Suppression of triple-negative breast cancer metastasis by a new glyco-humanized polyclonal antibody

Carine Ciron, Ophélie Dauphouy, Pierre-Joseph Royer, Pierre Morice, Odile Duvaux, Firas Bassissi

Context:

Triple-negative breast cancer (TNBC) is an aggressive subtype accounting for 15–20% of breast cancer cases, characterized by poor prognosis, high recurrence rates, and limited treatment options. The lack of hormone receptors associated with a high level of molecular heterogeneity of TNBC hinders the development of effective therapies. Chemotherapy remains the standard of care but is often compromised by resistance and toxicity, emphasizing the urgent need for innovative treatments. Here, we assess the efficacy of XON7, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), in preclinical models of TNBC.

Methods:

The anti-tumor efficacy of XON7 was assessed in TNBC cell lines for its capacity to induce complement-dependent cytotoxicity (CDC) and apoptosis, compared to paclitaxel and the ADC Sacituzumab Govitecan. Apoptotic mechanisms were explored by evaluating caspase 8/9 activation, reactive oxygen species (ROS) levels, and mitochondrial membrane potential. In vitro 3D cultures, XON7's ability to inhibit tumor growth, spheroid formation, and metastasis was tested. In vivo antitumor efficacy was evaluated in orthotopic and metastatic models in SRG rats inoculated with MDA-MB-231-Luc cells, using bioluminescence imaging.

Results:

XON7 demonstrated potent and selective cytotoxicity across human TNBC cell lines (80–100% cytotoxicity), sparing healthy PBMCs. Apoptotic induction involved both intrinsic and extrinsic pathways, evidenced by caspase 8/9 activation, ROS generation, and mitochondrial membrane potential loss. In 3D cultures, XON7 reduced spheroid size by 50% after two treatments (300 µg/ml) and significantly decreased cancer cell viability (28% remaining). Pre-treatment with XON7 prevented spheroid formation, outperforming paclitaxel and sacituzumab govitecan. XON7 also inhibited metastasis in transwell invasion assays. In vivo, bi-weekly XON7 treatments reduced tumor growth by 50% and eliminated lung metastases in an orthotopic rat model. In a metastatic model, tri-weekly XON7 treatments reduced total metastases (lung, liver, etc.) by 90%.

Conclusion

XON7 is a multi-target antibody currently under clinical evaluation, it represents a promising candidate for advancing treatment paradigms in TNBC.

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INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive subtype accounting for 15–20% of breast cancer cases, characterized by poor prognosis, high recurrence rates, and limited treatment options. The molecular heterogeneity of TNBC hinders the development of effective therapies. Chemotherapy remains the standard of care but is often compromised by resistance and toxicity, emphasizing the urgent need for innovative treatments.

Here, we assess the efficacy of **XON7**, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), in preclinical models of TNBC.

MATERIAL AND METHODS

XON7 was obtained by hyperimmunizing pigs genetically modified to lack Neu5GC and α1,3-Gal epitopes, using tumor-associated antigens.

In Vitro Assays

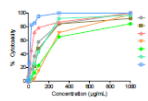
- Complement-dependent cytotoxicity (CDC) was evaluated on primary tumor cells and established cell lines using rabbit complement (1:12) and serial dilutions of XON7.
- 3D tumor spheroid models were used to assess the impact of repeated XON7 administrations and to study metastatic behavior.

In Vivo Studies

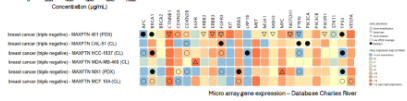
- ***Orthotopic TNBC model***: MDA-MB-231-Luc cells were injected into the mammary fat pad of immunodeficient rats. XON7 (40 mg/kg, twice weekly) was administered once tumors became palpable. Tumor volume was measured regularly. Groups: XON7 (n=6), untreated control (n=5).
- ***Intravenous induced Metastatic TNBC model***: MDA-MB-231-Luc cells were injected intravenously. Treatment with XON7 began on Day 1 post-injection. Metastatic progression was monitored by bioluminescence imaging three times per week. Groups: XON7 (n=5), untreated control (n=5).

RESULTS

XON7 induces a potent anti tumoral activity against a panel of TNBC cancer cell lines (CellTiter-Glo 24h)

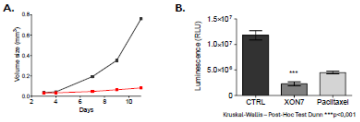


After 24 hours incubation with serial dilutions of XON7 and in the presence of rabbit complement, XON7 induced cytotoxic activity ranging from 84% to 100% at the highest dose



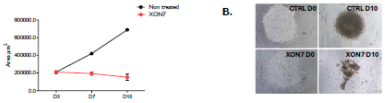
Evaluation of XON7 anti-tumor activity in 3D cultures

XON7 is incubated on each spheroid for 24 hours and then detected with a fluorophore-labeled secondary antibody (Alexa Fluor 488 – green/ DAPI-blue). This photomicrograph show the penetration of XON7 into a triple negative breast cancer (MDA-MB-231) spheroid.



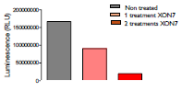
Spheroids were treated on day 3 (D3) with 300 µg/ml of XON7 in the presence of rabbit complement (1:6 dilution). This treatment fully inhibited tumor growth (A). Comparison between paclitaxel (300 µM) and two repeated treatments of XON7 (300 µg/ml on D3 and D7) revealed a significant decrease in cell viability at day 11 (D11) in the XON7 group, compared to untreated controls (CTRL) or paclitaxel-treated spheroids.

XON7 reduces the tumorogenicity of MDA-MB-231 cells by blocking sphere formation



MDA-MB-231 cells were treated or left untreated with 300 µg/ml of XON7. Subsequently, 1,000 viable cells from each condition were re-seeded to assess spheroid formation (A). XON7-treated cells exhibited a marked loss of spheroid-forming capacity, as illustrated in the representative photomicrographs (B).

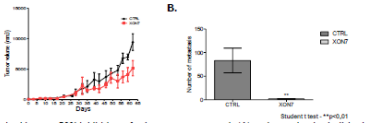
XON7 inhibits metastasis fomation in a transwell invasion assay



MDA-MB-231 spheroids were placed in the upper chamber of a transwell system; only invasive cells can migrate to the lower chamber. A single treatment with XON7 (300 µg/ml at D0) reduced metastasis formation by half, while two treatments (300 µg/ml at D0 and D3) completely abolished it. Metastatic cells in the lower chamber were quantified at day 18 (D18) using a CellTiter-Glo viability assay.

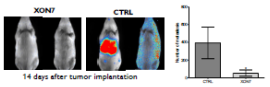
XON7 is effective and well tolerated in an orthotopic and a metastatic xenograft rat models

Orthotopic model



XON7 treatment resulted in over 50% inhibition of primary tumor growth (A) and completely abolished lung metastases compared to the control group (B).

Metastatic model



XON7 also strongly inhibits metastasis formation in an intravenously induced metastatic model.

REFERENCE

To learn more about polyclonal antibodies in oncology:

Ciron et al. JCI Insight 2024 Feb 8;9(3)

Poster high resolution

CONCLUSION

- XON7 shows potent cytotoxic activity against TNBC cells via complement-dependent mechanisms
- XON7 blocks tumor growth and metastatic spread in 3D cultures and in vivo models
- XON7 reduces tumor-initiating capacity by preventing spheroid formation

XON7 can provide a novel and promising therapy for fighting TNBC

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