TRICYCLO-DNA
a new generation of antisense oligonucleotides

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Tc-DNA have been designed as a **CONFORMATIONALLY CONSTRAINED** oligonucleotide analogue. Chemically, tc-DNA deviates from natural DNA by three additional C-atoms between C(5’) and C(3’). These chemical modifications change the properties of natural oligodeoxynucleotides in the following way:

- Increased RNA **AFFINITY** by 2-4°C / modification;
- Increased **HYDROPHOBICITY**;
- Increased **STABILITY** towards nucleolytic degradation for both, phosphate or thiophosphate internucleoside linkages;
- Inability to elicit **RNaseH** activity.

Unlike LNA, fully modified tc-oligonucleotides in the **LENGTH RANGE OF 11-25** nucleotides can easily be prepared and produce potent antisense effects.

Due to their properties, tc-oligonucleotides are particularly suited for biological and **THERAPEUTIC APPLICATIONS** where high target affinity and biostability is required and where the mechanism of action does not rely on RNaseH activity.
TcDNA design for exon 23 skipping in mouse models of DMD

M23D (+2-13)

skipped mRNA
rescued dystrophin (quasi-dystrophin)
Comparison study between AON chemistries

- **2′O-methyl modified ribose with phosphorothioate backbone (2′OMe)**
- **Phosphorodiamidate morpholino (PMO)**
- **Tricyclo-DNA with phosphorothioate Backbone (tc-DNA)**

**Design** of the experiment.

- IV injection;
- 12 weeks of treatment;
- time after analysis: 2 weeks after the last injection;
- 4 mice in each group (18 wks old at analysis)

- High dose: 200 mg/kg/wk.
- Equimolar dose (15 µmol/kg/wk):
  - 80 mg (tc-DNA / 15-mer, 5363 g/mol)
  - 100 mg (2′OMe / 20-mer, 6880 g/mol)
  - 125 mg (PMO / 25-mer, 8413 g/mol)
- Low dose comparison: 50 mg/kg/wk.
- Very low dose comparison: 20 mg/kg/wk.

**Analysis** at the end of the treatment.

1) Exon skipping levels in various tissues
2) Dystrophin restoration (WB and immunostaining);
3) Serum over the treatment (CK, ALT, AST);
4) Muscle force;
5) Respiratory function;
6) Cardiac function;
7) Fear response.
Comparison study: Exon skipping and dystrophin levels

**High dose** (200 mg/kg/wk for 12 weeks)

- Exon skipping levels

  ![Exon skipping levels graph](image1)

- Dystrophin restoration

  ![Dystrophin restoration graph](image2)

- TcDNA induce significantly higher levels of exon skipping and dystrophin restoration in all tissues

**Equimolar** (15 µmole/kg/wk: 80, 100, 125 mg/kg)

![Exon skipping levels graph](image3)

![Dystrophin restoration graph](image4)

Goyenvalle et al., Nat Med, 2015
Comparison study: Exon skipping levels in CNS

• Taqman quantification of exon 23 skipping in Hippocampus, Cortex and Cerebellum from treated mice

• Detection of dystrophin in the cerebellum by WB and hippocampus by Immunostaining
Comparison study: functional rescue

- Evaluation of muscle function in situ (200 mg/kg/wk – 12 weeks)

- DOSE-ESCALATION STUDY of tc-DNA M23D in mdx.

- TcDNA treatment improves muscle function in mdx mice
Respiratory function

- Evaluation of respiratory function using whole body PLETHYSMOGRAPHY

- Evaluation of the respiratory function in C57B10 and mdx mice (18 weeks of age)

<table>
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<th>PIF (ml/s)</th>
<th>PEF (ml/s)</th>
<th>EEP (ms)</th>
<th>TV/BW (ml/g)</th>
<th>EV/BW (ml/g)</th>
<th>MV (ml/min)</th>
<th>BR (bpm)</th>
<th>(TV/BW)/Ti</th>
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<td>1. E-05</td>
<td>1. E-04</td>
<td>0.86</td>
<td>1.6 E-05</td>
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T-test results indicate significance at p < 0.05.
Comparison study: Respiratory function

- Evaluation of the respiratory function in mdx mice (High dose 200 mg/kg/wk – 12 weeks)

- TcDNA treatment improves respiratory function in mdx mice
Cardiac function

- Evaluation of the cardiac function in **mdx** mice (80 and 200 mg/kg/wk – 12 weeks)

- TcDNA treatment improves cardiac function in mdx mice
Correction of emotional responses in mdx

- Dystrophin (Dp427) is found in Purkinje cells of the CEREBELLUM, and in pyramidal neurons of the HIPPOCAMPUS and AMYGDALA.

  ![Wild type (CA1 hippocampus)](image1) ![mdx](image2)

CA1 pyramidal neurons are thought to be critical in long-term memory; Amygdala is involved in attention to and perception and memory of dangerous situations (fear & stress).

- Dystrophin (Dp427) is localized at the level of INHIBITORY SYNAPSES where it is required to recruit GABA_A-Receptors and power synaptic plasticity.

  (Vaillend et al., Mol Ther, 2010. Rescue of a dystrophin-like protein by exon skipping in vivo restores GABAA-receptor clustering in the hippocampus of the mdx mouse)
  (Dallerac et al., Neurobiol Dis, 2011. Rescue of a dystrophin-like protein by exon skipping normalizes synaptic plasticity in the hippocampus of the mdx mouse)

It has been reported that defensive behaviour, in RESPONSE TO DANGER or a threat, is enhanced in mdx. Dystrophin-deficient mice consistently showed POTENT DEFENSIVE FREEZING RESPONSES to a brief restraint that never induced such responses in wild-type mice. This is thought to be due to an alteration of AMYGDALA local inhibitory neuronal circuits which enhance fear-motivated defensive behaviors.

  (Sekiguchi et al., Brain, 2009. A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behaviour in mice)
Quantification of the freezing response

BEHAVIORAL in mdx mice treated with AONs (Equimolar & High dose – 12 weeks)
Summary on TcDNA Efficacy

- Superior efficiency of TcDNA compared to other naked chemistries

- Efficient and widespread restoration of dystrophin in mdx and dKO mouse models

- TcDNA target the respiratory and cardiac muscles efficiently

- TcDNA induce exon skipping in the CNS -> ability to cross the blood brain barrier in adult mice

- Great therapeutic potential in the systemic treatment of DMD
CONCLUSIONS AND ON GOING WORK
PRE-CLINICAL DEVELOPMENT OF TcDNA FOR THE TREATMENT OF NEUROMUSCULAR DISORDERS

Safe toxicologic profile of TcDNA
- TcDNA treatment is well tolerated in all treated mice
- No significant changes in serum biochemistry (except creatinine)
- No significant changes in urinary total protein, creatinine and albumin
- No major histopathological findings
- Only specific early biomarkers of kidney toxicity are elevated

Development and validation of human specific TcDNA sequences for clinical application
- In vitro evaluation in human myoblasts (wt and appropriate DMD genotypes)
- In vivo validation of selected TcDNA in hDMD mice
- Evaluation of efficacy in mdx52 mice
- Tox profile of specific human sequences (Collab CitoxLab, France)

Evaluation of new generations of TcDNA oligonucleotides in mdx mice
- Test various modifications to the tcDNA backbone
- Evaluate tox profiles in mdx and WT mice
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