3.0
ICD XII	Diseases of the skin and subcutaneous tissue	2.4
ICD XVII	Injury and poisoning	1.2
ICD I	Infectious and parasitic diseases	0.6
ICD IV	Diseases of the blood and blood-forming organs	0.6

4 Future Developments and Conclusions

Micro-RNA research is expected to induce the development of new medicinal products showing specific effects in the cellular metabolism with high benefit in patients with degenerative diseases and cancer. These products will be based on well-characterised synthetic RNA entities, detailed descriptions of pharmacological properties and proven efficacy in clinical applications. Natural RNA extracts from animal tissues or yeast provide mixtures, like most natural extracts from plants, which were not yet characterised in detail but with clinically proven relevance and with a background of decades of experience in a safe application in patients with chronic and degenerative diseases. Since natural RNA extracts contain a variety of micro-RNAs, further progress in micro-RNA research might lead to a better understanding of the pharmacological properties of natural RNA extracts confirming decades of clinical experience with these products.

References

- Abhishek A, Doherty M (2013) Mechanisms of the placebo response in pain in osteoarthritis. *Osteoarthritis Cartilage* 21:1229–1235
- Abouheif MM, Nakasa T, Shibuya H, Niimoto T, Kongcharoensombat W, Ochi Mitsuo (2010) Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. *Rheumatology* 49:2054–2060
- Agrawal S, Tamsamani J, Galbraith W, Tang J (1995a) Pharmacokinetics of antisense oligonucleotides. *Clin Pharmacokinet* 28:7–16
- Agrawal S, Zhang X, Lu Z, Zhao H, Tamburin JM, Yan J, Cai H, Diasio RB, Habus I, Jiang Z, Iyer RP, Yu D, Zhang R (1995b) Absorption, tissue distribution and in vivo stability in rats of a hybrid antisense oligonucleotide following oral administration. *Biochem Pharmacol* 50:571–576
- Akhtar N, Haqqi TM (2012) MicroRNA-199a* regulates the expression of cyclooxygenase-2 in human chondrocytes. *Ann Rheum Dis* 71:1073–1080
- Aksenov OA, Golovin BP, Smorodintsev AA (1970) Antiviral action of the yeast RNA derivatives and the effect of polycation thereon. *Westn Akad Med Nauk SSSR* 25(8):51–58 (Russian)
- Altman RD (1991) Criteria for classification of clinical osteoarthritis. *Litera Rheumatol* 13:63–73
- Amos H, Moore MO (1963) Influence of bacterial ribonucleic acid on animal cells in culture, I. Stimulation of protein synthesis. *Exp Cell Res* 32:1–13
- Arct W (1982) Conservative treatment of severe osteoarthritis of the knee and hip joint with long-term injections of artemparon. *Chir Narzadow Ruchu Ortop Pol* 47:115–118 (Polish)
- Ashley FL, McNall EG, Dutt NR, Garcia EN, Sloan RF (1960) The effect of nucleic acids on homograft tolerance. *Ann NY Acad Sci* 87:429–444
- Babayan R, Bethge JFJ, von Fehrenthell R, ten Hoff H (1979) Versuch der biochemischen Beeinflussung der Frakturheilung im Tierexperiment. *Med Welt* 30:1725 (German)
- Babiichuk GA, Belous AM (1969) Effects of exogenous homologous RNA and hydrocortisone on the content of collagen and glycosaminoglycan in regenerating rat bone. *Bull Exp Biol Med* 68:1151–1154
- Baker CL Jr, Ferguson CM (2005) Future treatment of osteoarthritis. *Orthopedics* 28(2 Suppl):s227–s234
- Barry ELR, Gesek FA, Friedman PA (1993) Introduction of antisense oligonucleotides into cells by permeabilization with Streptolysin O. *Biotechniques* 15:1016–1020
- Batkin S (1966) The effect of RNA on the recovery of spinal-sectioned carp (*Cyprinus carpio*). *Proc Natl Acad Sci* 56:1689–1691
- Bazanova OM, Vlassov VV, Zarytova VP, Ivanova IM, Kuligina EA, Yakubov LA, Abdukayubov MN, Karamyshev VN, Zon G (1991) Oligonucleotide derivatives in organism: distribution in organs, rates of release and degradation. *Nucleotides Nucleosides* 10:523–525
- Becker S, Schühlein S, Meyer W (1995) Ribonukleinsäuren – Theorie und Therapie. *J Orthomolek Med* 3:83–106 (German)
- Bekman EM, Baranova OA, Vlasenko RYa, Arion VYa (2001) Effects of low-molecular-weight thymic RNA on T cell-dependent antibody formation. *Bull Exptl Biol Med* 4:971–974
- Beljaew DK, Videlets IJ, Matienko NA, Gruntenko EV (1974) The influence of early thymectomy and administration of heterologous RNA on the appearance of spontaneous mammary tumors in mice. *Vopr Onkol* 20(7):63–66
- Beljanski M, Plawecki M (1979) Particular RNA fragments as promoters of Leukozyte and Platelet formation in rabbits. *Expl Cell Biol* 47:218–225
- Beljanski M, Plawecki M, Bourgareil P, Beljanski M (1983) Leukocyte recovery with short-chain RNA fragments in cyclophosphamide-treated rabbits. *Cancer Treat Rep* 67:611–619
- Beljanski M (1991) Radioprotection of irradiated mice-mechanisms and synergistic action of WR-2721 and R.L.B. *Dtsch Zschr Onkol* 23:6
- Bellamy N (2005) WOMAC Osteoarthritis Index User Guide. Version VII. Brisbane, Australia

- Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G (2005a) Intraarticular corticosteroid for treatment of osteoarthritis of the knee. *Cochrane Database Syst Rev* 18: CD005328
- Bellamy N, Bell MJ, Goldsmith CH, Pericak D, Walker V, Raynauld JP, Torrance GW, Tugwell P, Polissin R (2005b) Evaluation of WOMAC 20, 50, 70 response criteria in patients treated with hylan G-F 20 of knee osteoarthritis. *Ann Rheum Dis* 64: 881–885
- Belous AM, Pankow EJ (1966) Strukturell-morphologische Besonderheiten der Regeneration des Knochengewebes unter dem Einfluss von Ribonucleinsäure. *Ortop Travmatol Protez* 27(8):14–19 (Russian)
- Belous AM, Pankov EY, Gusakova VA, Savenko NF, Timoshenko OP (1969) Effect of exogenous RNA and ultrasound on fracture healing in rats. *Bull Exp Biol Med* 67:542–544
- Belous AM (1971) Ribonucleinsäure stimuliert Heilung von Knochenbrüchen. *Zbl Pharm* 110:890 (German)
- Bennet RM, Hefeneider SH, Bakke A, Merritt M, Smith CA, Mourich D, Heinrich MC (1988) The production and characterization of murine monoclonal antibodies to a DNA receptor on human leukocytes. *J Immunol* 140:2937–2942
- Bennet RM, Cornell KA, Merritt MJ, Bakke AC, Hsu PH, Hefeneider SH (1991) Autoimmunity to a 28–30 kD cell membrane DNA binding Protein: occurrence in selected Sera from Patients with SLE and mixed connective tissue disease (MCTD). *Clin Exp Immunol* 86:374–379
- Berg PA, Kaboth U, Becker EW, Klein R (1992) The analysis of a severe side effect of a cartilage-protective agent by immunological studies. *Dtsch Med Wochenschr* 117:1589–1593 (German)
- Bethge JFJ, Babayan R, Borm HP, von Fehrentell R, ten Hoff H, Hose H, Mangels P, Piening H, Reimers C, Wilder U (1979) Versuch der biochemischen Beeinflussung der Frakturheilung im Tierexperiment. *Res Exp Med* 175:197 (German)
- Bogdanovsky D, Hermann W, Schapira G (1973) Presence of a new RNA species among the initiation protein factors active in eucaryotes translation. *Biochem Biophys Res Com* 54(1):25–32
- Borecky L, Buchvald J, Adlerova E, Stodola I, Obrucnikova E, Gruntova Z, Lackovic V, Duskocil J (1978) Results of a five-year study of the curative effect of double stranded ribonucleic acid in viral dermatoses and eye diseases. *Adv Exp Med Biol* 110:175–191
- Bormann v F, Reyher-Pauly v S (1972) RN 13, eine Kombination aus heterologen organspezifischen Ribonukleinsäuren. Untersuchungen über sein Verträglichkeit im Tierversuch. *Ztschr f Ther* 10:154–158 (German)
- Brandt KD, Flusser D (1991) Osteoarthritis. In: Bellamy N (ed) *Prognosis in the Rheumatic Diseases*. Kluwer Academic Publishers, Lancaster, pp 11–35
- Brandt KD, Mazzuca SA (2005) Lessons learned from nine clinical trials of disease-modifying osteoarthritis drugs. *Arthritis Rheum* 52(11):3349–3359
- Brenner SS, Klotz U, Alschner DM, Mais A, Lauer G, Schweer H, Seyberth HW, Fritz P, Bierbach U (2004) Osteoarthritis of the knee—clinical assessment and inflammatory markers. *Osteoarthritis Cartilage* 12:469–475
- Breslavskij AS, Maximov SV, Lapynina LA, Magkaeva LV (1978) Restoration process in cells of the insular apparatus of pancreas in Alloxan-diabetic rats under the effect of RNA. *Fiziol Zh* 24 (1):23–28 (Russian)
- Bruyere O, Pavelka K, Rovati LC, Deroisy R, Olejarova M, Gatterova J, Giacobelli G, Reginster JY (2004) Glucosamine sulfate reduces osteoarthritis progression in postmenopausal women with knee osteoarthritis: evidence from two 3-year studies. *Menopause* 11:138–143
- Burmeister G, Rainsford KD (1991) Discriminating effects of nucleotide-rich yeast extracts, probioticum, as an immunomodulator contrasted with actions in chronic immunoinflammatory disease (adjuvant-induced arthritis) in rodents. *Inflammopharmacology* 1:161–183
- Buchanan WW, Kean WF (2002a) Osteoarthritis IV: clinical therapeutic trials and treatment. *Inflammopharmacology* 10:79–155
- Buchanan WW, Kean WF (2002b) Osteoarthritis II: pathology and pathogenesis. *Inflammopharmacology* 10:23–52

- Buchanan WW, Kean WF, Kean R (2003) History and current status of osteoarthritis in the population. *Inflammopharmacology* 11:301–316
- Buchanan WW, Kean WF (2002c) Osteoarthritis III: radiological and clinical definition. *Inflammopharmacology* 10:53–78
- Caborn D, Rush J, Lanzer W, Parenti D, Murray C, Synvisc 901 Study Group (2004) A randomized, single-blind comparison of the efficacy and tolerability of hylan G-F 20 and triamcinolone hexacetonide in patients with osteoarthritis of the knee. *J Rheumatol* 31:333–343
- Cameron DE, Solyom L (1961) Effects of ribonucleic acids on memory. *Geriatrics* 16:74–81
- Cameron DE, Sved S, Solyom L, Wainrib B, Barik H (1963) Effect of ribonucleic acid on memory defect in the aged. *Am J Psychiatry* 120:320–325
- Cantley M, Haynes DR, Newton J, Clench MA, Rainsford KD (2010a) Functional osteoclast bioassay using human mononuclear precursors for determining control of bone resorption by ribonucleinate components of Osteochondrin® S. In: MipTec drug discovery conference, Basel, Switzerland, Abstract 133
- Cantley MD, Haynes DR, Rainsford KD (2010b) Effects of ribonucleinate components of Osteochondrin® S osteoarthritis therapy on formation and activity of human osteoclasts in vitro. In: 2010 World congress of osteoarthritis. Osteoarthritis Research Society International (OARSI), Osteoarthritis and Cartilage, 18 (Suppl. 2): Abstract 569, S254
- Cantley MD, Rainsford KD, Haynes DR (2013) Effects of Osteochondrin S and select connective tissue ribonucleinate components on human osteoclasts in vitro. *J Pharm Pharmacol* 65:1214–1222
- Caujolle F (1966) Rapport D'Expertise Toxicologique et D'Expertise Pharmacologique du Professeur F. Caujolle (in House Report, Dyckerhoff) (French)
- Chassany O, Sagnier P, Marquis P, Fullerton S, Aaronson N (2002) Patient-reported outcomes: the example of health-related quality of life—an European guidance document for the improved integration of health-related quality of life assessment in the drug regulatory process. *Drug Info J* 36:209–238
- Cheknev SB, Mikovskaya OI, Meshkova EN, Khaldin AA, Samgin MA, Malinovskaya VV (1994) Time-course of natural-killer-cell activity and interferon status in patients with relapsing herpes genitalis in the course of therapy with rhidostin. *Vopr Virusol* 39:125–128
- Chernukh AM, Vyshepan ED, Razumova IL, Alekseeva NN, Chinenova NS (1970) Effect of liver RNA on the course of experimental cirrhosis of the liver. *Bull Exp Biol Med* 70:1112–1114
- Chernukh AM, Vyshepan ED, Shukalova TF, Alekseeva NN, Razumova IL (1971) Effect of homologous hepatic RNA on liver collagen and tryptophan pyrrolase in rats poisoned with CCl₄. *Bull Exp Biol Med* 71:640–642
- Chevallard M, Galanti A, Paresce E, Wolf A, Carrabba M (1993) Efficacy and tolerability of galactosamino-glycuronoglycan-sulfate in osteoarthritis of the knee: an 11-month experience. *Int J Clin Pharmacol Res* 13(Suppl):49–53
- Chin DJ, Green GA, Zon G, Szoka FC Jr, Straubinger RM (1990) Rapid nuclear accumulation of injected oligodeoxyribonucleotides. *New Biol* 2:1091–1100
- Cook L, Davidson AB (1963) Ribonucleic acid: effect on conditioned behavior in rats. *Science* 141:268–269
- Cossum PA, Truong L, Owens SR, Markham PM, Shea JP, Crooke ST (1994) Pharmacokinetics of a ¹⁴C-labeled phosphorothioate oligonucleotide, ISIS 2105, after intradermal administration to rats. *J Pharmacol Exp Ther* 269:89–94
- Creamer P, Lethbridge-Cejku M, Hochberg MC (1998) Where does it hurt? Pain localization in osteoarthritis of the knee. *Osteoarthritis Cartilage* 6:318–323
- Crooke ST, Grillone LR, Tendolkar A, Garrett A, Fratkan MJ, Leeds J, Barr WH (1994) A pharmacokinetic evaluation of ¹⁴C-labeled afovirsen sodium in patients with genital warts. *Clin Pharmacol Ther* 56:641–646
- Dai L, Zhang X, Hu X, Zhou C, Ao Y (2012) Silencing of microRNA-101 prevents IL-1 β -induced extracellular matrix degradation in chondrocytes. *Arthritis Res Ther* 14:R268
- Davis RH, Forst MB, Rand SA, Bernhard L (1981) Prevention of adjuvant arthritis with ribonucleic acid. *J Am Podiatr Assoc* 71:482–486

- Davis RH, Shapiro E, Agnew PS (1985) Topical effect of Aloe with ribonucleic acid and vitamin C on adjuvant arthritis. *J Am Podiatr Assoc* 75:229–237
- Davis HP, Squire LR (1984) Protein synthesis and memory: a review. *Psychol Bull* 96:51–559
- DeCarvalho S, Rand HJ (1961) Comparative effects of liver and tumour ribonucleic acids on the normal liver and the Novikoff hepatoma cells of the rat. *Nature* 189:815–817
- Demin AA (1973) Clinical and experimental investigations into the action of the ribonucleic acid preparations in leukemia. *Sov Med* 36:23–27 (Russian)
- Deshpande AK, Jakowlew SB, Arnold H-H, Crawford PA, Siddiqui MAQ (1977) A novel RNA affecting embryonic gene functions in early chick blastoderm. *J Biol Chem* 252:6521–6527
- Deshpande AK, Siddiqui MAQ (1978) Acetylcholinesterase differentiation during myogenesis in early chick embryonic cells caused by an inducer RNA. *Differentiation* 10:133–137
- Díaz-Prado S, Cicione C, Muiños-López E, Hermida-Gómez T, Oreiro N, Fernández-López C, Blanco FJ (2012) Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. *BMC Musculoskelet Disord* 13:144
- Di Giovanna I, Hayes G (eds) (2001) Principles of clinical research. Wrightson Biomedical Publishing, Petersfield
- Doherty M, Dieppe P (2009) The “placebo” response in osteoarthritis and its implications for clinical practice. *Osteoarthritis Cartilage* 17(10):1255–1262
- Dong S, Yang B, Guo H, Kang F (2012) MicroRNAs regulate osteogenesis and chondrogenesis. *Biochem Biophys Res Commun* 418:587–591
- Dworkin RH, Tu DC, Peirce-Sandner S, He H, McDermott MP, Hochberg MC, Jordan JM, Katz NP, Lin AH, Neogi T, Rappaport BA, Simon LS, Strand V (2014) Meta-analysis of assay sensitivity and study features in clinical trials of pharmacologic treatments for osteoarthritis pain. *Arthritis Rheumatol* 66:3327–3336
- Ebel JP, Beck G, Keith G, Langendorff H, Langendorff M (1969) Study of the therapeutic effect on irradiated mice of substances contained in RNA preparations. *Int. J Radiat Biol* 16:201–209
- Engibarman AA (1977) Effect of yeast RNA on the phagocytic capacity of the leucocytes under conditions of experimental focal myocardial necrosis. *Eksp Klin Med* 17(6):40–43 (Russian)
- Esposito S (1964) Effect on leukaemic cells of ribonucleic acid extracted from calf's spleen. *Nature* 203:1078–1079 (Italian)
- Fencel MM, Villee CA (1971) Effect of RNA from estradiol-treated immature rats on protein synthesis in immature uteri. *Endocrinology* 88:279–285
- Filatov VP, Zagorueva LL, Leontieva FS (1977) The efficacy of using exogenous bone RNA in surgical treatment of chronic epitympanitis. *Vestn Otorinolaringol* 4:22–25 (Russian)
- Fornadi F (1993) from Korth A (ed) Fortbildungsseminare 1993 Regeneresen. Ralf Reglin Verlag, Köln 29–36 (German)
- Frolov VM, Razenkova AT (1980) Use of sodium nucleinate in the complex therapy of viral hepatitis in children. *Vopr Okhr Materin Det* 23(4):12–15 (Russian)
- Fujii T, Villee CA (1969) Partial characterization of the RNA stimulating growth of the seminal vesicle. *Biochemistry* 62:836–843
- Fuks BB, Shershevskaya SF, Popova LM, Shnaper AI (1969) Effectiveness of ribonucleotides for replacement therapy in certain diseases. *Bull Exp Biol Med* 68(9):971–973 (Russian)
- Fuks BB, Shershevskaya SF, Levina FG, Shabanova ME (1971) On a specific therapeutic effect of ribonucleotides in tapetoretinal dystrophies. *Vestn Akad Med Nauk SSSR* 26(7):63–68 (Russian)
- Fukuyama S, Yoshino I, Yamaguchi M, Osoegawa A, Kameyama T, Tagawa T, Maehara Y (2005) Blockage of the macrophage migration inhibitory factor expression by short interference RNA inhibited the rejection of an allogenic tracheal graft. *Transplant Intl* 1203–1209
- Galbraith WM, Hobson WC, Giclas PC, Schechter PJ, Agrawal S (1994) Complement activation and hemodynamic changes following intravenous administration of phosphorothioate oligonucleotides in the monkey. *Antisense Res Dev* 4:201–206
- Gao W-Y, Storm C, Egan W, Cheng Y-C (1993) Cellular pharmacology of phosphorothioate homooligodeoxynucleotides in human cells. *Mol Pharmacol* 43:45–50

- Gasparyan MG, Dzhagatspanyan NG, Gevorkyan RA, Kamalyan LA (1991) Anti tumour effect of the interferon inducer larifan experimentally and clinically. *Vopr Virusol* 36:127–130
- Germaniuk IaL, Minchenko AG, (1972) The effect of repeated injections of hydrocortisone and sodium ribonucleinate on RNA concentration in the mitochondria of white rat organs. *Bull Eksp Biol Med* (2):53–55 (Russian)
- Germaniuk IaL, Minchenko AG, Kozyriskii VG (1976) Ultrastructural changes in the livers of rats following multiple injections of corticotropin and sodium ribonucleinate. *Tsitol Genet* 10 (5):409–412 (Russian)
- Germaniuk IaL, Goidash MM (1976) Aminoacyl-tRNA formation in the liver and skeletal muscles of rabbits under the influence of multiple injections of ACTH alone and in combination with sodium ribonucleinate. *Probl Endokrinol (Mosk)* 22(2):83–86 (Russian)
- Germaniuk IaL, Minchenko AG (1982) Action of perorally administered sodium ribonucleinate on RNA biosynthesis in the aortic, cardiac, liver and kidney tissue of animals with experimental diabetes mellitus. *Farmakol Toksikol* 45(1):50–52. Russian
- Geselowitz DA, Neckers LM (1992) Analysis of oligonucleotide binding, internalization, and intracellular trafficking utilizing a novel radiolabeled crosslinker. *Antisense Res Dev* 2:17–25
- Ghosh P, Smith M, Wells C (1992) Second-line agents in osteoarthritis. In: Dixon JS, Furst DE (eds) *Second-line agents in the treatment of rheumatic diseases*. Marcel Dekker, New York, pp 363–427
- Gibson NW (2014) Engineered microRNA therapeutics. *J R Coll Phys Edinb* 44:196–200
- Gifford GE (1965) Inhibitory effect of mononucleotides on virus plaque formation. *P.S.E.B.M.* 119:9–12
- Giles RV, Spiller DG, Tidd DM (1995) Detection of ribonuclease H-generated fragments in human leukemia cells following reversible membrane permeabilization in the presence of oligodeoxynucleotides. *Antisense Res Dev* 5:23–31
- Goldberg VM, Buckwalter JA (2005) Hyaluronans in the treatment of osteoarthritis of the knee: evidence for disease-modifying activity. *Osteoarthritis Cartilage* 13:216–224
- Goldring MB, Marcu KB (2012) Epigenomic and microRNA-mediated regulation in cartilage development, homeostasis, and osteoarthritis. *Trends Mol Med* 18(2):109–118
- Goossens N, Gastpar H (1960) Über die Wirkungsweise und Toxizität von Regeneresen. *Med Welt* 32:1636–1640 (German)
- Gottwik HE (1989) Die Behandlung von Tinnituspatienten mit organspezifischen Ribonukleinsäuren. *Therapeutikon* 3:412–418 (German)
- Grabowska M, Brysch B, Jurka J, Chorazy M (1981) Stimulatory effect of hnRNA on template activity of isolated rat liver chromatin. *Acta Biochim Polon* 28:135–148
- Groth CG, Porter KA, Daloze PM, Huguet C, Smith GV, Brettschneider L, Starzl TE (1968) Effect of ribonucleic acid perfusion on canine kidney and liver homograft survival. *Surgery* 64:31–38
- Guyette CA, Chavis DM, Chearer DH (1980) The effect of ribonucleic acid injections upon the maze-learning ability of rats. *Physiol Behav* 24:971–974
- Hawley P, Gibson I (1992) The detection of oligodeoxynucleotide molecules following uptake into mammalian cells. *Antisense Res Dev* 2:119–127
- Held K, Schnitker J, Schulte-Körne-Kunsleben Ch (1989) Biometrische Auswertung einer kontrollierten Untersuchung der Wirksamkeit und Verträglichkeit von RN13 Regeneresen bei zerebraler Insuffizienz, In-House-Report, Dyckerhoff. German
- Högger P (1999) Kleine Ribonukleinsäuren. *DAZ* 139(3):83–84 (German)
- Hou S, Y-C Huang, S-S Ling (1988) Effect of ribonucleic acid on serum protein in patients of chronic active hepatitis. *Chin J Intern Med* 27:272–274
- Hydén H, Pigon A (1960) A cytophysiological study of the functional relationship between oligodendroglial cells and nerve cells of deiters' nucleus. *J Neurochem* 6:57–72
- Hydén H, Egyhazi E (1963) Glial RNA changes during a learning experiment in rats. *Physiology* 49:618–623
- Ignat'ev GM, Gribencha SV, Tazulakhova EB, Barinskii IF (1988) Antiviral activity of yeast dsRNA in experimental infection with human acute encephalomyelitis virus. *Voprosy Virusologii* 2:251–253

- Ikedo S, Neyts J, de Clercq E (1994) Host defense mechanisms against murine cytomegalovirus infection induced by poly I: C in severe combined immune deficient (SCID) mice. *Proc Soc Exp Bio Med* 204:191–196
- Iliescu R, Repanovici R, Mutiu A, Sahnazarov N, Danielescu G, Popa IM, Cajal N (1983) Investigation of the effect of cellular and viral nucleic acids on certain virus infections. Note 2. Effect of nucleic acids on virus multiplication in cell cultures. *Rev Roum Med-Virol* 34:191–196
- Iversen PL, Crouse D, Zon G, Perry G (1992) Binding of antisense phosphorothioate oligonucleotides to murine lymphocytes is lineage specific and inducible. *Antisense Res Dev* 2:223–233
- Jiang X, Dutton CM, Qi WN, Block JA, Garamszegi N, Scully SP (2005) siRNA mediated inhibition of MMP-1 reduces invasive potential of a human chondrosarcoma cell line. *J Cell Physiol* 202:723–730
- Jolley WB, Inshaw DB, Eterson M (1961) Effect of ribonucleic acid on homograft survival. *Surg Forum* 12:99–101
- Kanehisa T, Kitazume Y, Ikuta K, Tanaka Y (1977) Release of template restriction in chromatin by nuclear 4,5 S RNA. *Biochim Biophys Acta* 475:501–513
- Karabun PM, Yefimov AS (1975) Use of ribonucleic acid in the complex therapy of patients with diabetes mellitus. *Vrach Delo* 4:11–12 (Russian)
- Kataoka M, Wang D-Z (2014) Non-coding RNAs including miRNAs and lncRNAs in cardiovascular biology and disease. *Cells* 3:883–898
- Katona G (1987) A clinical trial of glycosaminoglycan-peptide complex ('Rumalon') in patients with osteoarthritis of the knee. *Curr Med Res Opin.* 10: 625–633
- Kawano S, Nakamachi Y (2011) miR-124a as a key regulator of proliferation and MCP-1 secretion in synoviocytes from patients with rheumatoid arthritis. *Ann Rheum Dis* 70(1):88–91
- Kelly SJ, Loria J, Gyves MT, Ilan J (1983) Effect of transfer RNA from various sources on placental messenger RNA translation. *Mol Cell Endocrinol* 29:181–195
- Kirschner S, Walther M, Bohm D, Matzer M, Heesen T, Faller H, Konig A (2003) German short musculoskeletal function assessment questionnaire (SMFA-D): comparison with the SF-36 and WOMAC in a prospective evaluation in patients with primary osteoarthritis undergoing total knee arthroplasty. *Rheumatol Int* 23:15–20
- Klyueva GF, Timoshenko OP, Kladchenko LA, Leontjeva FS (1977) Regulation of the restorative processes in the bone tissue of animals by various preparations of bone RNA. *Ortop Travmatol Protez* 7:19–23 (Russian)
- Kotevoglou N, Iyibozkurt PC, Hiz O, Toktas H, Kuran B (2006) A prospective randomised controlled clinical trial comparing the efficacy of different molecular weight hyaluronan solutions in the treatment of knee osteoarthritis. *Rheumatol Int* 26(4):325–330
- Kral VA, Solyom L, Enesco HE (1967) Effect of short-term oral RNA therapy on the serum uric acid level and memory function in senile versus senescent subjects. *J Am Geriatr Soc* 15:364–372
- Krieg AM, Gmelig-Meyling F, Gourley MF, Kisch WJ, Chrisey LA, Steinberg AD (1991) Uptake of oligodeoxyribonucleotides by lymphoid cells is heterogeneous and inducible. *Antisense Res Dev* 1:161–171
- Krieg AM, Tonkinson J, Matson S, Zhao Q, Saxon M, Zhang L, Banja U, Yakubov L, Stein CA (1993) Modification of antisense phosphodiester oligodeoxynucleotides by a 5' cholesteryl moiety increases cellular association and improves efficacy. *Proc Natl Acad Sci USA* 90:1048–1052
- Kulkarni AD, Farnsworth WB, Rudolf FB, van Buren CT (1986) Effect of dietary nucleotides on responses to bacterial infections. *J Parent Ent Nutr* 10:169
- Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, Gilchrist DS, Reilly J, Ierna M, Fraser AR, Stolarski B, McSharry C, Hueber AJ, Baxter D, Hunter J, Gay S, Liew FY, McInnes IB (2011) MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *PNAS* 108(27):11193–11198

- Lacour J, Lacour F, Spira A, Michelson M, Petit J-Y, Delage G, Sarrazin D, Contesso G, Viguier J (1980) Adjuvant treatment with polyadenylic-polyuridylic acid (PolyA.PolyU) in operable breast cancer. *The Lancet* 2(8187):161–164
- Lacour J, Lacour F, Spira A, Michelson M, Petit J-Y, Delage G, Sarrazin D, Contesso G, Viguier J (1984) Adjuvant treatment with polyadenylic-polyuridylic acid in operable breast cancer: updated results of a randomised trial. *BMJ* 288:589–592
- Lacour J, Lacour F, Ducot B, Spira A, Michelson M, Petit JY, Sarrazin D, Contesso G (1988) Polyadenylic-polyuridylic acid as adjuvant in the treatment of operable breast cancer: recent results. *Eur J Surg Oncol* 14:311–316
- Lapik AS, Matienko NA (1970) General action and toxicity of ribonucleic acid preparations. *Farmakol i toksikol* 1:94–95 (Russian)
- Largiadèr F, Träbert E, Senning A, Humbel R, Wegmann W (1968) Retarded rejection of renal transplants with nucleic acid. *Res Progr* 12:607–609
- Le LTT, Swingle TE, Clark IM (2013) The role of MicroRNAs in osteoarthritis and chondrogenesis. *Arthritis Rheum* 65:1963–1974
- Leopold SS, Redd BB, Warme WJ, Whrle PA, Pettis PD, Shott S (2003) Corticosteroid compared with hyaluronic acid injections for the treatment of osteoarthritis of the knee. A prospective, randomized trial. *J Bone Joint Surg Am* 85-A:1197–1203
- Lequesne MG, Maheu E (2003) Clinical and radiological evaluation of hip, knee and hand osteoarthritis. *Aging Clin Exp Res* 15(5):380–390
- Lequesne M (1994) Klinische und röntgenologische Verlaufsbeobachtung bei Hüft- und Kniearthrosen – Methoden und Ergebnisse. *Zeitschrift Rheumatol* 53:243–249
- Leuschner F (1974a) Akute Toxizität von Regeneresen VC5 an Sprague-Dawley Ratten bei intravenöser Verabreichung. In-House-Report, Dyckerhoff. German
- Leuschner F (1974b) Akute Toxizität von Regeneresen VC5 an Sprague-Dawley Ratten bei intramuskulärer Verabreichung. In-House-Report, Dyckerhoff. German
- Leuschner F (1974c) Über die akute Toxizität von Regeneresen VC5 an mischrassigen Hunden bei intravenöser Verabreichung. In-House-Report, Dyckerhoff. German
- Leuschner F (1974d) Über die akute Toxizität von Regeneresen VC5 an mischrassigen Hunden bei intramuskulärer Verabreichung. In-House-Report, Dyckerhoff. German
- Leuschner F (1975) Prüfung des Einflusses von Regeneresen VC5 (Ampullenflüssigkeit) - kurz "VC%" - auf die trächtige Ratte und ihren Foetus bei intramuskulärer Verabreichung. In-House-Report, Dyckerhoff. German
- Leuschner F (1984) Acute toxicity of osteochondrin-injection solution, Batch No. 314, in Sprague-Dawley rats by using intramuskular administration. In-House-Report, Dyckerhoff. German
- Leuschner F (1988) Mutagenicity study of RN13 Batch No. 7004 in the Ames Salmonella/Microsome plate test (in Vitro). In-House-Report, Dyckerhoff. German
- Levina LD, Ambalov YM, Ostrovskaya AA (1975) Clinico-biochemical assessment of a therapeutic effect of vitohepate in acute viral hepatitis. *Ter Arkh* 8:62–66 (Russian)
- Li X, Gibson G, Kim J-S, Kroin J, Xu S, van Wijnen AJ, Im H-J (2011) MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene* 480:34–41
- Li J, Huang J, Dai L, Yu D, Chen Q, Zhang X, Dai K (2012a) miR-146a, an IL-1b responsive miRNA, induces vascular endothelial growth factor and chondrocyte apoptosis by targeting Smad4. *Arthritis Res Ther* 14:R75
- Li Y-T, Chen S-Y, Wang C-R, Liu M-F, Lin C-C, Jou I-M, Shiau A-L, Wu C-L (2012b) Amelioration of collagen-induced arthritis in mice by lentivirus-mediated silencing of microRNA-223. *Arthritis Rheum* 64(10):3240–3245
- Liang Z-J, Zhuang H, Wang G-X, Li Z, Zhang H-T, Zhang B-D (2012) MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1b-stimulated human articular chondrocyte C28/I2 cells. *Inflamm Res* 61:503–509
- Liossis SN, Tsokos GC (1998) Cellular immunity in osteoarthritis: novel concepts for an old disease. *Clin Diagn Lab Immunol* 5:427–429

- Liu WC, Godbout R, Jay E, Yu KK-Y, Krause MO (1981) Tissue and species-specific effects of small molecular weight nuclear RNA's in isolated mammalian nuclei. *Can J Biochem* 59:343–352
- Lodemann E, Hochheimer G, Pilgram M (1989) Biologische Wirkungen eines Ribonucleisäure-haltigen Arzneimittels. *Erfahrungsheilkunde* 8:490–4 (German)
- Loke SL, Stein CA, Zhang XA, Mori K, Nakanishi M, Subasinghe C, Cohen JS, Neckers JM (1989) Characterization of oligonucleotide transport into living cells. *Proc Natl Acad Sci USA* 86:3474–3478
- Malpoix P (1964) Influence of extraneous ribonucleic acid on the differentiation of haematopoietic tissue in chick embryos. *Nature* 203:520–521
- Malpoix P (1967) Effects of polyadenylic acid and actinomycin on lysine incorporation in chick embryos. *Nature* 214:1125–1126
- Mansour AM (1968) The effect of exogenous ribonucleic acid on alkaline phosphatase activity in the ovariectomized mouse uterus. *Acta Endocr* 57:465–472
- Massardo L, Watt I, Cushnaghan J, Dieppe P (1989) Osteoarthritis of the knee joint: an eight year prospective study. *Ann Rheum Dis* 48:893–897
- Matienko NA, Ronichevskaya GM, Belyaev BK, Martynova RP, Salganik RI (1971) An inhibitory effect of homologous ribonucleic acid on the growth of spontaneous tumours in mice of mice of high cancer and C.H. Lines. *Patol Fiziol Exsp Tes* 15(1):45–47 (Russian)
- Martinez-Sanchez A, Dudek KA, Murphy CL (2012) Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by MicroRNA-145 (miRNA-145). *J Biol Chem* 287:916–924
- Matsukawa T, Sakai T, Yonezawa T, Hiraiwa H, Hamada T, Nakashima M, Ono Y, Ishizuka S, Nakahara H, Lotz MK, Asahara H, Ishiguro N (2013) MicroRNA-125b regulates the expression of aggrecanase-1 (ADAMTS-4) in human osteoarthritic chondrocytes. *Arthritis Res Ther* 15:R28
- McLean MJ, Renaud J-F, Niu MC, Sperelakis N (1977) Membrane differentiation of cardiac myoblasts induced in vitro by an RNA-enriched fraction from adult heart. *Exp Cell Res* 110:1–14
- Merritt K, Johnson AG (1965) Studies on the adjuvant action of bacterial endotoxins on antibody formation. *J Immunol* 94:416–422
- Minchenko AG, Germaniuk IaL (1976) The effect of multiple injections of ACTH (alone and combined with sodium ribonucleinate) on incorporation of P32-orthophosphate into the mitochondrial RNA of the liver, spleen and heart of white rats. *Probl Endokrinol (Mosk)* 22 (1):47–50 (Russian)
- Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, Kato Y, Sato T, Lotz MK, Asahara H (2009) MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. *Arthritis Rheum* 60(9):2723–2730
- Moskowitz RW, Hooper M (2005) State-of-the-art disease-modifying osteoarthritis drugs. *Curr Rheumatol Rep* 7:15–21
- Mu J-Y (1973) from: Niu and Segal (eds) The role of RNA in reproduction and development, North-Holland Publ. Co, pp 86–89
- Münzenberg KJ (1998) OST-Studie paravertebral kontra intramuskulär. Integrierter Abschlußbericht Osteochondrin S, In-House-Report Dyckerhoff. German
- Nakamachi Y, Kawano S, Takenokuchi M, Nishimura K, Sakai Y, Chin T, Saura R, Kurosaka M, Kumagai S (2009) MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 60(5):1294–1304
- Nakamura H, Yoshino S, Kato T, Tsuruha J, Nishioka K (1999) T-cell mediated inflammatory pathway in osteoarthritis. *Osteoarthritis Cartilage* 7:401–402
- Nakasa T, Nagata Y, Yamasaki K, Ochi M (2011) A mini-review: microRNA in arthritis. *Physiol Genomics* 43:566–570

- Neustadt D, Caldwell J, Bell M, Wade J, Gimbel J (2005) Clinical effects of intraarticular injection of high molecular weight hyaluronan (Orthovisc) in osteoarthritis of the knee: a randomized, controlled, multicentre trial. *J Rheumatol* 10:1928–1936
- Niimoto T, Nakasa T, Ishikawa M, Okuhara A, Izumi B, Deie M, Suzuki O, Adachi N, Ochi M (2010) MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients. *BMC Musculoskeletal Disord* 11:209
- Niu MC, Cordova MC, Niu LC (1961) Ribonucleic acid-induced changes in mammalian cells. *Proc N.A.S.* 47:1689–1700
- Niu MC, Niu LC, Yang SF, from: Niu and Segal (1973) The role of RNA in reproduction and development. North-Holland Publ. Co., pp 90–109
- Nosik NN, Ershov FI, Nikolaeva OV, Bukata LA, Nesterova GF (1984) Interferon-inducing and antiviral activity of dsRNA of yeast origin. *Voprosy Virusologii* 6:718–720 (Russian)
- Novakova V, Albrecht I, Linhart J (1979) Total RNA content and blood flow in rat brain after RNA administration. *Activ Nerv Sup (Praha)* 21:90–97
- Ozturk C, Atamaz F, Hepguler S, Argin M, Arkun R (2006) The safety and efficacy of intraarticular hyaluronan with/without corticosteroid in knee arthritis: 1-year, single-blind, randomized study. *Rheumatol Int* 26(4):314–319
- Pavelka K, Gatterova J, Gollerova V, Urbanova Z, Sedlackova M, Altman RD (2000) A 5-year randomized controlled, double-blind study of glycosaminoglycan polysulphuric acid complex (Rumalon) as a structure modifying therapy in osteoarthritis of the hip and knee. *Osteoarthritis Cartilage* 8:335–342
- Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacomelli G, Rovati LC (2002) Glucosamine sulphate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med* 162:2113–2123
- Pelletier J-P, Martel-Pelletier J, Abramson SB (2001) Osteoarthritis, an inflammatory disease. Potential implication for the selection of new therapeutic targets. *Arthritis Rheum* 44:1237–1247
- Pilgramm M, Schumann K (1985) Organspezifische Ribonukleinsäuren beim Hörsturz und M. Menière. *Med welt* 36:1046–1049 (German)
- Pilgramm M, Schumann K. (1986). Zur Notwendigkeit rheologisch wirksamer sowie vasoaktiver und stoffwechselaktiver Substanzen bei der Erstbehandlung des akuten Knalltraumas. *HNO* 34:424–28. German
- Plawewski M, Beljanski M (1981) Comparative study of Escherichia coli Endotoxin, Hydrocortisone and Beljanski Leukocyte restorer activity in cyclophosphamide-treated rabbits. *Proc Soc Exp Biol Med* 168:408–413
- Powanda MC, Sammons ML, Stephen EL (1977) Systemic metabolic alterations associated with repeated injections of a modified polyriboinosinic-polyribocytidylic acid complex. *Antimicrob Agents Chemother* 12:602–605
- Rainsford KD (1996) Mode of action, uses and side effects of anti-inflammatory drugs. In: Rainsford KD (ed) *Advances in anti-rheumatic therapy*. CRC Press, Boca Raton, pp 59–111
- Rainsford KD (ed) (1999) *Ibuprofen. A critical bibliographic review*. Taylor & Francis, London
- Rainsford KD, Bolten W, Schühlein K-H, Dempsey A, Schnitker J (2004) Effects of intramuscular sodium ribonucleinate (Osteochondrin® S) in osteoarthritis of the knee: identification of “Responders” and “Non-Responders”. *EULAR Congress, Berlin. Ann Rheum Dis* 63(Suppl. 1), Abst FRI0–406
- Rainsford KD, Jonas A, Ying C, Smith FC (2008) Ribonucleate sodium [Osteochondrin® S] inhibits cytokine-induced cartilage-bone degradation but not proteoglycan synthesis. *Inflammopharmacology* 16:321, Abstract No. P17
- Raynauld JP, Torrance GW, Band PA, Goldsmith CH, Tugwell P, Walker V, Schultz M, Bellamy N; Canadian Knee OA Study Group (2002) A prospective, randomized, pragmatic, health outcomes trial evaluating the incorporation of hylan G-F 20 into the treatment paradigm for patients with knee osteoarthritis (Part 1 of 2): clinical results. *Osteoarthritis Cartilage* 10:506–517

- Raynauld JP, Goldsmith CH, Bellamy N, Torrance GW, Polisson R, Belovich D, Pericak D, Tugwell P (2005) Effectiveness and safety of repeat courses of hylan G-F 20 in patients with knee osteoarthritis. *Osteoarthritis Cartilage* 13:111–119
- Razumova IL (1970) The effect of RNA on dystrophic processing the gastroncnemius and regeneration of the sciatic nerve after its section. *Patol Fiziol Eksp Ter* 14(5):78–79 (Russian)
- Razvorotnev VA, Sysoeva GM, Fadina VA (1987) Study of the effect of yeast dsRNA on humoral immune response and delayed type hypersensitivity. *Antibiot Med Biotechnol* 32(4):285–288 (Russian)
- Reginster JY, Deroisy R, Rovati LC, Lee RI, Lejeune E, Bruyere O, Giacobelli G, Henrotin Y, Dacre JE, Grosset C (2001) Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomized, placebo-controlled clinical trial. *Lancet* 357:251–256
- Repanovici R, Moisa I, Mihalache O, Iacobescu U, Burducea O, Iliescu R, Popa LM, Cajal N (1983) Investigation of the effect of cellular and viral nucleic acids on certain virua infections. Note 1. Protective effect of nucleic acids against experimental influenza virus A/PR8/34 (H1N1) infection in mice. *Rev Roum Med-Virol* 34:183–189
- Richy F, Bruyere O, Ethgen O, Cucherat M, Henrotin Y, Reginster JY (2003) Structural and symptomatic efficacy of glucosamine and chondroitin in knee osteoarthritis: a comprehensive meta-analysis. *Arch Intern Med* 163:1514–1522
- Rieber M, Urbina C, Strasberg Rieber M (1989) DNA on membrane receptors: a target for DNA antibody induced by a nucleoprotein shed in systemic lupus erythematosus. *Biochem Biophys Res Commun* 59:1441–1447
- Rollins E, Miyagi M, Moser CR, Flickinger RA (1966) Stimulation of protein synthesis of frog embryo cells by larval and adult frog liver ribonucleic acid preparations. *Nature* 209:509–510
- Rosenzweig MR (1984) Experience, memory and the brain. *Am Psychol* 39:365–376
- Rovetta G (1991) Galactosaminoglycuronoglycan sulfate (matrix) in therapy of tibiofibular osteoarthritis of the knee. *Drugs Exp Clin Res* 17:53–57
- Rudolph FB, Kulkani AD, Schandle VB, van Buren CT (1984) Involvement of dietary nucleotides in T lymphocyte function. *Adv Exp Biol Med* 165:175
- Rychnev VE, Frolov VM, Fedotova LT, Nikonova SI (1982) Experience with clinical use of regeneration stimulators in the therapy of fatty hepatosis. *Vratschebnoje delo* 10:81–83 (Russian)
- Saijo Y, Perlaky L, Wang H, Busch H (1994) Pharmacokinetics, tissue distribution, and stability of antisense oligodeoxynucleotide phosphorothioate ISIS 3466 in mice. *Oncol Res* 6:243
- Sakkas LI, Koussidis G, Avgerinos E, Gaughan J, Platsoucas CD (2004) Decreased expression of the CD3zeta chain in T cells infiltrating the synovial membrane of patients with osteoarthritis. *Clin Diagn Lab Immunol* 11:195–202
- Sakkas LI, Platsoucas CD (2002) Role of T cells in the pathogenesis of osteoarthritis. *Arthritis Rheum* 46(11):3112–3113
- Salaffi F, Leardini G, Canesi B, Mannoni A, Fioravanti A, Caporali R, Lapadula G, Punzi L: Gonarthrosis and Quality of Life Assessment (GOQOLA) (2003) Reliability and validity of the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index in Italian patients with osteoarthritis of the knee. *Osteoarthritis Cartilage* 11:551–560
- Salaffi F, Carotti M, Grassi W (2005) Health-related quality of life in patients with hip or knee osteoarthritis: comparison of generic and disease-specific instruments. *Clin Rheumatol* 24:29–37
- Sands H, Gorey-Feret LJ, Cocuzza AJ, Hobbs FW, Chidester D, Trainor GL (1994) Biodistribution and metabolism of internally 3H-labelled oligonucleotides. I. Comparison of a phosphodiester and a phosphorothioate. *Mol Pharmacol* 45:932–943
- Schadelin J, Sommer W, Straub PW (1981) Sterile abscesses after intramuscular injection of a mucopolysaccharide preparation. *Schweiz Med Wochenschr* 111:2011–2013 (German)
- Schlaak JF, Schwarting A, Knolle P, Meyer zum Buschenfelde KH, Mayet W (1995) Effects of Th1 and Th2 cytokines on cytokine production and ICAM-1 expression on synovial fibroblasts. *Ann Rheum Dis* 54:560–565

- Schroeder A, Dorn M, Schuehlein K-H, von Sulecki W (1989) Die Behandlung Degenerativer Gelenkerkrankungen. Therapiewoche 39:2310–2315 (German)
- Semina OV, Konoplayannikov AG, Pverennyi (1976) Effect of polyinosinic acid-polycytidylic acid on colony-forming capacity of the haemopoietic stem cell fraction during allogeneic inhibition. *AM Bull Exp Biol Med* 81:555–557
- Semochkin SV, Bekman EM, Arion VYa. (1999). Effect of rybothyme on delayed-type hypersensitivity and would process in mice with surgical trauma and burn disease. *Bull Exptl Biol Med* 128:945–947
- Semochkin SV, Bekman EM, Baranova OA, Arion VYA (2001) Regulatory effects of Ribotim on functional activity of neutrophils and wound healing during experimental burn trauma. *Bull Exptl Biol Med* 131:257–259
- Shershevskaya SF, Fuks BB, Potapova LN, Levina FG, Denisova TP (1971) Experience with the use of RNA in the complex treatment of tapetoretinal dystrophies. *Westa Oftamol* 3:59–63 (Russian)
- Shershevskaya SF, Levina FG (1978) Clinical experience in application of RNA preparations in the treatment of tapetoretinal dystrophies. *Westn Akad Med Nauk* 10:40–44 (Russian)
- Silvestrov VP, Provotorov VM, Zemskov AM, Nikitin AV, Buravleva IV (1981) Effect of sodium nucleate on the clinic and immunological characteristics of patients suffering from infectious-allergic bronchial asthma. *Ter Arch* 53:115–119 (Russian)
- Skuba ND, Levkova NA (1980) Effect of embryonic organ-specific RNA on hypertrophy of the myocardium. *Bull Exp Biol Med* 89:677–680
- de Smidt PC, Doan TL, de Falco S, van Berkel TJC (1991) Association of antisense oligonucleotides prolongs the plasma half-life and modifies the tissue distribution. *Nucleic Acids Res* 19:4695–4700
- Solyom L, Enesco HE, Beaulieu C (1967) The effect of RNA on learning and activity in old and young rats. *J Gerontol* 22:1–7
- Song J, Kim D, Lee CH, Lee MS, Chun C-H, Jin E-J (2013) MicroRNA-488 regulates zinc transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis. *J Biomed Sci* 20:31
- Stanczyk J, Pedrioli DML, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, Detmar M, Gay S, Kyburz D (2008) Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum* 58(4):1001–1009
- Stanczyk J, Ospelt C, Karouzakis E, Filer A, Raza K, Kolling C, Gay R, Buckley CD, Tak PP, Steffen Gay S, Kyburz D (2011) Altered expression of MicroRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation. *Arthritis Rheum* 63(2):373–381
- Stebbing N, Grantham CA, Kaminski F, Lindley IJD (1977) Protection of mice against encephalomyocarditis virus infection by preparations of transfer RNA. *J Gen Virol* 34:73–85
- Stebbing N, Lindley IJD (1980) Anti-viral effects of single-stranded polynucleotides against avirulent semliki forest virus infection of mice avirulent infection of rats with encephalomyocarditis virus. *Arch Virol* 64:57–66
- Steck E, Boeuf S, Gabler J, Werth N, Schnatzer P, Diederichs S, Richter W (2012) Regulation of H19 and its encoded microRNA-675 in osteoarthritis and under anabolic and catabolic in vitro conditions. *J Mol Med* 90:1185–1195
- Stommel G, Bolten WW, Rainsford KD, Schühlein K-H, Dempsey A, Schnitker J (2008) Safety and efficacy of intramuscular sodium ribonucleinate [Osteochondrin® S] for relief of pain and joint functions in knee osteoarthritis. *Inflammopharmacology* 16:310–311, Abstract No. P2
- Strayer DR, Carter WA, Brodsky I, Cheney P, Peterson D, Salvato P, Thompson C, Loveless M, Shapiro DE, Elsasser W, Gillespie DH (1994) A controlled clinical trial with a specifically configured RNA drug, Poly(I).Poly(C₁₂U), in chronic fatigue syndrome. *Clin Infect Dis* 18 (Suppl.):88–95
- Stucki G, Meier D, Stucki S, Michel BA, Tyndall AG, Dick W, Theiler R (1996) Evaluation of a German version of WOMAC (Western Ontario and McMaster Universities) arthrosis index. *Z Rheumatol* 55:40–49

- Sturmer T, Brenner H, Koenig W, Gunther KP (2004) Severity and extent of osteoarthritis and low grade systemic inflammation as assessed by high sensitivity C reactive protein. *Ann Rheum Dis* 63:200–205
- Sugahara T, Nagata H, Tanaka T (1966) Effect of an alkaline-hydrolyzed product of yeast RNA on the survival of repeatedly irradiated mice. *Radiat Res* 29:516–522
- Sula K, Nouza K (1984) Changes induced in the lymphoid system by the double-stranded RNA. *Folia Biol* 30:349–357
- Svirnovskii AI, Levin VI, Nikolaichik VV (1974) Comparative antileukemic action of RNA and histones from intact and regeneration homologous tissue. *Bull Exp Biol Med* 78:1055–1058
- Swingler TE, Wheeler G, Carmont V, Elliott HR, Barter MJ, Abu-Elmagd M, Donell ST, Boot-Handford RP, Hajihosseini MK, Münsterberg A, Dalmay T, Young DA, Clark IM (2012) The expression and function of MicroRNAs in chondrogenesis and osteoarthritis. *Arthritis Rheum* 64(6):1909–1919
- Taborsky I, Dolnik V (1977) Ability of human polymorphonuclear blood cells to produce interferon after induction with phage double-stranded RNA. *Acta Virol* 21:499–502
- Tardif G, Hum D, Pelletier J-P, Duval N, Martel-Pelletier J (2009) Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes. *BMC Musculoskelet Disord* 10:148
- Tchaika M, Labes D, Salama ZB (1999) Nachweis der Wirksamkeit und Verträglichkeit von Regeneresen Knochenmark und RN 13 Regeneresen zur Verbesserung der Verträglichkeit und Optimierung des Zeit- und Dosisplanes einer adjuvanten Chemotherapie bei weiblichen Patienten mit Mammakarzinom nach Operation. In-House-Report, Dyckerhoff. German
- Tew SR, Vasieva O, Peffers MJ, Clegg PD (2011) Post-transcriptional gene regulation following exposure of osteoarthritic human articular chondrocytes to hyperosmotic conditions. *Osteoarthritis Cartilage* 19:1036–1046
- Tomakova T, Petrek M, Gallo J, Kriegova E (2011) MicroRNAs: emerging regulators of immune-mediated diseases. *Scand J Immunol* 75:129–141
- Trenkmann M, Brock M, Gay RE, Michel BA, Gay S, Huber LC (2013) Tumor necrosis factor α -induced MicroRNA-18a activates rheumatoid arthritis synovial fibroblasts through a feedback loop in NF- κ B signaling. *Arthritis Rheum* 65:916–927
- Trutneva KV, Katsnelson LA, Bogoslovsky AI, Fuks BB, Milyavskaya TI, Averbakh IM, Shabanova ME, Shamshinova AM (1972) RNA and ribonucleotide agents in the treatment of tapeto-retinal dystrophy. *Vestnik oftal'mologii* 85(2):68–70 (Russian)
- Ukai T, Sato M, Akutsu H, Umezawa A, Mochida J (2012) MicroRNA-199a-3p, microRNA-193b, and microRNA-320c are correlated to aging and regulate human cartilage metabolism. *J Orthop Res* 30:1915–1922
- Vichikova AN (1982) Der Einfluss der Präparate der homologen RNS auf den Gehalt der Bindegewebe-Komponenten in den denervierten Muskeln. *Ukrainskij Biokhemieskij J* 54 (6):685–687 (Russian)
- Villee DB (1967) Ribonucleic acid: control of steroid synthesis in endocrine tissue. *Science* 158:652–653
- Vladimirov VG, Lukashin BP, Morozova IN, Kolosov AI (1985) Influence of synthetic polyribonucleotides on the immunologic and colony-forming ability of irradiated mouse cells. *Radiobiologija* 25:405–407
- Vlassov VV, Karamyshev VN, Yakubov LA (1993) Penetration of oligonucleotides into mouse organism through mucosa and skin. *FEBS* 327(3):271–274
- Von Sulecki W (1990) Symposium on Regeneresen, March 1990. Münster Gummersbach, Nuernberg. Laboratorium Dyckerhoff GmbH, Köln, Germany. German
- Wacker A, Eichler A (1981) Über die Interferon induzierte Wirkung von RN 13 Regeneresen®. *Erfahrungsheilkunde* 30:936–939 (German)
- Wang M, Liu C, Zhang Y, Hao Y, Zhang X, Zhang YM (2013) Protein interaction and microRNA network analysis in osteoarthritis meniscal cells. *Genet Mol Res* 12(1):738–746
- Westphal J. (1997) from Gerster (ed). *Expertengespräch 1997 über Regeneresen*. Ralf Reglin Verlag, Köln, pp 57–69 (German)

- Wieland HA, Michaelis M, Kirschbaum BJ, Rudolphi KA (2005) Osteoarthritis—an untreatable disease? *Nat Rev Drug Discov* 4:331–344. Erratum in: *Nat Rev Drug Discov* 4:543
- Williamson MB, Guschlbauer W (1961a) Metabolism of nucleic acids during regeneration of wound tissue. *J Biol Chem* 236:1463–1466
- Williamson MB, Guschlbauer W (1961b) Changes in the concentration of ribonucleic acid during regeneration of wound tissue. *Nature* 192:454–455
- Williamson MB, Guschlbauer W (1963) Metabolism of nucleic acids during regeneration of wound tissue. II. The rate of formation of RNA. *Arch Biochem Biophys* 100:245–250
- Wool IG, Stirewalt WS, Moyer AN (1968) Effect of diabetes and insulin on nucleic acid metabolism of heart muscle. *Am J Physiol* 214:825–831
- Wu CW, Kalunian KC (2005) New developments in osteoarthritis. *Clin Geriatr Med* 21:589–601
- Wu-Pong S, Weiss TL, Hunt A (1994) Antisense c-myc oligonucleotide cellular uptake and activity. *Antisense Res Dev* 4:155–163
- Yakubov LA, Deeva EA, Zarytova VF, Ivanova EM, Rytte AS, Yurchenko LV, Vlassov VV (1989) Mechanism of oligonucleotide uptake by cells: involvement of specific receptors? *Proc Natl Acad Sci USA* 86:6454–6458
- Yamasaki K, Nakasa T, Miyaki S, Yamasaki T, Yasunaga Y, Ochi M (2012) Angiogenic MicroRNA-210 is present in cells surrounding osteonecrosis. *J Orthop Res* 30:1263–1270
- Yu C, Chen W-P, Wang X-H (2011) MicroRNA in osteoarthritis. *J Int Med Res* 39:1–9
- Zamecnik P, Aghajanian J, Zamecnik M, Goodchild J, Witman G (1994) Electron micrographic studies of transport of oligodeoxynucleotides across eukaryotic cell membranes. *Proc Natl Acad Sci USA* 91:3156–3160
- Zeidler H (2011) Paracetamol and the placebo effect in osteoarthritis trials: a missing link? *Pain Res Treat* 2011:696791
- Zemskov VM (1977) RNA-induced intensification of antibacterial resistance and aggravation of infection. *J Hyg Epidemiol Microbiol Immunol* 21:195–202
- Zemskov AM, Provotorov VM, Nikitin AV (1979) Sodium nucleinate in the therapy of chronic pneumonia complicated by bronchial asthma. *Antibiotiki* 24(11):853–855 (Russian)
- Zemskov AM (1980) Stimulation of primary and secondary immune responses of yeast NaRNA. *Mikrobiol Zh* 42:219–225 (Russian)
- Zemskov AM, Zemskov VM, Peredery VG (1984) Correction of secondary immune deficiency with yeast RNA. *Mikrobiol Epidemiol Immunol* 9:77–82 (Russian)
- Zhang R, Diasio RB, Lu Z, Liu T, Jiang Z, Galbraith WM, Agrawal S (1995) Pharmacokinetics and tissue distribution in rats of a oligodeoxynucleotide phosphorothioate (GEM91) developed as a therapeutic agent for human immunodeficiency virus type-1. *Biochem Pharmacol* 49:929–939
- Zhang W, Robertson J, Jones AC, Dieppe PA, Doherty M (2008) The placebo effect and its determinants in osteoarthritis: meta-analysis of randomised controlled trials. *Ann Rheum Dis* 67:1716–1723

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