Treatment of Alport syndrome: beyond animal models

Oliver Gross[1] and Clifford E. Kashtan[2]

[1]Department of Nephrology and Rheumatology, University Medicine Goettingen, Goettingen, Germany and [2]Division of Pediatric Nephrology, Department of Pediatrics, University of Minnesota, Minneapolis, USA

Alport syndrome (AS) is a hereditary glomerulopathy due to abnormal composition of the glomerular basement membrane, leading to end-stage renal disease (ESRD). Studies of animal models of AS have suggested a variety of potentially effective therapies, but none of these has been definitely shown to prevent or delay ESRD in human AS. Studies in Alport mice suggest that angiotensin inhibition not only has antiproteinuric effects but suppresses cytokine and collagen production as well as tubulointerstitial fibrogenesis and inflammation. For these reasons, many Alport patients are treated empirically with angiotensin antagonists. Cyclosporine may reduce proteinuria in AS, but the risk of nephrotoxic side effects complicates long-term therapy in children. Current data on the role of HMG-CoA reductase inhibition are sparse, so therapy should be limited to adults with dyslipoproteinemia. Results of some, but not all, studies suggest that bone marrow–derived cells may ameliorate disease in Alport mice. However, until experimental doubts concerning the superiority of bone-marrow transplantation over other treatments are resolved by additional investigation, human research subjects should not be exposed to cell-based therapies that may carry substantial risks. In summary, all potential therapies are off-label use in children. As a consequence, initial therapeutic trials should focus on the safety and efficiency of medical treatment, as well as the optimal timing of therapy.

Correspondence: *Oliver Gross, Department of Nephrology and Rheumatology, University Medicine Goettingen, Robert-Koch Str. 40, Goettingen 37075, Germany. E-mail: gross.oliver@med.uni-goettingen.de*

Alport syndrome (AS) is an inherited basement membrane disorder characterized by a progressive hereditary nephropathy leading to end-stage renal disease (ESRD) in all affected males and many affected females, hearing loss in 60–80% and ocular lesions in 25–40% of affected males. Numerous mutations in the COL4A5 gene encoding the α5-chain of type IV collagen have been described in families with X-linked AS.[1] Autosomal recessive AS arises from mutations in both alleles of COL4A3, which encodes the α3(IV) chain, or COL4A4, which codes for the α4(IV) chain. Heterozygous mutations in COL4A3 and COL4A4 cause thin basement membrane nephropathy.[2] Hematuria is the cardinal symptom of thin basement membrane nephropathy and AS. Almost 1% of the population are heterozygous carriers for mutations in the autosomal Alport genes and have thin basement membrane nephropathy, progressing to renal failure in 5–20%.[2]

Mutations in COL4A5 that severely alter the α5(IV) chain (such as large rearrangements, premature stop, or frameshift mutations) result in an early-onset of ESRD at a mean age of 20 years and increased prevalence of extrarenal symptoms. In contrast, mutations altering the quaternary structure of type IV collagen (such as small in-frame mutations or glycine missense mutations) cause a later onset of ESRD (mean age about 30 years).[2,3]

Mutation analysis in patients and families with suspected AS adds significant information for genetic counseling and can replace diagnostic renal biopsy in many cases. Further, molecular genetic diagnosis before the onset of proteinuria would theoretically allow early effective medical intervention. Preemptive therapy with the ACE inhibitor ramipril in an Alport mouse model prolonged lifespan until death from renal failure by more than 100%.[4] These encouraging results raise the possibility that early therapy before the onset of proteinuria might also delay renal failure in humans with AS. As a consequence, mutation analysis of the very large collagen genes has begun to be offered on a professional basis.[5] The chain of events in AS leading from a defective basement membrane to progressive renal fibrosis, however, remains unclear and is a subject of further studies.

Although animal studies have presented us with a variety of potentially effective therapies for Alport kidney disease,[2,6] none of these potential treatments has been formally tested in

Alport populations, and no pharmacological or biological agent has been definitely shown to prevent or delay the development of terminal renal failure in people with AS. The purpose of this brief review is to assess the opportunities for clinical trials in AS and the challenges such trials will face.

Alport nephropathy is essentially cured by renal transplantation. The success of renal transplantation in Alport patients is similar – if not superior – to outcomes in patients with structural renal disease.[3,7] AS does not recur in the allograft as long as the organ donor does not have the disease. Antiglomerular basement membrane nephritis in the allograft, though devastating in individual patients, is fortunately a rare complication of transplantation for AS.[3] While remaining conscious of the morbidities of chronic renal failure and the limitations on the availability of donor organs, we should be cognizant of the high success rate of transplantation when we consider the potential risks of intervention for patients and the possible impacts on transplantation.

This review will discuss data generated by studies of pharmacological and biological interventions in animal and human AS. As the majority of animal studies involve transgenic mice, it is important to be aware of the strain effects on the Alport phenotype in mice.[8,9] The discussion of pharmacological intervention is divided into three categories – angiotensin antagonism, cyclosporine, and other treatments – because most of the 'other' treatments involve drugs with which there is relatively little clinical experience, particularly in children. The discussion of biological interventions will focus on stem cell therapy.

PHARMACOLOGICAL THERAPY
Angiotensin antagonism
Both angiotensin-converting enzyme inhibition (ACEI) and angiotensin receptor blockade (ARB) have been shown to suppress proteinuria, delay loss of renal function, and prolong survival in $Col4a3^{-/-}$ mice with autosomal recessive AS, raised on a 129 genetic background (Figures 1 and 2).[4,10] The most impressive results were observed in mice in which continuous treatment with ramipril was initiated before the onset of proteinuria; duration of survival was doubled in these mice.[4] The beneficial effects on urine protein excretion, renal function, and survival were associated with reduced glomerular and tubulointerstitial fibrosis. In addition, therapy resulted in the downregulation of proteins thought to be key players in human Alport pathogenesis, such as transforming growth factor-β1, connective tissue growth factor, matrix metalloproteinases 2 and 9, and type I collagen.[10] Studies of angiotensin antagonism in autosomal recessive AS mice on the B6 background or in mice with X-linked AS (XLAS) have not been reported. ACEI therapy slowed the decline of renal function in canine XLAS.[11]

To date, only uncontrolled, relatively short-term studies of the effect of angiotensin antagonism on established proteinuria have been reported in human AS.[12–14] ACEI appears capable of transiently reducing protein excretion,[12] an effect that may be augmented by aldosterone inhibition.[15]

Although the mechanisms by which angiotensin antagonism ameliorates Alport kidney disease remain uncertain, evidence from animal studies suggest that the suppression of cytokine and collagen production by podocytes and the antifibrotic and anti-inflammatory effects in the tubulointerstitial compartment may be more important than the antiproteinuric and antihypertensive effects.

Cyclosporine
Cyclosporine therapy slowed the progression of the glomerular basement membrane changes and the deterioration of renal function in canine XLAS.[16] Although there have been no randomized controlled trials of cyclosporine therapy in human AS, uncontrolled studies have shown that cyclosporine can reduce urine protein levels in Alport patients.[17,18] A study of eight Alport males who received cyclosporine for 7–10 years suggested slower progression to ESRD in comparison with related affected males who did not receive such treatment, without development of histological evidence of cyclosporine nephrotoxicity after 5 years of treatment.[17] However, another group of investigators found that cyclosporine therapy in similar doses resulted in reduced proteinuria and decreased inulin clearances in children with AS.[18] Four of five patients who underwent renal biopsy after 20–27 months of therapy exhibited cyclosporine nephrotoxicity.[18]

The mechanisms by which cyclosporine might have a beneficial or harmful effect on Alport kidney disease remain uncertain. Cyclosporine may interfere with alterations in the podocyte cytoskeleton or type IV collagen turnover.

Other pharmacological therapies
Studies of $Col4a3^{-/-}$ mice with autosomal recessive AS, raised on a 129 genetic background, have shown the beneficial effects of a variety of pharmacological approaches, including anti-transforming growth factor-β1 antibody,[19] inhibition of matrix metalloproteinases,[20] vasopeptidase inhibition,[21] chemokine receptor 1 blockade,[22] HMG-CoA reductase inhibition,[23] and bone morphogenetic protein-7.[24] There are no reported studies of these approaches in other murine or canine models of AS.

BIOLOGICAL INTERVENTION
Gene- and cell-based therapies aim at repairing the underlying defect in AS: the defective assembly of α3/4/5(IV) collagen. Replacement of the defective genes by gene therapy in models of AS has not been successful thus far. Delivery of sufficient copies of the normal collagen genes to the appropriate glomerular location is one of the difficult challenges that will need to be overcome to make gene therapy of AS successful.[25] Although new gene vectors (and their efficacy and safety) still have to be established, two research groups have reported that wild-type bone marrow-derived cells can ameliorate disease in Alport mice by differentiation of stem cells into podocytes secreting the missing collagen α3/4/5(IV) chains [26,27] – basically a curative

Therapy	Reduction of proteinuria		Delay of renal failure		Optimal start of therapy	
	Human	Animal	Human	Animal	Human	Animal
*ACE inhibition [6, 8–11]	(+++) (A)	(+++) (A)	ND	(+++) (A)	ND** (microalbuminuria?)	Hematuria (A)
*AT1 antagonist [7]	(++) (D)	(++) (A)	ND	(++) (A)	ND** (microalbuminuria?)	Hematuria (A)
*Aldosterone antagonist [12]	(+) (D)	(+) (D)	ND	ND	ND (proteinuria?)	ND
*HMG-CoenzymeA inhibition [20]	(+) (D)	(+) (A)	ND	(+) (A)	ND** (nephr. syndrome)	Nephr. syndrome (A)
*Cyclosporine [13–15]	(++) (B)	(++) (A)	NS (harm?)	(+) (A)	No therapy?	Unknown
***Vasopeptidase inhibition [18]	(+++) (D)	(+++) (A)	ND	(+++) (A)	ND (microalbuminuria)	Hematuria (A)
***TGFβ-antagonist [16]	ND	(+) (A)	ND	(+) NS (A)	ND	ND
***BMP7 [21]	ND	Yes (A)	ND	ND (A)	ND	ND
***Gene therapy [22]	ND	(++) (A)	ND	(+) (A)	ND	ND
***Bone marrow cells [23–25]	ND	NS (A)	ND	ND, NS (A)	ND	ND
***Mesenchymal stem cells [26]	ND	NS (A)	ND	ND, NS (A)	ND	ND
Endothelin receptor *	ND	(++) (A)	ND	(+) (A)	ND	ND
***Chemokine receptor [19]	ND	(++) (A)	ND	(+) (A)	ND	ND
Collagen receptor **	ND	(++) (A)	ND	(+) (A)	ND	ND

Figure 1 | Current therapeutic approaches in humans and animal models (mice and dogs). In bold: medications used off-label in children and young adults with AS. A = placebo-controlled study, B = controlled study, D = expert view, ND = no data, NS = not significant. *Off-label use, **under investigation, ***experimental therapy only, ****Licht C, Gross O, Scheffler M *et al*. ETB-receptor blockade reduces tubulointerstitial fibrosis caused by chronic proteinuria in COL4A3 knockout mice (abstract TH-PO471, poster presentation at American Society of Nephrology Renal Week 2005). *****Gross O, Vogel WF, Weber M *et al*. Loss of function of the collagen-receptor DDR1 delays renal fibrosis in the COL4A3 knockout mouse model (abstract TH-PO499, poster presentation at American Society of Nephrology Renal Week 2005). +, somewhat effective; ++, effective; +++, very effective.
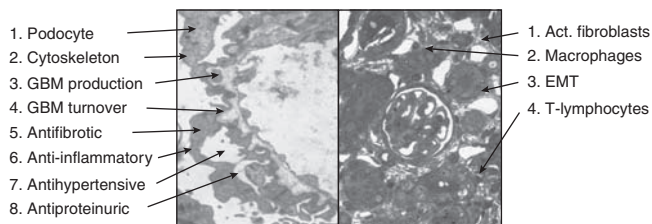

1. Podocyte
2. Cytoskeleton
3. GBM production
4. GBM turnover
5. Antifibrotic
6. Anti-inflammatory
7. Antihypertensive
8. Antiproteinuric

1. Act. fibroblasts
2. Macrophages
3. EMT
4. T-lymphocytes

Figure 2 | Synopsis of possible therapeutic targets in AS. *Left:* Podocyte damage and alterations of the glomerular basement membrane: Possible targets include (1) the podocyte itself (bone marrow-derived cells), (2) the podocyte cytoskeleton (cyclosporine, ACEI, collagen-receptor blockade), (3) GBM production (cyclosporine, collagen-receptor blockade), (4) GBM turnover (MMP antagonists), (5) antifibrotic effects (ACEI, ARBs, CSE inhibitors, TGFβ antagonists, collagen-receptor blockade), (6) anti-inflammatory effects (ACEI, ARBs, CSE inhibitors, collagen-receptor blockade), and (7 + 8) antihypertensive/antiproteinuric effects (ACEI, ARBs, aldosterone and CSE inhibitors, cyclosporine). Original magnification ×10,000. *Right:* Tubulointerstitial infiltration and fibrosis: possible targets include (1) activated fibroblasts (ACEI, ARBs, CSE inhibitors, TGFβ antagonists), (2) macrophages (ACEI, ARBs, CSE inhibitors, collagen-receptor blockade), (3) epithelial-mesenchymal transition (EMT) (BMP7) and (4) T lymphocytes (chemokine-receptor blockade, cyclosporine). Original magnification ×400.

cell-based therapy approach for treating Alport glomerulopathy. These groups postulate that circulating bone marrow-derived cells are recruited to damaged glomeruli where they can cross the glomerular basement membrane, become podocytes, secrete the missing collagen chains, repair the glomerular basement membrane defects, and slow (if not reverse) disease. However, although these authors reported

improvements in overall kidney histology compared with untreated or Col4a3^{−/−} bone marrow-treated Alport mice, the most meaningful end point, i.e. age at end-stage renal failure, was not tested in either study for reasons not explained.[26,27] Recently, Katayama *et al*.[28] reported that bone marrow transplantation following lethal irradiation with either wild-type or Col4a3^{−/−} bone marrow prolonged the lifespan of Alport mice with similar efficiencies. Sublethal irradiation alone also provided significant benefits. Further, a previous study of Alport mice that received bone marrow-derived mesenchymal stem cells also reported improved renal histology, but there was no delay of death from renal failure.[29]

Our current knowledge about AS and its possible therapy by bone marrow transplantation is as yet incomplete. Many open questions remain that need to be addressed experimentally before clinical bone marrow transplantation studies should be considered in Alport patients. Owing to the associated risks of the bone marrow transplantation procedure, it is currently being performed only in potentially lethal diseases. Given the perspective of renal transplantation, AS may not generally qualify for bone marrow transplantation in terms of improving patient survival. Thus, any experimental doubts that the effects of bone marrow transplantation are superior to those of other treatments (such as angiotensin inhibition) increase the need for more research before exposing research subjects to therapies that may carry greater risks.

SUMMARY OF CURRENT MEDICAL THERAPY
Empiric off-label treatment of Alport patients with overt proteinuria is widespread in North America and Europe.

A survey conducted through a pediatric nephrology listserv found that 97% of 107 respondents used medications to suppress proteinuria and/or prolong renal survival in children with AS (unpublished data). Medications used included ACEI (25%), ARB (10%), ACEI plus ARB (58%), and cyclosporine (6%). Indications for initiation of treatment included hematuria alone (3%), microalbuminuria (27%), and overt proteinuria (70%).

These observations have important implications. First, children with AS and proteinuria are being treated in a way that prevents an objective assessment of the risks and benefits of therapy. Second, if treatment of proteinuric Alport patients with ACEI/ARB represents the standard of practice, proposals to conduct placebo-controlled trials of such treatment may be challenged by research subjects protection programs; if approved, such studies may encounter resistance to recruitment efforts. On the other hand, observational studies of markers of disease progression in children who have not yet developed overt proteinuria, and placebo-controlled treatment trials in these patients, would not conflict with the current treatment practices of most pediatric nephrologists.

At present, no serious side effects of angiotensin antagonism have been reported to the North American or European AS registries. However, data showing that angiotensin antagonism, alone or in combination with other agents, alters the natural history of human AS are lacking. All biological interventions including gene and stem cell therapy are still in their infancy, but increase the hope for future therapeutic options. Current data on the role of HMG-coenzyme A reductase inhibition in human chronic renal disease are sparse. Therefore, in our opinion, therapy should be limited to adults with dyslipoproteinemia associated with to nephrotic syndrome. Data on cyclosporine are inconsistent as well; therefore, possible harm owing to nephrotoxic side effects should, at present, hinder any long-term therapeutic approach in children.

All potential therapies are off-label use in children. Trials should focus on safety and efficiency of medical treatment plus optimal start of therapy – microalbuminuria, overt proteinuria, or even before onset of microalbuminuria.

CHALLENGES OF CLINICAL TRIALS IN AS

Clinical trials in AS face difficult, but not insurmountable, challenges. First, the disease is not common. Approximately 40–45% of affected individuals are females with XLAS, in whom the disease course is difficult to predict because of X-inactivation.[30,31] In males with XLAS, rapidity of progression to ESRD is strongly influenced by COL4A5 genotype.[3] Consequently, treatment groups will need to be balanced in terms of genotype and phenotype. Finally, loss of renal function in AS occurs over decades; at this time, little is known about the early markers of disease progression in human AS.

Overcoming these obstacles will require an international collaborative effort. Registries have been developed in North America and Europe to identify Alport families and facilitate recruitment of affected individuals to natural history studies of early markers of disease progression and to clinical trials. Support for these efforts will require a combination of private, public, and industrial support. Our experience with Alport families has convinced us that subjects will eagerly participate in well-designed clinical trials.

REFERENCES

1. Hudson B, Tryggvason K, Sundaramoorthy M *et al.* Alport's Syndrome, Goodpasture's Syndrome, and Type IV Collagen. *N Engl J Med* 2003; **348**: 25.
2. Gross O, Weber M. From the molecular genetics of Alport's syndrome to principles of organo-protection in chronic renal diseases. *Med Klin* 2005; **100**: 826–831.
3. Jais JP, Knebelmann B, Giatras I *et al.* X-linked Alport syndrome: natural history in 195 families and genotype- phenotype correlations in males. *J Am Soc Nephrol* 2000; **11**: 649–657.
4. Gross O, Beirowski B, Koepke M-L *et al.* Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in COL4A3-knockout mice with Alport syndrome. *Kidney Int* 2003; **63**: 438–446.
5. Nagel Nagel M, Nagorka S, Gross O. Novel COL4A5, COL4A4 and COL4A3 mutations in Alport syndrome. *Hum Mutat* 2005; **26**: 60–65.
6. Cosgrove D, Kalluri R, Miner JH *et al.* Choosing a mouse model to study the molecular pathobiology of Alport glomerulonephritis. *Kidney Int* 2007; **71**: 615–618.
7. Kashtan CE, McEnery PT, Tejani A *et al.* Renal allograft survival according to primary diagnosis: a report of the North American Pediatric Renal Transplant Cooperative Study. *Pediatr Nephrol* 1995; **9**: 679–684.
8. Gross O, Borza DB, Anders HJ *et al.* Stem cell therapy for Alport syndrome: the hope beyond the hype. *Nephrol Dial Transplant* 2009; **24**: 731–734.
9. Andrews KL, Mudd JL, Li C *et al.* Quantitative trait loci influence renal disease progression in a mouse model of Alport syndrome. *Am J Pathol* 2002; **160**: 721–730.
10. Gross O, Schulze-Lohoff E, Koepke M-L *et al.* Antifibrotic, nephroprotective potential of ACE-inhibitor vs AT1 antagonist in a murine model of renal fibrosis. *Nephrol Dial Transplant* 2004; **19**: 1716–1723.
11. Grodecki KM, Gains MJ, Baumal R *et al.* Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting enzyme (ACE) inhibitor. *J Comp Pathol* 1997; **117**: 209–225.
12. Cohen EP, Lemann Jr J. In hereditary nephritis angiotensin-converting enzyme inhibition decreases proteinuria and may slow the rate of progression. *Am J Kidney Dis* 1996; **27**: 199–203.
13. Proesmans W, Knockaert H, Trouet D. Enalapril in paediatric patients with Alport syndrome: 2 years' experience. *Eur J Pediatr* 2000; **159**: 430–433.
14. Proesmans W, Van Dyck M. Enalapril in children with Alport syndrome. *Pediatr Nephrol* 2004; **19**: 271–275.
15. Kaito H, Nozu K, Iijima K *et al.* The effect of aldosterone blockade in patients with Alport syndrome. *Pediatr Nephrol* 2006; **21**: 1824–1829.
16. Chen D, Jefferson B, Harvey SJ *et al.* Cyclosporine a slows the progressive renal disease of alport syndrome (X-linked hereditary nephritis): results from a canine model. *J Am Soc Nephrol* 2003; **14**: 690–698.
17. Callis L, Vila A, Carrera M *et al.* Long-term effects of cyclosporine A in Alport's syndrome. *Kidney Int* 1999; **55**: 1051–1056.
18. Charbit M, Gubler MC, Dechaux M *et al.* Cyclosporin therapy in patients with Alport syndrome. *Pediatr Nephrol* 2007; **22**: 57–63.
19. Cosgrove D, Rodgers K, Meehan D *et al.* Integrin alpha1beta1 and transforming growth factor-beta1 play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy. *Am J Pathol* 2000; **157**: 1649–1659.
20. Zeisberg M, Khurana M, Rao VH *et al.* Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. *PLoS Med* 2006; **3**: e100.

21. Gross O, Koepke ML, Beirowski B *et al.* Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. *Kidney Int* 2005; **68**: 456–463.

22. Ninichuk V, Gross O, Reichel C *et al.* Delayed chemokine receptor 1 blockade prolongs survival in collagen 4A3-deficient mice with Alport disease. *J Am Soc Nephrol* 2005; **16**: 977–985.

23. Koepke ML, Weber M, Schulze-Lohoff E *et al.* Nephroprotective effect of the HMG-CoA-reductase inhibitor cerivastatin in a mouse model of progressive renal fibrosis in Alport syndrome. *Nephrol Dial Transplant* 2007; **22**: 1062–1069.

24. Zeisberg M, Hanai J, Sugimoto H *et al.* BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; **9**: 964–968.

25. Heikkilä P, Tryggvason K, Thorner P. Animal models of Alport syndrome: advancing the prospects for effective human gene therapy. *Exp Nephrol* 2000; **8**: 1–7.

26. Prodromidi EI, Poulsom R, Jeffery R *et al.* Bone marrow-derived cells contribute to podocyte regeneration and amelioration of renal disease in a mouse model of Alport syndrome. *Stem Cells* 2006; **24**: 2448–2455.

27. Sugimoto H, Mundel TM, Sund M *et al.* Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci USA* 2006; **103**: 7321–7326.

28. Katayama K, Kawano M, Naito I *et al.* Irradiation prolongs survival of Alport mice. *J Am Soc Nephrol* 2008; **19**: 1692–1700.

29. Ninichuk V, Gross O, Segerer S *et al.* Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. *Kidney Int* 2006; **70**: 121–129.

30. Bell RJ, Lees GE, Murphy KE. X chromosome inactivation patterns in normal and X-linked hereditary nephropathy carrier dogs. *Cytogenet Genome Res* 2008; **122**: 37–40.

31. Kashtan CE: Alport syndrome and the x chromosome: implications of a diagnosis of Alport syndrome in females. *Nephrol Dial Transplant* 2007; **22**: 1499–1505.