Focusing New Ataxia Telangiectasia Therapeutic Approaches

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Abstract

Ataxia Telangiectasia (AT) is a rare worldwide disease inherited as autosomal recessive with a poor prognosis in its classical form. It is characterized by neurological impairment (progressive cerebellar ataxia, axonal peripheral neuropathy, oculomotor apraxia, and movement disorders such as dystonia, choreoathetosis, myoclonus, tremor, Parkinsonism), telangiectasias, recurrent sino pulmonary infections, proneness to cancer, increased alpha-fetoprotein and decreased IgA levels and radio hypersensitivity. AT is caused by biallelic mutations in ATM gene, which plays a pivotal role in the control of cell cycle and in the response to DNA double strand break damage and chromatin changes. The management of patients, as well as prognosis, depends on the severity of the phenotype; only symptomatic therapies are by now available. Here we discuss the classical and the new therapeutic approaches in the light of the most recent reports in the literature.

Keywords: Ataxia telangiectasia; Rare disease; Neurological impairment

Introduction

Ataxia Telangiectasia (AT, OMIM #208900) or Louis-Bar syndrome is a rare (1:10,000-1:40,000 new births per year over the world) multisystemic autosomal recessive disease characterized by neurological impairment (progressive cerebellar ataxia, axonal peripheral neuropathy, oculomotor apraxia, and movement disorders such as dystonia, choreoathetosis, myoclonus, tremor, parkinsonism), telangiectasias, recurrent sinopulmonary infections, proneness to cancer, increased alpha-fetoprotein and decreased IgA levels and radio hypersensitivity. AT is caused by biallelic mutations in ATM gene, which plays a pivotal role in the control of cell cycle and in the response to DNA double strand break damage and chromatin changes. As ATM is a large gene, the laboratory diagnosis is primarily made by determining the levels of ATM protein by Western Blot analysis, and then confirmed by direct sequencing. Due to the heterogeneity of the phenotype, differential diagnosis with AT-LD (Ataxia Telangiectasia-Like Disorders), AOA1 and AOA2 (Apraxia with Oculomotor Apraxia type 1 and 2) must be performed on the basis of clinical and laboratory data. Molecular analysis and segregation of mutations in AT families allows prenatal diagnosis. The management of patients, as well as prognosis, depends on the severity of the phenotype; only symptomatic therapies are by now available and will be discussed in this review.

Background and Natural History

The primary features of AT include progressive gait and truncal cerebellar ataxia with onset between one and four years of age; progressively slurred speech; oculomotor apraxia; movement disorders (dystonia, choreoathetosis, myoclonus, tremor, parkinsonism); mild to severe symptoms of axonal peripheral neuropathy; oculocutaneous telangiectasia, usually by six years of age; frequent infections, with accompanying evidence of serum and cellular immunodeficiency; susceptibility to cancer, usually leukemia or lymphoma; and hypersensitivity to ionizing radiation. Other features include premature aging with graying of the hair. Endocrine abnormalities, such as insulin-resistant diabetes mellitus, have also been observed. The AT syndromic pathway varies little from family to family in its late stages [1-3].

Lifespan

Over the past twenty years, the expected lifespan of individuals with AT has increased considerably; most individuals now live beyond 25 y of age. Some have survived into their 40s and 50s [4, 5]. In AT patients' pulmonary failure, with or without identifiable infections, is the major cause of failing health and death. Tumors are the second cause of death; one third of the patients will

develop a neoplastic pathology during their life. The overall risk for tumors in AT patient is 38%, leukemia and lymphomas being the 85% with a frequency of 70 and 250 times respectively vs. wild type age-matched individuals. Youngest patients present with an acute T lymphocytic leukemia, the oldest ones with a very aggressive T cell leukemia; 10% of patients suffer a chronic T prolymphocytic leukemia [6].

The increased survival allowed the observation of solid tumors like ovarian, breast and gastric carcinomas; melanomas, leiomyoma's and sarcomas (Chessa and Lederman: Personal communication). After the age of 20 the frequency of solid tumors is slightly but not significantly increased vs. matched wild type population [7].

Epidemiology

AT is a disease found in all races and its frequency varies considerably from country to country depending upon the degree of inbreeding and the ability to distinguish it from other neurological disorders [8]. The incidence in general population has been variously estimated at between 1 in 40,000 (in United States) and 1 in 400,000 live births. The carrier frequency is estimated at 0.5%-2.0% in the general population.

An epidemiological study was conducted on 72 Italian AT families from the Italian Registry for Ataxia Telangiectasia applying the Dahlberg's formula. On the basis of the consanguinity rates a theoretical disease frequency of 1 patient in 7090 conceptions and heterozygotes frequency from 1.69% to 3.43% were obtained [9].

Clinical Findings

AT is clinically characterized by progressive severe neurological impairment (early onset cerebellar ataxia, oculomotor apraxia, extrapyramidal involvement, axonal peripheral neuropathy), oculocutaneous telangiectasias, recurrent sinopulmonary infections, and proneness to cancer.

Most patients die in the second or third decade of life, primarily from pulmonary infections and cancer, but neurological degeneration is the major contributor to the severe outcome of the disease.

The hallmarks of the neurological involvement are:

1. Progressive cerebellar ataxia (100% of patients) of the trunk and limbs, starting at walking age (is the presenting symptom in 90% of patients) and leading to wheelchair toward the second decade of life. An important clue to diagnosis is the peculiar narrow gait ataxia, which is probably a mixture of cerebellar ataxia and gait apraxia, with an extrapyramidal component (impairment of postural responses). Typically the patient is better running than walking. A second clue to diagnosis is neck posturing with anterior or posterior bending (a forward head tilt in 70% of patients, as opposed to lateral posturing, which is common in posterior fossa lesions) [2, 10-13].

2. Extra-piramidal symptoms (both hyperkinetic movement disorders such as choreoathetosis, myoclonus, polymyoclonus, dystonia, kinetic tremor in 60% of patients, and parkinsonism such as bradykinesia, bradylalia, hypomimia, resting tremor in 70% of patients). Extrapyramidal symptoms are especially prominent and may overshadow ataxia as a cause of disability, especially during the second decade of life, when cerebellar symptoms plateau while movement disorders are still progressive [2, 10-15].

3. Eye movement abnormalities (80% of patients) with characteristic oculomotor apraxia, with nystagmus and various defects in saccade and gaze control (usually, abnormalities in head and neck posturing have been related to gaze impairment, because of an oculocephalic dissociation: during head rotation, the head reaches the target before the eyes, which lag) [16, 17].

4. Sensorimotor axonal peripheral neuropathy of various degrees, from a mild form characterized only by hypotony and absence of tendon reflexes, to a severe hypotony and weakness with dramatic ankle and foot tendon retractions requiring surgery [6].

Cognitive abilities are relatively preserved but, due to severe neurological involvement, academic achievement is impaired [18].

Biological Findings

The instrumental diagnosis is based on increased alpha-fetoprotein levels (95% of patients), reduced or absent IgA levels (70%) and ATM protein (98%), spontaneous and radiation-induced chromosomal instability or breaks, and molecular diagnosis on ATM gene mutations.

Etiopathogenesis

The gene mutated in Ataxia Telangiectasia patients has been mapped in 1988 [19] on the long arm of chromosome 11 at region q22-q23. In the following years, the joined efforts of an international Consortium allowed the progressive restriction of this region to a 500 kb interval [20-22] leading, in 1995, to the identification of the ATM gene by positional cloning [23].

The AT Mutated gene (ATM) extends over 150 kb of genomic DNA, includes 66 exons (62 coding), and has an open reading frame of 9168 nucleotides. The coded ATM protein contains 3056 amino acids and is a member of the phosphatidylinositol (PI) 3-kinase family, with the kinase domain in its C-terminal region [24]. The patients are mostly compound heterozygotes for two different mutations inherited by their parents, but the homozygotes for the same mutation are not infrequent, especially in highly inbred populations. More than 500 unique mutations, spread all over the ATM gene, have been identified in AT patients, without evidence of any mutational hot spots. Most of these changes are predicted to give rise to a truncated protein that is highly unstable, effectively producing a null phenotype (~ 85%). However, a significant number of missense mutations has been recorded (~ 10%) and data suggest that many of these have dominant interfering effects [25]. Recurrent mutations are reported in Norway, the Netherlands, Costa Rica, the English Midlands, Italy, Japan, Poland, and among people of Irish English, Utah Mormon, African American, Israeli Jewish, and Amish/Mennonite descent [6, 26-30]. For several of these mutations the carriers share common haplotypes, indicating a founder effect. A

regularly updated ATM mutation Database Web site is available (http://chromium.liacs.ml/loud).

The ATM protein plays a key role in several pathways involved in cell-cycle control, oxidative stress, and DNA repair [31]. It acts recognizing and facilitating the repair of a subcategory of double strand breaks (DSBs) or a form of damage, like oxidative stress, that is converted into a DSB in DNA; this recognition would probably be the trigger for ATM to activate a number of cell cycle checkpoints. The ATM protein is present in the nucleus as an inactive dimer and is activated in response to DNA DSBs or chromatin changes by an auto-phosphorylation on ser-1981 that causes the dissociation of the dimer to form active monomeric forms, which are capable to initiate the phosphorylation of multiple intermediates involved in DNA repair and cell cycle control [32]. The double strand break is generally regarded as the most toxic DNA lesion. DSBs are induced by a number of different mechanisms, including exposure to ionizing radiations and radiomimetic drugs, collapse of replication forks when the replication machinery encounters single-stranded breaks (SSBs) in the template DNA, and programmed cleavage by specific endonucleases during meiotic recombination and immunoglobulin gene rearrangements. SSBs are mainly generated directly by oxidative stress and, if not rapidly processed, are converted to DSBs. The major types of repair pathways are homologous recombination repair (HRR) and non-homologous end joining (NHEJ), which are fundamentally different because of the dependence on DNA homology in HHR [33]. Errors in DSBs repair generate small deletions or insertions at the site of the lesion and can result in chromosome translocations and genomic instability, finally leading to cancer.

Progressive neuro-degeneration, a major characteristic of AT, has been reported to be associated with cerebellar defects involving ectopic migration and loss of Purkinje cells. However, it is evident that other regions of the brain and the CNS are affected by loss of ATM [34]. Given the important role for ATM in recognising and signalling DNA double strand breaks, it is understandable that post-mitotic cells would be vulnerable in AT patients. It has been suggested that a checkpoint defect in AT post-mitotic cells would be responsible for this vulnerability [35]; this hypothesis is supported by the evidence of a mitotic spindle defect in AT cells post-irradiation [36]. The existence of a surveillance mechanism ATM-dependent for inhibiting DNA reduplication downstream of the spindle assembly checkpoint has been suggested by the data showing that ATM is essential for p53 centrosomal localization and is required for the activation of the postmitotic checkpoint after spindle disruption [37].

The initiating event in the damage response is the change in the chromatin structure that rapidly triggers the activation of ATM, as indicated by the auto phosphorylation of ATM at serine 1981 and subsequent ATM dimer dissociation [38]. After DSBs induction Mre11 activates ATM which phosphorylates numerous players in various pathways of the DSB response, as many sensors and other protein kinases, originating a multi-layered phosphorylation network [39, 40].

More recently, the role of ATM in the redox state of the cell has been highlighted indicating another important role of ATM protein in cellular homeostasis and metabolism [41-43].

Differential Diagnosis

Establishing the diagnosis of Ataxia Telangiectasia is most difficult in very young children, primarily because the full syndrome is not yet apparent. The most common misdiagnosis is cerebral palsy. Diagnosis of AT is questionable when accompanied by severe mental retardation, seizures, non-progressive ataxia, or microcephaly. Not unexpectedly, mutations in genes specifying proteins in pathways controlled by or involving ATM leads to phenotypes overlapping with AT [39]. Mutations in Nbs1 and Mre11 give rise to Nijmegen Breakage Syndrome (NBS) and AT-LD respectively, both of which show considerable overlap in clinical and cellular phenotype with AT [44-46]. ATM protein is dependent upon the MNR (NBS1, Mre11 and RAD50) complex as a sensor of double strand breaks and in turn phosphorylates these proteins to enhance their activity in DNA repair and as an adaptor molecule for phosphorylation of other downstream substrates of ATM. Patients presenting Ataxia with Oculomotor Apraxia type 1 (AOA1) share with AT patients a common neurological phenotype but no multisystemic involvement neither elevated alpha-fetoprotein, decreased IgA levels, chromosomal instability and hypersensitivity to ionising radiations. The AOA1 patients show ataxia with onset in childhood, choreoathetosis, dystonia, severe oculomotor apraxia; the disease is slowly progressive and the prognosis is favourable due to the absence of recurrent infections and neoplasia. The gene responsible for the disease in Japanese as well in Portuguese patients was identified s in 2001 and called APTX [47, 48]. This gene encodes for a protein called ataxin, which is a member of the HIT superfamily, involved in DNA Single Strand Breaks repair and response to genotoxic agents causing oxidative stress [49, 50]. Ataxia with Oculomotor Apraxia type 2 (AOA2) presents with the same neurological and cellular phenotypes but elevated alphafetoprotein levels; the onset is between 10 y and 22 y [51, 52]. The gene defective in AOA2 was cloned in 2004 and called SETX [53]; it encodes for a protein, the senataxin, which plays an important role in RNA processing and homeostasis [54]. Both the AOA new syndromes could be misdiagnosed as AT, but the clinical features together with normal levels of ATM protein will help for a correct diagnosis, then confirmed by molecular analysis.

Recently, using whole exome sequencing, mutations in ATM gene have been identified in patients suffering dopa-responsive cervical dystonia [55].

Therapeutic Approaches

No established therapy for AT is currently available: treatments are symptomatic and supportive only, and since last few years no controlled study existed regarding the pharmaceutical reduction of ataxia symptoms in AT. Interventional trials are hampered by the lack of universal rating scales to assess the complex neurological impairment in AT, and the variable contribution of different movement disorders to the morbidity of the patients. The prevention of primary manifestations of AT has been so far unsuccessful. Therapeutic interventions such as early and continued physical therapy minimize contractures, which appear in almost all individuals with time and lead to other physical problems, whereas IVIG replacement therapy appears to reduce

the number and severity of infections in patients presenting with them. Rehabilitation and supportive care includes physical, occupational and speech/swallowing neuro-rehabilitation. Adaptive equipment used includes braces, walkers, orthotics, wheelchairs and computers. A wheelchair is necessary by 10 y of age in patients with the classical form of the disease.

The therapeutic and prophylactic approach to managing infections involves:

- Early antibiotic treatment and continuous prophylactic therapy;
- Use of vaccines (*Streptococcus pneumonia*, *Neisseria meningitides*, *Haemophilus influenzae*);
- Regular (every 3-4 weeks) injection of immunoglobulin.

Fetal thymus implants and stimulants of the immunological system have given inconclusive results.

The predisposition to malignancy suggests the need for regular medical checks to watch for early signs. The use and doses of radiation therapy and chemotherapy are controversial. Due to the hypersensitivity to ionizing radiations, the use of radiotherapy and some radiomimetic chemotherapeutic agents should be monitored carefully; conventional doses are potentially lethal and specific protocols must be applied. Some reports indicate that standard-dose chemotherapy should be given to each AT patient with lymphoid malignancies, whereas others advise reduced doses, especially for alkylating agents. According to some references, bleomycin, actinomycin D and cyclophosphamide should be avoided. A positive effect of treatment with dexamethasone has been reported both *in vitro* and *in vivo* [56, 57].

Neuroprotective Treatments

Antioxidants

The compound phenotype of AT is linked to a continuous state of oxidative stress leading to an increase of programmed cell death. Oxidative damage to lipids and DNA has been found to be markedly increased in AT patients [58]. Antioxidants (e.g. vitamin E or a-lipoic acid) are recommended, although there has been no formal testing for their efficacy in AT individuals [59].

An *in vivo* adaptive response to a pro-oxidant state in AT-related target organs was suggested by Degan et al. [60] measuring a set of oxidative stress end points in nine AT homozygotes, on the basis of a significant decrease in blood glutathione disulfide (p=0.012) and in methylglyoxal plasma concentrations (p=0.012) in patients versus controls.

Several studies have examined the effect of antioxidants in ATM-deficient mice used as an animal model of AT; it has been demonstrated that these treatments correct the neurobehavioral deficit in mice [61].

The biochemical basis for the antioxidant treatment is that oxidation of ATM directly induces ATM activation in the absence of DNA DSBs. The oxidized form of ATM is a disulfide cross-linked dimer, and mutation of a critical cysteine residue involved in

disulfide bond formation specifically blocks activation through the oxidation pathway. Identification of this pathway explains the observations of ATM activation under conditions of oxidative stress and shows that ATM is an important sensor of reactive oxygen species in human cells [62-64] demonstrated that ATM promotes an antioxidant response by regulating the pentose phosphate pathway and protects the cells from ROS accumulation by stimulating NADPH production and promoting the synthesis of nucleotides required for the repair of DSBs.

Desferrioxamine has been shown to increase the genomic stability of AT cells and therefore may present a promising tool in AT treatment [65, 66].

Gene therapy

In the last several years, a new therapeutic strategy has suggested the use of Premature Termination Codon suppression in the treatment of PTC induced genetic disorders. This therapeutic strategy, also called "suppression therapy" or "translational read-through", utilizes small molecule pharmacological agents to selectively suppress translation termination at PTCs but not at normal stop codons to restore translation of full-length, functional proteins [67]. *In vitro* studies suggest the possibility of a successful therapy for the 15% of patients carrying single-nucleotide changes that introduce premature termination codons. The *in vitro* use of aminoglycoside antibiotics has shown their potential to read through premature termination codons inducing full-length ATM protein and the restoration of ATM functions [68-70]. Despite the great potential, AGs use is quite restricted due to relatively high toxicity values observed upon their administration. In consequence, *in vitro* testing has been carried out on a range of new molecules, such as antisense morpholino oligonucleotides [71-73], SMRT (small molecules read-through) compounds [67, 74, 75] and micro-RNA [76, 77].

Although these derivatives hold great promise to serve as therapeutic candidates they also demonstrate the necessity to further understand the molecular mechanisms of which aminoglycosides and other small molecules confer their biological activity in eukaryotic cells for further rational drug design. In fact, all these approaches appear promising but are still in an early phase for application in humans.

Symptomatic Treatments

Antiepilepticic drugs

Gazulla et al. [78] treated a 34 y old man with adult onset AT with a combination of pregabalin (225 mg/day) and tiagabine (7.5 mg/day). Pregabalin treatment improved the patient's gait (patient was able to walk for >10 m without support), and the addition of tiagabine increased the distance of unsupported walking to >20 m and facilitated execution of half turns. Such improvements were sustained for an unspecified period, after which they disappeared, and the drugs were withdrawn.

Antidyskinetic drugs

The literature on the treatment of movement disorders in AT is scarce. The main encountered problem is that drugs that increase

dopamine in the striatum treat Parkinsonism (bradykinesia, bradylalia, hypomimia) but exacerbate hyperkinetic movements (choreoathetosis, myoclonus, polymyoclonus, dystonia), and vice versa. Nissenkorn et al. [12] treated 17 children with the dopaminergic and anti-NMDA agent amantadine sulfate administered at doses up to 7 mg/kg/day for 8 weeks. Overall, 76.5% of patients were responders, showing more than 20% improvement on the neurologic scores; the drug affected to the same degree ataxia, involuntary movements and Parkinsonism without exacerbating movement disorder and with mild and transient sides effects. Interestingly, after completing the study, 9 patients chose to continue with amantadine chronic treatment, but follow-up visits at 6 and 12 months showed no significant change in neurological score as compared with 8 week's visit. Otherwise follow-up assessments in 7 patients who discontinued the drug revealed significant neurological deterioration.

The improvement in Parkinsonism that Nissenkorn and colleagues documented is probably explained by a dopaminergic mechanism well known for more than 40 y. In children, there is a long experience of L-dopa treatment in primary deficiencies of the dopamine system, as in dopa-responsive dystonia or in juvenile Parkinson's disease. Treatment usually requires very low doses, and if the dose is raised very slowly, then children seem to tolerate the drug with relatively few side effects.

Two subgroups of Italian AT patients with movement disorders have been treated with the dopaminergic drugs amantadine (at the dose of 5 to 7 mg/kg/day for 8 weeks) and levodopa (at the dose of 4 to 10 mg/kg/day for 12 weeks), showing no relevant beneficial effects on both cerebellar ataxia and extrapyramidal involvement (Leuzzi and Micheli: personal communication).

Glucocorticoids

Glucocorticoids are the most effective anti-inflammatory therapies, widely applied in the treatment of many diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, and so on. Most recently, glucocorticoids have been found to be useful also in the treatment of neurometabolic and neurogenetic diseases, but their overuse is associated with long-term side effects, including osteoporosis, diabetes, obesity, cataracts, hypertension and pneumonia, which are dose and time dependent [79-81].

In the study of Buoni et al. [82, 83] a 3 y old boy with classic AT symptoms and molecular evidence of AT experienced a marked reduction in neurological signs following corticosteroid therapy for asthma. Subsequent treatment with betamethasone (BETA), at 0.05 mg/kg every 12 h for 4 weeks, showed improvements in the child's neurological symptoms 2 or 3 days after the beginning of therapy; after 4 weeks of treatment, the improvement was dramatic: the disturbance of stance and gait was clearly reduced, and the control of the head and neck had increased, as had control of skilled movements. The neurological improvement was so great that the child was able to go up- and downstairs. The most notable adverse effects were increased appetite and body weight and fluid retention, resulting in a moonlike face.

This serendipitous observation was then confirmed in several observational studies [82-87].

In 2011 a multicenter, double-blind, randomized, placebo controlled crossover trial was performed in Italy [88]. The trial compared oral treatments with BETA and placebo in terms of their reduction of ataxia symptoms as assessed with the International Cooperative Ataxia Rating Scale (ICARS). In this study of 13 AT children, BETA reduced the ICARS total score by a median of 13 points in the intent-to-treat population and of 16 points in the per-protocol population, with a median percent decrease of ataxia symptoms of 28% and 31%, respectively. There were no compulsory withdrawals. Minor side effects did not require medical intervention. Small increases in body weight were observed in 12 patients on BETA and in 4 patients on placebo. Moon face was present in eight patients on BETA. Out of the 13 patients enrolled, 10 completed the trial: 1 resulted not to respond to the drug; the 9 responding were classified as low responders (2), certain responders (4) and good responders (3).

In order to avoid the side effects of long-term administration of steroids, a method for the encapsulation of dexamethasone sodium phosphate (DSP) into autologous erythrocytes (EryDex method), allowing the slow release of dexamethasone for up to 1 month, has been established [89]. Among the glucocorticoids, dexamethasone is the most similar to BETA, and its high anti-inflammatory potency, together with its lack of mineralcorticoid activity, make it a good choice for low-dose/long-term treatment as needed in chronic inflammatory diseases, especially in those, such as AT, characterized by immunodeficiency. The ability to incorporate (encapsulate) DSP (an inactive pro-drug) into autologous erythrocytes allows the slow release of low doses of dexamethasone (active drug) [90]. Preliminary results obtained in 10 patients with chronic obstructive pulmonary disease, 18 patients with cystic fibrosis and in a pilot study of 5 patients with Crohn's disease and 5 with ulcerative colitis showed in all patients a significant improvement in the indices of inflammation; no side effects from steroids were registered [91-93].

Following this, a single-arm, open-label, Phase II clinical trial was conducted in two Italian centers (Brescia and Roma) to assess the effect of EryDex method on twenty-two children and adolescents with AT, which underwent for 6 months monthly infusion of EryDex [94]. Compared with baseline, a significant improvement in ataxia symptoms (assessed by ICARS) was detected, both in the intention-to-treat (ITT) population (p=0.02) as well as in patients completing the study per protocol (PP) (n=18; p=0.01), without association with the typical steroid side effects. Similarly, significant improvements were noted in adaptive behavior, as assessed by the Vineland Adaptive Behavior Scales (VABS, p<0.0001). Considering that most of the patients had an advanced form of the disease, these results were noteworthy and the improvement was more remarkable in less neurologically impaired patients, suggesting the efficacy of treatment as function of the stage of the disease. Moreover, the clinical improvement was even more relevant in patients with the most efficient dexamethasone loading in the erythrocytes. The efficiency of erythrocyte loading was related to a greater improvement in responding females than males.

The trial demonstrated that steroid treatment led to a significant improvement in ataxia symptoms, and EryDex method has been proposed as an effective treatment option to avoid the side effects of long-term administration of steroids.

At the end of the trial, four patients chose to continue with monthly EryDex infusions. After an adjunctive 24-month period of treatment, the ICARS scores of these patients were compared with those of 7 age matched AT subjects who have discontinued the treatment after the first 6 infusions but continued to be evaluated according to the same protocol [95]. Compared to the patients who stopped the treatment, patients in the extended study experienced a continuous neurologic improvement with respect to their pre-treatment status (p=0.01) and to the scores detected at the end of the 6 month trial (p=0.00), whereas controls showed a progressive neurologic deterioration, according to the natural history of the disease after the discontinuation of the treatment. No steroid-dependent adverse reactions were observed during the extension of the study. The persistent neurological improvement observed in the treated patients in the additional 24 month period of infusions supported the hypothesis that a protracted EryDex treatment may modify the natural progression of the disease (**Table 1**).

So far, the same four AT subjects have been treated in compassionate use with monthly infusions for an overall 48 month period. In follow-up assessments, performed every three months, patients showed no further improvements on ICARS scores with respect to the 24 month period of treatment. From a clinical perspective, this observation probably corresponds to a plateau in cerebellar symptoms, while extrapyramidal movement disorder and bradykinesia progress, causing a deterioration of patients' global neurologic conditions (Micheli R, unpublished data).

In vitro dexamethasone has been shown to induce a noncanonical splicing that leads to the translation of a short ATM variant retaining kinase activity, called mini ATM. Thus, ATM may be restored by a new molecular mechanism that overcomes most of the mutations so far described in the ATM gene [96]. If confirmed in other studies, this observation would provide the molecular basis for dexamethasone action in AT patients. However, other mechanism (s) of dexamethasone that could help explain the observed clinical improvement, such as its anti-inflammatory effects, need to be considered and are currently under investigation.

The Authors currently consider EryDex treatment as a safe and well-tolerated treatment that is effective on both cerebellar ataxia and oculomotor apraxia in AT; conversely, in our experience, steroids had no effects on extrapyramidal involvement (both Parkinsonism and hyperkinetic movements) and on peripheral neuropathy. In the near future, we look forward hopefully to a long-term treatment combining both steroids and dopaminergic/NMDA antagonists to improve ataxia/oculomotor apraxia and Parkinsonism/movement disorders. We have at present no chances to treat axonal peripheral neuropathy, except for the symptomatic therapies as in common peripheral neuropathies (motor rehabilitation and prevention/surgical treatment of the contractures).

Conclusion

The efficacy of steroids in improving ataxia in AT patients has been proven in the last 5 y. To avoid the side effects of long-term administration of steroids, the EryDex method appears a promising approach. A potentially pivotal worldwide double-blind trial with EryDex in AT patients, planned to start in 2016, is being prepared. Nevertheless, there are problems to be addressed. The encapsulation of the drug into the patient's erythrocytes must be performed in transfusion centers. As patients are widely spread across their countries, often far from a big hospital, this could cause logistical difficulties to their families.

The mechanism by which steroids act to improve neurological symptoms in AT patients is also not yet clarified. The two current main hypotheses are a read-through mechanism and an anti-inflammatory mechanism. Steroids are well known and widely used anti-inflammatory agents, able to pass the blood-brain barrier. It is possible to envisage effects on the functionality of the neuro-glio-vascular unit [97]. Recently, Biagiotti et al. [98] constructed cDNA libraries containing those genes that are transactivated by dexamethasone in AT human cells. By means of suppression subtractive hybridization and in silico analyses, they identified genes coding for transcripts involved in metabolic processes and the regulation of cellular processes. Moreover, Liston and Gan [99] have demonstrated a critical role for glucocorticoids in the development and maintenance of dendritic spines in the living cortex in mice. Together, these results may help to unravel the mechanism of glucocorticoid action in reversing the AT phenotype.

Extrapyramidal symptoms, on the other hand, may be ameliorated by dopaminergic or NMDA antagonists. However, long-term, placebo-controlled studies are needed. With regard to treatments with read-through compounds or SMRT molecules, these are still experimental and while very promising but limited to patients with a specific type of mutation.

The author's opinion is that both current therapeutic approaches, that is, dopaminergic drugs and glucocorticoids, show promise in helping patients and slowing progression of the disease. However, when we look at the results obtained so far, we need to take into account that we have little knowledge about the basis of individual responses to a given therapy. Due to the great heterogeneity of AT, a number of subjects have been found who are not responding to both therapeutic approaches. Understanding the reasons that make an individual a good responder will be of great help for planning the best individual therapy.

Early treatment in asymptomatic children could be speculated to delay neurological degeneration.

Acknowledgment

Table 1 Chronic treatment with ERY-DEX: Long term benefit confirmed with compassionate treatment up to 2 y.

Case/born	V1 (Baseline) 03/2011	V7 (End of trial) 08/2011	C1 (Start of compassionate treatment) 11/2011	C8 (After 24 months of compassionate treatment) 01-11-2013	Improvement	
2.01 20/02/2002	57	53	49	45	21%	ICARS TOTAL SCORE
2.02 09/03/2002	55	58	58	51	7%	
2.05 31/08/1996	58	56	50	39	33%	
2.08 18/08/2000	49	42	37	40	18%	

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