

## **Sensitive and specific detection of Ewing sarcoma minimal residual disease in ovarian and testicular tissues**

Ewing sarcoma (EWS) is a common pediatric solid tumor with high metastatic potential. Due to toxic effects of treatments on reproductive functions, the cryopreservation of ovarian tissue (OT) or testicular tissue (TT) is recommended to preserve fertility. However, the risk to reintroduce residual metastatic tumor cells should be evaluated before fertility restoration. Our goal was to validate a sensitive and specific approach for EWS minimal residual disease (MRD) detection in frozen germinal tissues. Thawed OT (n=12) and TT (n=14) were contaminated with tumor RD-ES cells (10, 100 and 1000 cells) and *EWS-FLI1* tumor-specific transcript was quantified with RT-qPCR. All contaminated samples were found to be positive, with the strong correlation between RD-ES cell numbers and *EWS-FLI1* levels in OT ( $r=0.93$ ) and TT ( $r=0.96$ ) ( $P<0.001$ ). No transcript was detected in uncontaminated control samples. The invasive potential of Ewing cells was evaluated using co-culture techniques. After co-culturing, tumor cells were detected in OT/TT with histology, FISH and RT-qPCR. In addition, 4 OT and 4 TT samples from children with metastatic EWS were tested, and no MRD was found using RT-qPCR and histology. We demonstrated the high sensitivity and specificity of RT-qPCR to detect EWS MRD in OT/TT samples.

**Clinical trial: NCT 02400970.**

**Key words:** Fertility preservation - Ewing sarcoma - Ovarian tissue - Testicular tissue - Minimal residual disease detection – RT-qPCR

Chaput Laure<sup>1</sup>, Grèze Victoria<sup>2,3,4</sup>, Halle Pascale<sup>4</sup>, Robin Nina<sup>5,6</sup>, Pereira Bruno<sup>7</sup>, Véronèse Lauren<sup>6,8</sup>, Lejeune Hervé<sup>9,10</sup>, Durand Philippe<sup>11</sup>, Martin Guillaume<sup>11</sup>, Sandra Sanfilippo<sup>1</sup>, Canis Michel<sup>1</sup>, Kanold Justyna<sup>2,3,4</sup>, Tchirkov Andrei<sup>6,8</sup> and Brugnon Florence<sup>1,6\*</sup>.

**\* Corresponding author:**

Florence BRUGNON, M.D., Ph.D.

Assistance Médicale à la Procréation, CECOS

CHU Estaing, 1 place Lucie et Raymond Aubrac

63003 Clermont-Ferrand cedex 1, France

Telephone number: +33 4 73 75 02 31

Fax number: +33 4 73 75 02 19

E-mail: [fbrugnon@chu-clermontferrand.fr](mailto:fbrugnon@chu-clermontferrand.fr)

**Affiliations:**

1. CHU Clermont-Ferrand, CHU Estaing, Biologie et Médecine de la Reproduction, AMP, CECOS, France
2. CHU Clermont-Ferrand, CHU Estaing, Service d'Hématologie et d'Oncologie Pédiatrique, France
3. INSERM-CIC 1405, Unité CRECHE, Clermont-Ferrand, France
4. CHU Clermont-Ferrand, CHU Estaing, Centre de Biothérapie d'Auvergne, France
5. LBM OncoGenAuvergne, Unité Pathologie, Centre Jean Perrin (CLCC de la région Auvergne), Clermont-Ferrand, France
6. Université Clermont Auvergne, Unité INSERM 1240, Imagerie Moléculaire et Stratégies Théranostiques Faculté de Médecine, Clermont Ferrand, France
7. CHU Clermont-Ferrand, Unité de Biostatistiques, Délégation à la Recherche Clinique et l'Innovation, Clermont Ferrand, France
8. CHU Clermont-Ferrand, Service de Cytogénétique Médicale, Clermont Ferrand, France
9. Hospices Civils de Lyon, Service de Médecine de la Reproduction, Hôpital Femme Mère Enfant, Bron, France
10. Université Claude Bernard Lyon 1, Unité INSERM U 1208 Laboratoire de Biologie de la Reproduction, Lyon, France
11. Kallistem, Ecole Normale Supérieure de Lyon, Lyon, France

**Emails:**

lchaput1@chu-clermontferrand.fr  
vgreze@chu-clermontferrand.fr  
phalle@chu-clermontferrand.fr  
nina.robin@cjp.fr  
bpereira@chu-clermontferrand.fr  
lveronese@chu-clermontferrand.fr  
herve.lejeune@chu-lyon.fr  
philippe.durand@kallistem.com  
guillaume.martin@kallistem.com  
sanfilipposandra@gmail.com  
mcanis@chu-clermontferrand.fr  
jkanold@chu-clermontferrand.fr  
atchirkov@chu-clermontferrand.fr  
fbrugnon@chu-clermontferrand.fr