Steroids have been used since two decades and several trials were conducted to establish their efficacy in DMD patients with various regimens. The clinical outcomes showed increased function in the treated boys, and in a single trial with deflazacort, prolongation of ambulation but with different side effects. Steroids clinical efficacy is now established. The main concern is to increase steroid efficacy and decrease side effect and toxicity. A trial comparing daily prednisone, deflazacort and intermittent glucocorticoids (prednisone 10 days on/10 days off) (FOR-DMD) is starting under NIH grant. The primary outcomes will be muscle strength, forced vital capacity and patient/parents satisfaction.

Key words: Steroids, Duchenne, DMD, side effect, quality of life

Introduction

Duchenne muscular dystrophy (DMD) has an incidence in the general population is 1/3500 born males. Mutations that preclude the synthesis of the dystrophin protein are the basis of DMD muscle pathology (1). However even before the exact pathogenesis was known, several therapeutical trials were conducted under the support of the Muscular Dystrophy Association and in Italy and Germany by various collaborative groups.

In 1974 Drachman et al. (2) first used the steroids in DMD in an open-label study with positive outcome. In the same year an independent trial by Siegel et al. (3) reported the only negative controlled trial of prednisolone/ prednisone in DMD, but the outcome measures were not quantitative and the results were heavily criticized.

Since then, many studies have been undertaken and patients in many countries are now offered steroid treatment, though there is still a lack of international consensus on the most effective steroid and optimal dosage regimen. There is evidence, from randomized controlled trials, that steroids do improve muscle strength and functional outcome in DMD (4-9).

In 1981 the Clinical Investigation group of Duchenne Dystrophy (CIDD) was formed. This consortium of US-based specialists performed a series of randomized trials in DMD patients aged 5 years or over. Most of the CIDD work, notably the measurement of the downward slope of strength loss over time, was done before the discovery of dystrophin gene. In the randomised, double-blind, placebo controlled trials were started in DMD with different agents and natural history of the disease was studied and well established. Of 14 tested drugs, steroids were the only ones to provide any benefit. Mendell and CIDD group (9) in a short-term study showed the increase of strength in children obtained with prednisone.

Several clinical trials have established both the effect of steroids in DMD and the well-known risk of side effects associated with their daily use. The CIDD showed a daily dose of 0.75 mg/kg to be most effective regime in their randomized controlled trials, in which dose-response analysis showed that 0.3 mg/kg per day was not as effective (8) whereas 1.5 mg/kg daily gave no additional benefit.

Moreover, disease milestones were established by the Italian collaborative group (10) in 131 DMD (Fig. 1).

An Italian group first used deflazacort (DFZ), an oxazolidine derivative of prednisone, which has an equivalent dose of 6 mg for each 5 mg of prednisone, DFZ 0.9 mg/kg is the equivalent daily dose to 0.75 mg/kg of prednisone (4, 10).

Additional studies have demonstrated equal effectiveness with prednisone, possibly with less risk of weight gain (11, 12) (Fig. 2). Another study (13), which used DFZ, led to preserved of lung function compared with untreated controls. A long-term benefit in cardi-
ac function with DFZ was found after treatment for 5 years (13). Echocardiography showed a mean fractional shortening of 33% compared with 21% in the untreated group. Side-effect profiles of the two steroids differ slightly. Deflazacort appears to cause less weight gain and less effect on loss of vertebral bone mass: this effect seems particularly important in growing children and DFZ has therefore been used in DMD because of its potential ‘bone-sparing’ effect but it is more likely to be associated with the development of asymptomatic cataracts (1, 4).

It is difficult to assess the long-term differences in these regimens with respect to their effect on bone mineral density. Some studies have reported a high incidence of vertebral fractures with deflazacort, whereas others did not have this experience. Only a few randomized controlled trials of steroids in DMD have been presented in sufficient detail to enable efficacy to be compared for several outcomes. These trials presented evidence that use of daily prednisone (0.75 mg/kg) or the equivalent dose of deflazacort (0.9 mg/kg) stabilizes strength in DMD (11, 12). Several studies then confirmed that in patients treated with one or other of these regimes that this increase in strength was followed by improvement in function; open follow-up studies have demonstrated that age at loss of ambulation was postponed to the mid-teens and there was better preservation of respiratory and cardiac function. Thus, there is controlled evidence that in the short-term, e.g., 6 months to 2 years, steroids significantly improve muscle strength and function in DMD and there is increasing evidence of long-term benefits.

**Figure 1.** Natural history of untreated DMD. Note a correlation between MRC score, strength of quadriceps muscle force by myometry and worsening of grading in 10 meters walking (1 to 7).

**Figure 2.** (A) Medical Research Council (MRC) score in deflazacort and prednisone treated groups shows stabilization of strength after initial, slight improvement. Natural history controls show a continuous decline in strength. (B) Functional score in both the deflazacort- and prednisone-treated groups shows a slight improvement at the beginning of therapy, whereas the natural history group tends to increase in grade and worsen progressively. (C) Percentage of body weight increase in the deflazacort- and prednisone-treated groups. A less significant increase in weight was found in patients treated with deflazacort after 6 months. Filled square indicates prednisone group, open triangle indicates deflazacort group, and a cross indicates natural history group.
Many studies have suggested useful benefits from steroids; the recommended daily dose is now prednisone 0.75 mg/kg or deflazacort 0.9 mg/kg. As reported by Fenichel et al. (7) there is less effect on alternate-day prednisone therapy. Other studies have been performed using steroids on alternate days (4), or with an intermittent schedule for 10 days a month, for 10 days on/10 days off (14), or at the weekends only (15), and some have demonstrated benefit in functional parameters and fewer side effects. The weekend-only regimen allowed linear growth to be maintained in boys (15), but did not prevent contractures, which resulted in loss of ambulation about at age 10 years in 25% of treated DMD. Other regimens (daily low dosing 0.35 mg/kg prednisone day) aim to reduce the cumulative steroid dose (16).

How do steroids act?

Steroids may act on the regulation of signal transduction and have a direct nuclear effect. The beneficial effect of DFZ on muscle has been associated with activation of the calcineurin/NF-AT pathway (17). Studies at transcriptome level by microarray analysis and at proteomic level are in progress in DMD. Such studies might identify gene and protein targets for steroid response. A direct effect on muscle degeneration has been observed in *Caenorhabditis elegans* (18), in which prednisone reduced muscle cell degeneration by 40%.

Other hypotheses are that steroids reduce muscle necrosis and inflammation, although possible alternative actions may be in modulating the cell response to inflammation (19). In fact it was demonstrated that complement deposition occurs in dystrophic fibers. Alternatively, steroids may enhance the proliferation of myogenic precursor stem cells or myoblasts and thereby increase muscle regeneration and growth due to their anabolic effect (20-22) (Fig. 3).

Skeletal muscle is made of multinucleated postmitotic fibers that arise from mononuclear precursors such as satellite cells located between the plasma membrane and extracellular matrix. Another possible explanation for steroid beneficial effect might be a reduction in the rate of muscle breakdown. A study on protein metabolism concluded that the beneficial effect of steroids in DMD is associated with increased muscle mass mediated by inhibition of proteolysis (21).

Steroids may act as direct transcriptional modifiers to increase dystrophin expression in “reverted fibers”, or increase synergistic molecules, such as muscle glycoproteins, that complement the action of dystrophin. Methylprednisone selectively affects dystrophin in culture and increases utrophin and satellite cells (22). It is unlikely that steroids have a purely immunosuppressive function since azathioprine does not have a positive effect in DMD (24) and mononuclear cell populations are differentially expressed in controls and patients treated with prednisone or azathioprine (25). However the mechanism of action of steroids and azathioprine are profoundly different and might not be compared easily in this way. Immunohistochemical studies on biopsies from DMD (26) boys treated with placebo or prednisone showed that the total number of T-cells was less in the prednisone-treated group, but B-cells, natural killer cells, macrophages, and necrotic fibers did not differ.

How to follow up patients in steroids

Disease milestones are the most meaningful end-points to measure changes in muscle function. One functional index that is easy to monitor is the loss of ability to arise from the floor (Gowers’ maneuver) (Fig. 4). The most useful index would be the age at loss of ambulation (wheelchair-bound-WCB), since a change in this end-point implies a better quality of life. Only one randomized study with DFZ (4) used prolongation of ambulation as an outcome measure.

Myometry and dynamometry

Quantitative strength measurements by different techniques have been performed: dynamometry was first used in the Drachman trial (2) where specific application were described. Such measurements correlate with loss of force in the natural history of DMD. Quantitative muscle testing by standard techniques (27) has been validated and direct assessment of muscle strength is a valid outcome measurement not only in DMD but in all chronic neuromuscular disorders.
Corrado Angelini, Enrico Peterle

Graded and timed testing

Many clinicians use timed and graded functional tests as a measure of disease progression. The commonly validated measures include time and grading of: gait (G); climb a set of stairs (S); rise from a chair (C); rise from the floor (Gowers’ maneuver) (G) (Fig. 4, Table 1). The sum of grades to perform such functional parameters vary from 1 to 7 and each of these sums are grouped together to form the GSGC score (28, 29). These are simple, and reproducible functional tests that vary with age. Several trials have shown that the tests are standardized and the timed activities are truly the same (4, 10-12). Gowers’ time was analyzed and improved significantly in two trials with prednisone (5, 9). Walking and stair-climbing time showed statistically significant improvement in these two trials (5, 9). Various composite scores of function have been defined and consider not only the time taken to perform an activity, but also the quality of its performance and include both the GSGC score and Brooke’s score (5). This latter analyzes in detail lower and upper limb measures and might be particularly valuable in non-ambulant children.

Table 1. GSGC score.

| Gait (G)                          |                        |
|----------------------------------|------------------------|
| 1. Normal                        |                        |
| 2. Mild waddling, lordosis and/toe walking |                        |
| 3. Moderate waddling, lordosis and/toe walking |                        |
| 4. Severe waddling, lordosis and/toe walking |                        |
| 5. Walks only with assistance (i.e. braces, cane, crutches) |                        |
| 6. Stands, but unable to walk    |                        |
| 7. Confined to wheelchair        |                        |

Time to walk 10 meters: __ seconds

| Climbing stairs (S)              |                        |
|----------------------------------|------------------------|
| 1. Climbs without assistance     |                        |
| 2. Supports one hand on thigh    |                        |
| 3. Supports both hands on thighs |                        |
| 4. Climb stairs in upright position but with aid of railing |                        |
| 5. Climbs while clinging to the railing with both hands |                        |
| 6. Manages to climb only a few steps |                        |
| 7. Unable to climb steps         |                        |

Time to climb steps: __ seconds

| Gowers’ maneuver (G)             |                        |
|----------------------------------|------------------------|
| 1. Normal                        |                        |
| 2. Butt-first maneuver, one hand on floor |                        |
| 3. Butt-first maneuver, two hands on floor |                        |
| 4. Unilateral hand support on thigh |                        |
| 5. Bilateral hand support on thighs |                        |
| 6. Arises only with aid of an object (table, chair, etc.) |                        |
| 7. Unable to arise               |                        |

Time to standing from sitting: __ seconds

| Arising from a chair (C)         |                        |
|----------------------------------|------------------------|
| 1. Normal                        |                        |
| 2. With wide base and/or difficulty, but without support |                        |
| 3. With support on one thigh     |                        |
| 4. With support on both thighs   |                        |
| 5. With support on arms of chair or on a table |                        |
| 6. Not possible                  |                        |

Time to standing from sitting: __ seconds

Total GSGC Score: __ of 27

with daily prednisone (0.75 mg/kg) demonstrated after 6 months an improvement which was in average of 0.17 L or higher in the prednisone-treated group than in the placebo group (9) compared 1.5 mg/kg prednisone daily with placebo and found a mean improvement of only 0.14 L.

Muscle imaging

Muscle computerized tomography scanning might be useful to qualitatively document muscle atrophy and fat degeneration (30). Magnetic resonance imaging offers a good index of muscle mass and of myoedema in STIR sequences. Magnetic resonance spectroscopy can also be used to assess metabolism in individual DMD cases (31).
Ophthalmological examination is necessary to monitor for the development of cataracts and increased intraocular pressure. Cataracts in children with DMD treated with DFZ have been mostly asymptomatic and in few surgical treatment was required.

Effects and safety of steroids in long-term use

Given the long periods that young patients with DMD may be treated with steroids, it is important to address the prevalence and management of long-term side effects, which may include weight gain, behavioural changes, vertebral fractures secondary to osteoporosis and cataracts (33, 34, 36, 39, 40). Bone mineral density has to be followed by DXA to monitor the level of vitamin D and collect the history of fracture is useful. The frequency, the site and the type of trauma should be recorded (32). In general, the beneficial response to steroids in functional tests is particularly evident in younger children. However, non-randomized cohort studies, using both prednisolone and deflazacort, record long-term improvement in functional outcomes, including prolongation of walking from a mean age of 10 years to a mean age of 14.5 years (Fig. 5), preservation of lung function, reduction in the need for scoliosis surgery, and possibly a reduced incidence of cardiomyopathy.

Cardiological involvement

It is important to know and follow-up the effect of steroids on cardiac function, since cardiomyopathy is almost constantly present in DMD. Moreover, a long-term cohort studies of boys treated with deflazacort suggest that steroids may have a cardioprotective effect (37). In DMD patients treated with perindropril before the development of any signs of left ventricular dysfunction, the drug was well tolerated and after follow up for 5 years, a smaller proportion of treated boys had left ventricular dysfunction than those in the placebo group (38). Furthermore it was demonstrated that is important to monitor annually the cardiac function by electrocardiography and echocardiography, and the management of any deterioration in cardiac function should be promptly treated with angiotensin converting enzyme inhibition and beta blockers.

Steroids side-effects

Weight gain is the most frequently reported side effect for DMD children on steroids. To monitor body weight and clearly define when weight gain occurs is very important in DMD patients during follow up, because it constitutes a substantial adverse event. It is very important to give the parents a diet advice to limit weight gain, and this should include the suggestion to cut down on high calorie foods and maintain a healthy diet. It was demonstrated that DFZ is usually associated with less weight gain than prednisone (10, 12). Parents should be warned that appetite can increase dramatically at the onset of steroid treatment. However, many DMD children gain excessive weight even in the absence of steroid treatment, a tendency probably exacerbated by relative lack of activity and parental indulgence. Excessive weight can causes reduced the mobility, so it is important to control this factor. Long-term daily use of steroids has an effect on linear growth and a loss of final adult height with DFZ was often observed, although this might confer an additional advantage on muscle strength.

Bone mineral density is one of the most important side effect related to the steroids; in fact vertebral fractures are rarely seen in DMD patients who are not treated with steroids (32-35). However it was also demonstrated that the bone density reduction occurs frequently in DMD even before steroid use (36) and it is associated with an increased risk of bones fractures. This is probably associated to relatively low levels of activity, though recent studies also show that children with DMD may have abnormally low levels of vitamin D and osteoporosis even at diagnosis. Then it is important to measure and monitor bone mineral density. Nowadays, many techniques are available but many problems might occurs with interpretation of the results of single bones on the entire body.

Figure 5. Steroid treated DMD patient walking at age 17 years.
and prolongs age of ambulation. Untreated DMD patients however, showed delayed loss of ambulation if they had reverted fibers and faint dystrophin in biopsy (41). Another modifier of natural history and possibly of steroid response is a polymorphism of osteopontin (SPP1). Therefore future studies should to consider these factors in the course of the disease (42).

Other possible side effects of long-term daily steroid use are adrenal suppression, susceptibility to infection, hypertension, impaired glucose tolerance, gastrointestinal irritation, and skin fragility, but these complications are less frequently observed, perhaps because the daily dose of corticosteroid is usually reduced over time (43).

New trials and future perspectives

NIH has approved a FOR-DMD trial where prednisone, deflazacort and 10 days on/10 days off prednisone will be compared for 3 years. This trials, that now is in progress in several countries, will compare 3 corticosteroids regimens to address the pragmatic hypothesis that daily deflazacort and prednisone are of greater benefit in terms of function and patient/parent satisfaction than intermittent corticosteroids (prednisone). The primary outcome will consist of the following three components (time to stand up from lying, forced vital capacity, subject parent global satisfaction), each averaged over all post-baseline follow up examination through month 36. Secondary outcome variables will include regimen tolerance, other timed function tests, cardiac function, quality of life, and adverse event profile. The trial will randomize 300 DMD boys (100 for group) who have not been on steroids before (44).

The last year, two therapeutic trials have addressed the issue of exon 51 skipping (45, 46) in DMD. In both trials steroids were allowed. In DMD the open reading frame of dystrophin gene can be affected by deletions, duplications or point mutations; in the milder Becker muscular dystrophy (BMD), dystrophin mutations do not disrupt the open reading frame. Conversion of the DMD dystrophin to a shorter but functional protein (BMD-like) using antisense oligonucleotides (i.e. a complementary sequence of 20-30 nucleotides that allows skipping of exon 51) is an attractive therapeutic strategy. Goemans et al. (45) started by subcutaneous injection first with 4 different doses and then in an extended open trial at a systemic administration with 6 mg/kg/bodyweight of PRO051 (Prosensa, Netherlands), that brought stabilization of muscle function and a modest improvement (35 meters) of 6 minute walk test (6MWT), from the baseline 384 meters.

In the UK trial (46), several cohorts of DMD boys were injected systemically with phosphoro-diamidite morpholino oligomers (AVI-4658). From the third cohort on (dose 2 mg/kg) and in the followings cohorts there was a significant increase in fluorescence intensity of dystrophin positive fibers and dystrophin amount by Western blotting, which increased to 8-18% in the 3 best responders. Analysis of post treatment biopsies showed a reduction of cytotoxic T cells and increased dystrophin expression, consistent with the fact that most patients received contemporary steroids.

In these two complementary studies, more than 30 boys were systematically injected with antisense oligonucleotides, with no significant side effects.

Antisense technology has therefore shown to be promising, and it is likely to be used in other neuromuscular disorders, e.g. myotonic dystrophy. For DMD, a long term study that measures a series of functional parameters is needed, since no study has measured both functional daily activity and muscle strength. The age of patients at the beginning of treatment is also important to verify the effect of such treatment after 2-3 years, to check if the improvement in 6MWT is maintained. In fact some boys started treatment at 7 years of age when natural history and concomitant steroid treatment might justify some of the clinical efficacy.

Acknowledgments

We thank patients and their families. This paper was supported by Telethon GUP 07001 and Eurobiobank.

References

1. Angelini G. The role of corticosteroids in muscular dystrophy: a critical appraisal. Muscle Nerve 2007;36:424-35.
2. Drachman DB, Toyka KV, Myer E. Prednisone in Duchenne muscular dystrophy. Lancet 1974;14:1409-12.
3. Siegel IM, Miller JE, Ray RD. Failure of corticosteroid in the treatment of Duchenne (pseudo-hypertrophic) muscular dystrophy. Report of a clinically matched three year double-blind study. III Med J 1974:145:32-3.
4. Angelini C, Pegoraro E, Turella E, et al. Deflazacort in Duchenne dystrophy: study of long-term effect. Muscle Nerve 1994;17:386-91.
5. Brooke MH, Fenichel GM, Griggs RG, et al. Clinical investigation of Duchenne muscular dystrophy. Interesting results in a trial of prednisone. Arch Neurol 1987;44:812-7.
6. Fenichel GM, Mendell JR, Moxley RT, et al. Comparison of daily and alternate-day prednisone therapy in the treatment of Duchenne muscular dystrophy. Arch Neurol 1991:48:575-9.
7. Fenichel GM, Florence JM, Pestronek A, et al. Long-term benefit from prednisone therapy in Duchenne muscular dystrophy. Neurology 1991;41:1874-7.
8. Griggs RC, Moxley RT III, Mendell JR, et al. Prednisone in Duchenne dystrophy. A randomized, controlled trial defining the time course and dose response. Clinical Investigation of Duchenne Dystrophy Group. Arch Neurol 1991:48:383-8.
9. Angelini C, Pegoraro E, Perini F, et al. A trial with a new steroid in Duchenne muscular dystrophy. In: Angelini C, Danieli GA, Fontanari D, eds. Muscular dystrophy research: from molecular diagnosis toward therapy. Amsterdam: Excerpta Medica 1991, pp. 173-9.
11. Bonifati DM, Ruzza G, Bonometto P, et al. A multicenter double-blind randomized trial of deflazacort versus prednisone in Duchenne muscular dystrophy. Muscle Nerve 2000;23:1344-7.

12. Bonifati DM, Ruzza G, Bonometto P, et al. A multicenter double-blind trial of deflazacort versus prednisone in Duchenne muscular dystrophy: analysis after 2 years. Basic Appl Myol 2000;10:171-5.

13. Biggar WD, Gingras M, Fehlings DL, et al. Deflazacort treatment of Duchenne muscular dystrophy. J Pediatr 2001;138:45-50.

14. Kinali M, Mercuri E, Main M, et al. An effective, low-dosage, intermitent schedule of prednisolone in the long-term treatment of early cases of Duchenne dystrophy. Neuromuscul Disord 2002;12(Suppl 1):S169-74.

15. Connolly AM, Schierbecker J, Renna R, et al. High dose weekly oral prednisone improves strength in boys with Duchenne muscular dystrophy. Neuromuscul Disord 2002;12:917-25.

16. Backman E, Henriksson KG. Low-dose prednisolone treatment in Duchenne and Becker muscular dystrophy. Neuromuscul Disord 1995;5:233-41.

17. St-Pierre SJ, Chakkalakal JV, Kolodziejczyk SM, et al. Glucocorticoid treatment alleviates dystrophic myofiber pathology by activation of the calcineurin/NF-AT pathway. FASEB J 2004;18:1937-9.

18. Gaud A, Simon JM, Witzel T, et al. Prednisone reduces muscle degeneration in dystrophin-deficient Caenorhabditis elegans. Neuromuscul Disord 2004;14:365-70.

19. Spuler S, Engel AG. Unexpected sarcomemal complement membrane attack complex deposits on nonnecrotic muscle fibers in muscular dystrophies. Neurology 1998;50:41-6.

20. Pasquini F, Guerin C, Blake D, et al. The effects of glucocorticoids on the accumulation of utrophin by cultured normal and dystrophic human skeletal muscle satellite cells. Neuromuscul Disord 1995;5:105-14.

21. Rифаль Z, Welle S, Moxley RT III, et al. Effect of prednisone on protein metabolism in Duchenne dystrophy. Am J Physiol 1995;268:E67-74.

22. Sklar RM, Brown RH Jr. Methylprednisolone increases dystrophin levels by inhibiting myotube death during myogenesis of normal human muscle in vitro. J Neurol Sci 1991;101:73-81.

23. Hardiman O, Sklar RM, Brown PH. Methylprednisolone selectively affects dystrophin expression in human muscle cultures. Neurology 1993;43:342-5.

24. Griggs RC, Moxley RT, Mendell JR, et al. Duchenne dystrophy: randomized controlled trial of prednisone (18 months) and azathio-prine (12 months). Neurology 1993;43:520-7.

25. Kissel JT, Lynn J, Rammoah KW, et al. Mononuclear cell analysis of muscle biopsies in prednisone- and azathioprine-treated Duchenne muscular dystrophy. Neurology 1993;43:532-6.

26. Kissel JT, Burrow KL, Rammoah KW, et al. Mononuclear cell analysis of muscle biopsies in prednisone-treated and untreated Duchenne muscular dystrophy. Neurology 1991;41:667-72.

27. Mayhew JE, Florence JM, Mayhew TP, et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. Muscle Nerve 2007;35:36-42.

28. Angelini C, Ringel SP, Micaglio GF, et al. Italian multicentertherapeutic trials in Duchenne dystrophy. I. Protocol. Ital J Neurol Sci 1984;4:137-42.

29. Bonifati DM, Witchel SF, Ermani M, et al. The glucocorticoid receptor N363S polymorphism and steroid response in Duchenne dystrophy. J Neurol Neurosurg Psychiatry 2006;77:1177-9.

30. Pichiechio A, Ugetti C, Egito MG, et al. Quantitative MR evaluation of body composition in patients with Duchenne muscular dystrophy. Eur Radiol 2002;12:2704-9.

31. Stramare R, Beltrame V, Dal Borgo R, et al. MRI in the assessment of muscular pathology: a comparison between limb-girdle muscular dystrophies, hyaline body myopathies and myotonic dystrophies. Radiol Med 2010;115:585-99.

32. Bothwell JE, Gordon KE, Dooley JM, et al. Vertebral fractures in boys with Duchenne muscular dystrophy. Clin Pediatr 2003;42:353-6.

33. Larson CM, Henderson RC. Bone mineral density and fracture in boys with Duchenne muscular dystrophy. J Pediatr Orthop 2000;20:71-4.

34. McDonald DG, Kinali M, Gallagher AC, et al. Fracture prevalence in Duchenne muscular dystrophy. Dev Med Child Neurol 2002;44:695-8.

35. Vestergaard P, Grurup H, Steffenson BF, et al. Fracture risk in patients with Duchenne muscular dystrophy and spinal muscular atrophy. J Rehabil Med 2001;33:150-5.

36. Bianchi ML, Mazzanti A, Galbiati E, et al. Bone mineral density and bone metabolism in Duchenne muscular dystrophy. Osteoporos Int 2003;14:761-7.

37. Silversides CK, Webb GD, Harris VA, et al. Effects of deflazacort on left ventricular function in patients with Duchenne muscular dystrophy. Am J Cardiol 2003;91:769-72.

38. Duboc D, Meuné C, Lerebours G, et al. Effect of perindopril on the onset and progression of left ventricular dysfunction in Duchenne muscular dystrophy. J Am Coll Cardiol 2005;45:855-7.

39. Quinlivan R, Roper H, Davie M, et al. Report of a Muscular Dystrophy Campaign funded workshop, Birmingham, UK, January 16, 2004. Osteoporosis in Duchenne muscular dystrophy; its prevalence, treatment and prevention. Neuromuscul Disord 2005;15:72-9.

40. Talim B, Malaguti C, Gnudi S, et al. Vertebral compression in Duchenne muscular dystrophy following deflazacort. Neuromuscul Disord 2002;12:294-5.

41. Fanin M, Danieli GA, Cadalini M, et al. Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. Muscle Nerve 1995;18:1115-20.

42. Pegoraro E, Hoffman EP, Piva L, et al. SPP1 Genotype is a determinant of disease severity in Duchenne muscular dystrophy. Neuromuscul Disord 2002;44:851-8.

43. De Silva S, Drachman DB, Mellits D, et al. Prednisone treatment in Duchenne muscular dystrophy. Long-term benefit. Arch Neurol 1995;42:231-5.

44. Issuu.com/parentprojectmd/docs/connect2010_bushby/1?mode=a_p

45. Goemans NM, Tulinius M, van den Akker JT, et al. Systemic administration of PRR051 in Duchenne’s muscular dystrophy. N Engl J Med 2011;364:1513-22.

46. Cirak S, Arechavala-Gomeza V, Guglieri M, et al. Exon skipping restores dystrophin expression in human muscle cultures in Duchenne muscular dystrophy. Muscle Nerve 1995;18:1115-20.

47. Panza F, Guglieri M, Pellicciari M, et al. Exon skipping restores dystrophin expression in human muscle cultures in Duchenne muscular dystrophy. Muscle Nerve 1995;18:1115-20.

48. Panza F, Guglieri M, Pellicciari M, et al. Exon skipping restores dystrophin expression in human muscle cultures in Duchenne muscular dystrophy. Muscle Nerve 1995;18:1115-20.