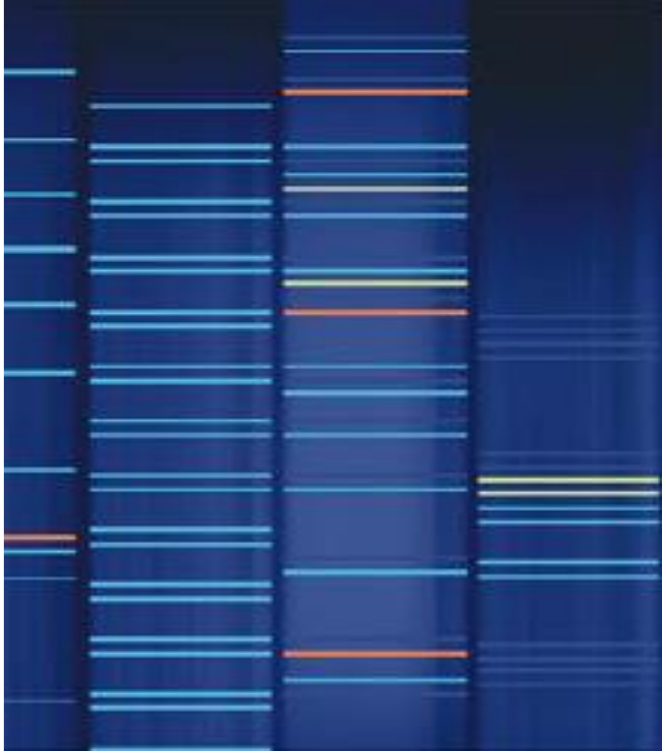


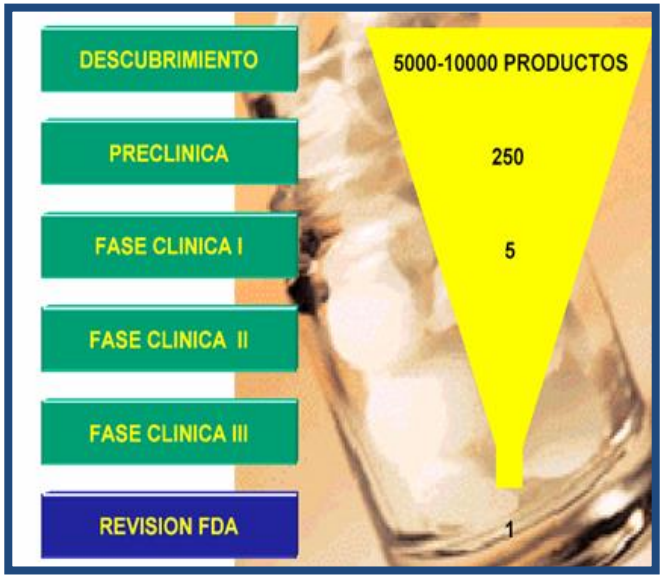
VALENTIA BIOPHARMA

A Drosophila high-throughput drug screening platform identifies inhibitors of misregulated alternative splicing events in myotonic dystrophy

Irma Garcia-Alcover

www.valentiabiopharma.com

Consequences of traditional HTS drug discovery



"Poor selection of hits with limited predictive value for clinical outcome"

"Slow-down in the development of new and innovative medical products"

"Waste of funds (millions of $) and efforts (years of research)"

VLT BIOPHARMA

Weakness of hits from traditional HTS drug discovery

- Unpredicted toxicity
- Off-target interactions leading to undesirable side effects
- Therapeutic effects not translating to traditional mammalian models and humans in the clinic (i.e. neuromuscular diseases) when cell-organ physiological connections critical in development of disease
- Time component of disease progression not recapitulated

VLT BIOPHARMA

Drug discovery problems

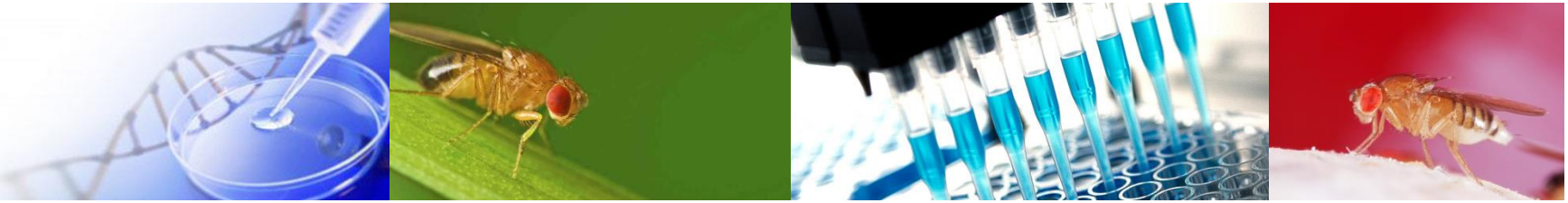
Traditional drug development

- Between 12 and 15 years
- Investment ~800 millions $
- 80% of cost in preclinical assays

Model organisms

Toxicity and activity evaluation in the same experimental approach: <u>entire organism</u>

VLT BIOPHARMA



in vivo drug discovery benefits

"shift from one disease-one target to multifactorial disease paradigm: drug discovery in whole animals"

VLT BIOPHARMA

in vivo drug screening

- **Information of the therapeutic effect of a compound in a complete organism**

- **Several advantages vs *in vitro***

  Compounds identified by positive screening have two main characteristics:

  - **Ameillorate a phenotype that mimics hallmarks of the human disease**

  - **Their pharmacological properties are compatible with life**

Toxicity and activity evaluation in the same experimental approach: <u>entire organism</u>

VLT BIOPHARMA

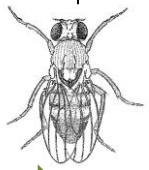
Process & output

Drosophila

Vector design

Transformation

Confirmation



start R&D

3-6 months, HTS format, and low cost

Disease model development time

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

1st year

2nd year

>1,5 years, no HTS format, and high cost

Vector design

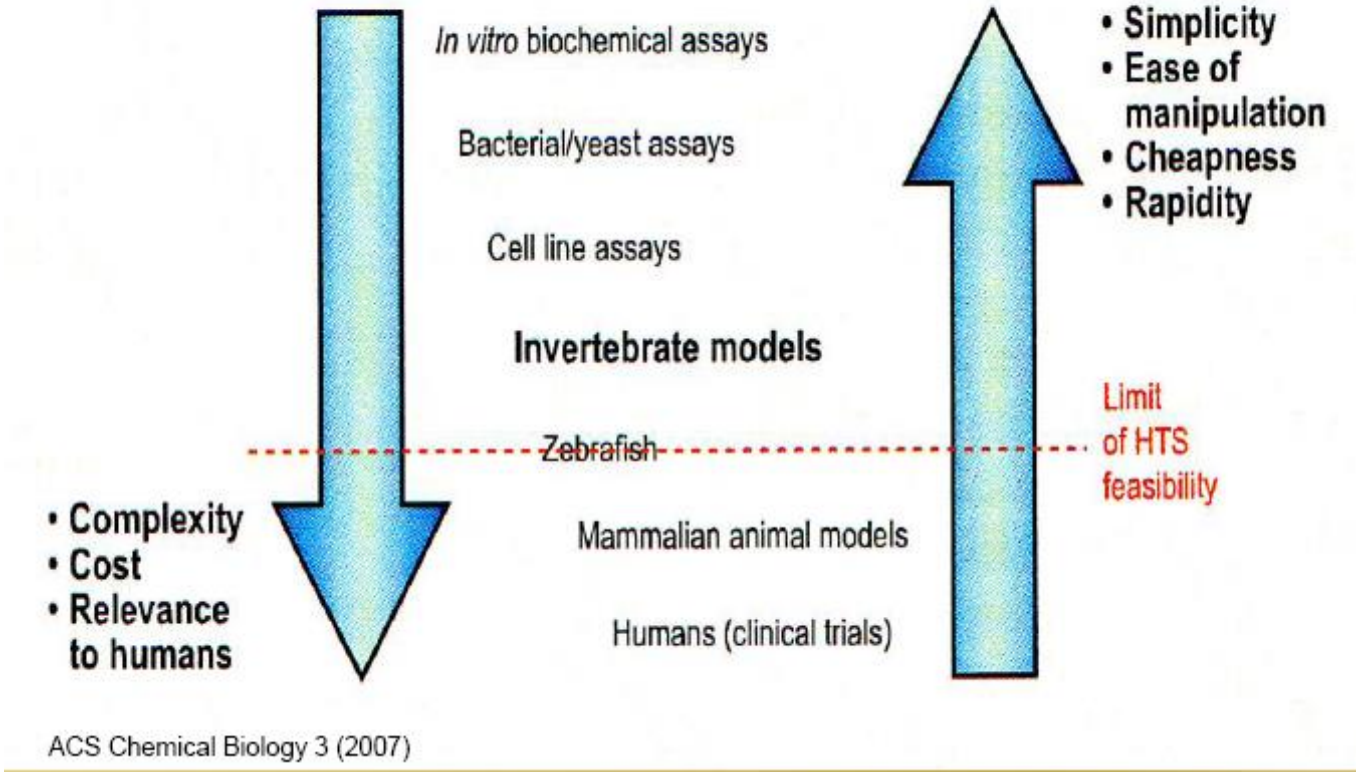
Transformation

Confirmation

Mouse

VLT BIOPHARMA

In vivo Drug Discovery



ACS Chemical Biology 3 (2007)

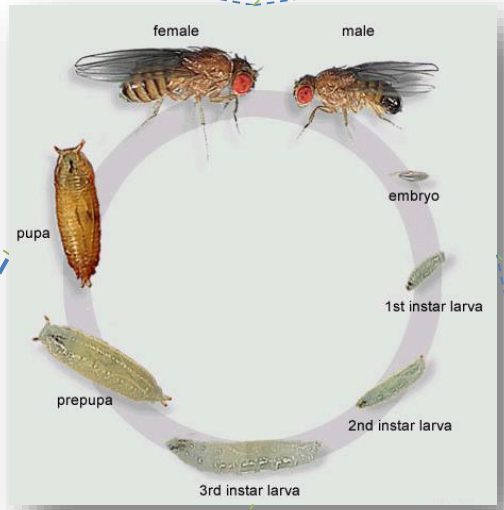
VLT BIOPHARMA

75% of human genes causing disease are conserved in *Drosophila*

Culture conditions compatible with large-scale screens.

No ethical issues.

Short time frame from drug dosing to results



Large, sophisticated number of genetic tools/fly stocks available

Identification of new drugs in WHOLE organism

Fundamental aspects of cell biology conserved from *Drosophila to humans*

Low variability at low costs (Flies are CHEAP)

VLT BIOPHARMA

Toxicity and activity evaluation in the same experimental approach: entire organism

Short time frame from drug dosing to results
Large number of genetic tools available
Low variability at low costs

VALENTIA BIOPHARMA

Examples of diseases modeled into the fly:

(reviewed in Pandey and Nichols, Pharmacol. Rev. (62) 2:A-Z, 2011)

Diseases Afecting CNS

- Alzheimer
- Parkingson
- Hungtington Disease
- Epilepsy
- Cognitive and affective disorders as depression

Inflammatory diseases

- Asthma
- Inflammatory mechanism related with inflammatory bowel disease and necrotizing enterocolitis
- Related pathways – Toll and toll-like receptos, nuclear factor kB, TNF and JAK/STAT

Metabolic diseases

- Obesity
- Diabetes
- Glycerol kinase deficency

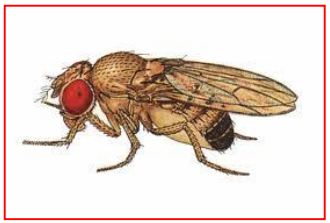
VLT BIOPHARMA

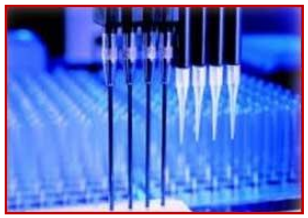
How Valentia (VLT) flies can help

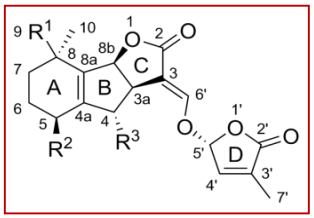
- VLT can build custom “humanized” *Drosophila* disease models and evaluate compound activity
- After VLT modeling (2-3 months), results in weeks not months/years
- Study large numbers of functional pathways, risk genes and human mutations
- Provide fast, simple assays (phenotypic and biochemical/molecular) for drugs response
- Test chemical libraries on a whole organism, estimating drugs response in the same way that human do

VLT BIOPHARMA

Drug Discovery







Drosophila model generation and validation → **Drug screening** → **Hits validation**

4-6 months | 1000 comp./week | 1-6 months

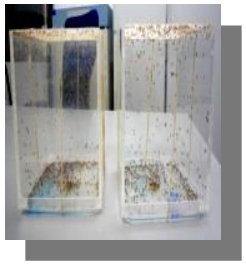
- **Target identification**
- **Readout selection**
- **Model generation**
- **Model validation**

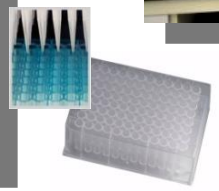
- **Robustness of the assay statistically validated**
- **Throughput:1000 compounds/week**

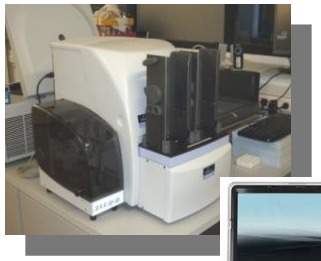
- **Additional Drosophila assays**
- **Validation studies in other biological systems (mice, human cells)**

VLT BIOPHARMA

In vivo drug *screening* platform







1. FLY CROSSES
2. DRUG PLATES PREPARATION (Robot with stackers)
3. SEEDING (Sorter Cytometry)
4. HOMOGENIZATION (Robot with stackers)
5. READING (Envision Reader /Scanner)
6. ANALYSIS

F0: Adults

F1: Embryo/Larvae

F1: Adults

VLT BIOPHARMA

A case study: Myotonic Distrophy type 1 (DM1)

VLT BIOPHARMA

www.valentiabiopharma.com

Home | About Us › | Technology › | Drug Pipeline › | Investors › | News | C

VLT Biopharma gets the Orphan Drug designation for VLT015

25th Feb, 2014

by VLT Biopharma

1

VLT Biopharma gets the Orphan Drug designation for VLT015, a compound for the treatment of Myotonic Dystrophy

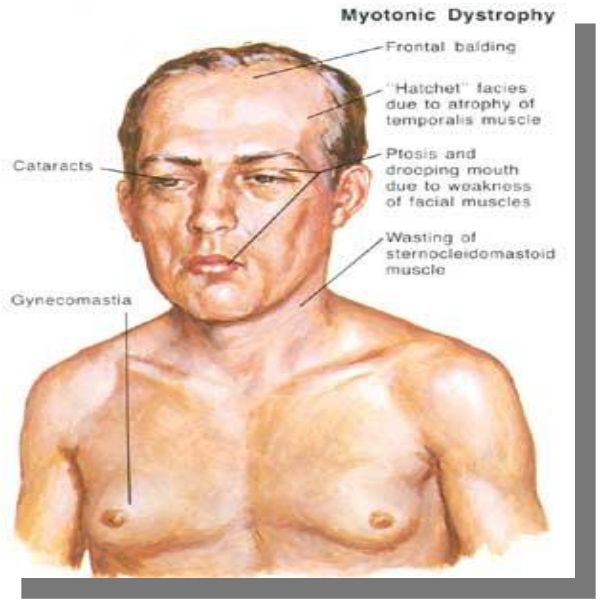
Valencia, February 2014. Valentia Biopharma announced today that the European Commission has granted Orphan Drug Designation to VLT015 for the treatment of Myotonic Dystrophy type 1 (DM1) and type 2 (DM2) after a positive opinion of the Committee for Orphan Medicinal Products (COMP) from the European Medicinal Agency (EMA)

Myotonic Dystrophy is a rare inherited muscle disorder…

VLT BIOPHARMA

Myotonic Dystrophy type 1

•Overall worldwide prevalence: 1 / 8,000. Higher in some populations like in Quebec (Canada). High penetrance.



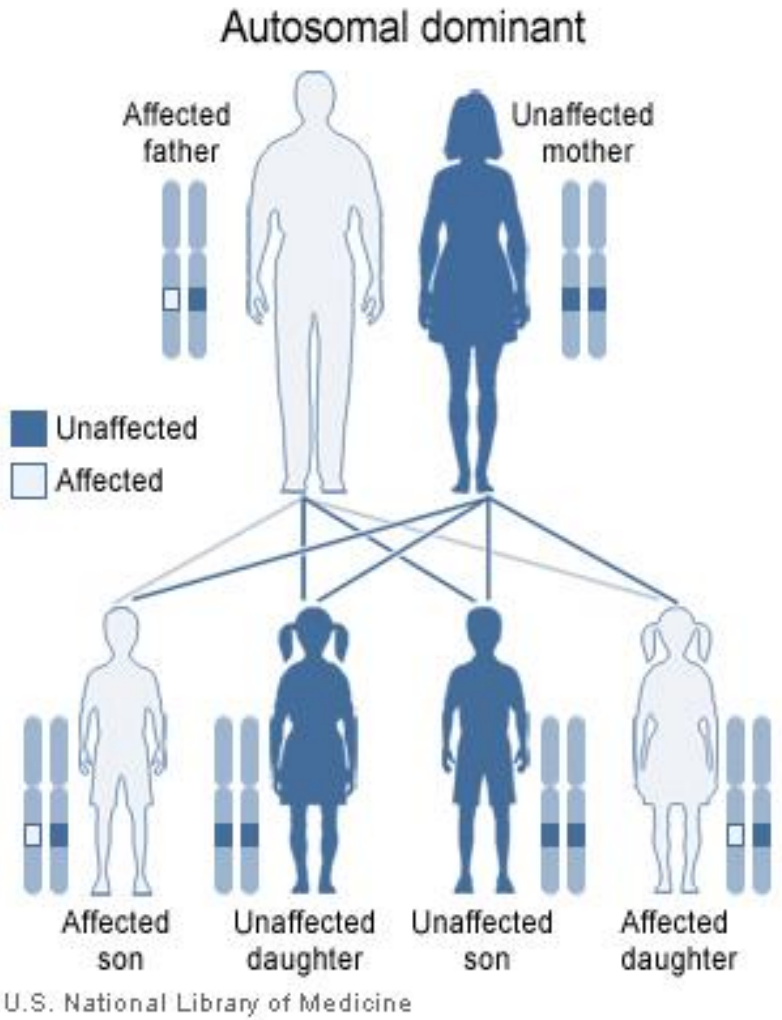
•Multifactorial disease. Mainly a muscular disorder: Myotonia, progressive muscular wasting and weakness.

•But also cataracts, hypogonadism, arrythmias, infertility, cognitive dysfunction, mental retardation…

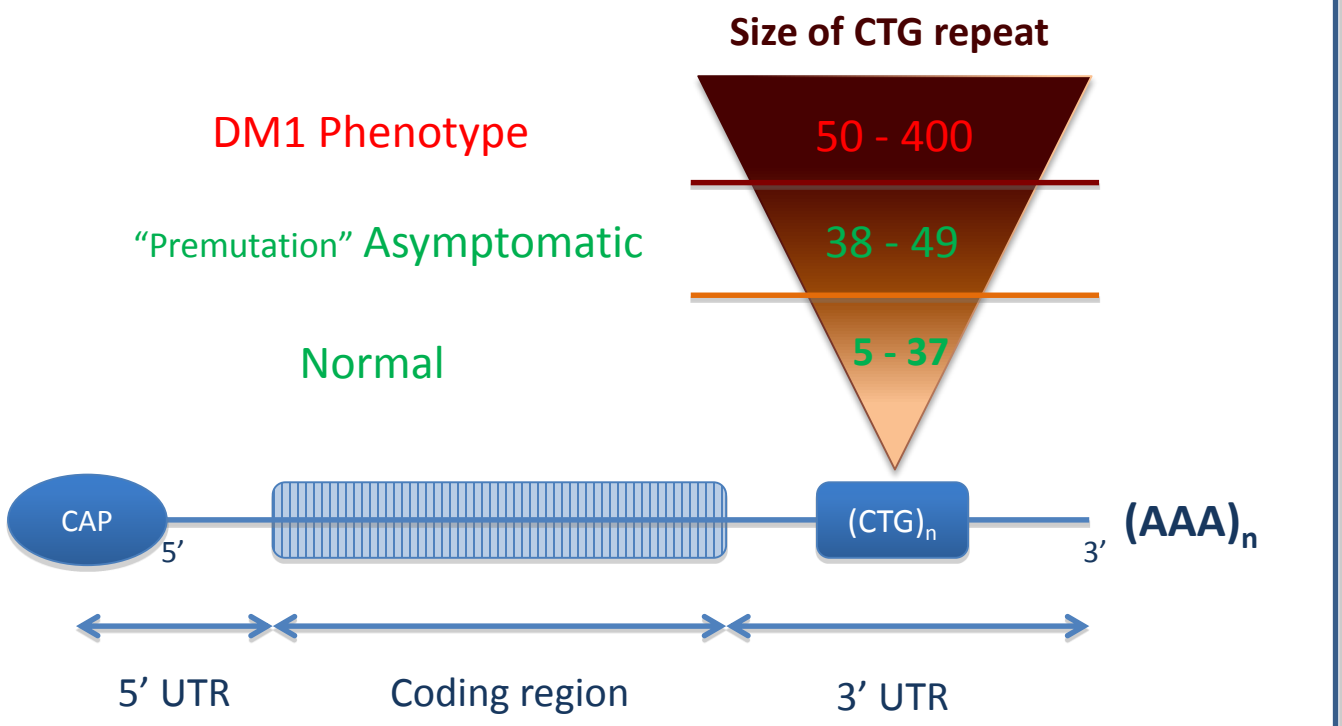
Source:http://omim.org/entry/160900

VLT BIOPHARMA

Myotonic Dystrophy type 1 inheritance



Molecular cause



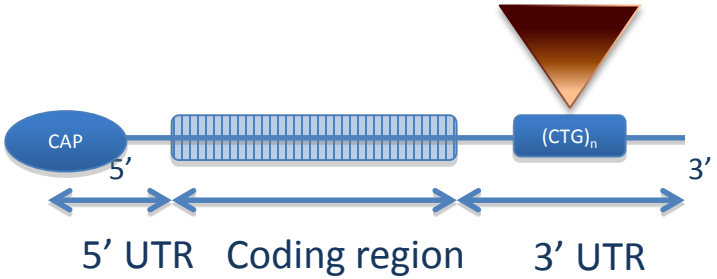
Myotonic Dystrophy Mutation: An Unstable CTG Repeat in the 3′ Untranslated Region of the Gene

MANI MAHADEVAN, CATHERINE TSILFIDIS, LUC SABOURIN, GARY SHUTLER, CHRIS AMEMIYA, GERT JANSEN, CATHERINE NEVILLE, MONICA NARANG, JUANA BARCELÓ, KIM O'HOY, SUZANNE LEBLOND, JANE EARLE-MACDONALD, PIETER J. DE JONG, BÉ WIERINGA, ROBERT G. KORNELUK*

***Science*, 1992**

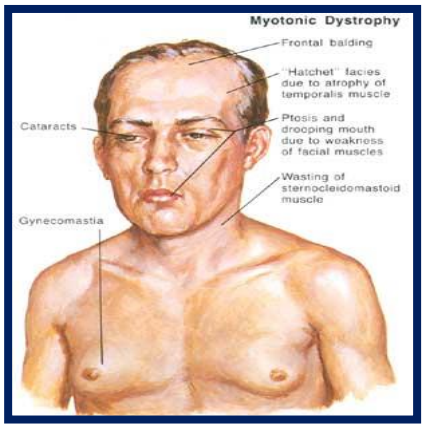
VLT BIOPHARMA

But is a non-coding DNA expansion…



DNA expansion

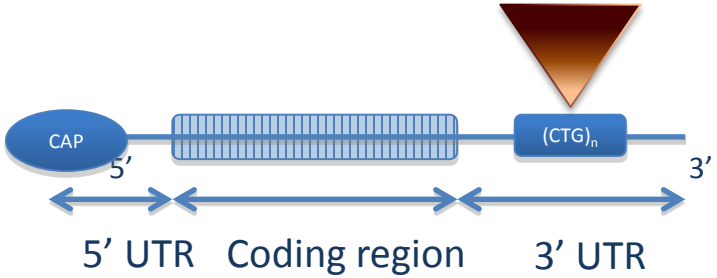
???

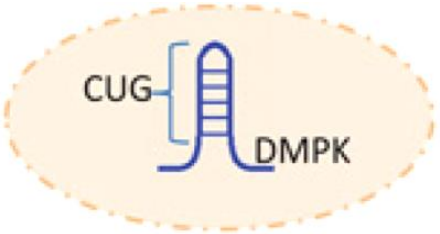


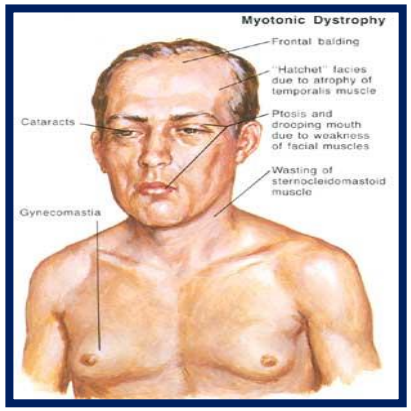
Disease symptoms

VLT BIOPHARMA

But is a non-coding DNA expansion…







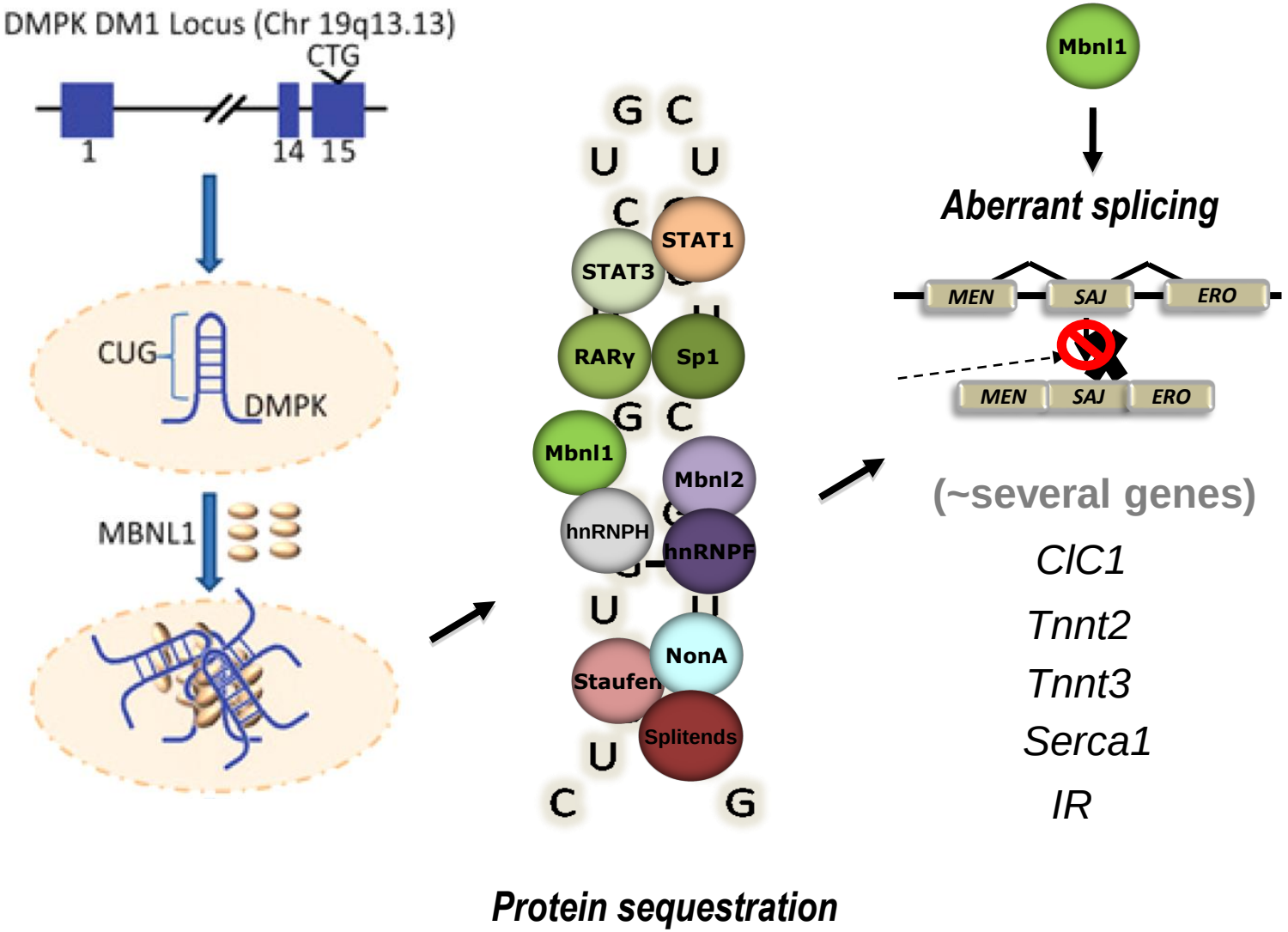
DNA expansion

RNA gain of function
(toxic RNA formation)

Disease symptoms

VLT BIOPHARMA

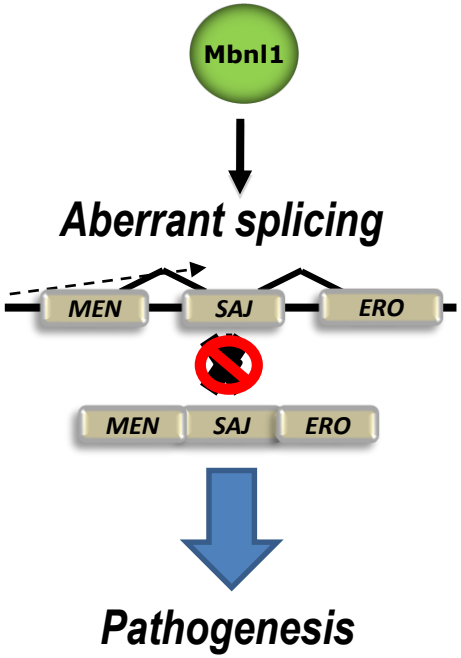
Molecular pathogenesis

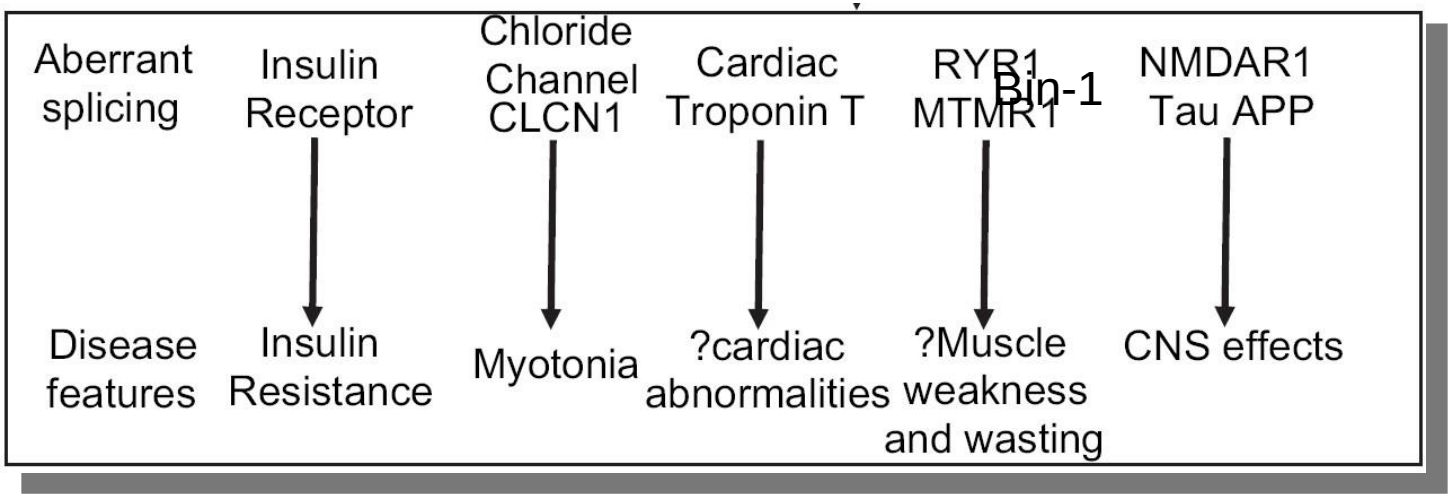


From Mastroyiannopoulos et al. Biol Cell 2010

VLT BIOPHARMA

Molecular pathogenesis

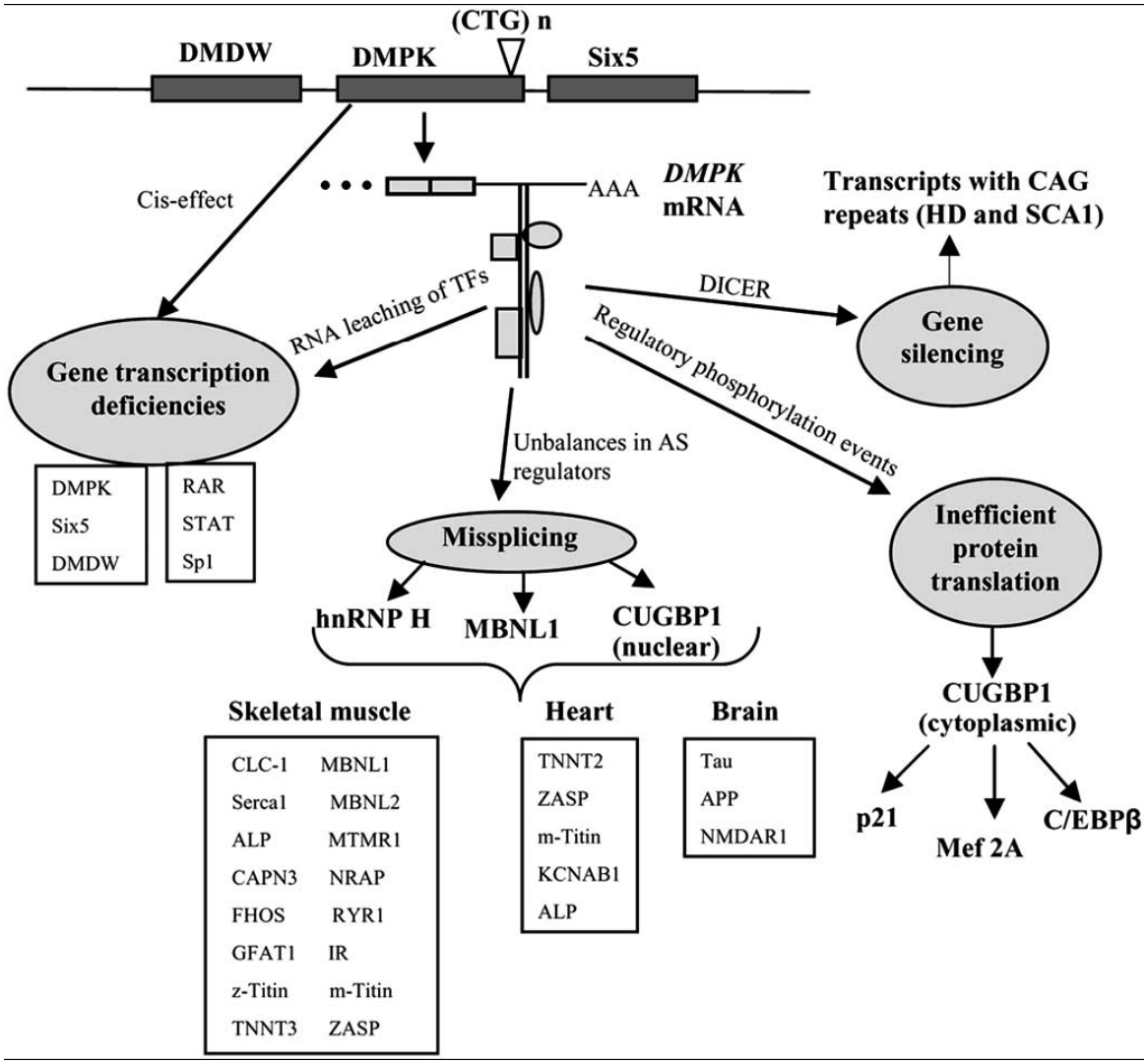




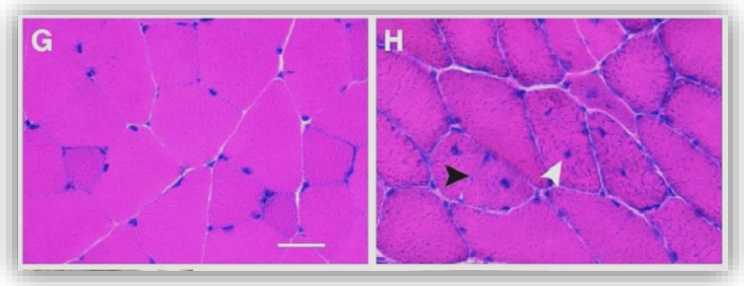
From Turner and Hilton-Jones (2010). J Neurol Neurosurg Psychiatry 81(4):358-67

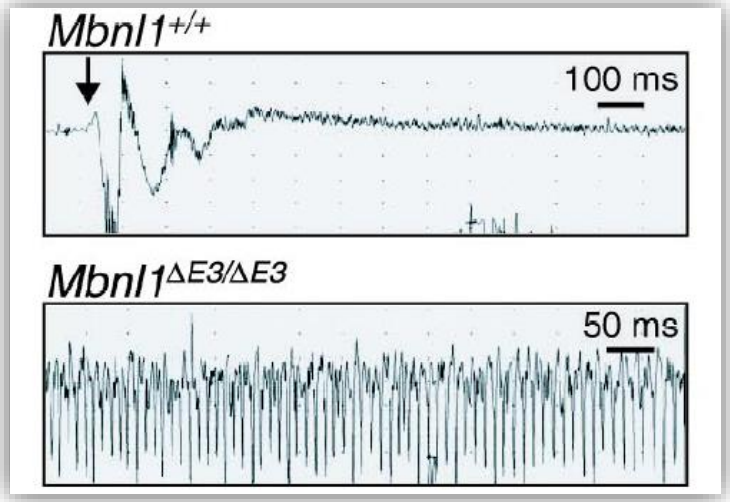
VLT BIOPHARMA

DM1 is a very complex disease



Mbnl1 importance in DM1





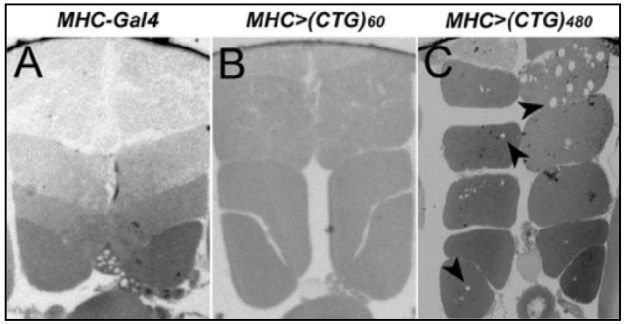
Kanadia et al., 2003

	***Mbnl1*$^{-/-}$**	**DM1 patients**
Clcn1	+	+
Sercal	+	+
Tnnt3	+	+
ZASP	+	+
z-Ttn	+	+
z-Ttn	+	+
m-Ttn	+	+
Capn3	+	+
Alp	+	+
Fhos	+	+
Gfat1	+	+
Mbnl1	+	+
Mbnl2	+	+

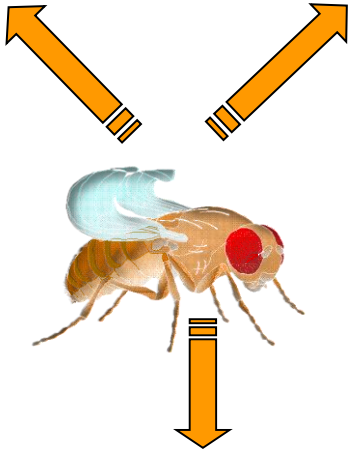
VLT BIOPHARMA

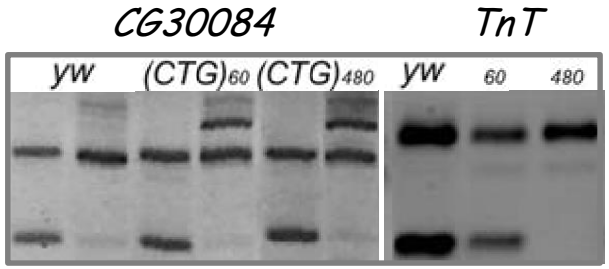
A DM1 fly model

$(CTG)_{480}$ repeticiones

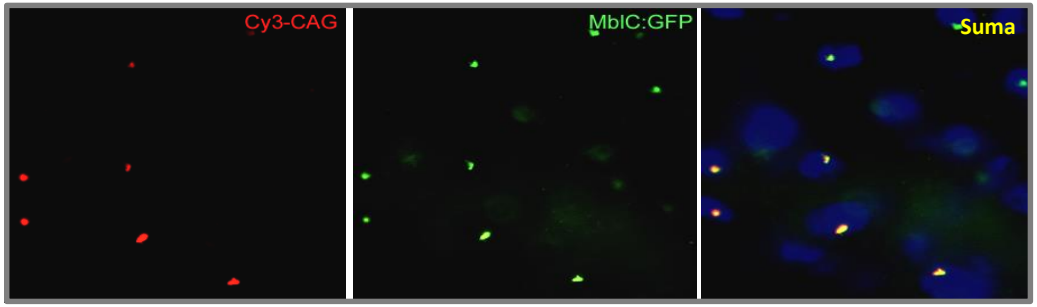


Defectos histológicos





Defectos de splicing

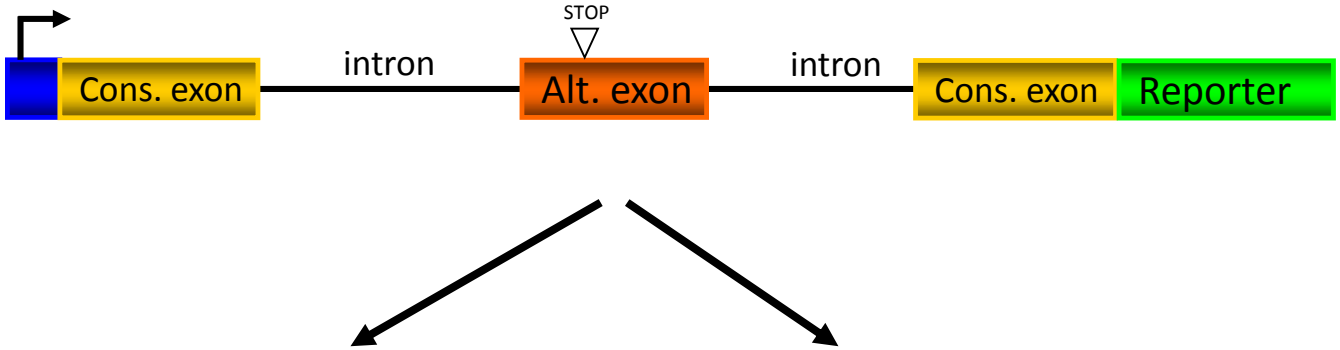


Secuestro de Mbl en forma de foci

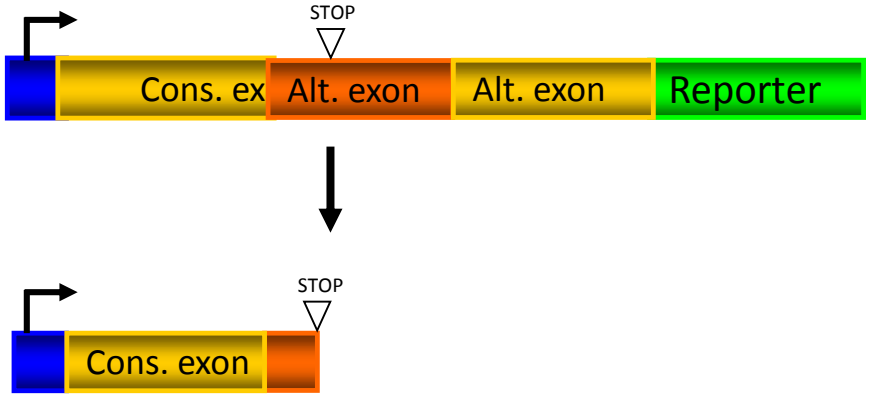
Garcia-Lopez y Monferrer et al., 2008

VLT BIOPHARMA

Human minigen-reporter construct

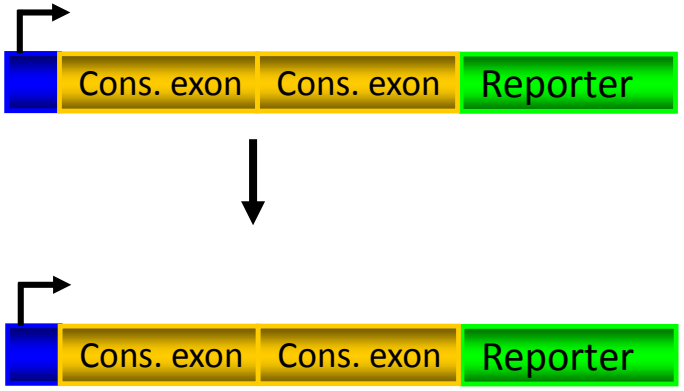


Exon inclusion



No reporter expression

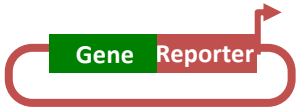
Exon exclusion

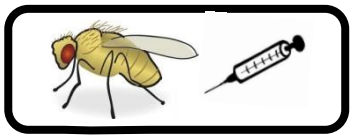


Reporter expression

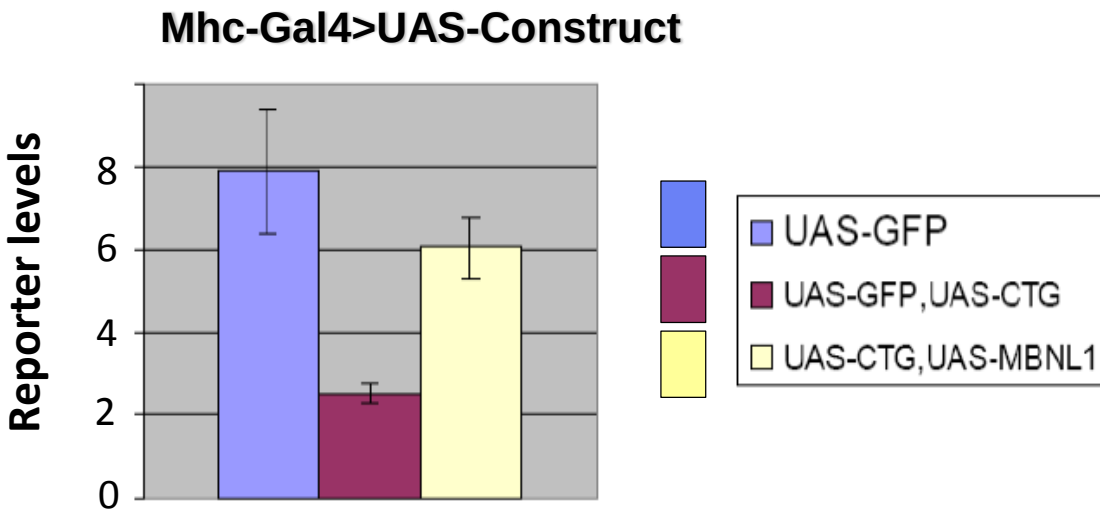
VLT BIOPHARMA

Spliceosensor flies

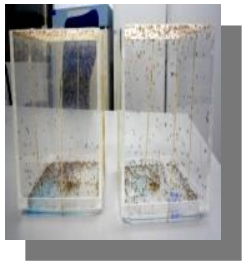


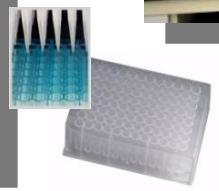


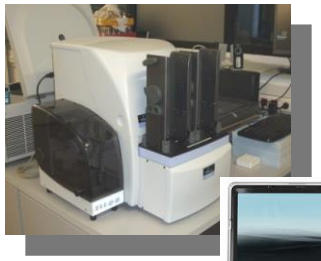
CUG toxic RNA



In vivo drug *screening* platform







1. FLY CROSSES
2. DRUG PLATES PREPARATION (Robot with stackers)
3. SEEDING (Sorter Cytometry)
4. HOMOGENIZATION (Robot with stackers)
5. READING (Envision Reader /Scanner)
6. ANALYSIS

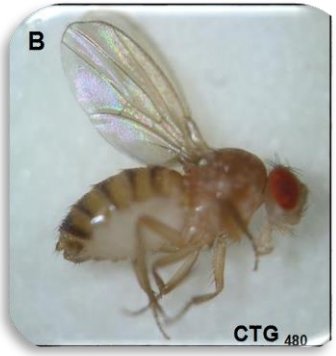
F0: Adults

F1: Embryo/Larvae

F1: Adults

VLT BIOPHARMA

Rastreo de compuestos



Modelo DM1

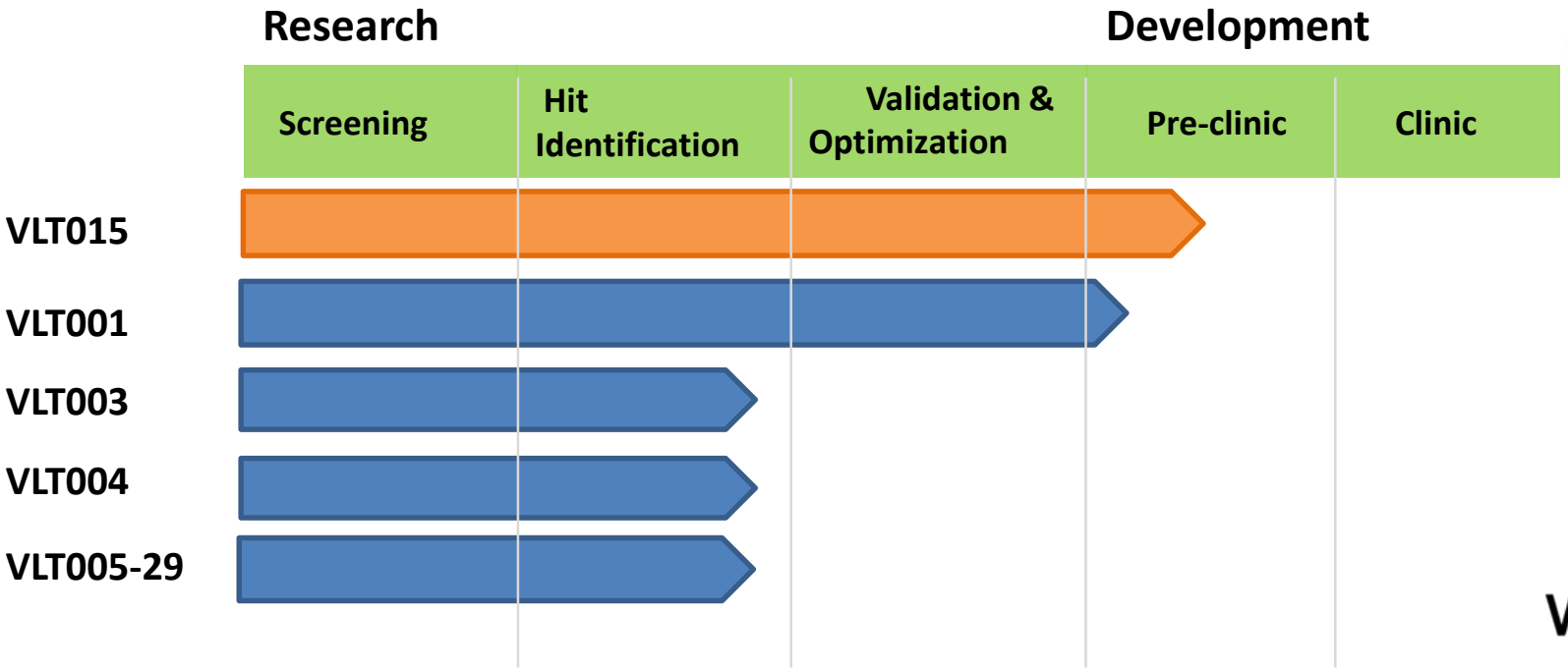
> 15,000 small molecules

screening analysis and validation

30-35 hits

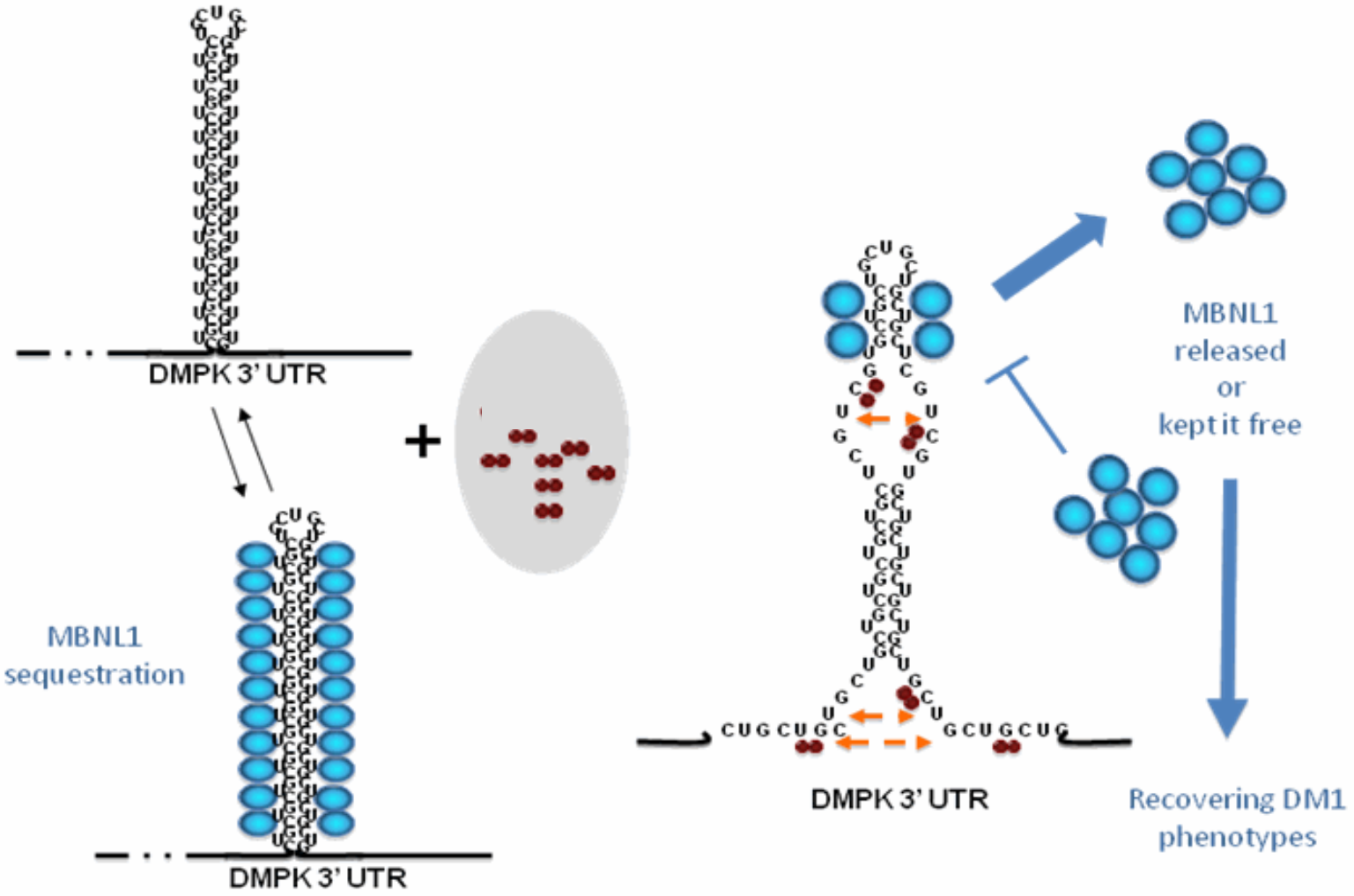
Secondary assays validation

1 lead (VLT015)



VLT BIOPHARMA

Mechanism of action



Hairpin destructuration and MBNL1 release

Examples of diseases modeled into the fly:

(reviewed in Pandey and Nichols, Pharmacol. Rev. (62) 2:A-Z, 2011)

Diseases Afecting CNS

- Alzheimer
- Parkingson
- Hungtington Disease
- Epilepsy
- Cognitive and affective disorders as depression

Inflammatory diseases

- Asthma
- Inflammatory mechanism related with inflammatory bowel disease and necrotizing enterocolitis
- Related pathways – Toll and toll-like receptos, nuclear factor kB, TNF and JAK/STAT

Metabolic diseases

- Obesity
- Diabetes
- Glycerol kinase deficency

"Models mostly available for *in vivo* HTS approaches"

VLT BIOPHARMA

THANKS FOR YOUR TIME

Aknowledgements:

VLT staff
(Scientific Park of Valencia)
Arturo López-Castel
Dr. Jordi Colonques
Juanma Fernández-Costa
Mari Carmen Álvarez
Isabel Campillo
Aida Tomás
José Rubén Tormo

Department of Pharmacology
(University of Valencia)
Dr. Diego Cortés
Laura Moreno

Department of Genetics
(University of Valencia)
Dr. Rubén Artero
Dr. Amparo García-López

irma.garcia@valentiabiopharma.com

IOPHARMA