

LB01

Haemophilia B gene therapy study in the UK

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Our study differs from previous HB clinical trials with AAV vectors in three important aspects. Firstly, AAV8 pseudotyped vectors will be used instead of AAV2 primarily because of the substantially lower prevalence of pre-existing humoral immunity to this AAV serotype in humans. The second difference relates to the use of a vector containing a self complementary genome which, is more potent than conventional single stranded AAV vectors and offers a unique opportunity to mediate efficient therapeutic gene transfer potentially at a low dose of vector. Finally, because the biodistribution of vectors pseudotyped with AAV8 serotype capsid is predominantly to the liver, regardless of the route of administration, scAAV particles will be administered via a peripheral vein. This dispenses with the need for invasive surgical procedures, making vector administration safer for patients with severe HB.

Our Phase I/II clinical trial therefore entails peripheral vein administration of a single dose of our novel self complementary AAV (scAAV2/8-LP1-hFIXco) vector into adult subjects with severe HB, starting with a dose of 2×10^{10} vg/kg and then escalating to the intermediate (6×10^{10} vg/kg) and high dose (2×10^{11} vg/kg) levels in the absence of toxicity.

The first subject was recruited to this study in early March 2010 and he received a single peripheral vein infusion of 2×10^{10} vg/kg without any side effects with a follow-up period now extending beyond six weeks. This dose was defined as the subtherapeutic dose by the regulators and is 100 fold lower than the dose that transiently (<6 weeks) mediated therapeutic level of transgene expression in the previous liver directed rAAV haemophilia B study. We have observed stable human FIX expression in our first subject at between 1.5-2% of normal levels over a period that extends beyond 6 weeks following vector infusion. Importantly, this subject did not have neutralising antibodies to AAV8 and we have not observed any evidence of vector induced hepatitis despite the fact that he did not receive any immunosuppressive treatment. Furthermore he has not required any treatment or prophylaxis with FIX concentrate over this period and remains free of spontaneous joint bleeds.

These data are highly promising and suggest that our novel self complementary AAV vector encoding hFIX, may be more potent in human than conventional single stranded rAAV vector used previously. Additionally it suggests that low doses of scAAV vector, when pseudotyped with serotype 8 capsid can mediate therapeutic levels of hFIX without provoking an immunological response of the type seen in the previous trial.

We are planning to treat another patient at this low dose level but we feel that it is important to share these early promising results with the Haemophilia B community. We would, therefore, welcome an opportunity to present our data at the upcoming Hemophilia World congress in Buenos Aires, in July, as a late breaking abstract. In fact my colleague Professor Edward Tuddenham is planning to attend this important meeting and is more than happy to present the data on behalf of our group.

LB02

Role of duplications in the molecular mechanisms of haemophilia :

New insights provided CGH array

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Inversions between duplicated regions represent well-known molecular mechanisms responsible for haemophilia A (HA). More than 45 % of severe cases are due to inversions involving intrachromosomal homologous recombination between the segmental duplications int22h-1 located in intron 22 of the *F8* gene and one of the two duplicons int22h-2 or int22h-3 situated approximately 400 and 500 kb more telomerically. Inversion of intron 1 present in 1 to 3% of severe cases is secondary to a similar mechanism between other duplicated sequences. Sequencing of the complete human genome has shown that ~ 5% is composed of duplicated sequences. Several segmental duplications are implicated in many genomic disorders (Charcot-Marie-Tooth, Smith Magenis). Several other duplications represent polymorphisms that are neutral suggesting that they have played a role in the genomic evolution. With respect to HA, besides intron 22 and 1 inversions, the presence of duplicated sequences in the *F8* gene and their pathogenic implications have not been studied. Using microarray-based comparative genome hybridization assay, we delimited duplications of the 5' position of *F8* gene (including exons 1 to 22 and exon 1 only) in normal and HA patients harbouring different severities of HA. The causal effects of the duplications could be explained by different rearrangements inside *F8* gene. These findings show that duplications resulting from a recombination between homologous sequences at Xq28 may be present in both normal subjects and HA patients. These duplications may be neutral in function except if they are accompanied by a more complex rearrangement disturbing the *F8* gene.

LB03

First in Human Clinical Experience of a High Purity Factor X Concentrate

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Introduction: Severe factor X deficiency is a rare (1:~1,000,000) and potentially life-threatening bleeding disorder. BPL has developed a high-purity factor X concentrate (FACTOR X) specifically for the management of this condition. **Objectives:** To evaluate the pharmacokinetics (PK), safety and efficacy of FACTOR X in patients with severe and moderate hereditary factor X deficiency (<5% normal FX:C). PK parameters for FX:C (one-stage clotting assay) and FX:Ag are assessed at baseline and 6 months post-baseline with sampling timepoints up to 144 hours (6 days) post-infusion. Efficacy in bleed management is assessed over at least 6 months. **Results:** PK data: Data from the first 2 patients' baseline FX:C PK profiles give incremental recoveries of 1.64 and 1.92 IU/dL per IU/kg, and half-lives of 25.1 and 39.4 hours (non-compartmental analysis). Efficacy data: One patient has experienced a shoulder haemarthrosis, starting 7 days after the PK dose. 24 hours following the first dose to treat the bleed (25 IU/kg) the patient felt less pain, mobility was improved and the efficacy of the product for this event was judged to be excellent. A further 13 preventative doses (total 326 IU/kg) were given, with no adverse effects. **Conclusion:** These are the first robust factor X PK data in patients with factor X deficiency. The half-lives of factor X are similar to those in another study following infusion of a prothrombin complex concentrate in healthy volunteers. In addition, FACTOR X appears to be safe and efficacious based on the management of one bleed treated to date.

LB04

Phase I, Randomized, Double-Blind, Placebo-Controlled, Single-Dose Escalation Study of the rFVIIa Variant (BAY 86-6150) in Hemophilia A or B With or Without Inhibitors

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Introduction: BAY 86-6150, a human recombinant FVIIa (rFVIIa) variant, was developed to provide a longer-acting activated factor VII (FVIIa) in the management of bleeds in haemophiliacs with inhibitors.

Objectives: To investigate safety, tolerability, pharmacodynamic/pharmacokinetic profiles, and immunogenicity of BAY 86-6150 in nonbleeding patients with haemophilia

Methods: The population of this randomized, double-blind, placebo-controlled, single-dose escalation study comprised nonbleeding patients aged 18 to 65 years with moderate or severe

haemophilia A or B with or without inhibitors. Sixteen patients were randomized 3:1 to escalating doses of BAY 86-6150 at 6.5, 20, 50, or 90 µg/kg (n=3 each) or placebo (n=4). Patients were followed up for 50 days postdose.

Results: BAY 86-6150 was not associated with clinically significant adverse events or dose-limiting toxicities. BAY 86-6150 pharmacokinetics were linear over the dose range, with a half-life of 5–7 hours. Patients demonstrated consistent, dose-dependent thrombin generation ex-vivo in platelet poor plasma (mean peak effect 26–237 nM FII from 6.5–90 µg/kg). Peak thrombin levels over time paralleled the presence of BAY 86-6150 by PK analysis, indicating drug in circulation retained activity. There were corresponding decreases in activated partial thromboplastin time and prothrombin time.

Conclusions: The data safety monitoring board recommended progression to the highest proposed dose (90 µg/kg). Further safety and efficacy will be evaluated in Phase II/III studies.

Contribution to the practice/evidence base of hemophilia and bleeding disorders:

BAY 86-6150 is a novel rFVIIa agent with increased potency and a longer half-life designed to improve treatment of haemophilic patients with inhibitors.

Key Words: BAY 86-6150, rFVIIa, haemophilia

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