Development of an ALK Lymphoma-Derived Autophagosomal and Dendritic Cells Vaccine.

1D Sorrentino*, 2R Chaikle, 3S Manenti, 5G Giuriato. 1Cancer Research Center of Toulouse CRCR, Haute-garonne, Toulouse, France; 2Harvard Medical School, Department of Pathology- Children’s Hospital Boston, Boston, USA; 3Cancer Research Center of Toulouse CRCR, Haute Garonne, Toulouse, France

Introduction ALK-positive Anaplastic Large Cell Lymphoma (ALCL) accounts for 10% to 15% of paediatric lymphomas. Their current treatments, based on chemotherapy or targeted therapy, are not optimal since relapses are invariably observed in 30% of the cases. Facing these therapeutic failures, the development of ALK lymphoma immune-therapies represents a promising research field. Indeed, the ALK oncogene has been proposed as an effective antigen for vaccination.

Autophagy, a process of cell self-digestion, has important roles in immunity, notably through its participation in antigen processing and presentation through both MHC I and II molecules. Therefore, our project aims at isolating pure autophagosomes from ALK+lymphoma cells, to incubate them with dendritic cells specialised for cross-presentation, and to use this preparation as a vaccine to stimulate anti-ALK immune responses.

Material and methods
1. Isolation of autophagosomes from murine ALK+ tumour cells.

The cell line (VAC) was treated with Chloroquine a well-known blocker of Autophagosome-Lysosome fusion. The goal of the treatment is to keep intact the autophagosomal fraction (AF). The AF purity will then be assessed by western-blot and by electron microscopy.

2. Production of dendritic cells (DC) specialised for crosspresentation.

After extracting the mice bone marrow, we decided to use mouse FLT3 ligand to generate preferentially CD8 alpha like DC cells, which are the most prone for antigen crosspresentation.

3. In-vitro immunological assays to assess the effective ability of the DCs to crosspresent ALK tumour specific antigens (in progress).

4. In-vivo immunological assays to evaluate the efficiency of the vaccine (in progress).

Results and discussions
1. Our preliminary results indicate that an autophagosome rich fraction (as assessed by LC3-II and p62 enrichment) could be recovered from the tumour cell culture medium since autophagosome exocytosis has been reported. Of utmost importance, NPM-ALK could be detected in this fraction. However, we are concerned by the quantity and also the purity of this ‘autophagosome preparation’ and we expect to improve this step using cells (VAC) homogeneous lysates, followed by centrifugation on a Nycodenz gradient.

2. Our preliminary results are encouraging but the percentage of CD8 DC cells need to be improved.

Conclusion The use of autophagosomes for a new formulation of anti-ALK vaccine may represent new weapons to improve the therapy of ALK lymphoma, and possibly other ALK oncogene-associated cancers.