Current and potential therapeutic strategies for the treatment of ataxia-telangiectasia

Martin F. Lavin[*†‡]**, Nuri Gueven**[†]**, Stephen Bottle**[¶]**,** and **Richard A. Gatti**[§]

[†]*Queensland Institute of Medical Research, Brisbane, Queensland, Australia,* [‡]*School of Medicine, The University of Queensland, Brisbane, Australia,* [¶]*Science Research Centre, Queensland University of Technology, Brisbane, Australia, and* [§]*UCLA School of Medicine, Los Angeles, CA, USA*

Ataxia-telangiectasia (A-T) is a rare autosomal recessive genetic disorder characterized by progressive neurodegeneration, a high risk of cancer and immunodeficiency. These patients are also hypersensitive to radiotherapy. The gene product defective in this syndrome, ATM (*a*taxia-*t*elangiectasia *m*utated), normally recognizes DNA damage and signal to the DNA repair machinery and the cell cycle checkpoints to minimize the risk of genetic damage. No curative strategy for this disease exists. Treatment has focused on slowing the progress of the neurodegeneration; devising approaches for the treatment of tumours while minimizing side effects and treatment with immunoglobulin for the immunodeficiency. The most debilitating feature of this disorder is the progressive neurodegeneration due to loss of Purkinje cells in the cerebellum and malfunction of other neuronal cells. Correcting for the loss of Purkinje cells is technically very difficult and would require transplantation of embryonic stem cells. However, since it seems likely that oxidative stress may contribute to the neurodegeneration in A-T, potential therapies based on the use of antioxidants offer some hope. We describe the natural course of disease, some supportive therapeutic approaches already in use and those with potential based on our knowledge of molecular and cellular characteristics of this disorder.

Keywords: Ataxia-telangiectasia/neurodegeneration/ATM/antioxidants/ mutation-targeted therapy

Background

Ataxia-telangiectasia (A-T) is a complex multisystem disorder characterized by progressive neurological impairment, immunodeficiency and ocular and cutaneous telangiectasia. It was first described by Syllaba and Henner[1] and established as a disease entity 30 years later by Boder and Sedgwick.[2] An adverse response to radiotherapy was reported by several groups a decade later[3,4] and hypersensitivity to X-rays and γ-rays was later established for A-T cells in culture.[5,6] Gatti *et al.*[7] used linkage analysis to map the gene defective in A-T to chromosome

11q22-23, and the gene was eventually identified by positional cloning by Savitsky *et al.*[8] The identification of the gene *ATM* (*A*taxia-*T*elangiectasia *M*utated) as a member of a family of phosphatidylinositol-3-kinase (PI3K)-related genes, involved in cellular responses to DNA damage and cell cycle control, provided further insight into the pleiotropic nature of A-T. This disorder is inherited as an autosomal recessive trait with full penetrance[9] and has been reported in all races throughout the world, and, as expected from the type of inheritance, it is represented equally in males and females. The first estimate of disease prevalence was provided by Boder and Sedgwick[10] as one in 40 000 live births. A more comprehensive case-finding study by Swift *et al.*[11] produced a maximum incidence of one case of A-T per 88 000 births. The incidence from studies conducted in UK between 1970–1972 and 1980–1984 was estimated to be four-fold lower, i.e. closer to three per 10^6 live births.[12]

Functioning of the ATM protein

ATM is predominantly a nuclear protein with protein kinase activity similar to that of other DNA damage recognition and repair proteins that constitute the phosphoinositide-3 kinase-like kinase family.[13] ATM is activated by autophosphorylation at several sites in response to DNA double strand breaks.[14,15] Activation is also dependent on the presence of the Mre11 complex (Mre11/Rad50/Nbs1) which acts as a sensor of breaks in DNA.[16] Once activated ATM then phosphorylates a series of substrates that participate in signalling to the cell cycle checkpoints and in facilitating DNA repair.[17] These substrates include the tumour suppressor protein p53 which is activated during ATM signalling to prevent the passage of cells from G1 to S phase or to induce apoptosis. Other ATM substrates are the checkpoint kinase Chk2; the breast cancer susceptibility protein BRCA1; and Nbs1 mutated in the human genetic disorder Nijmegen Breakage Syndrome.[18] Disrupting the function of this protein in mice gives rise to a similar phenotype to that of A-T patients; however, these animals do not exhibit neurodegeneration.[19] On the other hand, these animals have reduced catalase activity, reduced levels of NAD(P)H, increased activities of superoxide dismutase (SOD) and hemoxygenase and increased production of reactive oxygen species (ROS) in the cerebellum.[20–22] Further evidence for cerebellar impairment is provided by the observation that cell cycle proteins are re-expressed in Purkinje cells from $Atm^{-/-}$ mice and that DNA replication accompanies this reappearance.[23] Furthermore, *in vitro* survival of cerebellar Purkinje cells of *Atm*-mutant mice is significantly reduced when compared with cells from their wild-type

littermates and most neurons from these animals have dramatically reduced dendritic branching. These neurological abnormalities can be corrected by exposure of mice to an isoindoline nitroxide antioxidant, as further discussed below.[24,25]

Clinical features

In the first report of A-T, Syllaba and Henner[1] observed progressive choreoathetosis and ocular telangiectasia in three members of a single family. Louis-Bar[26] subsequently described progressive cerebellar ataxia and cutaneous telangiectasia in a Belgian child and this disorder was called the Louis-Bar Syndrome. A-T was not described as a distinct clinical entity for a further 16 years, when Boder and Sedgwick[2] and Biemond,[27] assisted by autopsies, reported organ developmental abnormalities, neurological manifestations and a third major characteristic of the disease, recurrent sinopulmonary infection. The major features of this syndrome were confirmed in many subsequent reports.

Ataxia and telangiectasia

A-T is stereotyped in its neurological symptomatology with ataxia, generally the presenting symptom in this syndrome, being evident when the child begins to walk at the end of the first year of life, manifesting ataxic gait and truncal movements.[28] As is the case for other major characteristics of A-T, ataxia is progressive, spreading to affect the extremities and then speech. Eventually, involuntary movements become evident, and the child usually requires a wheelchair by the end of the first decade of life. Cortical cerebellar degeneration in A-T involves primarily Purkinje and granular cells, but adjacent basket cells (GABAergic interneurons in the molecular layer) remain unaffected. While degenerative changes in the central nervous system (CNS) are seen predominantly in the cerebellum, changes have also been described, including changes in the dentate and olivary nuclei, the spinal cord and spinal ganglia, the cerebrum, the basal ganglia and the brain stem. Telangiectasia is a second major clinical manifestation of the disease. It usually has a later onset than ataxia, occurring between 2 and 8 years of age.[28,29] Telangiectasia is due to dilation of blood vessels, primarily in the ocular sclerae, and often gives the impression of 'bloodshot' eyes. Telangiectasis is not exclusively ocular and may also appear in the butterfly regions of the face and on the ears. Patches of telangiectasia elsewhere in the skin are less common. Ocular

telangiectasis may be mistaken for conjunctivitis but can be readily distinguished because they are characterized by dilated vessels against a white background, whereas in conjunctivitis the background is pink.

Immunodeficiency and chromosomal instability

A-T is a highly variable primary immunodeficiency, involving both cellular and humoral immunity.[29–33] In the latter case a reduction in IgA and IgE levels as well as abnormalities in IgM and IgG subclasses are widely observed. Some patients have a history of chronic sinopulmonary infections, others have repeated infections and the incidence of infections in a third group is no higher than in their unaffected siblings.[29] Infections include otitis and sinusitis as well as recurrent pneumonia, which may progress to bronchiectasis and pulmonary fibrosis, severe enough to cause clubbing of fingers and toes, and, eventually, to respiratory insufficiency and death.[30] A-T patients are susceptible to common bacterial pathogens and viruses, and not opportunistic infections, as seen in other primary immunodeficiencies. They do not appear to be subject to generalized or persistent fungal or protozoan infections. There appears to be no direct correlation between the severity and frequency of infections and the degree of immunodeficiency.

Faulty development of the thymus is highly characteristic of A-T.[29] The thymus often cannot be identified grossly at autopsy, but only recognized microscopically as a scattered collection of thymic reticular elements with a marked paucity of thymocytes and absence of Hassell's corpuscles and corticomedullary demarcations. These abnormalities appear to reflect a developmental defect rather than atrophy of the thymus.[31] Approximately one-third of patients display unequivocal lymphocytopenia, but this is usually mild in degree. The CD4/CD8 ratio in A-T patients is reversed when compared with controls because of a decrease in the total number of $CD4^+$ T cells.[32,33] Furthermore, A-T patients have a relative increase in T cells bearing γ/δ antigen receptors compared with those bearing α/β receptors, unlike T cell populations in most other immunodeficiency syndromes. Fiorilli *et al.*[32] suggested that a defect in recombination or DNA rearrangement may explain the defects in both T and B cell differentiation. Such a defect could also account for the high incidence of chromosomal rearrangements involving primarily chromosomes 7 and 14.[34] Four common sites of chromosome breakage have been described in A-T patients: 7p14, 7q35, 14q11.2 and 14q32. The T cell receptor genes map to the first three of these sites, and the Tcl-1 gene was cloned from within the 14q32 breakpoints of such translocations in T cell malignancies of A-T and non-AT patients.[35] However, neither

recombination or V(D)J rearrangements are grossly abnormal in A-T cells. T cell leukaemias in A-T patients and lymphomas in Atm mutant mice are characterized by chromosomal translocations involving T-cell receptor loci.[36,37] More recently, Matei *et al.*[38] have shown that ATM deficiency increases the frequency of T-cell receptor α deletion events which compromise $CD4^+$/$CD8^+$ thymocyte maturation and may be responsible for oncogenic T-cell receptor translocations. Thus, a defect in DNA processing with persistence of some double-strand breaks would best explain the immunodeficiency and the lymphoid malignancies seen in A-T.

Cancer

The lifetime prevalence of cancer in A-T patients is 10–30%[39] which is the second most common cause of death.[37] This incidence is approximately 100-fold greater than expected for an age-matched population. Of these cancers, 40% are non-Hodgkin's lymphomas, 25% are leukaemias, 25% are assorted solid tumours and 10% are Hodgkin's lymphomas.[39] Most leukaemias and lymphomas are of T-cell origin[37], a pattern that is similar to that observed in *ATM* knockout mice [19,40], but is remarkably different from the B-cell predominance of lymphoreticular malignancies in children without A-T. The solid tumours in A-T patients include adenocarcinoma of the stomach, dysgerminoma, gonadoblastoma and medulloblastoma. The range and frequency of tumours[41] are probably best explained by genome instability arising from defective recognition and repair of double-strand DNA breaks, as well as defective cell cycle checkpoints. There is also an increased risk of cancer among A-T heterozygotes, particularly in breast cancer,[42] suggesting prevalence/dominance of the defective gene even though this is an autosomal recessive disorder.

Phenotypic heterogeneity

Phenotypic heterogeneity has long been described in both the laboratory and clinical features of A-T. Susceptibility to pulmonary infection, presence and degree of mental retardation and predisposition to leukaemia is variable in A-T and represents a tentative means of subclassifying A-T. Even among classical A-T patients there is some variability in the rate of progression of clinical symptoms. Some patients are intermediate in their radiosensitivity and others have typical radiosensitivity.[43] Several studies on *ATM* mutations predict that 70–80% give rise to truncated proteins, while the remainder represent small to large

in-frame deletions and missense mutations.[8,44,45] Most patients are compound heterozygotes with different mutations in the two *ATM* alleles. This information has been useful in accounting for milder forms of the disease in some A-T patients. A missense mutation in *ATM* that activates a cryptic splice/donor acceptor site resulting in the insertion of 137 nucleotides of intronic sequence (5762ins 137) was present in the heterozygous state in 15% of A-T patients in the UK with a milder phenotype.[46] A second missense mutation 7271T > G, that produces a kinase-dead ATM protein, is also associated with a mild-variant A-T phenotype.[46] Thus, allelic diversity might explain much of the heterogeneity in clinical severity in A-T.

Diagnosis

Clinical diagnosis of A-T is relatively easy once the characteristic neurodegeneration and ocular telangiectasia have developed. In these cases the diagnosis can usually be confirmed by finding an elevated serum α-fetoprotein level (Table 1). It is not clear why α-fetoprotein remains high in A-T patients since there is no obvious liver damage; but may be due to abnormal regulation of RNA transcription in the absence of ATM. It is much more of a challenge to make the diagnosis in young children without progressive ataxia, in the minority of children who never develop telangiectasia or in those children with atypical or mild phenotype. The differential diagnosis includes a variety of conditions,

Table 1 Clinical and laboratory diagnosis

Diagnostic criteria	A-T phenotype	Reference
Clinical		
Cerebellar ataxia	Present	Sedgwick and Boder[30]
Telangiectasia	Present	Sedgwick and Boder[30]
Oculomotor apraxia	Present	Sedgwick and Boder[30]
Sinopulmonary infection	Variable	Sedgwick and Boder[30]
Cellular and molecular		
α-fetoprotein	Elevated	Waldman and McIntire[58]
Radiation response	Hypersensitive	Taylor *et al.* (1976),[59] Huo *et al.* (1994),[60] Sun *et al.* (2002)[61]
p53 stabilization	Defective	Kastan *et al.* (1992),[62] Khanna and Lavin (1993)[63]
ATM mutations	Yes	Savitsky *et al.* (1995)[8]
ATM protein	Absent/Detectable	Watters *et al.* (1997),[64] Becker-Catania *et al.* (2000),[65] Chun *et al.* (2003)[66]
ATM kinase activity	Absent	Banin *et al.* (1998),[67] Canman *et al.* (1998),[68] Khanna *et al.* (1998)[69]
ATM signalling pathways	Defective	Multiple reports

including infectious encephalitis, Friedreich's ataxia which has a later onset, ataxia associated with a number of metabolic diseases of infancy and childhood (such as Gaucher disease and Niemann-Pick disease), A-T like disorder (A-TLD) (Mre11 deficiency) and the autosomal recessive syndromes with ataxia and oculomotor apraxia AOA1 and AOA2. For a more detailed description of differential diagnosis see Lavin and Shiloh[13]. The identification of the *ATM* gene has facilitated diagnosis, but because the gene is so large and there are no mutational hot spots, mutational analysis has not been practical for clinical screening. Other confirmatory laboratory test results include absence of the ATM protein on immunoblots, lack of ATM protein kinase activity, increased frequency of chromosomal breaks after exposure to γ-radiation, radioresistant DNA synthesis and decreased colony survival after γ-radiation. None of these methods is 100% specific or 100% sensitive, and clinical correlation is essential. See references for all of these characteristics and methods in Table 1.

Features requiring treatment

Neurodegeneration

The earliest clinical manifestation of A-T is ataxia.[47] By approximately 4 years, deterioration of gross and fine motor skills occurs; eye movements are characterized by a series of small jumps rather than in a single smooth motion (oculomotor apraxia); choreiform movements of the hands and feet are observed, difficulty with chewing and swallowing occur and drooling is common.[48] A-T is primarily a syndrome of progressive cerebellar ataxia but more diffuse changes to the CNS are also evident. Microcephaly is usually not observed in A-T unlike that in NBS patients.[18] Cerebellar degeneration in A-T manifests as dystrophic changes involving the dendrites and axons of Purkinje cells and ectopic Purkinje cells are evident. Of all the features of A-T the progressive cerebellar neurodegeneration is the most debilitating. By the end of the first decade ataxia has progressed to the extent that the child is confined to a wheelchair and may have poor control of head and torso. This progresses to include peripheral neuropathy and eventually to spinal muscular atrophy. Thus, any effective treatment for A-T would ideally involve prevention or at least slowing of the progressive neurodegeneration. It would of course be a bonus to increase the latency period or markedly reduce the risk of lymphoid malignancies.

Modest improvements can sometimes be achieved for treating the associated neurological symptoms of A-T. Basal ganglia dysfunction may respond to L-DOPA derivatives, dopamine agonists and,

occasionally, to anticholinergics. The latter may also reduce drooling. The loss of balance may respond to amantadine, fluoxetine or buspiron. These may also improve speech and coordination. Tremors are often controlled with gabapentin, clonazepan or propanalol. A recent case report points out a long known observation that steroids produce a short-term improvement in ataxia.[49] However, the long-term complications of steroid use far outweigh this short-term benefit. Furthermore, although deficiencies of thiamine, vitamin B_{12} and vitamin E can cause ataxia, multivitamin supplements do not correct the ataxia of A-T patients.

Immunodeficiency

As in other disorders, frequent infections accompanied by hypogammaglobulinaemia with antibody deficiency can be treated with immune globulin replacement. Sinopulmonary complications are associated, but not directly correlated, with the immunodeficiency and chronic aspiration. In addition, patients with recurrent lower respiratory tract infections should be assessed for dysfunctional swallowing and aspiration (preferably without undue exposure to diagnostic X-rays). These defects are associated with pulmonary symptoms, which are exacerbated by poor mucociliary function, defective inspiration and weak cough. Conventional methods for assessing pulmonary function are unreliable in A-T, so it is necessary to develop tests that will accurately determine lung function to aid intervention and initiate treatment. Treatment for dysfunctional swallowing and aspiration includes the addition of thickeners to thin liquids and the placement of a gastroscopy tube to facilitate feeding in severe cases of malnutrition. Sinopulmonary infections usually respond well to antibiotics.

Malignancy

Lymphoid haematopoietic malignancies develop in 10–30% of A-T patients, primarily non-Hodgkin lymphomas. The nature of the treatment for these tumours is very much influenced by the extreme sensitivity to radiation in A-T patients. Clinical radiosensitivity in the form of severe and adverse response to X-irradiation was first described for A-T patients in the late 1960s.[3] Clearly, it is desirable to reduce the intensity of radiotherapy or dosage of radiomimetic chemotherapeutics to minimize tissue damage in patients while retaining effectiveness of treatment of the tumour. Other considerations are the selection of agents that minimize muscle weakness in a background of ataxia; the

risk of late onset haemorrhagic cystitis caused by cyclophosphamide, ifosamide and possibly vincristine; gastrointestinal toxicity caused by methotrexate requiring aggressive rescue with leucovorin and the potential hypersensitivity to topoisomerase inhibitors.[50]

Potential therapies

At present there is no therapy available to cure or prevent the progress of A-T. As mentioned above it is possible to alleviate some of the symptoms associated with immunodeficiency and deficient lung function but neither the cancer predisposition nor the progressive neurodegeneration can be prevented. Extensive research into A-T, since the ATM gene was identified, has provided several potential approaches for treatment. These are reviewed in the following sections.

Effects of myo-inositol on neurological and immune functions in A-T

Yorek *et al.*[51] demonstrated that uptake and incorporation of *myo*-inositol into phosphoinositides, as well as free *myo*-inositol content, was low in some A-T fibroblasts. Phospholipid metabolism was also less active in A-T than in normal cells. They suggested that these abnormalities might impact on cellular signalling pathways and membrane formation and in this way account for some of the A-T phenotype. Dr. Gerald Berry, an author on that paper, conducted the first A-T clinical study to investigate the effects of *myo*-inositol on neurological and immune functions. They referred to promising changes in certain immune cells in some A-T children (www.treatAT.org). While the study was described as revealing 'valuable information about the progression of A-T in the brain', the study size was not large enough to have statistical power to draw conclusions about the efficacy of this approach.

Evaluation of mitochondrial-generated ROS in patients with A-T

The overall aim of this study is to determine whether lymphocytes from A-T patients show abnormal levels of ROS and increased apoptosis and whether chronic broad antioxidant therapy retards dysfunction of lymphocytes and cerebellar neurons. The proposed study is a pilot to be conducted over 3 months, recruiting A-T patients older than 2 years. A cocktail of antioxidants will be employed. The study developed from the combined efforts of Treat-AT, Dr. Berry and his colleagues at duPont Children's Hospital of Philadelphia, and SHS International Ltd (Liverpool, UK). SHS covered the costly and lengthy

process to secure FDA approval, and is donating the treatment formula. SHS develops specialized metabolic formulas and therapies for many diseases, such as cystic fibrosis, liver and renal disease. In the 1950s, SHS produced one of the first infant formulas to manage PKU (www.treatAT.org).

Use of antioxidants

It has been reported that antioxidant capacity is reduced in the serum of A-T patients and A-T cells in culture show evidence of increased oxidative stress.[52] In addition, inhibition of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells.[53] It is not clear why absence of ATM leads to a build-up of oxidative stress. However, it is possible that the loss of ATM leads to dysregulation of transcription, which then leads to oxidative stress. Alternatively, the persistence of breaks in the chromosomes of A-T patients could indirectly lead to stress through alterations in gene expression or it may interfere with mitochondrial function causing an imbalance in O_2 metabolism and as a consequence oxidative stress (Fig. 1). Oxidative stress could then lead to macromolecular damage and in turn genome instability, cancer and neurodegeneration. Thus, one approach to treating A-T would be to use antioxidants. At present two clinical trials employing antioxidants are underway or have been completed.

Combined antioxidant and PARP inhibitor trial in A-T patients

This trial is being conducted by Dr. Howard Lederman and his colleagues at Johns Hopkins Hospital (Baltimore, MD, USA). The project was designed to test the hypothesis that some of the abnormalities seen in patients with A-T are due to oxidative stress. The A-T Clinical Center at Johns Hopkins conducted a randomized, double blind, double dummy trial of an anti-oxidant (alpha-lipoic acid 200 mg TID) and a poly ADP-ribose polymerase (PARP) inhibitor (nicotinamide 12.5 mg/kg BID) to control some of the secondary consequences of oxidative stress in adolescents and adults with A-T. The primary objectives of the trial were to look for change in markers of oxidative stress, as assessed by measuring lipid peroxidation and oxidative DNA damage in blood and urine. Secondary objectives were to look for changes in neurologic or pulmonary function, and to assess the safety of the two drugs. The trial required participants to make 10 visits to the clinical centre over a 9-month period, and to swallow as many as 15 very large capsules per day for 6 months. Seventeen subjects were recruited and
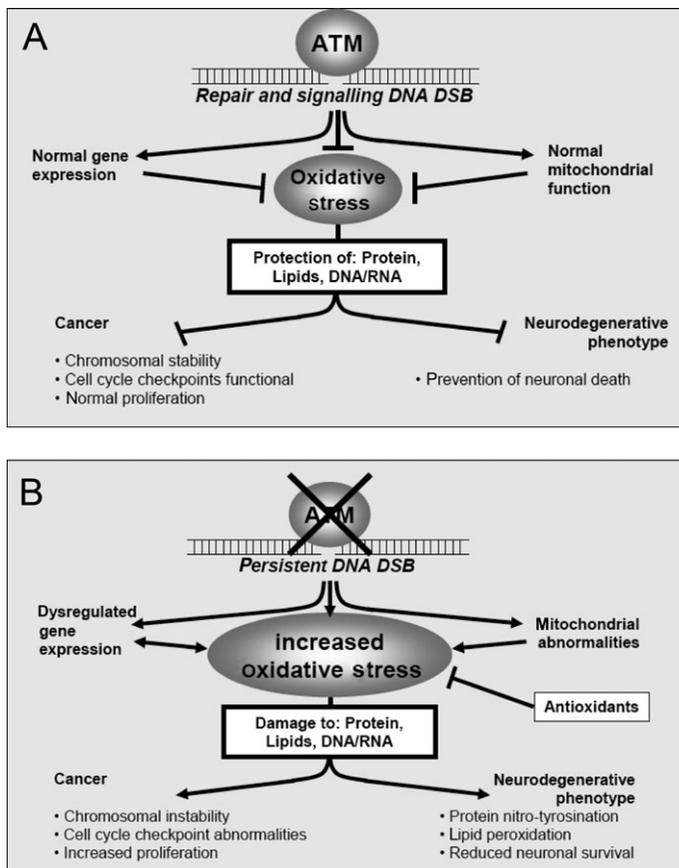
Fig. 1 Mechanism of oxidative stress in A-T cells. (A) Depiction of normal function of ATM in recognition and repair of DNA double-strand breaks to minimize oxidative stress, genome instability, cancer and neurodegeneration. (B) Mutation or loss of ATM leads to abnormal signalling of DNA breaks. There is increasing evidence from A-T patients, A-T cells in culture and in *Atm* gene-disrupted animals that loss of ATM function is associated with signs of oxidative stress.[20–22,24,25,47] It remains uncertain as to why this is the case and how much of the A-T phenotype can be explained by effects of oxidative stress. Loss of ATM has been shown to alter transcriptional profiles[70] and lead to gene silencing on the inactive X chromosome.[71] Changes in gene expression could lead to oxidative stress and contribute to the phenotype. It is also possible that residual chromosomal breaks in A-T cells could be responsible for accumulation of ROS. Continued presence of breaks might downregulate genes required to prevent oxidative stress or have an impact on mitochondrial function that in turn would increase the amount of ROS. The presence of ROS would lead to protein, lipid and DNA damage and in the longer term this could increase the risk of cancer and/or neurodegeneration.

14 successfully completed the entire trial. There were no significant adverse effects attributed to the drugs.[54]

Two markers of oxidative stress (levels of urine total alkanes and serum fast oxygen reduced absorbance capacity, ORAC) improved compared with baseline. The levels of urine total alkanes improved

when subjects were taking either drug, but were most statistically significant when subjects took both alpha-lipoic acid and nicotinamide ($P < 0.01$). The levels of serum fast ORAC were statistically improved only when subjects took both drugs ($P = 0.06$). A trend toward increased lymphocyte counts was observed when subjects took both drugs, but the difference did not quite achieve statistical significance ($P = 0.08$). The study allowed testing of the reproducibility of multiple parameters of neurologic (measures of tremor, tone, saccadic latency and A-T index score) and pulmonary (spirometry) function. There did not appear to be any important changes in any of the measured parameters. However, because subjects returned many times for these assessments within a relatively short period of time, they have been able to determine which of the parameters will be sufficiently reproducible for use in future clinical trials.

Pre-clinical trials with a novel isoindoline nitroxide antioxidant

Administration of the antioxidant 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (CTMIO) to *Atm*-deficient mice reduces the rate of cell death of Purkinje cells and enhances dendritogenesis to wild-type levels.[24] Intraperitoneal administration of this antioxidant throughout pregnancy enhanced survival of Purkinje cell neurons from *Atm*-disrupted animals and protected against oxidative stress in older animals, as determined by levels of nitrotyrosinated proteins and amount of catalase activity in the cerebellum.[24] This antioxidant, a member of the nitroxide group, is a stable, free radical, capable of scavenging ROS and may also circumvent Fenton-derived pathways by oxidizing the metals involved. Nitroxides act catalytically as SOD mimics through 1-electron redox cycles, allowing reversible formation of an ox-ammonium cation or the hydroxylamine. These properties make them suited for the treatment of disease states characterized by oxidative stress. As outlined above, results to date provide strong support for a role of oxidative stress in the development of the neurological abnormalities in *Atm*-mutant mice. They also strongly suggest that CTMIO may protect against neurodegeneration and cancer development that have a major impact on the progression of the disease. Gueven *et al.*[25] have shown this to be the case in their demonstration that CTMIO dramatically delays the onset of thymic lymphomas in $Atm^{-/-}$ mice which is not due to an enhancement of apoptosis by CTMIO. They also showed that this compound corrects neurobehavioural deficits in these mice. It appears likely that this effect was due to a reduction in oxidative stress since protein damage (3-nitrotyrosination) and lipid damage (immunoreactivity to

4-hydroxy-2 noneal Michael adducts) were significantly reduced in Purkinje cells from $Atm^{-/-}$ mice after CTMIO treatment. The likely mechanism of action of CTMIO is due to a reduction in oxidative stress, which is protective against both the tumour progression and the development of neurological abnormalities. These data suggest that antioxidant therapy has considerable potential in the management of ataxia-telangiectasia and possibly other neurodegenerative disorders where oxidative stress is implicated.

Mutation-targeted therapies

The majority of A-T patients inherit two distinct mutations. More than 500 mutations, spread over the entire coding region have been described for ATM.[55] Most of these changes (>80%) in A-T patients are predicted to give rise to truncated proteins, either through nonsense or splicing mutations, or through secondary premature terminations resulting from frameshift mutations. Thus, any attempt to restore normal function to mutant ATM through mutation-targeted therapy would require read-through of the termination codon or concealment of the cryptic splice site. Clearly, either of these approaches would have to be tailored to individual mutations. Any such approach does not necessarily have to restore normal levels of protein since even low levels of ATM ($\sim$5–10%) in some A-T patients result in a considerably milder phenotype.[43]

Correction of ATM gene function by read-through of premature termination codons

Lai et al.[56] employed aminoglycosides to achieve read-through expression of functional ATM protein. Aminoglycoside antibiotics bind to the internal loop of helix 44 of the 16S ribosomal RNA subunit, the decoding site, inducing a local conformational change that compromises the integrity of the codon–anticodon proofreading and allows translation through an otherwise terminating codon. Gatti's group showed that geneticin and gentamycin produced detectable 'read-through' ATM protein. They also demonstrated that the read-through-induced ATM protein was functional in a number of ATM-dependent signalling pathways, including activation of the S phase checkpoint, correcting radiosensitivity and giving rise to ATM pSer1981 autophosphorylation foci, indicating that ATM was being activated. As a proof of principle, this methodology is very promising; however, it requires the use of aminoglycosides that are toxic to cells and humans at concentrations that would be effective for read-through.

Identification of other compounds with less toxicity and effectiveness in reading through the terminating codon will be required to make this a therapeutic option. Promising new read-through compounds have recently been identified from a high-throughput screen. None of these compounds are aminoglycosides (Gatti *et al.*, unpublished).
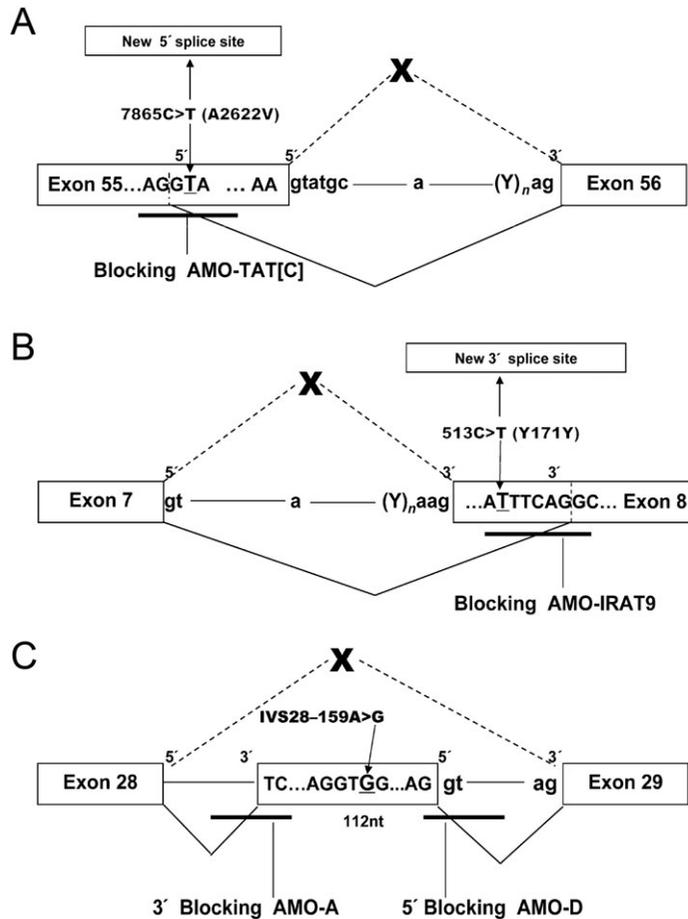
Fig. 2 Schematic representation of splicing mutations in *ATM* gene and correction with AMO. 'X' refers to normal splicing. (A) 7865 C > T leads to the deletion of the last 64 nucleotides of exon 55. AMO-TAT(C) was employed to block the mutation-generated 5′ splice site. (B) 513 C > T leads to the deletion of the first 22 nucleotides in exon 8. AMO-IRAT9 was designed to block the mutation-activated 3′ splice site. (C) IVS28-159A > G (IVS, intervening sequence) This mutation causes 112 nucleotides of intron 28 to be included in the mRNA by activating a downstream 5′ cryptic splice site and/or an upstream cryptic 3′ splice site in intron 28. Two blocking AMO were used, AMO-A and AMO-D, to restore normal splicing between exons 28 and 29.

Correction of ATM splicing mutations with antisense morpholino oligonucleotides

The Gatti laboratory has also used antisense morpholino oligonucleotides (AMOs) to redirect and restore normal splicing in the *ATM* gene.[57] Two of three mutations tested activated cryptic 5′ and 3′ splice sites in exons, while the third activated a downstream 5′ splice site causing a pseudo-exon inclusion of a portion of intron 28. The AMOs were targeted to conceal these aberrant splice sites and enable expression of normally spliced full-length *ATM* mRNA (Fig. 2). The efficiency of translation of this mRNA into protein was ∼30% of normal after 3.5 days exposure of the cells to the AMOs, and this protein had functional activity. ATM was autophosphorylated on ser1981 in response to DNA double-strand breaks and it phosphorylated downstream substrates (p53, Nbs1 and SMC1). Exposure of these cells to radiation was manifested as increased resistance. Again while this method provides proof of concept, a number of issues need to be addressed before it could be employed as a human therapeutic. Its effectiveness in animal models of A-T needs to be determined and whether there is any toxicity associated with the use of these AMOs. Another issue relating to use in A-T patients is the method or vehicle for delivery. Therapy with AMOs requires that the 'corrected' ATM be induced in the cerebellum where it needs to be effective in restoring normal functioning of Purkinje cells. Getting oligonucleotides or indeed any other construct to this site presents impediments such as the blood brain barrier and the small numbers of Purkinje cells that populate the cerebellum.

Conclusions

A-T is a multisystem disease requiring intervention to: (i) halt progressive neurodegenerative changes; (ii) reduce the risk or treatment of tumours; (iii) correct immunodeficiency; (iv) alleviate bronchial complications. There is no cure for the progressive neurodegeneration with conventional therapies but some promise exists in the use of antioxidants and the development of methodology designed to target specific prototypes of mutations in the ATM gene *in situ*. An effective antioxidant might be expected to simultaneously slow the progress of the neurological phenotype and perhaps reduce the risk of cancer as well. It may well be possible to combine these therapies. Normal stem cells have great promise but delivering them to the regions of the brain most in need presents a formidable challenge. While the number of potential options for treating A-T patients is limited at present, the great advances achieved over the past decade in identifying mutations and

understanding how ATM functions in response to DNA damage provide additional hope for the future.

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