Chardon Pharma

Chardon Pharma is a company associated with the Radboud University Nijmegen (The Netherlands).

Chardon's scientists have an extensive experience within big Pharma (Organon, Schering Plough, Merck).

Chardon Pharma performs high quality proof of concept (efficacy) studies.

Chardon Pharma combines a number of activities:

1. We scout for new ideas that require proof of concept studies before they can be commercialized *(scouting)*.
2. We invest intellectually in these ideas by bringing in our scientific and drug development expertise *(consultancy)*.
3. We invest in these ideas financially *(investment)*.
4. We perform the proof of concept studies (preclinical, phase I, phase IIA) *(CRO)*.
5. We write and file patents for the ideas *(patent application)*.
6. We bring the inventor in contact with an industrial partner *(match making)*.

The proof of concept studies of Chardon Pharma are either animal studies (zebrafish, rodents) or small human studies (phase I, healthy volunteers or phase IIA, pilot efficacy with biomarkers in patients).

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Zebrafish research

Chardon Pharma has a strategic partnership with Noldus IT (Wageningen, The Netherlands) in the area of zebrafish *(Danio rerio)* research.

Chardon Pharma has a full permit to perform animal experiments, with both wild-type and genetically modified organisms (GMO).

Chardon Pharma has a fully equipped zebrafish facility. This includes highly specialized laboratory equipment and housing and breeding systems.

Chardon Pharma can:

- Genetically modify zebrafish (micro-injections).
- Perform behavioural and physiological studies in zebrafish larvae (eg. cardiovascular, pharmacological and toxicological analyses).
- Perform behavioural and physiological studies in adult zebrafish (eg. cardiovascular, pharmacological and toxicological analyses).

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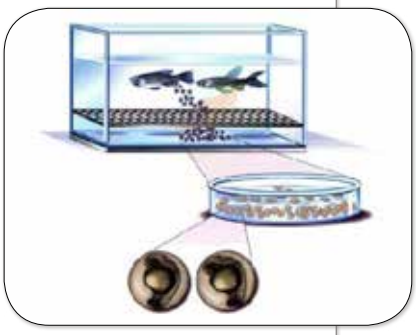
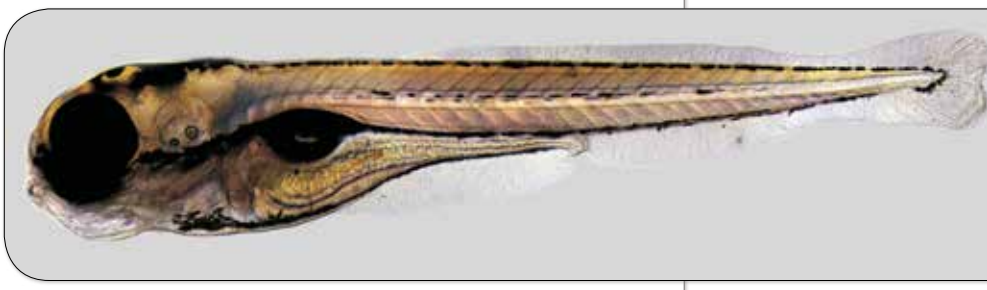
Why Zebrafish (Danio rerio)?

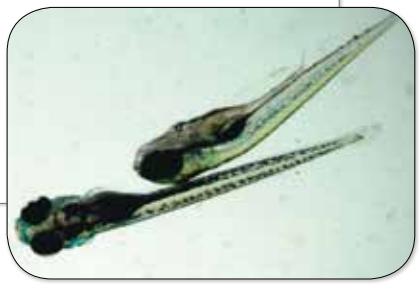
- Small, robust freshwater fish
- Easy to maintain, high fecundity (200 eggs / female / week)
- Several strains available
- Wild-type and genetically modified fish available



- Most organs fully functional between 3 – 5 dpf.
- Larvae are transparent
- Easy to handle
- Fit for high or medium throughput assays



- Genome fully sequenced
- Genome, genetic pathways and development highly conserved between zebrafish and humans
- Easy genetic manipulation
- Large behavioural repertoire
- **Low costs and timelines**



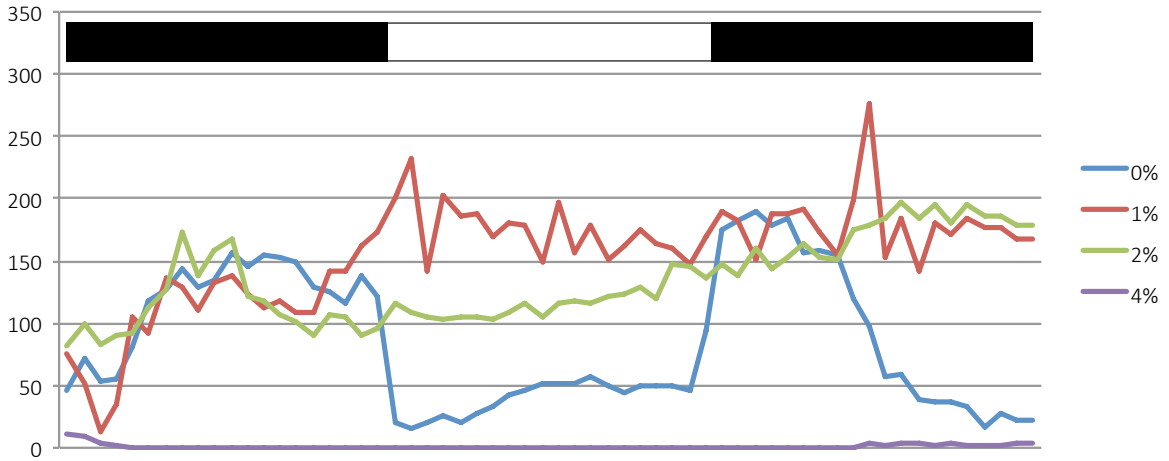
High throughput drug screening in Zebrafish larvae

Using Danio vision equipment and Ethovision software (both from Noldus IT, Wageningen, The Netherlands) the behaviour of 96 zebrafish larvae can be recorded simultaneously.

This technique is very powerful for high throughput drug screening in whole animals in vivo.

- Light intensity and water temperature can be varied
- Both light and acoustic startle stimuli can be applied

Example of results



Effects of ethanol at different concentrations on the spontaneous behaviour (mm moved per min) of 5 day old zebrafish larvae. Two dark periods (of 20 min, black bar) were separated by a light period (of 20 min). Per group 12 larvae were treated.

A database of reference compounds is available for comparisons.

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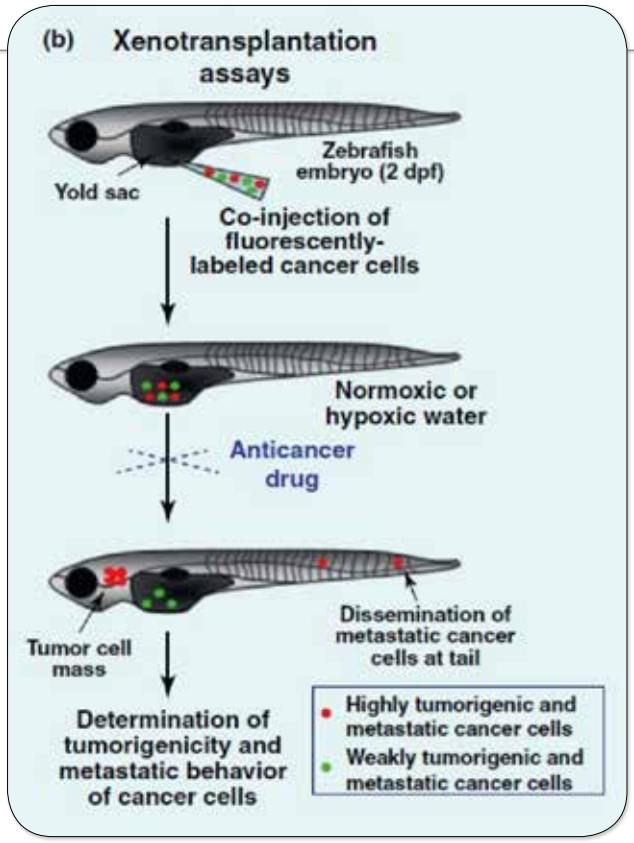
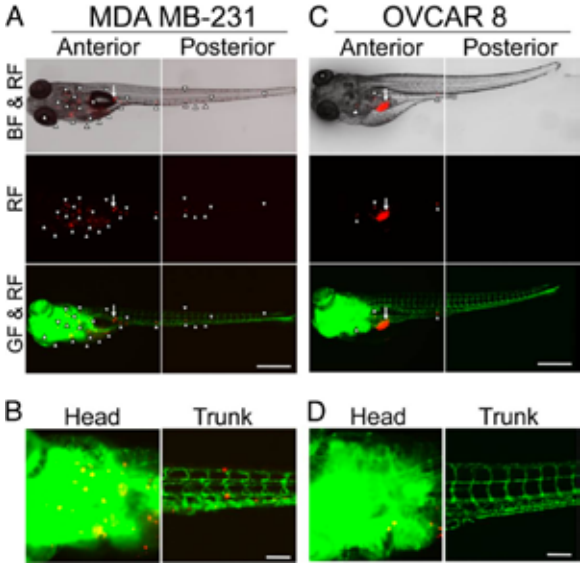
Oncology studies in Zebrafish larvae

The immune system of zebrafish matures after day 11 post fertilization.

Tumor cells that are injected before day 11 are not rejected.



Mimeault & Batra, 2013



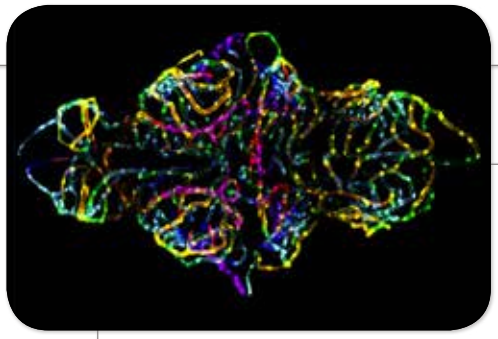
Both tumor growth and metastases can be measured; and can be manipulated by experimental treatments.

Of special interest for this purpose is the transparent Casper strain.

Medium throughput oncology studies are running in the area of breast cancer, melanoma and glioma.

Investigating the passage of drugs through the blood-brain-barrier in Zebrafish larvae

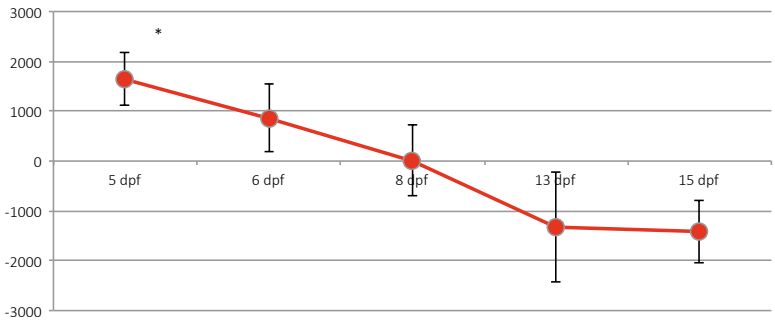
The blood-brain-barrier of zebrafish closes around day 10 post fertilisation (pf). This makes zebrafish larvae a very suitable model to identify central and peripheral effects of drugs.



By comparing drug effects in 5 and 15 day old larvae, the contribution of central effects in the overall activity can be estimated.

An example:

Behavioural activity (mm /min), relative to placebo scores, of methylphenidate (5µM) in zebrafish larvae of 5, 6, 8, 13 and 15 days old (N=16 per group).



On day 5 (pf) both central and peripheral effects are observed (resulting in behavioural activation). On day 15 (pf) the central component is reduced and effects are mainly caused by peripheral actions (resulting in behavioural inhibition)

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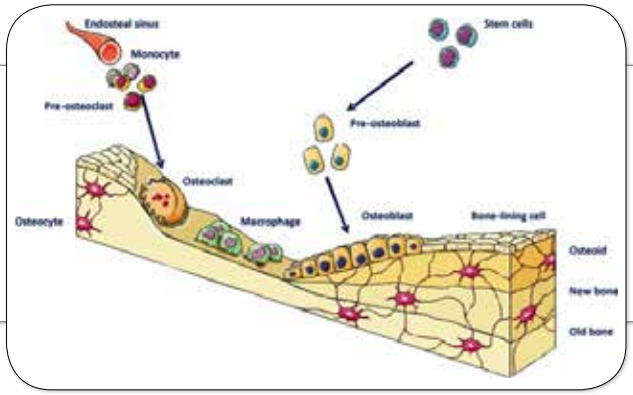
Osteoporosis research in Zebrafish scales



Osteoporosis (loss of bone mass) is caused by too little bone formation (osteoblast activity) or a too high bone resorption (osteoclast activity).

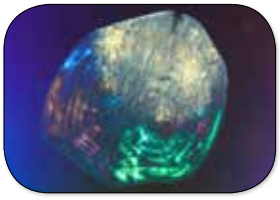
Most new osteoporosis treatments are based on osteoblast stimulation.

Screening of new osteoporosis treatments is performed in human osteoblast cultures (but this is not a dynamic system with bone formation and resorption).

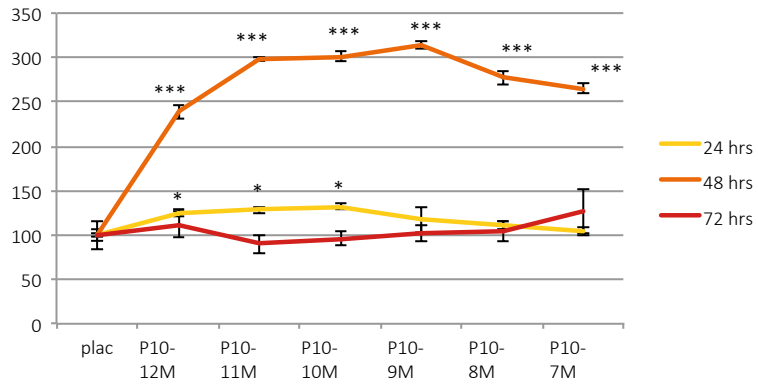
Animal models for osteoporosis (rodents) have high discomfort, are invasive, costly and time-consuming.

In zebrafish, bone formation does not only occur in the skeleton but also in the scales.

Chardon Pharma developed, together with the Hubrecht Laboratory (Utrecht, The Netherlands), a genetically modified zebrafish model in which osteoblasts light-up when they are activated.



Scales of these fish can be used for high throughput screening (96 well format) of potential osteoporosis drugs.



Effects of the known osteoblast stimulator PTH (in different concentrations and different treatment times) on luminiscence of zebrafish scales.

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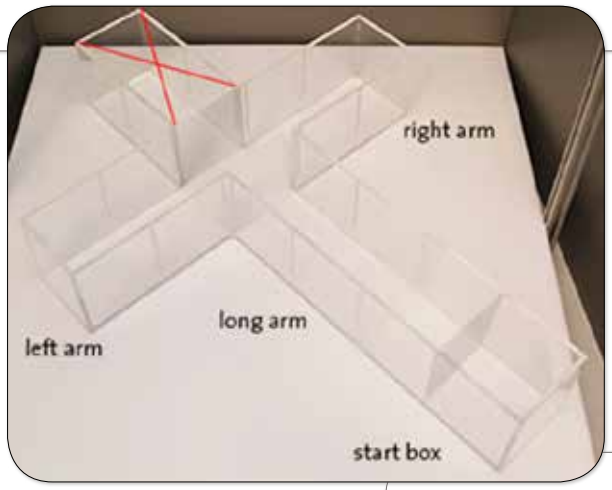
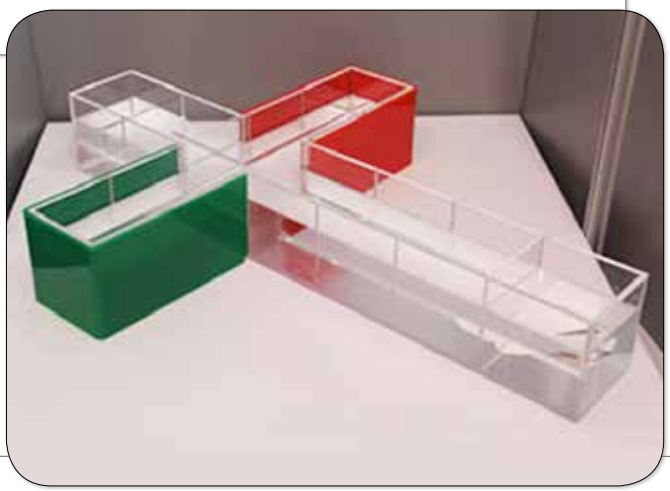
Research in adult Zebrafish

The flexible plus-maze set-up in combination with Ethovision software (developed by Noldus IT, Wageningen, The Netherlands) can be used for different paradigms:

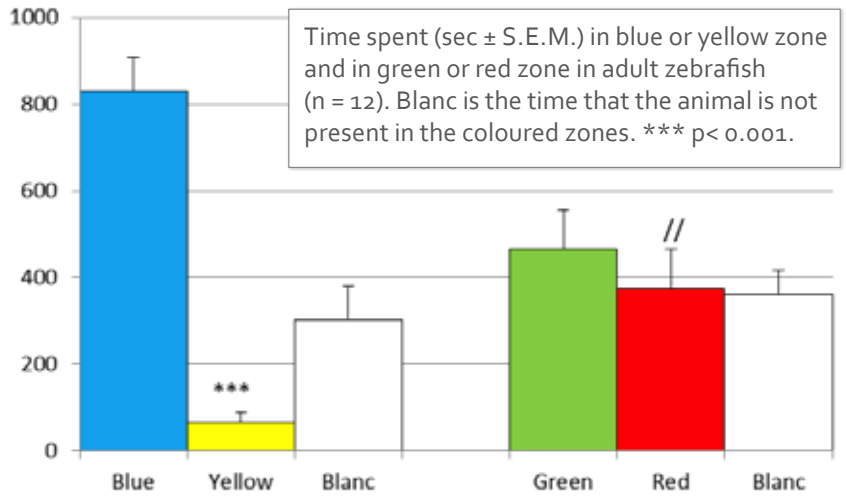
- Colour preference testing
- Learning and memory experiments
- Social interaction testing





An example:

Colour preference of adult zebrafish in the T-maze.



Time spent (sec ± S.E.M.) in blue or yellow zone and in green or red zone in adult zebrafish (n = 12). Blanc is the time that the animal is not present in the coloured zones. *** p< 0.001.

Adult zebrafish have a clear aversion for the colour yellow.

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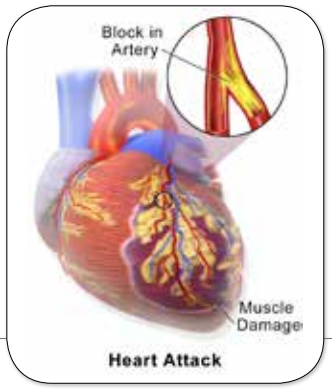
Heart tissue regeneration in Zebrafish



A heart attack causes heart-muscle damage and scar formation; resulting in heart failure. Human heart tissue cannot regenerate.

Zebrafish heart tissue has a regenerative capacity.

The zebrafish heart can be used as a model to investigate which factors are responsible for the heart regeneration (and scar tissue removal).

These factors can be potential leads for medicines to treat heart failure.

Chardon Pharma has three experimental models available:

1. Dissection of heart tissue in adult zebrafish (Poss et al., 2002).
2. Cryoinjury of heart tissue in adult zebrafish (Gonzalez-Rosa & Mercader, 2012).
3. Aristolochic acid induced heart failure in zebrafish larvae (Huang et al., 2007).