Current Progress with the Systemic Administration Trial of AVI-4658, a Novel Phosphorodiamidate Morpholino Oligomer (PMO) Skipping Dystrophin Exon 51 in Duchenne Muscular Dystrophy (DMD)

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Abstract

Objective: AVI BioPharma in collaboration with the MDEX consortium have identified a PMO to skip in DMD patients, restore reading frame and enable expression of dystrophin protein. Here, we test 6 PMO doses to select an effective, well tolerated dose for subsequent registration.

Method: Open label, dose escalation study in ambulant DMD boys aged 5-15 years with relevant deletions, of 12 weekly administrations of AVI-4658; 14 week follow up with muscle biopsy to assess dystrophin expression. Clinical efficacy (including 6 minute walk and North Star assessment), skeletal muscle, pulmonary and cardiac function is being assessed. Safety assessment includes adverse events, physical examinations and laboratory tests – including hematology, coagulation studies, chemistry and anti-dystrophin antibodies. A DSMB guided dose escalation decisions (across 6 doses: 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg).

Results: Study fully enrolled 19 patients by Dec 2009. All doses well tolerated (ongoing at 20mg/kg). No Drug Related SAEs or severe AEs reported so far. To date, maximum single dose is 900mg and cumulative PMO dose exceeds 8100mg (Table 1). Biopsies from first 4 cohorts showed exon 51 skipping at 2 and 4 mg/kg and 1 patient with 20% increase in number of dystrophin positive fibres.

Conclusion: Study drug well tolerated to date. Dosing and follow up continues on schedule. These preliminary data bode well for safe, long-term administration of AVI-4658 in DMD boys, and suggests clinically meaningful dystrophin expression can be expected following systemic administration. Preliminary, laboratory data from the remaining cohorts are due in 2Q 2010.

Methods::

The (now completed) trial evaluated the systemic delivery of AVI-4658 once per week for 12-weeks by slow intravenous infusion. The study includes measures of drug efficacy and pharmacokinetics. The clinical costs for the trial are provided, in part, by the UK Medical Research Council.

- 6 Cohorts Dose Escalation
  - 0.5, 1.0, 2.0, 4.0, 10.0 and 20.0 mg/kg IV
  - Weekly x 12 weeks
  - Open-label, no randomization
- Follow-up
  - First review and post Rx Biopsy at 13 wks (2 weeks after last dose)
  - Further F/U at weeks 18, 22 and 26

Trial entry criteria:

Inclusion

- An out of frame deletion(s) that could be corrected by skipping exon 51 [45-50; 47-50; 48-50; 49-50; 50; 52; 52-63], based on DNA sequencing data.
- Male ≥ 5 years and ≤ 15 years of age.
- Muscle biopsy analysis showing < 5% revertant fibers present.
- DNA sequencing of exon 51 confirms that no DNA polymorphisms occur that could compromise PMO duplex formation or there is confirmation of in vitro dystrophin production after exposure of fibroblast or myoblast to AVI-4658 cultures.
- Intact right and left biceps muscles or alternative arm muscle group.
- Ability to walk independently for at least 25 meters.
- Forced vital capacity (FVC) ≥ 50% of predicted
- No ventilatory support or supplemental oxygen required.

Exclusion:

- A DNA polymorphism within exon 51 that may compromise PMO duplex formation.
- Known antibodies to dystrophin.
- A calculated creatinine clearance < 70% of predicted normal for age based on the Cockroft and Gault Formula.
- A left ventricular ejection fraction (EF) of < 35% and/or fractional shortening < 25% based on echocardiogram (ECHO) during screening.
- A history of respiratory insufficiency as defined by a need for intermittent or continuous ventilatory support and/or supplemental oxygen.
- A severe cognitive dysfunction rendering the potential subject unable to understand and comply with the study protocol.
- Surgery within 3 months of entry or planned for anytime during the duration of the study.

Patient demographics, genotypes and cumulative exposure to AVI-4658 are shown in **Table 1**. Drug administration and dose escalation for cohorts is shown in **Figure 1.**

Figure 1. Study drug administration and dose escalation

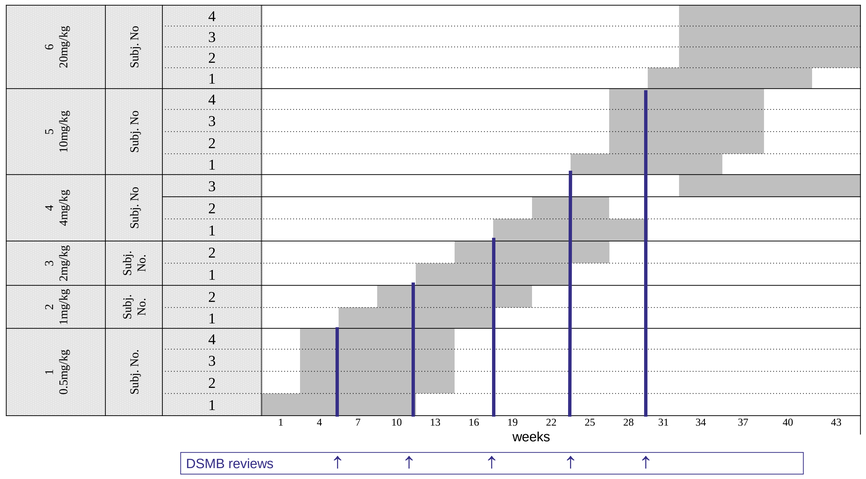

Table 1. Patient demographics, genotypes and exposure

Cohort	Subject No.	Age	Genotype	GCS	ACEi	Doses received	Cumulative dose (mg)
1	101	10	Del 48-50	Yes	Yes	12	186
1	102	9	Del 45-50	Yes	No	12	171
1	103	8	Del 49-50	Yes	No	12	218
1	104	8	Del 48-50	Yes	No	10 of 12	180
2	105	6	Del 45-50	Yes	No	12	326
2	106	6	Del 48-50	Yes	No	12	255
3	201	13	Del 49-50	Yes	No	12	1113
3	107	10	Del 49-50	Yes	No	12	924
4	108	10	Del 48-50	Yes	No	11 of 12	2801
4	202	10	Del 52	Yes	No*	7*	864
4	206	10	Del 45-50	Yes	No	12	1342
5	109	6	Del 49-50	Yes	No	12	3036
5	203	13	Del 47-50	Yes	No	12	6207
5	204	13	Del 49-50	Yes	No	12	4866
5	110	7	Del 48-50	Yes	No	12	2664
6	205	10	Del 49-50	Yes	Yes	12	10813
6	111	10	Del 45-50	Yes	No	12	6982
6	112	7	Del 45-50	Yes	No	12	5673
6	207	10	Del 45-50	Yes	TBC	12	6710

Results:

All 19 DMD patients who enrolled have now completed the initial 12 week dosing phase and have also now completed the observation follow up period. Dosing escalation is shown in **Figure 1**. In patients dosed with total exposures of up to 10,813mg of AVI-4658, no drug infusion related adverse events have been observed Adverse events tended to be mild or moderate, unrelated to treatment and resolved readily (**Table 2**). 2 Serious Adverse Events (SAEs) were reported, both unrelated to the drug: 1 post-operative nausea and vomiting; 1 ankle fracture; Both in 14 week follow-up. Subject 202 had treatment discontinued after 7 doses when his cardiomyopathy was noted to deteriorate, but he remained under observation and was stabilized on ACEi and β blockade

Results of immunohistochemical analysis of muscle biopsies taken 2 weeks after last dose were compared with samples taken pre treatment (and normal control) and are presented in **Figures 2 and 3**.

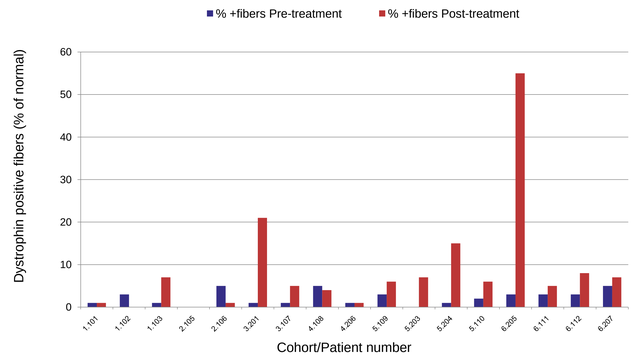

Figure 2. Dystrophin positive fibers comparing pre and post treatment muscle biopsies

Preliminary results for the biopsies obtained from all patients, revealed 3 patients (#201, 204 and 205 at 2.0, 10 and 20mg/kg respectively), with an increase in dystrophin expression exceeding 10%. In the case of #205 (20mg/kg), dystrophin positive fibers surpassed 50%. In addition exon skipping was consistently confirmed in all patients from cohort 3, 4, 5 and 6.

In addition, dystrophin intensity per fiber (compared to normal non-DMD intensity) and protein expression by Western blot were assessed (not reported here).

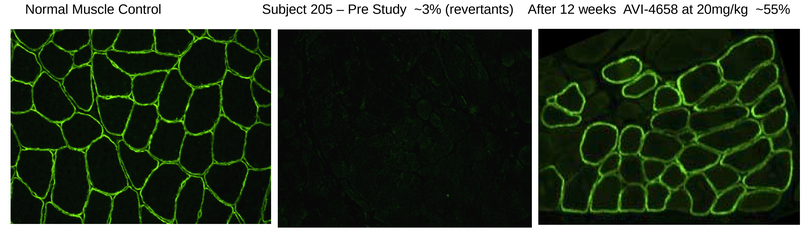

Figure 3. Immunohistochemical (Mandys 106 Antibody) comparing patient 205 pre and post treatment muscle biopsies to normal

Table 2. Adverse events (reported by last Safety Review)

Description of AE *	No. Subjects *	No. Events *
Headache	8	14
Rhinitis	4	5
URTI/viral	6	8
Abdominal pain	3	6
Hordeleum/Skin infection (tinea)	1	2
Osteopenia	1	1
Backache	1	1
Sunstroke	1	1
Insect bite/Sting	2	2
Muscle pain	4	5
Deteriorating cardiomyopathy**	1	1
Fatigue	3	3
Painful joint	1	1
Nausea/Vomiting	3	3
Fever	2	2
Dizziness	2	2
Pallor	1	1
Bruise	3	5

*At last safety meeting (Cohort 6 expansion): 23 November 2009

* *Previously, Subject 202 had several episodes of sinus tachycardia reported as AEs. A reassessment of pre-trial Echos has revealed evidence of cardiomyopathy in 2008. (not an exclusion criterion for this study) Fluctuation and elevation of cardiac troponin and decrease ejection fractional fractional shortening on Echo were new events.

Conclusions (tolerability, exon skipping and dystrophin expression)

Cohort	Patients Enrolled	Dose	No Severe Drug Related AEs	Dystrophin mRNA (Exon 51 Skipping)	Dystrophin Expression
1	4	0.5 mg/kg	✓	-	-
2	2	1.0 mg/kg	✓	-	-
3	2	2.0 mg/kg	✓	✓	✓
4	3	4.0 mg/kg	✓	✓	-
5	4	10.0 mg/kg	✓	✓	✓
6	4	20.0 mg/kg	✓	✓	✓

Conclusions (study)

- AVI-4658 well tolerated by IV administration
- No adverse safety signal
  - Max Single dose of 900mg
  - All 48 doses to 4 patients at 10mg/kg and all 48 doses to 4 patients at 20mg/kg well tolerated
  - Max Cumulative dose of 10,813mg
  - 220 infusions administered without any infusion associated reactions
- Exon skipping, RNA and dystrophin expression confirms “proof of concept”
- Further clinical studies are planned to determine a consistently effective dose