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To the editor of *World Journal Of Gastroenterology*

Manuscript title: “The use of zebrafish embryos as avatar of patients with pancreatic cancer: a new xenotransplantation model towards personalized medicine”. (Manuscript type: basic study)

### **Animal Care and Use Statement**

#### **Species of animals**

zebrafish, lines: AB line (WT)

#### **General description**

In contrast to sentient mammals, zebrafish is classified as insentient from fecundation to the time at which they become capable of independent feeding (rather than being dependent on the food supply from the yolk), which has been established at 120 hours post-fertilization (hpf), therefore during this time it does not require a license according to the Directive 2010/63/EU. Embryo will be strictly employed in this experimental window (0-120 hpf). Embryos will be obtained by natural and spontaneous fertilization by coupling males and females in appropriate conditions. This procedure will not require sacrificing animals or their hormonal stimulation; embryos will be directly collected from the tanks. The animals are housed in specific aquaria, according to the guideline provided by ZFIN. The colonies are maintained by a technician (Salvatore Di Maria), under the directions of expert researchers. The colonies are routinely visited by the veterinary (Dr Alberto Sbrana) and bioethic service and monitored yearly by an official of the local sanitary authority.

#### **Main adverse effect for the animals**

Experimental manipulations of larvae will be done under deep anesthesia (anesthetized in 0,05% tricaine).



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### **Ethical documents and authorizations**

Experiments will be performed at the Animal Facility of the Unit of Cell and Developmental Biology, Dipartimento di Biologia, UNIPI located in S.S. 12 Abetone e Brennero, 4 - 56127 Pisa, Italy (scientific responsible: Prof V Raffa) (authorization n. 99/2012-A, 19.04.2012, Annex I). Animal procedures will be performed in strict compliance with protocols approved by Italian Ministry of Public Health and of the local Ethical Committee of University of Pisa, in conformity with the Directive 2010/63/EU. The breeding of the animals for scientific purposes has been authorized by the “Comune di Pisa” (N. atto DN-16 / 43 del 19/01/2015).

### **Level of suffering of procedures**

No suffering (larvae at these stages are classified as not sentient)

Yours sincerely

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