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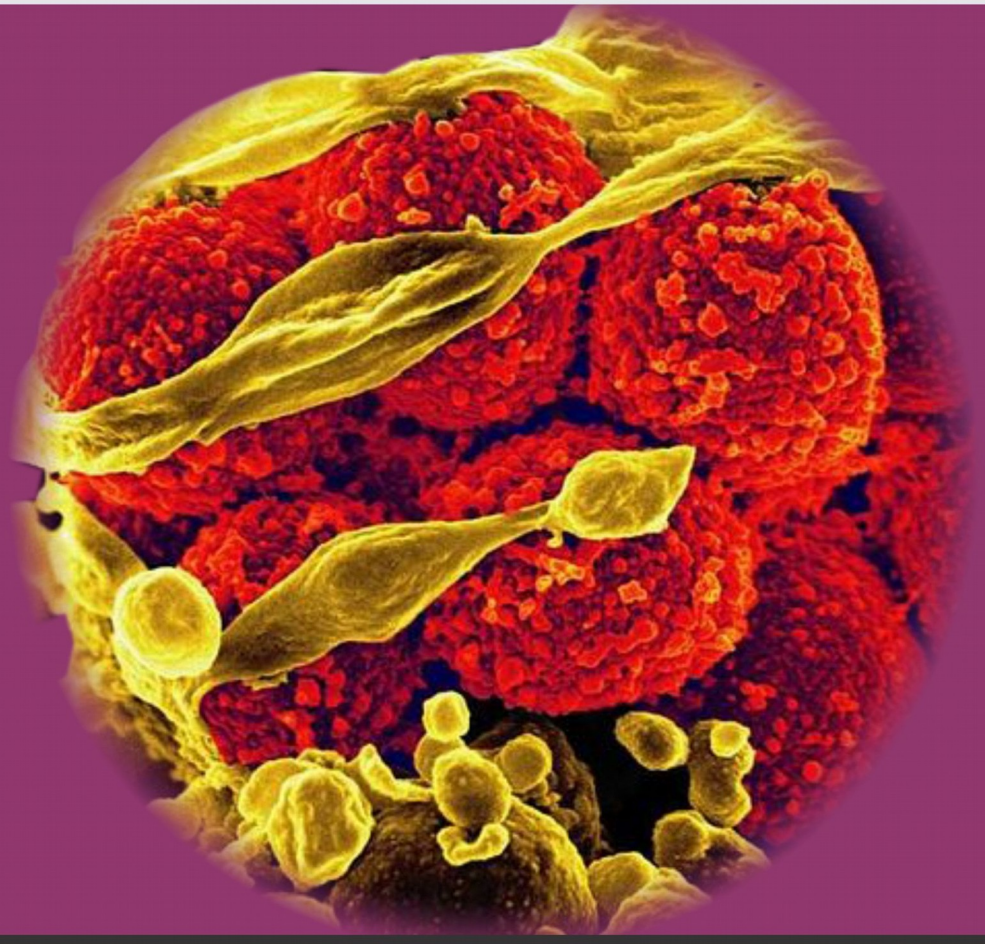

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Special Feature:
Guidelines for the Management of Venous Thromboembolism in Nigeria.

Advances in Treatment for Haemophilia

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SUMMARY

Traditional replacement therapy for haemophilia is limited by development of inhibitors, parenteral administration and short half-life of the protein. A number of new therapies are in development and reaching the clinic which can address all these problems. Firstly, modifications have been made to Factor VIII (FVIII) and Factor IX (FIX) which prolong their half-life without impairing function. The prolongation of approximately 1.5 times control for Factor VIII is relatively modest but for Factor IX it is 3-5-fold which allows prophylactic injections to be given every 7-14 days. A step further is the development of a bispecific antibody which mimics the cofactor function of FVIII by binding to both Factor IXa and Factor X. A completely novel approach is to remedy the procoagulant deficiency in haemophilia by balancing it with a corresponding reduction in anticoagulant activity. All three anticoagulant pathways (TFPI, Protein C-Protein S and antithrombin) have been identified as targets for this 'rebalancing' approach to therapy, which has been successful in preliminary trials. These new therapies have the advantage that they will not stimulate anti-FVIII or FIX antibodies and will be able to work effectively even if they are present. Moreover, they can be given by subcutaneous injection and have much longer half-lives that FVIII or FIX. However, they may be difficult to monitor and to combine safely with other treatments. Finally, the success of early trials with gene therapy offer the prospect of freeing patients from injections entirely and elevating their FVIII or IX levels into the range sufficient to secure normal haemostasis.

INTRODUCTION

Descriptions of haemophilia as a severe and frequently intractable bleeding disorder, inherited in a sex-linked manner, date back for thousands of years [1) but it was not until well into the 20th century that the cause was identified as a missing component of the blood. In the subsequent years limited therapy was possible using blood and plasma to treat acute bleeding events but the labile nature of Factor VIII (FVIII) meant that stored blood rapidly lost its potency. From these beginnings the most notable step forward in treatment was the discovery by Judith Pool in 1965 that the cryoprecipitate formed when frozen plasma was thawed, contained an increased concentration of the factor now identified and named as FVIII. This made more complete replacement possible and also allowed storage of the product in freezers at home for prompt administration when needed. [2]

A natural progression was the increasing purification of the missing factor with the development of lyophilised concentrates containing large amounts of FVIII. In 1952 the first report of several families with deficiency of Factor IX (FIX) had led to the name Christmas disease being coined for what is now more frequently referred to as Haemophilia B. [3] Similar advances using different methods led to the development of lyophilised concentrates of FIX. [4] Tragically, although the purity of the coagulation factors increased relative to other blood proteins, it was accompanied by an increased infectious risk due to transmissible viral diseases as plasmas from many donors had to be pooled to achieve sufficient amounts of purified clotting factors. The widespread transmission of hepatitis C and HIV by coagulation factor concentrates caused the death of many patients, but also triggered the search for more effective treatment and treatment regimens. [5,6] Nonetheless, by the late 1980s, donor screening, sample testing and viral inactivation procedures had made plasma derived products very safe and they remain so today. The cloning of FIX in 1981 [7] and FVIII in 1984 [6] enabled the production of

these proteins in vitro and the manufacture of concentrates free from transmission of donor diseases, theoretically enabling the production of unlimited amounts of the proteins. Notwithstanding concerns about rates of inhibitor formation, recombinant concentrates are now the mainstay of therapy in many parts of the world, even becoming cheaper than the plasma derived form. Development of recombinant manufacture has facilitated the production of modified molecules with altered properties and the same molecular techniques will support the introduction of gene therapy in the longer term.

INTRODUCTION OF PROPHYLAXIS

Once the principle of replacement as therapy for haemophilia had been established, effective treatment was limited by a number of problems. First was the limited amount of purified protein available and secondly its intrinsic pharmacokinetic properties: a plasma half-life of approximately 8 -12 hours for FVIII (18-24 hours for FIX) meant that maintaining effective levels required relatively frequent injections. Moreover, the injections must be administered by the intravenous route making treatment more difficult especially in children. Another major problem, the development of neutralising antibodies (inhibitors) against the infused protein remains unsolved today, although some of the novel therapies described here may be helpful. [8] A further problem was conceptual: should treatment be given for bleeding episodes as they occurred, or should it be given regularly in order to prevent bleeds from occurring? The latter, prophylactic, approach is a much bigger, more challenging and expensive enterprise but more likely to prevent patients from trauma and progressive joint damage. IM Nilsson and colleagues in Sweden realised as early as 1958 that patients with mild or moderate haemophilia, sometimes with only a few percent of normal FVIII levels, had significantly fewer problems with spontaneous bleeding and so much less debility from progressive joint damage. They therefore introduced the idea of prophylactic infusions of FVIII to convert patients from severe to moderate haemophilia and so reduce the frequency of bleeding. [9] Although initiated in Sweden at this time, the concept took a long time to become an accepted practice in the rest of the world, perhaps held back by concerns over disease transmission but also because of the cost burden for health care systems.

Introduction of prophylactic treatment regimens has greatly reduced the burden of joint damage and other morbidities and mortality in countries where this is affordable. However, to date these have not been completely abolished and this remains the outstanding goal, to achieve a normal life for people born with haemophilia. Debate continues regarding the optimum prophylactic trough level but the target is undoubtedly increasing in pursuit of 'zero bleeds'. [10] To achieve this more readily, a considerable number of new therapies are emerging with broadly similar aims: to achieve higher levels of FVIII or FIX for greater periods of time with the minimum of intrusion into patients' lives. Some approaches also avoid the problem of inhibitors (anti-FVIII or FIX antibodies) and some will be applicable to other bleeding disorders. [11]

TARGETS FOR TREATMENT:

1. Extended Half –Life (EHL) Products

The plasma half-life of FVIII is approximately 8-12 hours and although this varies widely between individuals, FVIII prophylaxis is generally administered every other day. The half-life of FIX is rather longer, 18-24 hours and so prophylaxis can be given every third day. [12] If it were possible to increase the half-lives of these molecules then it should be possible to achieve higher trough levels or increase the intervals between injections, or both. Two other proteins in the blood have much longer half-lives of several weeks: IgG and albumin. They achieve this by binding to the neonatal Fc receptor after being taken up by endothelial cells and this interaction diverts them away from degradation in the endosome and towards return to plasma thus prolonging their survival. [13] Factor VIII and FIX have both been successfully fused to the Fc portion of IgG to

take advantage of this effect without impairing their procoagulant function and resulting in prolongation of plasma half-life. [14] Factor IX has also been covalently fused with albumin, again with prolongation of survival but fusion of albumin to FVIII seems to impede its function and so has not been pursued.

The inorganic molecule polyethylene glycol (PEG) has been used to prolong the half-life of many molecules and has also been applied to FVIII and IX. This large polymeric molecule and its cloud of water molecules makes any protein to which it is attached much less likely to be cleared by the kidney or other mechanisms and thus prolongs plasma half-life. Random attachment of PEG can, not surprisingly, interfere with function and so methods have been developed to attach it to specific sites. Several targeted methods, for example linking to glycans or to cysteine residues have been successful, taking care not to impair functional interactions with other molecules. [15-18]

The results of these modifications to FVIII were generally somewhat disappointing. All the different methods achieved roughly the same half-life prolongation of approximately 1.5-1.7 times control (wild type). [15,19,20] In retrospect it was realised that this was the inevitable result of the association of FVIII with VWF. Over 90% of plasma FVIII is bound to VWF which in large part determines its half-life, so that only clearance of the free unbound fraction is extended by the added molecules. In a sense FVIII-VWF is already an extended half-life preparation. The practical consequence is that dosing intervals of FVIII can be increased from 2 days to 3 days whilst maintaining the same trough level. Equally, maintaining the same interval will increase the trough: either may be useful and beneficial. Recent studies have shown that the dominant effect of VWF can be circumvented by combining FVIII with the FVIII binding domain of VWF and additional segments preventing clearance. It may also be possible to administer by a subcutaneous route.

The half-life of FIX is not determined by a plasma binding partner and so similar modifications of Factor IX as described above for FVIII were generally much more successful. Addition of albumin, Fc and PEG resulted in half-life prolongations of 3-5 times wild type. This has allowed successful extension of prophylactic dose intervals to one or two weeks with maintenance of acceptable trough levels. [21-23] However, a review of the recovery and efficacy of these modified molecules has drawn attention to a previously known, but underappreciated aspect of FIX biology, its distribution outside plasma. [24] Earlier studies demonstrated that FIX is able to bind to endothelial cells and to extravascular collagen IV via residues in its GLA domain and that this results in a substantial reservoir of non-circulating FIX. Experiments using transgenic mice expressing a FIX variant that cannot bind to collagen showed increased bleeding times. [25] The ability of modified FIX molecules to replace this FIX reservoir may alter the relationship between their assayed plasma activity and their haemostatic effect.

2. Factor VIII Mimetic – Bispecific Antibody

Although the molecules described above have altered pharmacokinetics, they remain conventional in that the core FVIII molecule is retained with its ability to bind FIXa and FX, bringing them together on a phospholipid surface. Several years ago, scientists at Chugai began the development of a bispecific antibody with the ability to reproduce this action. After screening of many thousands of antibodies and combinations of light and heavy chains an active antibody named ACE910 was developed. The two arms bound FIXa and FX (the antibody can, however, not differentiate between FIX and FIXa and FX and FXa) but not phospholipid and thus increased the rate of FX cleavage into FXa. Although the molecule is considerably less efficient than native FVIII, at a concentration of 300nM (compared to the plasma FVIII concentration of 0.4-0.7nM), it supported thrombin generation that was similar to that of FVIII at 0.1 IU/ml in their thrombin generation system. [26,27] This molecule has the obvious advantages that it will not generate, or be neutralised by, anti-FVIII antibodies and that it can be administered sub-cutaneously.

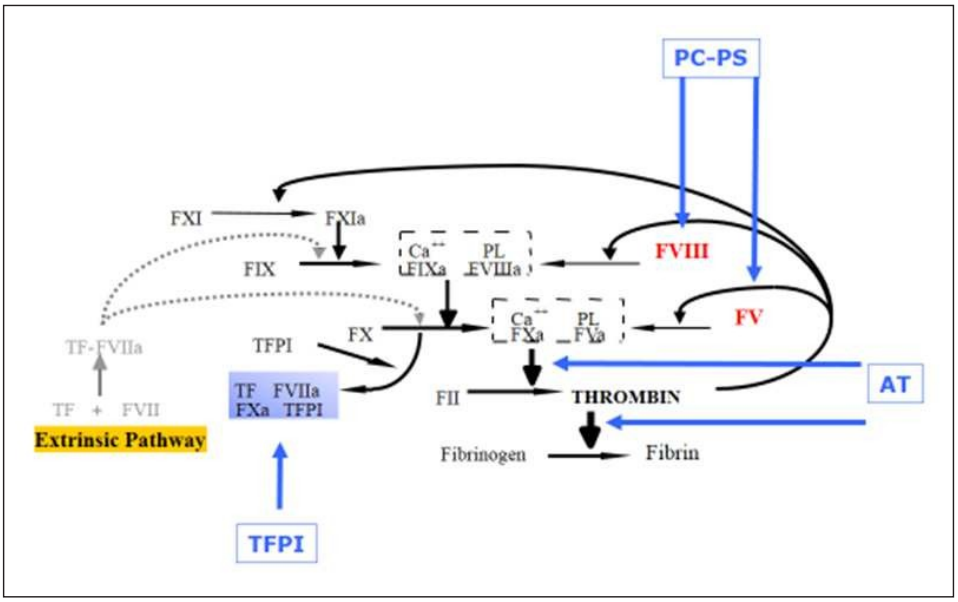
Furthermore, its half-life of several weeks allows long periods between injections. In the Haven 4 trial it appears that injections can be once monthly. The long half-life and sub-cutaneous injection effectively smooths out the peak and trough effect which characterises conventional and even extended half-life (EHL) therapy. This molecule has proven extremely effective in prophylaxis regimens for Haemophilia A patients with and without inhibitors. [28,29]

An important feature of the molecule is that it does not undergo an activation step comparable to the thrombin or FXa cleavage of FVIII because it is constitutively active: this renders conventional methods of assaying FVIII unsuitable for measuring its level in blood, although this will generally not be necessary. The permanent 'on' state plus the lack of any natural inhibitory or regulatory pathway has raised some concerns of increased coagulation activity and risk of thrombosis. In general, this does not seem to have been realised but a series of unusual thrombotic events including thrombotic microangiopathy, were seen in early trials when the antibody was administered in combination with activated prothrombin complex concentrate. [29] and supplementary data] It seems likely that the increased supply of FIXa led to unregulated coagulation activation. Normally the generation of FIXa would be restricted to the site of haemostatic activation by TF exposure and so too would the activity of ACE910 (now known as emicizumab or Hemlibra). These problems have not been seen in the absence of APCC and will not apply to non-inhibitor patients. [29]

In keeping with the different structure, emicizumab has been remarkably successful in preventing bleeding in patients with haemophilia A and inhibitors (anti-FVIII antibodies) for whom there was previously no truly effective therapy. Licensing is now being sought for patients without inhibitors. For these patients the advantage of infrequent subcutaneous injections may make it attractive. The principal remaining question is what level of haemostatic function can be achieved? It seems that it will provide a good level for prophylaxis, probably better than the trough level on most standard regimens, but not necessarily sufficient for surgery or major trauma. Laboratory methods may not be able to give an accurate answer to this question which, therefore, may only be answered by clinical observation. [30] A method for laboratory monitoring is not yet established but this is not a barrier to adoption. Antibodies against the antibody itself may occur but have been infrequent so far.

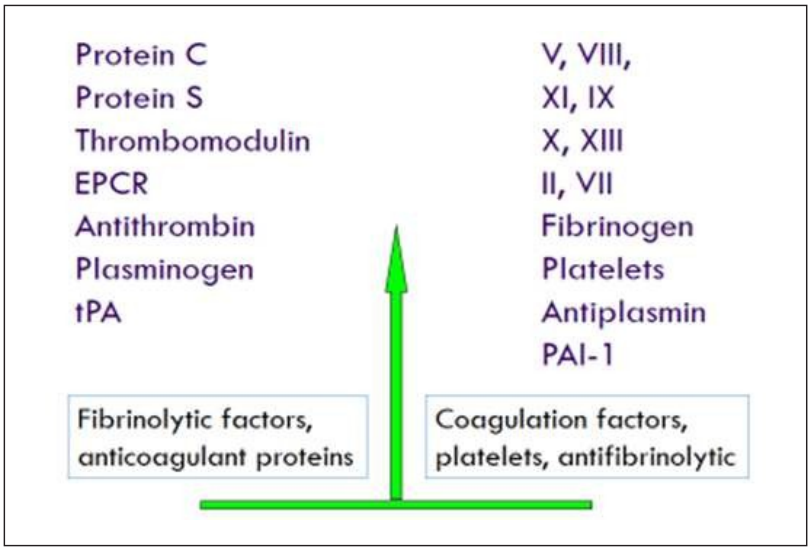
3. Rebalancing Hypothesis

The relatively high population prevalence of the Factor V Leiden polymorphism meant that its co-inheritance with haemophilia was occasionally encountered and raised the question as to whether this prothrombotic molecule could ameliorate the bleeding tendency of haemophilia. [31] From this developed the concept of correcting the procoagulant defect of haemophilia by reducing the effect of the natural anticoagulant molecules. In fact, the ability of TFPI inhibition to reduce bleeding in an animal model of haemophilia was demonstrated as early as 1995, [32] but each of the three natural anticoagulant pathways have subsequently been explored (Figures 1 & 2). Demonstration of the effect in vitro was facilitated by the development of the thrombin generation assay and showed that reduction of antithrombin (AT) concentration in haemophilic plasma resulted in partial restoration of thrombin generation. [33]



Key: TF= tissue factor; FVII= factor VII; FX=factor X; FVIII= factor VIII; FIX= factor IX, FXI= factor XI, FV= factor V; FII= prothrombin; TFPI= tissue factor pathway inhibitor; AT=antithrombin; PC= protein C; PS= protein S; PL=phospholipid. 'a' indicates the activated form of the protein.

Figure 1: The coagulation network showing the sites of action of the three anticoagulant systems.



Key: FVII= factor VII; FX -=factor X; FVIII= factor VIII; FIX= factor IX, FXI= factor XI, FV= factor V; FII= prothrombin; FXIII= factor XIII; EPCR= endothelial protein C receptor; tPA= tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor-1.

Figure 2: Normal haemostasis: a state of equilibrium.

Subsequently a method of reducing plasma AT concentration was developed using a small interfering RNA (siRNA) administered subcutaneously. The nucleic acid is coupled to a glycan molecule so that it is taken up by the asialoglycoprotein receptor on hepatocytes. Once in the cell it binds to the antithrombin mRNA and utilises the cell's own mechanisms for destruction of doublestranded mRNA to downregulate AT synthesis. The result is that monthly injections of the siRNA can reliably reduce plasma AT concentration to around 20% of normal. This would likely be lethal in a normal subject but in patients with haemophilia

restores thrombin generation close to normal. The phase 1 study noted that this was associated with a significant reduction in bleeding, although this was not a primary endpoint. [33]

The role of TFPI in shutting off the initiation of coagulation by the extrinsic pathway and the resulting dependence on FVIII-FIX and the intrinsic pathway for thrombin generation suggests that it would be a good target for the rebalancing strategy. Indeed, this seems to have been the most popular approach and numerous methods are currently under development. The most frequent approach is to inhibit TFPI using a monoclonal antibody. So far this has only reached phase I-II stage but data show that complete inhibition of plasma TFPI can be achieved. [34] The complicating factor is that 80% of vascular TFPI is bound to the surface of endothelial cells (TFPIβ) and so the effect on this pool cannot easily be measured. It is only after complete depletion of plasma TFPI has been achieved that further increases in antibody concentration result in a rise in F 1.2 or D-dimer indicating an increase in thrombin generation. [34] TFPI can also be targeted using an aptamer or non-anticoagulant sulphated polysaccharide, derived from seaweed. [35,36]

Huntington and colleagues have chosen activated protein C as a suitable target and have developed a novel engineered serpin based on alpha 1 antitrypsin. It has been shown to be effective in reducing bleeding in an animal model of haemophilia, but has not yet been administered to humans. [37] An early model of protein S suggests that it too might be an effective target. [38]

A general advantage of these rebalancing approaches is that they will work for both haemophilia A or B and will not be affected by the presence of antibodies. In fact, they might theoretically work for any coagulation factor deficiency, including disorders such as Factor V deficiency for which there currently no concentrates available. Moreover, they all offer the prospect of infrequent subcutaneous injection. At the same time, they have the problem that there are no established or validated methods for monitoring their effect in plasma, except perhaps thrombin generation assays, but as noted above this may not be representative of their effect in vivo. There is also the possibility of off-target effects because the molecules also participate in other pathways, particularly inflammation. Finally, the treatment of breakthrough bleeds may be difficult to judge with the risk of overcompensating and precipitating thrombosis.

1. Gene Therapy

Gene therapy is defined by the FDA as the 'Delivery of nucleic acid polymers into cells to treat disease'. It was noted early on that haemophilia was an excellent target for gene therapy. The key points in its favour are that haemophilia is a simple single gene disorder affecting a plasma protein. This means that expression in any tissue would be satisfactory as long as it could be secreted into the circulation. Moreover, tight regulation of expression is not required and clinical experience showed that even modest levels of expression would have significant therapeutic benefit. Several early trials were unsuccessful but current programmes have settled on the use of adeno-associated virus (AAV) vectors as a means of delivering the corrective gene to hepatocytes: this is the normal site of synthesis for FIX, but FVIII is normally synthesised in endothelial cells. Preliminary experiments showed safe and sustained expression of the therapeutic gene in animal models, but it has been found that responses in animal models including non-human primates are poorly predictive of the effects in man. In particular, it was found that the capsid protein used to encapsulate the therapeutic construct could elicit a cytotoxic T-cell response, which destroyed the transduced cells with consequent loss of the therapeutic effect. [39] In the first successful gene therapy trial for haemophilia (B) conducted by Nathwani *et al*, this effect was effectively avoided by prompt administration of steroids as soon as evidence of liver damage began to appear. [40]

Initial trials utilised FIX since the smaller cDNA fitted more readily into the AAV vector and because it was thought to be less immunogenic. Nathwani *et al* demonstrated that levels of approximately 5% could be achieved and sustained steadily over several years. [41] A recent innovation has been to use the FIX Padua variant whose specific activity is approximately 8 fold more than wild type FIX and was originally identified in an Italian family with familial thrombosis. [42] Utilising F1X Padua as the transferred gene allows functional levels of FIX that are >0.3IU/ml with antigen of only 0.05IU/ml. This has the added advantage of allowing smaller doses of vector to be administered. [43]

The FVIII cDNA is too large to fit into the AAV5 capsid, but fortunately the B domain can be removed without loss of function, as has been demonstrated with commercial concentrates. Removal of the B domain allows the FVIII cDNA to fit (just) into an AAV vector. Using a substantially higher vector dose than in previous studies, the Biomarin programme achieved elevation of plasma FVIII into the normal range (and beyond in some subjects). The levels then fell somewhat, but remained substantially elevated at one year. [44] All the gene therapy patients will be followed closely over the coming years to determine the duration of effect and its safety. Notably the dogs treated with gene therapy sustained expression for >10 years but it will be many years before the durability and safety of this approach is known. So far initial concerns of an immune reaction against the FVIII or FIX have not been realised, but the trial participants have all been heavily treated in the past. In fact, emerging data from animals suggest that gene therapy may even produce tolerance even when the animal has been immunised.

The AAV vectors in current trials are all non-integrating viruses so that the therapeutic gene usually remains as an episome and does not integrate into the recipient genome. This is beneficial with regard to safety. Gene therapy with integrating viruses for other diseases have induced leukemia due to insertion in the critical region of oncogenes. However, non-integrating gene therapy limits its use to adults because the episomes will not be replicated as the cells divide in a growing child. High antibody titres against the viral vector after treatment mean that it cannot be repeated in its current form. Gene therapy in children will either require a means to give repeated doses of the gene therapy or the use of an integrating vector so that it grows with the recipient. Trials are now under way using zinc finger nucleases to insert the FIX gene into the 'safe harbour' albumin locus. [45]

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