

## **Preclinical validation of AXL receptor as a target for antibody-based pancreatic cancer immunotherapy.**

Wilhem Leconet, Christel Larbouret, Thierry Chardès, Gaëlle Thomas, Madeline Neiveyans, Muriel Busson, Marta Jarlier, Nina Radosevic-Robin, Martine Pugnière, Florence Bernex, et al.

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# SupplementaryInformation

## Materials and Methods

### Generation of monoclonal antibodies

Balb/c mice were subcutaneously immunized with 10  $\mu$ grhAXL-EDC at day 0 in complete Freund adjuvant, 14 and 28 in incomplete Freund adjuvant. Three days after a last intravenous boost, spleen cells were fused with non-secreting P3X63Ag8.653 murinemyeloma cells using polyethylene glycol, as previously described<sup>(21)</sup>. Antibodies in hybridoma supernatants were screened by ELISA. Hybridoma cells that secreted anti-AXL antibodies were cloned by limiting dilution and cryopreserved. Antibodies were purified on protein G-agarose (Sigma-Aldrich, St Louis, MO) columns, as recommended by the manufacturer, sterilized through 0.2  $\mu$ m filters, quantified and stored at -20°C in phosphate buffered saline (PBS).

### Fluorescence-Activated Cell sorting (FACS)

Cells were trypsin-detached and incubated with 20 $\mu$ g/ml of purified anti-AXL mAbs in PBS/0.1%BSA on ice for 1.5h. After washing, cells were incubated with FITC-conjugated anti-mouse IgGs (Fc specific) (Sigma-Aldrich) on ice in the dark for 1h. Antibody binding to the cells was then quantified (minimum of 10000 events) using a Beckman-Coulter FC500 MPL Flow Cytometer.

### ELISA

Maxisorb 96-well plates (Nunc, Paisley, UK) were coated with 200 ng/ml rhAXL, rhMER, rhTYRO3 or rmAxl in PBS at 4°C overnight and then saturated with PBS/0.1% BSA. Purified anti-AXL mAbs were added at 37°C for 2h. After washes, horseradish peroxidase-conjugated anti-mouse antibodies (Sigma-Aldrich) were

added for 1h and *o*-Phenylenediaminedihydrochloride(Sigma-Aldrich) was used as substrate as recommended by the manufacturer. Absorbance was measured at 450 nm with an ELISA plate reader.

### **Phosphorylated AXL measurement by ELISA**

$1 \times 10^6$  cells were grown in 6-well plates for 24h and then switched to serum-free medium. Cells were then incubated, or not, with 100  $\mu\text{g/ml}$  purified anti-AXL mAbs for 1.5h, followed by 200 ng/ml rhGAS6 for 30 minutes. Cells were then lysed and protein concentration determined with the BC assay Protein Quantitation Kit (Interchim, Montluçon, France). The PathScan® Phospho-Axl (PanTyr) Sandwich ELISA Kit (Cell Signaling Technology) was used for quantifying phosphorylated AXL according to the manufacturer's instructions. Absorbance was measured at 450 nm with an ELISA reader.

### **Surface Plasmon Resonance analysis by BIACORE**

Analysis of the interaction of the anti-AXL mAbs with AXL ECD and of their competition with GAS6 was performed on a BIACORE 3000 instrument (BIACORE AB, Uppsala, Sweden) at 25°C with HBS-EP (10 mM HEPES, 150 mM NaCl, pH 7.4, 0.005% surfactant P20 buffer) as running buffer. Recombinant human AXL-Fc fusion protein was immobilized on a CM5 sensor chip using an amine coupling kit (Biacore AB). The control flow cell was treated only with chemical reagents without protein. For competition studies, a saturating concentration (625 nM) of rhGAS6 was injected onto the AXL-Fc-coated chip before injection of 666 nM anti-AXL mAbs. To validate our results, the reverse experiment (first injection of anti-AXL mAbs and then of rhGAS6) was carried out.