Abstract 2739

XON27, a novel and potent immunotherapy against hematologic malignancies

Carine Ciron[1], Pierre Morice[1], Françoise Shneiker[1], Odile Duvaux[1], Firas Bassissi[1]

[1] Xenothera, Nantes, France

XENOTHERA

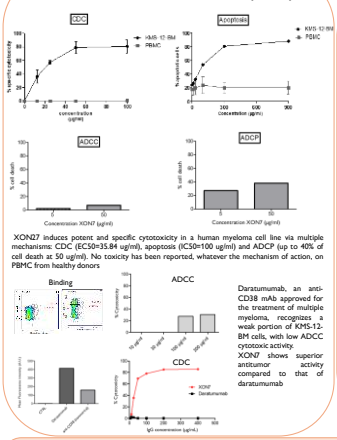
INTRODUCTION

Over the past decades, intensive research has led to the development of innovative therapies (monoclonal and bispecific antibodies, CAR T cells) that have increased the survival rate of patients with multiple myeloma (MM) and B cell lymphoma. However, despite this remarkable progress, the majority of patients relapse and become refractory due to the development of resistance mechanisms. Polyclonal antibodies, with their ability to target multiple antigens, can counteract this resistance. Herewe evaluate the safety and efficacy of **XON27**, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), in MM)

METHODS AND MATERIALS

- XON27 is obtained by hyperimmunizing pig double knock-out for the two main xenoantigens (NeuSGC and α1,3 galactosidase) with a human tumor cell lines property of Xenothera
- ***In vitro Assays***
  - Anti-tumor activity was assessed by complement dependent cytotoxicity (CDC), apoptosis , and antibody dependent cellular phagocytosis (ADCP)using serial concentrations of XON27 in a multiple myeloma cell line (KMS-12-BM), andtibody dependent cellular cytoxicity and healthy PBMCs
  - Cytotoxicity has been assessed in multiple myeloma cell line after 24h of incubation in presence of rabbit complement (dil 1:12) and a serial dilution of XON27
  - Repeated administrations of XON27 was performed on KMS-12-BM seeded at 0.410^5 cells per ml. The cells were incubated for 24 h with XON27, then the medium was changed, and cell density was monitored over time.
  - Immunohistology on tumoral patients'biopsies (Oncotest^{TM} PDX tumor TMA slides – Charles River Discovery Research Services). XON27 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***
  - A myeloma xenograft mice model was obtained by subcutaneous injection of 1.10^6 tumoral cells (KMS-12-BM). Treatment was initiated at the onset of tumor growth (approximately 50 mm^3) and was performed twice weekly for a total of 28 days. Treatment consisted of intraperitoneal injection of XON27 at 35mg/kg (n=10); no treatment for the "Control" group (n=10). Tumor growth was assessed by measuring tumor volume.

XON27 – Mechanism of action and tumor specificity

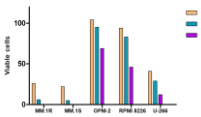


XON27 induces potent and specific cytotoxicity in a human myeloma cell line via multiple mechanisms: CDC (EC50=35.84 ug/ml), apoptosis (IC50=100 ug/ml) and ADCP (up to 40% of cell death at 50 ug/ml). No toxicity has been reported, whatever the mechanism of action, on PBMC from healthy donors

Daratumumab, an anti-CD38 mAb approved for the treatment of multiple myeloma, recognizes a weak portion of KMS-12-BM cells, with low ADCC cytotoxic activity.
XON7 shows superior antitumor activity compared to that of daratumumab

Evaluation of XON27 anti-tumor several multiple myeloma cell lines or primary tumors

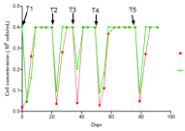
Cancer type	Available Data	KRAS	EGFR expression	VEGF expression	Prior therapy	Primary/ Metastatic/ Recurrent
Multiple myeloma	MM.1R MM.1R	mutated	mutated/ no expression	moderate	Carmustine, melphalan, cytoxan, vincristine, prednisone	Not known
Multiple myeloma	MM.1S MM.1S	mutated	mutated/no expression	moderate	Carmustine, melphalan, cytoxan, vincristine, prednisone	Not known
Multiple myeloma	OPM-2 OPM-2	not mutated	no expression	moderate	Not known	relapse, terminal
Multiple myeloma	RPMI 8226 RPMI 8226	mutated	mutated/no expression	moderate	Not known	n.a.
Multiple myeloma	U-266 U-266	gene loss	no expression	moderate	Not known	refractory, terminal



Rabbit complement Dil 1:12 – 24h incubation with serial dilution of XON27 Cell titer Glo analysis

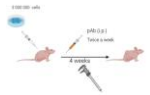
The cytotoxic activity of XON27 was evaluated in several cell lines or primary multiple myeloma tumors. The table summarizes EGF and VEGF receptor expression and prior treatment, if known. XON27 recognizes and kills tumor cells regardless of EGFR and VEGF expression levels or mutations. Interestingly, XON27 is also able to effectively kill tumor cells from multiply treated patients who developed resistance to their chemotherapy

XON27 induces persistent tumor cell cytotoxicity after repeated incubation without tumor cell resistance

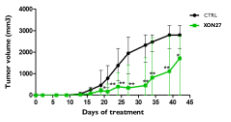


When cells reach a cell density of 10^6 cells/mL, XON27 is administered in the presence of rabbit complement (Dil 1/6). A similar cytotoxicity of 75% is observed after each treatment (except for treatment n°3), demonstrating the absence of treatment resistance due to repeated administration.

Evaluation of XON27 anti-tumor activity *in vivo*



Design of experimental protocol:
2 groups with n=10 NMRI nude mice per group
Group 1: control group without treatment
Group 2: XON27 treatment



Although nude NMRIs have a hemolytic complement, this is not active with porcine IgG. Thus, in this model, the cytotoxic activity of XON27 is essentially apoptotic. The targeted therapeutic dose is 300 µg/ml. In this Multiple lyeloma xenograft model (KMS-12-BM cells) the median tumor size of XON27-treated mice remained less than 500 mm^3 throughout the 28-day treatment period, whereas untreated mice had a median tumor size of more than 2000 mm^3 at the same time. One week after cessation of treatment, corresponding to a decrease in plasma XON27 concentration below the therapeutic threshold of 300 ug/mL, tumors escaped, and tumor growth continued.

CONCLUSION

XON27 is a new immunotherapy against Multiple myeloma:

- Acts against hematological malignancies mainly via plural MOA : CDC, Apoptosis, and ADCP.
- Selectively and effectively eliminates various multiple myeloma cells lines and primary tumors, while preserving healthy PBMCs.
- Induces a persistent tumor cell cytotoxicity after repeated in vitro dosing without tumor cell resistance induction
- Effectively inhibits tumor growth (up to 81%) in a multiple myeloma xenograft model.

Human cancer biopsies patient's biopsies	Number of stained	Mean % of stained area in positive biopsies
Leukemia	1/1	84,5%
Acute lymphoblastic leukemia	1/1	83,1%
Acute myeloid leukemia	10/10	79,4% ± 5,4%
Diffuse large B Cell lymphoma	7/7	70,5% ± 9,7%

XON27 also recognizes and targets other hematological malignancies

Context: Over the past decades, intensive research has led to the development of innovative therapies (monoclonal and bispecific antibodies, CAR T cells) that have increased the survival rate of patients with multiple myeloma (MM)and B cell lymphoma. However, despite this remarkable progress, the majority of patients relapse and become refractory due to the development of resistance mechanisms. Polyclonal antibodies, with their ability to target multiple antigens, can counteract this resistance. Here we evaluate the safety and efficacy of XON27, a first-in-class glyco-humanized polyclonal antibody (GH-pAb), in MM and other B-cell blood cancers.
Material and methods: The ability of XON27 to induce antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) and apoptosis was tested in a panel of hematological malignancy cell lines and peripheral blood mononuclear cells (PBMC). Specific binding to human B-cell blood cancers was assessed by immunochemistry on tissue microarrays. To study resistance mechanisms, MM cell lines were cocultured with macrophages in the presence or absence of XON27 and macrophage. To study relapse mechanisms and tumorigenicity, repeated in vitro administration of XON27 was evaluated in KMS-12-BM. The in vivo efficacy of XON27 was evaluated in NMRI nude mice using the KMS-12-BM cancer cell line. Pharmacokinetics and safety of this drug were assessed in non-human primate after repeated IV dosing up to 60mg/kg.
Results: XON27 induces potent and specific cytotoxicity in several blood malignancy cell lines including human MM cells via multiple mechanisms: CDC (EC50=35.84 ug/ml), apoptosis (IC50=100 ug/ml) and ADCP (up to 40% of cell death at 50 ug/ml). The cytotoxicity induced by XON27 in the KMS-12-BM was significantly more potent compared to daratumumab in both CDC and apoptosis assays, XON27 was able to kill up to 100% of cancer cells without affecting PBMC. XON27 showed cross-reactivity with B-cells from blood cancer patient biopsies without staining healthy tissue. In addition, repeated administrations of XON27 demonstrate its ability to effectively kill tumor cells after each administration. No resistance is induced after repeated treatments. Interestingly, XON27 blocked the proliferation of residual tumor cells for up to 45 days after the second treatment at 50 ug/ml compared to the control condition, demonstrating sustainable effect of XON27 on tumorigenicity. The in vitro efficacy of XON27 was translated into in vivo efficacy in a murine MM

xenograft model. It induced a significant reduction in tumor growth of up to 75%. XON27 was also characterized by a high tolerability profile and satisfactory exposure in primates.
Conclusion: Although many therapeutic advances have been made in the field of blood cancers, myeloma remains an incurable disease with a real need for new innovative therapies. XON27 represents a promising and selective immunotherapy which may provide an effective and safe treatment against refractory myeloma.