Abstract 2738

XON7, a new Glyco-humanized polyclonal antibody, first in class immunotherapy against several solid tumors

Carine Ciron[1], Gwenaelle Evanno[1], Pierre Morice[1], Pierre-Joseph Royer[1], Françoise Shneiker[1], Odile Duvaux[1], Bernard Vanhove[1], Firas Bassissi[1]

[1] Xenothera, Nantes, France

XENOTHERA

INTRODUCTION

Cancer immunotherapy has recently generated much excitement after the success of the immunomodulating anti-CTLA-4 and anti-PD-1 antibodies against various types of cancers. However, for many cancers, there is still a lack of effective treatment that can result in long-term cancer-free survival and lower metastatic and relapse risks. The emergence of cancer resistance could be minimized by drug combination or by multi-targeting of tumor cells. Polyclonal antibodies can target several tumor-associated antigens simultaneously and would be more efficient than a conventional mAbs. Here we evaluate the safety and efficacy of **XON7**, a first in class glyco-humanized polyclonal antibody (GH-pAb),in cancer preclinical models.

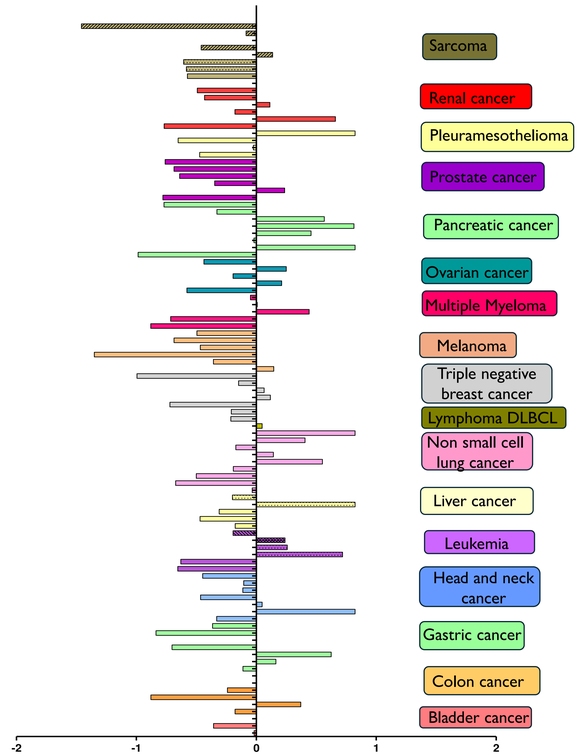
METHODS AND MATERIALS

- XON7 is obtained by hyperimmunizing pig double knock-out for the two main xenoantigens (Neu5GC and α1,3 galactosidase) with a human tumor cell lines property of Xenothera
- ***In vitro Assays***
  - Anti-tumor activity was assessed in a panel of primary patients and tumor cell lines or healthy PBMCs in a complement dependent cytotoxicity assay in presence of rabbit complement (1:12) and serial dilution of XON7
  - 3D culture of HCT116, A549, MDA-MB-231 have developed to study repeated administrations of XON7 and metastasis.
  - Immunohistology on healthy tissues and tumoral patients'biopsies (Oncotest™ PDX tumor TMA slides – Charles River Discovery Research Services). XON7 was used at a concentration of 5µg/mL. For bright-field microscopy, we used goat anti-pig secondary antibody with HRP-conjugate (1:1000; Mabtech AB) stained with ImmPACT® VIP Substrate Kit (Vector Laboratories).

- ***In vivo studies***

- Xenograft mice models were obtained by subcutaneous injection of 1.10^6 tumoral cells (HCT-116, LNCAP, A549) to generate a model of colon, prostate and non small cell lung cancer respectively. All tumoral cells were purchased at DSMZ. Treatment was initiated at the onset of tumor growth (approximately 50 mm3) and was performed twice weekly for a total of 28 days. Treatment consisted of intraperitoneal injection of XON7 at 35mg/kg for the "XON7" group (n=10); no treatment for the "Control" group (n=10). Tumor growth was assessed by measuring tumor volume.
- Human lung carcinoma chicken egg model was performed by Inovotion to evaluate combination of XON7 and Pembrolizumab (10 mg/kg and 2,5 mg/kg respectively). 1.10^6 H460 cells were added onto the CAM of each egg (E9) (n=20) and treatments begun at E10 and then E12, E14, E15, E17. Experiments stop at E18.
- Rat xenograft metastatic model of TNBC, MDA-MB-231-Luc cells were injected into the mammary gland. XON7 was administered as soon as tumors were palpable at a concentration of 40 mg/kg 2 times a week

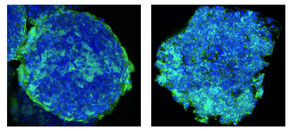
Screening of XON7 on a panel of tumor cell lines and patient primary tumors



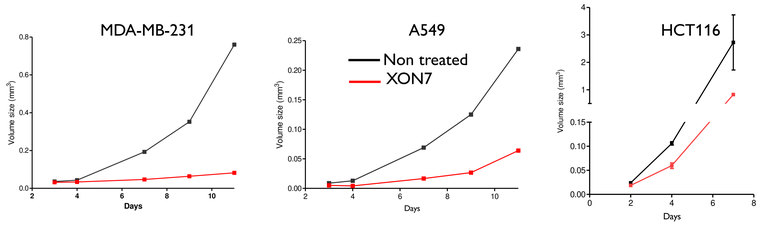
XON7's cytotoxic activity was evaluated in 100 tumor lines or primary patient tumors representing 17 cancers. Cells were incubated for 24h in the presence of rabbit complement (Dil 1:12) and a serial dilution of XON7. This graph shows cell lines and/or biopsies with EC50 < 150 µg/ml on the left, and those with EC50> 150 µg/ml on the right. 5 cancers appear to be highly sensitive to XON7: sarcoma, prostate cancer, melanoma, triple negative breast cancer and colon cancer.

Evaluation of XON7 anti-tumor activity in 3D cultures

1.

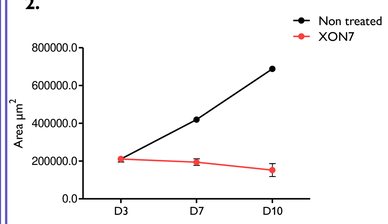


XON7 is incubated on each spheroid for 24 hours and then detected with a fluorophore-labeled secondary antibody (Alexa Fluor 488 – green/ DAPI-blue). These photomicrographs show the penetration of XON7 into the non-small cell lung cancer (A549) and triple negative breast cancer (MDA-MB-231) spheroids.

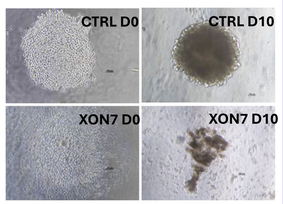


MDA-MB-231 (TNBC), A549 (NSCLC) and HCT116 (colon cancer) spheroids were treated at D3 with 300 µg/ml XON7 in the presence of rabbit complement (dil 1:6). Treatment completely blocked tumor growth in MDA-MB-231 spheroids, dramatically slowed tumor growth in A549 spheroids, and reduced the size of HCT116 spheroids by 70% at D7 (red curve) compared to untreated spheroids (black curve).

2.

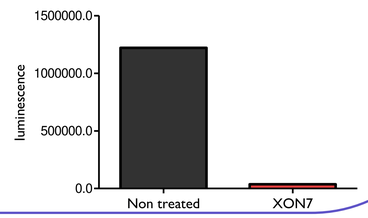


MDA-MB-231 cells are treated or not with XON7 at 300 µg/ml. 1000 live cells of each condition are re-cultured for spheroid formation (graph on the left). XON7-treated cells lost their ability to form spheroids, as shown in the photomicrographs.

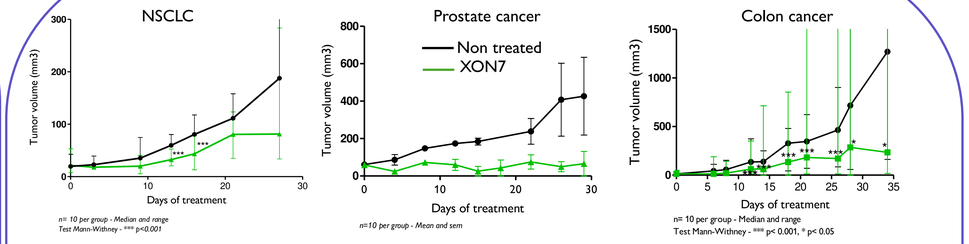


3.

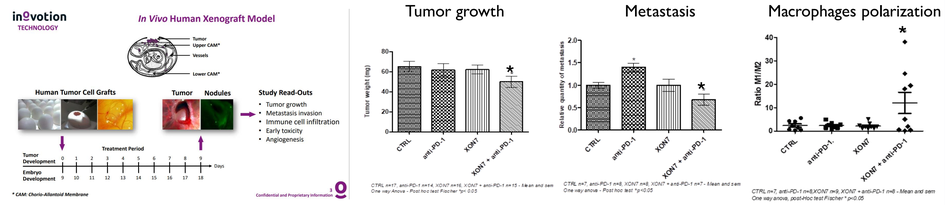
In a model invasion assay with HCT116 spheroids, metastasis formation is measured by luminescence after treatment or not with XON7. Only untreated wells show metastasis. Thus, XON7 blocks cell proliferation and metastasis.



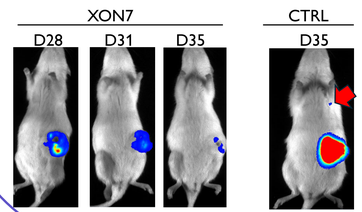
Evaluation of XON7 anti-tumor activity *in vivo*



Anti-tumor activity was evaluated in 3 murine xenograft models of prostate cancer (LNCAP cells), NSCLC (A549) and colon cancer (HCT116). Tumor cells were injected subcutaneously and XON7 treatment (35 mg/kg i.p.) was started when the tumor reached 50 mm3 and continued for 4 weeks at a frequency of twice a week. XON7 induced a significant reduction in tumor growth in the 3 models tested, ranging from 60% to 82% reduction.



The aim of the xenograft model in the egg was to evaluate the combination of XON7 with an ICI (anti-PD-1). The concentrations chosen for monotherapy are deliberately sub-optimal so as not to mask the possible synergistic effect of their combination. Thus, no tumor regression is achieved by monotherapies alone. In contrast a significant regression of tumor growth and tumor metastasis were observed with the combination of XON7 and the anti-PD-1. The combination of XON7 with anti-PD-1 led to an increase in the M1/M2 ratio, indicating a decrease in the polarization of macrophages into the M2 phenotype and thus an increase in M1, anti-tumor macrophages.



In a rat xenograft model of TNBC, MDA-MB-231-Luc cells were injected into the mammary gland. XON7 was administered as soon as tumors were palpable at a concentration of 40 mg/kg 2 times a week, and luminescence was measured by a bioimager. Here on the left, the photos show the gradual decrease of luminescence in treated rats between D28 and D35, reaching almost total disappearance at D35 compared to a control rat. XON7 treatment also prevents the secondary appearance of lung metastases.

CONCLUSION

XON7 is a new immunotherapy against solid cancers:

- **Demonstrates strong activity against 5 major cancers: Sarcoma, triple-negative breast cancer, prostate, melanoma and colon cancer.**
- **Selectively targets tumor tissues without affecting healthy tissues**
- **Inhibits tumor growth up to 82% in different mice xenograft models**
- **Blocks cell proliferation and reduces tumorigenicity**
- **Prevents tumor invasion and metastases and sensitize the tumor microenvironment to immuno-check point treatments**

Tissues	Diagnostic	XON7 binding
Breast	healthy	-
	Invasive ductal carcinoma	+
Pancreas	healthy	-
	adenocarcinoma	+
Prostate	healthy	-
	adenocarcinoma	+
Skin	healthy	-
	melanoma	+
Intestine	healthy	-
	Non-Hodgkinien lymphoma	+
Stomach	healthy	-
	adenocarcinoma	+

XON7 specifically targets and recognizes tumor tissue, not healthy tissue.