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Therapeutic targets and investigated treatments for Ataxia-Telangiectasia

Martin F. Lavin a, Abrey J. Yeo a, Amanda W. Kijas a, Ernst Wolveetang b, Peter D. Sly c,d, Claire Wainwright c,d and Kate Sinclair a

ABSTRACT

Introduction: Ataxia-Telangiectasia (A-T) is an autosomal recessive multisystem disease affecting the brain, immune system, lungs, liver and also characterised by an enhanced risk of lymphoid and other tumours. At present there is no cure for A-T with management relying on supportive care using symptom-specific medications. Identification of the gene defective in this syndrome, ATM, and further characterization of the disorder together with greater insight into the function of the ATM protein has provided greater opportunity for the development of potential therapies.

Areas covered: Here we review conventional as well as more recently developed approaches to manage the symptoms of patients with A-T. In addition we explore ongoing and potential strategies for therapy involving gene correction, stem cells and use of antioxidants and anti-inflammatory agents.

Expert opinion: Prevention or arrest of the progressive neurodegeneration, the most debilitating feature of A-T, represents a major goal in the development of a cure for this disorder. However, since lung disease and increased risk of cancer are responsible for the majority of mortality in A-T, a greater understanding of these pathologies together with more effective approaches to treatment is required in the overall management of patients.

1. Introduction

1.1. Clinical presentation and pathology

Ataxia-telangiectasia (A-T) is a rare, autosomal recessive disease caused by mutations in the ataxia-telangiectasia mutated (ATM) gene [1]. The ATM protein is a serine/threonine kinase that plays a major role in sensing of DNA double-strand breaks (DSB). It phosphorylates several key repair proteins that initiates arrest at cell cycle checkpoints, DNA damage repair and/or apoptosis. A-T was first described as a distinct clinical entity, assisted by autopsies, reported organ developmental abnormalities, neurological manifestations and a third major characteristic of disease, recurrent sinopulmonary infection [2,3]. See Figure 1 for major characteristics.

1.1.1. Neurodegeneration

A-T is characterized by its neurological symptomatology with ataxia, the major presenting symptom in this syndrome, evident when the child begins to walk toward the end of the first year of life, exhibiting ataxic gait and truncal movements [4]. Ataxia is progressive, spreading to affect the extremities and then speech. Involuntary movements become evident eventually, and by the end of the first decade of life the child usually requires a wheelchair but milder forms of the disease have been described where symptoms develop more slowly [5]. These involuntary movements include twitching and jerking of the hands and feet (chorea), slower twisting movements of the upper body (athetosis), adoption of stiff and twisted postures (dystonia), occasional uncontrolled jerks (myoclonic jerks), and various rhythmic and nonrhythmic movements with attempts at coordinated action (tremors). Cortical cerebellar degeneration in A-T involves primarily Purkinje and granule cells, but while degenerative changes in the central nervous system (CNS) are seen predominantly in the cerebellum, changes have also been described in the dentate and olivary nuclei, the spinal cord and spinal ganglia, the cerebrum, the basal ganglia and the brain stem [6]. The latter changes are usually seen in older patients.

Telangiectasia is the second major clinical manifestation of the disease with a later onset than ataxia, occurring between 2 and 8 years of age [4]. This is due to dilation of blood vessels, primarily in the ocular sclera, and often gives the impression of ‘bloodshot’ eyes. These are not confined to the eyes but may also appear in the butterfly regions of the face, ears and on the popliteal region.

1.1.2. Immunodeficiency

A-T is a highly variable primary immunodeficiency, involving both cellular and humoral immunity [7,8]. In the latter case, a reduction in IgA, IgG and IgE levels as well as abnormalities in IgM and IgG subclasses is widely observed. The infection history is variable, with some having chronic sinopulmonary infections, others have repeated infections and some having no more infections than their unaffected siblings [7]. Infections include otitis and sinusitis as well as bronchitis and recurrent pneumonia [9,10]. Patients with A-T are susceptible to common bacterial pathogens and viruses
but rarely to opportunistic infections, as observed in other primary immunodeficiencies. 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 also characteristic of A-T and frequently this organ cannot be identified at autopsy [4]. More recently, evidence has been provided for an inflammatory phenotype in A-T [11] which has been linked to a defect in the innate immune response [12,13]. This may explain the failure to observe a correlation between frequency of infection and B and T cell immunity. Recurrent upper and lower respiratory tract infections may progress to bronchiectasis and interstitial lung disease characterized by fibrosis and chronic inflammation [9,10]. Respiratory disease causes significant morbidity and mortality in patients with A-T, with up to 40% of deaths due to lung complications [14,15].

1.1.3. Cancer predisposition

The second most common cause of death in patients with A-T is cancer with a lifetime prevalence of 30% which is approximately 100-fold greater than expected for an age-matched population [16]. The breakdown of cancers observed are 40% non-Hodgkin’s lymphomas, 25% leukemias, 25% assorted solid tumors and 10% Hodgkin’s lymphomas [17]. Most leukemias and lymphomas are of T-cell origin [18] a pattern that is similar to that observed in Atm knockout mice [19,20] but is distinct from the B-cell predominance of lymphoreticular malignancies in children without A-T. A number of solid tumors have also been found in patients with A-T including adenocarcinomas, gonadoblastomas and medulloblastomas [21]. There is also an increased risk of cancer amongst A-T heterozygotes, particularly breast cancer pointing to some penetrance of the defective gene [22].

1.1.4. Lung disease

Recurrent infection, immune deficiency, aspiration and impaired clearance of respiratory tract secretions combine to increase susceptibility to lung disease in A-T [14,15]. Recurrent infections are a major clinical feature of A-T with up to 80% of patients being affected [9]. These include both upper and lower respiratory tract infections associated with the development of bronchiectasis or persistent pleural abnormalities [10,23]. A second major form of lung disease, interstitial lung disease or pulmonary fibrosis develops later in life and treatment with antibiotics only partially resolved the infections whereas treatment with corticosteroids led to patient improvement. Progressive neurological decline may also contribute to lung disease in patients through muscle impairment which in turn may affect swallowing and lead to aspiration [14]. It is clear that immunodeficiency involving both humoral and cell-mediated immunity makes a significant contribution to susceptibility to infection and the resulting lung disease. The recent observation of a defect in innate immunity in A-T cells and in Atm-deficient mice is compatible with pro-inflammatory changes which would be expected to respond to steroid therapy as described [12].

1.1.5. Liver disease

Alpha-fetoprotein (AFP) represents a useful biomarker to support the diagnosis of A-T since this protein remains elevated in the serum of most patients [24]. It remains unclear why AFP is elevated in patients with A-T, however, a report by Jung et al. [25] suggested that a small alternatively spliced product from the AT motif-binding factor 1 (ATBF1) gene, that interacts with an AT-rich element located upstream of AFP promoter to suppress transcription of this gene, is defective in its translocation to the nucleus in A-T cells. There is also evidence of increased liver transaminases in the serum of patients with A-T which might point to subclinical chronic liver involvement in A-T. Hepatic dysfunction was first described in five patients with A-T in 1970 as indicated by bromsulphthalein retention and serum enzyme elevations [26]. Hepatic insufficiency...
was subsequently described in patients with histological evidence of veno-occlusive disease and cirrhosis [27,28]. More recently in the case of a 22-year-old patient with A-T, with a 3 year history of hyperlipidemia and liver test alterations, histological examination of a liver biopsy revealed characteristic features of nonalcoholic steatohepatitis with moderate macrovesicular and multivesicular steatosis, ballooning hepatocytes and fibrosis [29]. They attributed the pathogenic changes in the liver to longer survival and oxidative stress. A recent investigation of hepatic involvement in a large cohort of patients with A-T (n = 53, mean age 14.6 ± 5.2 years) revealed abnormal liver enzyme levels in 43.4% (age 9.98 ± 5.1 years) of patients [30]. Fatty liver was detected in 39% [10] of these by ultrasonography and liver biopsy revealed mild-to-moderate steatosis in two patients and fibrosis in one of these. The authors recommended screening for fatty liver in all patients with A-T. Deficiency in ATM is also associated with hyperlipidemia, metabolic syndrome and mitochondrial dysfunction [31].

1.1.6. Diabetes
Schlack et al. [26] first described an unusual form of diabetes in five of eight patients with A-T characterized by marked hyperglycemia, resistance to ketosis, no glycosuria and elevated plasma insulin levels after glucose administration. The blood glucose response to insulin was decreased suggesting insulin resistance. It is of interest that all five patients also had hepatic disease. It was subsequently shown that monocytes from two patients consistently demonstrated an approximately 80% decrease in insulin receptor affinity which could account for the insulin resistance [32]. This relationship was further substantiated when Yang and Kastan [33] demonstrated a role for ATM in an insulin-signaling pathway that controls initiation of protein translation. They followed this up providing evidence that ATM-dependent stress pathways mediate susceptibility to the metabolic syndrome and that chloroquine or related agents promoting ATM activity could modulate insulin resistance and decrease vascular disease [34]. Takagi et al. [35] found that Atm−/− mice were insulin resistant and possessed less subcutaneous adipose tissue as well as a lower level of serum adiponectin than Atm+/+ mice. Furthermore, in vitro studies revealed impaired adipocyte differentiation in Atm−/− cells caused by the lack of induction of C/EBPα and PPARγ, crucial transcription factors involved in adipocyte differentiation. Interestingly, ATM was activated by stimuli that induced differentiation, and the binding of ATM to C/EBPα and p300 was involved in the transcriptional regulation of C/EBPα and adipocyte differentiation.

1.2. Laboratory characteristics of A-T
Prior to the cloning of the ATM gene a patient presenting with ataxia and suspected of having A-T would undergo a serum alpha-fetoprotein determination which is elevated in ~95% of patients [24]. Other abnormalities include absence of or marked reduction of IgA, IgG2 and IgE as well as elevated levels of IgM monomer and lymphopenia [7]. Deficiencies in antibody production which is variable in this syndrome have been associated with intrinsic defects in B lymphocytes primarily class switching [36]. This is also observed in Atm-deficient mice [37]. Depression of T-cell function, spontaneous cytogenetic abnormalities, including chromatin gaps, chromosomal breaks, translocations, rearrangements and inversions are observed in lymphocytes from the majority of patients [38-40]. Chromosomal translocations have long been reported to occur predominantly in the T cell receptor (TCR) loci and the immunoglobulin heavy chain gene (IGH) locus [41]. High rates of spontaneous intrachromosomal recombination (~30–200 times increase) have also been reported [42]. Increased chromosomal breakage postirradiation is also characteristic of A-T cells [43]. Reduced cell survival postirradiation is also a laboratory hallmark of patients’ cells and radiosensitivity has been widely used as a screening tool for A-T well before the gene was discovered. It is also of note that hypersensitivity to radiation was observed in patients exposed to radiotherapy for lymphoid malignancy well before the cellular sensitivity was reported [44-46]. A complete list of the laboratory characteristics and abnormalities in A-T cells appears in Figure 2.

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**Figure 2.** Laboratory characteristics observed in A-T.
1.2.1. Identification of the ATM protein and diagnosis

Mapping of the A-T locus [47] and subsequent identification of the gene mutated in A-T [1] provided the opportunity for more rapid diagnosis of this disorder. The ATM protein is a member of the phosphatidylinositol 3-kinase like-kinase (PIKK) family of proteins capable of phosphorylating a multitude of cellular proteins in response to DNA DSB [48,49]. These proteins function in cell cycle checkpoint control, DNA repair, apoptosis, transcriptional control and regulation of other cellular processes [50]. In short this protein functions to maintain the integrity of the genome and minimize the risk of cancer, lung disease and neurodegeneration that characterizes this disease [51,52]. ATM is predominantly present in the nucleus which fits with its role in maintaining genome stability. However, it has been shown that ATM is also present in the cytoplasm where it is associated with peroxisomes [53,54], and mitochondria [55]. Cytoplasmic localization of ATM is in keeping with the observation that this protein is also activated in response to oxidative stress by a mechanism not dependent on DNA damage [56]. Since both peroxisomes and mitochondria produce excess reactive oxygen species (ROS) when under stress, association of ATM with these organelles would represent a ‘front line of defense’ against potential oxidative damage. In the case of peroxisomes ATM bind to the peroxisome receptor PEX5 where it becomes activated in response to oxidative stress to induce pexophagy a process required for peroxisome hemostasis [54]. A fraction of ATM protein is also localized to mitochondria and mitochondrial damage (elevated mROS) can directly activate ATM in the absence of DNA damage [55,57]. As in the case of peroxisomes it appears likely that ATM also plays a role in mitochondrial homeostasis since mitophagy is defective in cells lacking ATM.

As indicated above the majority of ATM mutations are compound heterozygote and are largely indels, missense or splice site mutations resulting in premature stop codons giving rise to unstable ATM protein [58]. The great majority of patients with A-T are defined by lack of ATM protein or low levels of missense mutated protein but the ATM protein kinase activity is always lacking in patients. Of interest it has been found that some of the missense mutations for which a low level of ATM protein is detectable, results in a milder form of the disease [59,60]. Thus the absence of ATM protein and/or lack of ATM protein kinase activity represent a solid laboratory assay in conjunction with ATM gene mutation analysis and clinical diagnosis of A-T.

2. Treating different aspects of the defect

2.1. Clinical trials

Information on clinical trials for a wide range of diseases can be found under https://clinicaltrials.gov/. While there are many trials described for hereditary cerebellar ataxias such as the use of riluzole to improve Scale for the assessment and Rating of Ataxia (SARA) scores [61] the focus here will be on A-T. At present there are 15 trials listed under A-T as either completed, recruiting, not yet recruiting, enrolled by invitation or unknown (Table 1). Other trials reported in the literature are also referred to.

2.2. Respiratory tract infections

Recurrent respiratory tract infections affect the majority of patients with A-T, becoming more prevalent with age [9,23]. Microbiological evaluation of respiratory secretions from patients with A-T identified Pseudomonas aeruginosa, Haemophilus influenza, Streptococcus pneumoniae and Staphylococcus aureus as the most common bacterial pathogens [9,10]. Opportunistic infections are uncommon in A-T unlike that in other immunodeficiency disorders [66], rather the pattern of infection resembles that observed in patients with cystic fibrosis [9]. Infections with viral pathogens such as respiratory syncytial virus and Epstein–Barr virus have also been reported. As in other disorders, frequent infections accompanied by hypogamma-globulinemia with antibody deficiency can be treated with immune globulin replacement therapy. Airway clearance strategies are used along with antibiotic therapies and some groups have also instituted azithromycin to reduce the frequency of exacerbations of underlying bronchiectasis although there are no trials to date that have examined the evidence for these approaches. Patient management is best overseen in a specialist multidisciplinary clinic. There is also evidence that pneumococcal conjugate antigen may offer some protection.

2.3. Lung structure and function

Children with A-T often present in the first few years of life with recurrent respiratory infections and suppurative lung disease but assessment of this is limited by the inability of children to cope with conventional lung function tests [67,68]. Patients have increased sensitivity to ionizing radiation and repeated X-rays or computed tomography scans are not acceptable. In addition, with progression of the neurological disease coordination and respiratory function testing become more challenging. Magnetic Resonance Imaging (MRI) has generated interest as it does not involve ionizing radiation. MRI also enables functional imaging (particularly pulmonary perfusion), which is important as dilation of bronchial arteries. The reversibility of perfusion defects after therapeutic intervention could be a method to differentiate regions with reversible and irreversible disease. The application of current MRI sequences is limited due to the low proton density of lung tissue; difficulty in differentiating between bronchial wall thickening and bronchiectasis and the occurrence of susceptibility artifacts in air-tissue interfaces. Standard MRI sequences are not currently able to identify trapped air, emphysema or increased lung volume. Restricted spatial resolution, increased set up and acquisition time and higher cost have also been reported as challenges. Since methods for assessing pulmonary function are unreliable in A-T, it is necessary to develop tests that will accurately determine lung function to aid intervention and initiate treatment. A sensitive technique for measuring respiratory function during tidal breathing that does not require any complex respiratory manoeuvres has been described recently. This technique, known as T-FOT is a variation of the conventional forced oscillation technique (FOT) that is highly sensitive and specific for detecting airway obstruction [69]. The lung disease in A-T is likely to be patchy and involve airway obstruction/infection that can be
detected by T-FOT. The outcome of a forced Spirometry manœuvre longitudinal study with patients with A-T conducted at the Sheba Medical Center, Israel remains unreported. Lung imaging by computed tomography (CT) is limited by an increased sensitivity to ionizing radiation, making repeated X-rays or CT scans unacceptable. 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. 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 [17].

In addition to antibiotic administration and immunoglobulin replacement, respiratory therapy can be employed to reduce the development of bronchiectasis and interstitial lung disease. Since maximum inspiratory and expiratory pressures are good markers of respiratory muscle strength, especially in patients with A-T, they have been employed in patients to improve quality of life. Inspiratory muscle training has been shown to improve ventilatory pattern, lung volume

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Table 1. Ataxia-telangiectasia clinical trials.

<table>
<thead>
<tr>
<th>Name of trial</th>
<th>Sponsor</th>
<th>ID No.</th>
<th>Study start date</th>
<th>Estimated completion</th>
<th>Patients enrolled</th>
<th>Outcome or expected outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility to Infections in Ataxia Telangiectasia</td>
<td>Johann Wolfgang Goethe University Hospital</td>
<td>NCT02345135</td>
<td>September 2012</td>
<td>January 2016</td>
<td>41</td>
<td>Last update: January 2015</td>
</tr>
<tr>
<td>Body Composition and Hormonal Status in Ataxia Telangiectasia</td>
<td>Johann Wolfgang Goethe University Hospital</td>
<td>NCT02345200</td>
<td>April 2013</td>
<td>April 2014</td>
<td>52</td>
<td>See Pommerring et al. [62]</td>
</tr>
<tr>
<td>Conjugate Pneumococcal Vaccine in Ataxia Telangiectasia (AT)</td>
<td>Institute of Child Health, Great Ormond St Hospital</td>
<td>NCT00656409</td>
<td>June 2006</td>
<td>March 2008</td>
<td>30</td>
<td>Immunogenicity of vaccine and adverse outcomes</td>
</tr>
<tr>
<td>Response of Individuals With Ataxia-Telangiectasia to Metformin and Pioglitazone (RAMP)</td>
<td>NHS Tayside and University of Dundee</td>
<td>NCT02733679</td>
<td>June 2016</td>
<td>February 2018</td>
<td>30</td>
<td>Change in insulin sensitivity after taking metformin in A-T compared to controls</td>
</tr>
<tr>
<td>Status of Growth Hormone/Insulin-like Growth Factor-1 (GH/IGF-1) Axis and Growth Failure in Ataxia Telangiectasia (AT) (GHT)</td>
<td>Johann Wolfgang Goethe University Hospital</td>
<td>NCT01052623</td>
<td>January 2010</td>
<td>September 2012</td>
<td>23</td>
<td>To evaluate the GH increase after arginine provocation test. See Voss et al. [63]</td>
</tr>
<tr>
<td>Immunogenicity of Pneumococcal Vaccines in Ataxia-telangiectasia Patients</td>
<td>Sheba Medical Centre, Tel Aviv</td>
<td>NCT01075438</td>
<td>March 2010</td>
<td>February 2011</td>
<td>20</td>
<td>Primary end point will be levels of antibodies against 13 serotypes of Streptococcus pneumoniae. Outcome unclear</td>
</tr>
<tr>
<td>Oxidative Stress, Low Grade Inflammation, Tissue Breakdown and Biomarkers in Cerebrospinal Fluid of A-T</td>
<td>Johann Wolfgang Goethe University Hospital</td>
<td>NCT02285348</td>
<td>April 2013</td>
<td>May 2015</td>
<td>40</td>
<td>Alterations in protein expression related to A-T using proteomic studies. See Dzieciatkowska M et al. [64]</td>
</tr>
<tr>
<td>Cell-Based Approaches for Modeling and Treating Ataxia-Telangiectasia</td>
<td>Sidney Kimmel Comprehensive Cancer Centre, Johns Hopkins University</td>
<td>NCT02246491</td>
<td>September 2014</td>
<td>September 2018</td>
<td>20</td>
<td>Fibroblasts from patients with A-T will be collected for reprogramming and iPSC analysis in the laboratory but not for therapy</td>
</tr>
<tr>
<td>Study for Treatment of Cancer in Children with Ataxia-telangiectasia</td>
<td>St. Jude Children’s Research Hospital</td>
<td>NCT00187057</td>
<td>September 2002</td>
<td>June 2013</td>
<td>6</td>
<td>To determine the feasibility of delivering modified intensive chemotherapy to children with A-T who present with cancer. See Sandlund JT et al. [65]</td>
</tr>
<tr>
<td>Baclofen Treatment of Ataxia Telangiectasia to check usefulness for treating neurological problems</td>
<td>Johns Hopkins University</td>
<td>NCT00640003</td>
<td>April 2007</td>
<td>February 2011</td>
<td>12</td>
<td>Improvement in the decay constant for velocity storage as assessed by quantitative video examination of eye movement during and after rotation. Outcome not clear</td>
</tr>
<tr>
<td>The Validity of Forced Expiratory Manoeuvres in Ataxia Telangiectasia Studied Longitudinally</td>
<td>Sheba Medical Centre</td>
<td>NCT00951886</td>
<td>July 2009</td>
<td>July 2009</td>
<td>28</td>
<td>Determining ability to perform spirometry. Outcome not clear</td>
</tr>
<tr>
<td>EDS in Ataxia Telangiectasia Patients (ATTeST)</td>
<td>Erydel</td>
<td>NCT02770807</td>
<td>June 2016</td>
<td>December 2018</td>
<td>180</td>
<td>Steroid infusion trial underway. Change from baseline analyzed using a Mixed Model Repeated Measures (MMRM) approach</td>
</tr>
<tr>
<td>Amantadine for Improving Neurological Symptoms in Ataxia-Telangiectasia</td>
<td>Sheba Medical Centre</td>
<td>NCT00950196</td>
<td>November 2008</td>
<td>November 2009</td>
<td>30</td>
<td>Improvement in ataxia</td>
</tr>
</tbody>
</table>
and respiratory muscle strength in patients and has the potential to act as an adjuvant to drug treatment [70].

2.4. Neurological defects

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 Nijmegen breakage syndrome (NBS) and Rad50-deficient patients [71,72], although one report described a small head circumference in 60% of patients in a retrospective study of 57 patients [73]. Cerebellar degeneration in A-T manifests as dystrophic changes involving the dendrites and axons of Purkinje cells and ectopic Purkinje cells are evident [4]. 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 [17]. This progresses to include peripheral neuropathy and eventually to spinal muscular atrophy [6]. Neurological outcome measures have relied in the past on the more general tools for assessing ataxia, SARA and International Cooperative Ataxia Rating Scale (ICARS). More recently two scoring systems, designed to capture the special features of A-T, have been developed for the assessment of neurological features in A-T patients, the A-T Index or Crawford Score that consists of a 39 point scale derived from 10 separate parameters [74], and the A-T neurological Examination Scale Toolkit (A-T Nest). The A-T Nest is more comprehensive with 53 neurological parameters and also includes a communication domain [75]. While a good correlation was obtained between these two systems performed on the same day the A-T Nest may prove to be more discriminatory in the more comprehensive set of parameters tested. Thus, any effective treatment for A-T would ideally involve prevention or at least slowing of the progressive neurodegeneration and an ability to increase the latency period or markedly reduce the risk of lymphoid malignancies.

2.5. Dystrophy and nutritional studies

Failure to thrive has been reported in several studies with A-T patients [76,77]. Low body mass index (BMI) is also observed in 50–80% of patients which may be explained by dysphagia, aspiration or lung disease [78]. Patients exhibiting aspiration were older and had lower body weight for height z-scores (WHZ) and premature death appeared to be the result of secondary complications of the disorder. Pronounced deficiency of the GH/IGF-1 axis accompanied by markedly reduced body weight and high ataxia scores has also been reported for patients [79], pointing to a role for IGF-1 and nutritional status in neuroprotection.

A recent assessment of the nutritional status in Australian patients with A-T showed that they were vulnerable to issues that influenced their nutritional status [80]. Almost 80% had short stature and 50% were underweight with evidence of significant malnutrition in approximately 70% of patients. Poor food intake and diet quality suggested the need for early intervention. This would require ongoing support for families and discussions on the introduction of gastrostomy tube feeding. High rates of malnutrition and reduced lean body mass were also reported elsewhere [81,82].

2.6. Bone marrow transplantation

A-T is characterized by immunodeficiency and an increased risk of developing leukemias and lymphomas [83]. Thus these patients would be expected to benefit from bone marrow transplantation to treat the hematopoietic phenotype especially since there is no current cure for the disorder and up to 30% of patients die from malignancy. However, ethical issues exist since patients are hypersensitive to agents using an ablative therapy such as ionizing radiation and alkylating agents. Replacement of the bone marrow compartment in Atm−/− mice with clinically relevant nonmyeloablative host-conditioning regimes (anti-CD4, anti-CD8 antibodies) overcame the immune deficiency and prevented the development of malignancy in these mice [84]. However, treatment with cyclophosphamide was also necessary for engraftment which might be predicted to have increased toxicity in patients. Not surprisingly very few A-T patients have had this form of treatment given the associated risks. Ghosh et al. [85] reported the first BMT for an A-T patient presenting with Hyper IgM while a full lympho-hematopoietic reconstitution was observed the patient died of encephalopathy and liver failure 8 months later. There is only one report of long-term survival of a 3 year old A-T child with T-ALL that received allogeneic-matched sibling donor peripheral blood stem cells [86]. To minimize toxicity a modified form of the German regime used in Fanconi anemia (GEFA02) was employed which lacked myeloablative properties. Cyclophosphamide was not used. More recently, an A-T patient with immunodeficiency and recurrent EBV-associated lymphoproliferation was cured by chemotherapy followed by allogeneic-matched sibling stem cell transplantation [87]. In this case also, a conditioning regime based on the Fanconi anemia protocol GFFA-02 was used. This patient developed severe mucosal toxicity during the early transplant course as well as veno-occlusive disease and respiratory distress syndrome all of which responded to management. After experiencing deteriorating ataxia he is now able to sit and stand without support and can walk with assistance. It is evident from these studies that A-T patients are particularly susceptible to toxicity after exposure to chemotherapy and ablative conditioning prior to transplantation. Removing cyclophosphamide from the conditioning regime may have benefit but its reduction in the treatment of lymphoid malignancies is not recommended [88]. Recent advances in the generation of pluripotent stem cells from A-T patients also offers the possibility of patient-specific cell transplantation [89,90].

2.7. Therapy for malignancy in children with A-T

Up to 30% of children with A-T are diagnosed with malignancy during their lifetime [91]. The tumors involved tend to be primarily lymphomas and leukemias but with better management patients are living longer and developing a spectrum of solid tumors [92]. Prior to understanding the sensitivity to radiation and agents used in chemotherapy, patients with A-T showed severe adverse reactions to treatment [44]. Accordingly, reduced intensity therapy has been employed to reduce the risk of adverse effects. Seidemann et al. [93]
treated patients with A-T for non-Hodgkin’s lymphoma (B-NHL) and showed that curative treatment was possible with intensity of therapy adjusted to individual risk factors and tolerance. They suggested omission of alkylating agents and epipodophyllotoxins and limitation of methotrexate dose. At the time of the report 5/9 patients with either A-T or NBS were in continuous complete remission (CCR). In a later study on 19 patients with A-T with malignancy where 10 were diagnosed with B-NHL, it was evident that dose reduction attenuated the toxicity side effects of chemotherapy such as mucosal inflammation and infectious complications [94]. Earlier reports showed poor results on the treatment of A-T patients for lymphoid malignancies [88,95]. The former study demonstrated that patients treated with standard chemotherapy doses had a significantly better median survival than those treated with reduced dose. However, Bienemann et al. [94] revealed long-term survival in >50% of patients and at least intermittent cure for second malignancies. The study also demonstrated that there was no disadvantage for disease control for patients in whom chemotherapy was significantly reduced.

More recently a curative intent approach was employed for advanced stage high grade mature B-cell malignancies in five children with A-T [65]. They used a modified form of the SFOP LMB-B9 regime for B-cell lymphoma described previously [96]. This modification was made taking into account the unique toxicity profile observed in A-T patients receiving chemotherapy (see Table 1 [65]). Sustained CCR was observed in two patients, one patient had CCR at 6 years and a second patient maintained CCR for 3 years prior to symptoms as imbalance-dying from pulmonary complications. Two patients died from toxicity during induction and the other failed induction because of progressive disease. Clearly reduction of chemotherapeutic dose is not in itself sufficient to obtain optimal outcome for patients with A-T being treated for malignancy. Sandlund et al. [93] suggest the introduction of novel targeted therapeutic agents such as monoclonal antibodies or small molecule inhibitors within the less intensive chemotherapeutic approach. Other issues include drug resistance in some patients and a greater understanding of the biology of the tumors.

3. Treatment for the neurological defects

Modest improvements can sometimes be achieved by 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 (a tricyclic amine that releases dopamine and other monoamines at central synapses) [97], fluoxetine (selective inhibitor of serotonin re-uptake) or buspiron (serotonin 5-HT1A receptor partial agonist with moderate affinity for brain dopamine D2 receptors [98]. A second 5-HT1A receptor agonist has also been shown to be successful in treating patients with other forms of spinocerebellar ataxia [99]. A more comprehensive list of pharmacotherapeutic interventions for neurological disease symptoms of A-T appear in an excellent review by Hoche et al. [100]. They address such symptoms as imbalance, motor incoordination, dysarthria, tremor, nystagmus, choria, dystonia, drooling, spasticity and peripheral neuropathy and the drugs used to treat these symptoms. Cerebellar tremors may also be treated with propranolol (a beta-adrenergic blocking agent), gabapentine (a GABA analogue) or clonazepam (a benzodiazepine). Some of these agents may also improve speech and coordination. Furthermore, although deficiencies of thiamine, vitamin B and vitamin E can cause ataxia, multivitamin supplements do not correct the ataxia of patients with A-T [17].

3.1. Betamethasone

There is evidence from several studies that steroids produce short-term improvement in A-T. Amelioration of neurological signs, assessed by SARA, has been reported after short-term treatment with oral betamethasone [101,102]. In a follow-up study using significantly lower doses of betamethasone (0.03 and 0.01 mg/kg/day) with six patients responsive to betamethasone, SARA scores significantly improved in all patients at the higher dose and some improvement was also observed at the lower dose [103]. These authors suggest that antioxidative mechanisms may be at play in improving cerebellar functions in A-T [102]. In a separate randomized trial of 13 patients with A-T, betamethasone reduced ICARS total score significantly (−30%) in both intent-to-treat (all patients randomized) and in per-protocol patients [104]. Unfortunately, long-term complications of steroid use far outweigh the short-term benefits of this treatment.

3.2. Intra-erythrocyte dexamethasone delivery

The EryDex System (EDS) permits the encapsulation of dexamethasone sodium phosphate to autologous erythrocytes derived from a 50 ml blood sample, processed ex vivo with hypotonic saline which permits osmotic opening of the erythrocyte (RBC) pores and diffusion of dexamethasone phosphate into the cells (www.erydel.com). EDS was developed by EryDel (Urbino, Italy). Chessa et al. [105] employed EDS in single-arm, open-label, 6 month extended phase II trial where patients received a monthly treatment of 50 ml of autologous red blood cells (RBC) loaded with 2 vials of 250 mg of dexamethasone sodium phosphate. In this study which enrolled 22 patients (18 completed), after six infusions, a mean reduction of 5 points in ICARS was reported. In an extension of this study for an additional 24 months, patients experienced a continuous neurologic improvement with respect to pretreatment status whereas controls showed a progressive neurologic deterioration after discontinuation of the treatment [106]. Evidence has been provided for the mechanism involved by the induction of a noncanonical splicing event which generated a miniATM variant with partial restoration of ATM activity. More recently, dexamethasone has been shown to increase the production of the antioxidants, GSH and NADPH, which improved the antioxidant capacity of A-T cells to counteract oxidative stress [107]. This can be explained by the promotion of nuclear accumulation of the transcription factor NRF2 which drives expression of antioxidant pathways. In summary, EDS proved to be safe and well-tolerated and none of the side effects associated with chronic administration of steroid
were observed. Based on the success of the initial trial with EDS, an international, noninterventional observational study was performed in 6 centers specialized in treating pediatric ataxia patience. This study indicates that the ICARS and the CGI-S can be used as measures of severity in patients with A-T 6 years of age and older [108]. EryDel is also in the process of recruiting patients with A-T for an international, multicentre, 1-year, randomized, prospective, double-blind, placebo-controlled, phase III study which will assess the effect of two nonoverlapping dose ranges of EDS-EP, administered by IV infusion once per month, with assessment of neurological symptoms of enrolled patients with A-T (NCT02770807). Criteria for inclusion include meeting clinical criteria for diagnosis of A-T; 6 years and older of either sex; in autonomous gait or assistance by periodic use of support and having a body weight >15 kg.

3.3. Treatment with amantadine sulfate

Nissenkorn et al. [109] treated 17 children over 8 weeks with the dopaminergic and anti-IV-methyl-d-aspartate (NMDA) agent amantadine sulfate assessing ataxia using the International Cooperative Ataxia Scale, Parkinsonism by the Unified Parkinson Disease Rating Scale and chorea/myoclonus by the Abnormal Movement Scale. Overall, 76.5% of patients responded with a mean improvement of approximately 30%. The drug was well tolerated with mild and transient side effects that did not require discontinuation of the trial. Amantadine increases dopaminergic transmission by inhibiting synaptic uptake of the neurotransmitter. A larger follow-up study is required to further test the efficacy of the use of amantadine.

4. Treatment for oxidative stress

4.1. Oxidative stress and biomarkers in cerebrospinal fluid

Since it is neither safe nor ethical to surgically biopsy brain tissue from patients with A-T, cerebrospinal fluid (CSF), which is directly in contact with brain tissue, is relatively easy to sample safely by lumbar puncture. The aim by Zielen et al., at the Johann Wolfgang Goethe University Hospital is to investigate oxidative stress, low grade inflammation and tissue breakdown in the brains of patients with A-T by analyzing CSF. Alterations in protein expression related to A-T will be quantified by liquid chromatography/mass spectrometry (LC/MS)-based proteomic analysis of CSF from healthy individuals and patients with A-T to determine candidate proteins (new biomarkers) which might be used as surrogate markers of disease progression (NCT02285348).

4.2. Oxidative stress clinical trial

This was conducted by Dr Howard Lederman, John’s Hopkins Hospital, to test safety and identify markers for treatment of patients with a combination of nicotinamide and the antioxidant α-lipoic acid to investigate effect on progress of neurodegeneration. Two laboratory markers of oxidative stress significantly improved when participants took both α-lipoic acid and nicotinamide. A trend toward increased lymphocyte counts was also observed, but did not reach statistical significance. They suggest that these findings will form the basis for examination of other laboratory markers of oxidative stress in future trials of novel antioxidant treatments.

4.3. Use of myo-inositol on neurological and immune function

Dr. Gerald Berry (Philadelphia) 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 [110].

5. Potential new therapies

In addition to the therapeutic approaches described above extensive research into A-T, since the ATM gene was identified, has provided several potential approaches for treatment.

5.1. Use of stem cells

Stem cells play a key role not only in development and growth of organisms but also in building new tissues and replacing others after injury. Thus they have great potential in regenerative medicine for the development of new therapies in a variety of different disease types. Clearly A-T is a good candidate since different tissues are involved and it would be very beneficial to be able to correct the decline in neurological function with patient-specific stem cells. Reports on the regeneration of pluripotent stem cells (iPSC) from different cell types (e.g. skin fibroblasts and blood mononuclear cells) have elicited considerable interest since they can be produced from children/adults and avoid ethical considerations associated with use of human embryos. We employed fibroblasts from patients with A-T to reprogram into bona fide A-T induced pluripotent stem cells (A-T iPSCs) [90]. We showed that A-T iPSCs recapitulate key features of the A-T cellular phenotype, including radiation-induced cell cycle defects and increased sensitivity to radiation. A-T iPSC can be cultured for prolonged periods of time without acquisition of karyotypic instability, in agreement with the observation that ATM knockout in embryonic stem cells does not interfere with DSβ repair and genomic stability [111]. Fukawatase et al. also reported that AT-iPSCs did not show any chromosomal instability in vitro for at least 80 passages (560 days) despite their parental A-T cells showing significant chromosomal abnormalities [112]. Several groups have now shown that A-T iPSC can be successfully differentiated into neural progenitors an appropriate experimental system for modeling A-T. Olfactory neurosphere-derived multipotent stem cells have also been generated from patients with A-T which also represent a good disease model for A-T [89]. The challenge ahead is to differentiate these stem cells into the cell types that are most affected in A-T, for example into cerebellar cells which might be used for
regenerative medicine approaches with patients. However, before this can be attempted it is necessary to correct the ATM mutations in each patient. This can now be achieved using gene editing to correct the defective gene. One such approach is the CRISPR-Cas9 assisted homologous recombination mediated gene repair [113, 114]. CRISPR-Cas9 operates through targeted introduction of dsDNA breaks that allow strand invasion of a repair template (Figure 3). Since ATM is centrally involved in dsDNA break repair, homologous recombination in A-T stem cells may prove to be particularly challenging. These resulting, corrected stem cells will need to be checked for their efficacy and safety especially with respect to genomic sequence in animal models to confirm their therapeutic use in patients. These corrected stem cells from patients represent an important resource for use as an isogenic cell model for differentiation into neuronal cells of interest to screen for therapeutic agents.

5.2. Use of read-through of premature termination codons

Reading of the genetic code across nonsense mutations to express full-length normal protein and restore protein function has been employed in a range of disease states [115]. Important targets have included genes encoding the cystic fibrosis transmembrane conductance regulator (CFTR) and dystrophin defective in Duchenne muscular dystrophy. Du et al. [116] used high-throughput screening of small-molecule libraries to identify novel read-through compounds (RTCs) that suppress all three stop codons (TGA, TAG, and TAA) in A-T. The two top candidates were RTC 13 and RTC 14 that were shown to induce low-levels of full-length functional ATM. Since the efficiency of read-through was low (<15%) and there was some toxicity associated with RTC 13 their clinical benefit was questionable. Subsequent studies by this group identified two novel compounds (GJ071 and GJ072) with low toxicity, capable of read-through in cells derived from patients with A-T with three different types of nonsense mutations [117]. These compounds produced full-length ATM protein capable of being activated by radiation exposure and also which abrogated radiosensitivity in these cells. Future studies are planned in a mouse model prior to considering use in patients with A-T (Gatti RA, personal communication).

5.3. Use of antioxidants

There is increasing evidence for a role for oxidative stress in A-T that includes increased levels of ROS in a variety of A-T cell types and in cells from Atm-deficient mice; high levels of oxidative damage in A-T cells in culture; reduced antioxidant capacity in the serum of patients with A-T and the capacity of antioxidants to rescue several aspects of the A-T cellular...
phenotype \cite{118,119}. These observations together with the presence of ATM in the cytoplasm, where it is associates with both mitochondria and peroxisomes, and its activation by agents that cause oxidative damage, at concentrations not causing DNA damage, support a role for this protein in protecting against oxidative stress \cite{53,54,57}. While one trial with lipoic acid has been conducted with little success, it is evident from animal studies with several different antioxidants that protection against neurological changes and cancer development occurs as well as extended life-span \cite{120–122}.

6. Expert opinion

At present there is no established treatment that prevents or slows the progress of symptoms for the human genetic disorder A-T. This is a multisystem disease that involves the brain, immune system, the lungs, the endocrine systems, and the liver and is also characterized by a high susceptibility to developing lymphoid and other tumors. Notwithstanding this, recent data on intra-erythrocyte delivery of dexamethasone (EryDex) provide evidence of continuous neurologic improvement in patients with A-T. Since this is a multisystem disease it is not surprising that a number of specialist A-T clinics have been established in Australia (Brisbane), Israel (Tel Aviv), Italy (Rome), Germany (Frankfurt), UK (Nottingham) and US (Baltimore). Patients are best managed under a multidisciplinary team covering the many areas of expertise required for treatment, including the obvious ones in immunology, neurology, respiratory medicine etc. as well as physiotherapy and speech therapy.

Patients with A-T are primarily detected either by an immunologist or a neurologist although sometimes present to respiratory physicians with recurrent respiratory infections. This is a primary immunodeficiency disorder affecting humoral immunity, cellular immunity and there is a growing body of evidence for a defect in innate immunity in A-T. While numbers of B-cells are largely normal in A-T there is an intrinsic defect in these cells due to defective class switch recombination \cite{36}. This in turn leads to reduced synthesis of immunoglobulins with a marked deficiency in IgA and poor antibody response in many cases. Prophylactic treatment with immunoglobulin replacement therapy (IVIG) is often recommended for patients with a history of recurrent infections, which reduces the number and severity of these infections. Although IVIG may improve the ability of some patients to handle infections, blind treatment with antibiotics such as trimethoprim-sulfamethoxazole (TMP-SMX) is also prescribed as a prophylactic measure \cite{123}. However, it is evident that respiratory disease including bronchiectasis and interstitial lung disease arises in a significant number of patients accounting for up to 40% of the morbidity and mortality in this disorder. The progress of lung disease in A-T resembles that in cystic fibrosis (CF) and it has been suggested that therapies used to improve the quality of life in CF (e.g. bronchodilators, intravenous antibiotics and anti-inflammatory agents) be employed for patients with A-T \cite{9}. Prophylactic antibiotics are prescribed for patients with recurrent infection but this is done blindly in most cases since microbiological evaluation will not have taken place and there is the added risk of bacterial resistance developing. Very few studies have been conducted on microorganisms infecting the respiratory tract of patients with A-T. Cultures established from respiratory secretions performed during acute pulmonary exacerbation identified Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa and other gram negative bacilli \cite{9,10}. It remains unclear what the etiology of the lung disease is and how persistent or repeated infections from these microorganisms, together with complications from dysfunctional swallowing and aspiration, contribute to the progress of lung disease. Evidence for the involvement of oxidative stress is accumulating in investigations of patients with A-T, studies in cultured A-T cells and in Atm-deficient animal models \cite{118,124–127}. These include elevated levels of reactive oxygen species (ROS) in cells and tissues, protection by antioxidants and the capacity of ATM to recognize and be activated by stimuli that lead to oxidative stress \cite{56,128}. A recent report demonstrated that one of the microorganisms identified in the respiratory tract of patients with A-T, S. pneumoniae, caused DNA damage and death of lung epithelial cells by a mechanism that induces oxidative stress \cite{129}. These data suggest that S. pneumoniae together with other microorganisms invading the respiratory tract may contribute to death of airway epithelial cells and thus to the progressive lung disease in patients.

A separate investigation has shown that loss of ATM impairs inflammasome-dependant anti-bacterial innate immunity as determined by diminished caspase 1 and IL-1β responses after bacterial infection \cite{13}. The authors further demonstrated that oxidative stress was responsible for inhibition of the inflammasome, impaired innate immunity and higher susceptibility to bacterial infection. This is consistent with increased levels of ROS in A-T cells and in Atm-deficient mice \cite{130,131}. However, these results appear to be at variance with data from a previous study Hartlova et al. \cite{12} where they revealed the presence of cytoplasmic DNA in A-T cells and in Atm-deficient mice which enhanced antiviral and antibacterial responses. Contrary to the Erttmann et al. \cite{13} report these results point to a consistently high innate immune response in A-T cells and Atm mutant mice. Such an inflammatory phenotype is consistent with the capacity of steroids to alleviate the neurological symptoms in patients with A-T \cite{103–105}.

Overall oxidative stress and inflammation are emerging as important hallmarks of patients with A-T. Whether both of these are interrelated or whether they represent threats to different tissues in patients remains to be resolved. There have also been a number of reports of liver disease in A-T which are further supported by a recent clinical trial that reports fatty liver and abnormal liver enzymes in approximately 40% of patients. This is particularly interesting in the context of oxidative stress since it has been shown previously that Atm-mutant mice have increased hepatic iron, serum iron and ferritin \cite{132}. Furthermore, iron chelators have been shown to reduce chromosomal breaks in A-T cells and when combined with antioxidants were even more effective in maintaining genome stability \cite{133}. If this were to translate to patients the presence of excess iron in the liver and indeed in other tissues would lead to enhanced levels of ROS generated through the Fenton reaction \cite{134}. The association of oxidative stress and
inflammation with different aspects of this multisystem disease (e.g. neurodegeneration, lung disease) offer new possibilities for therapy.

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**Declaration of interest**

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

**References**

Papers of special note have been highlighted as either of interest (-) or of considerable interest (–) to readers.


- Describes treatment protocol for A-T patients with cancer and the need for treatments with less toxicity


- Designed A-T scores with a multidimensional index to demonstrate a very high correlation of progression of disease with age.


