Characterization of XON9, an effective glyco-humanized polyclonal antibody against hepatocarcinoma.

Pierre-Joseph Royer, Carine Ciron, Ophelie Dauphouy, George Graur, Odile Duvaux, Firas Bassissi

Context: Hepatocarcinoma is the second leading cause of cancer-related death, and its incidence is increasing. Multikinase inhibitors (Sorafenib or Lenvatinib) are the conventional first line treatment for unresectable HCC, with limited response rates and serious side effects. Although immune base therapies such as checkpoint inhibitors have recently showed promising results, they remain applicable to a small fraction of patients. Thus, new treatment and strategies are desperately needed to improve the management of hepatocarcinoma. We evaluate here the in vitro and in vivo efficacy and safety of XON9, first-in-class glyco-humanized polyclonal antibody (GH-pAb) against hepatocarcinoma.

Material and methods: Cytotoxic activity of XON9 against HUH7, HepG2 and Hep3B cell lines was investigated and compared to the activity of standards of care Sorafenib and Lenvatinib. Primary hepatocytes were used as control. Mechanisms of apoptosis were further explored. Activity of caspases 8 and 9, level of reactive oxygen species (ROS) and mitochondrial membrane potential was investigated after treatment with XON9. The *in vivo* efficacy of XON9 was then assessed in NMRI nude engrafted with HUH7 cancer cells while pharmacokinetics and safety parameters were evaluated in a non-human primate after once weekly dosing at 50mg/kg over 2 weeks.

Results: XON9 shows a potent complement dependent cytotoxicity (CDC) against HUH7, HepG2 and Hep3B cells (EC50<10µg/ml). By contrast, primary hepatocytes were relatively spared by XON9 (EC50>50µg/ml). Moreover, XON9 treatment induced apoptosis of the three cell lines tested with activation of caspases 8 and 9, increase of ROS levels and drop of mitochondrial membrane potential. Overall, *in vitro* lytic activity of XON9 towards HUH7, HepG2 and Hep3B was superior to that of Sorafenib and Lenvatinib, the current standards of care for hepatocarcinoma. *In vivo,* XON9 significantly reduced tumour progression in a mouse xenograft model (40% reduction at day 25). No toxicity was observed after repeated injections of 50mg/kg of XON9 in a non-human primate.

Conclusion: Although recent immunotherapy advances have been made, hepatocarcinoma remains an incurable disease with a real need for innovative therapies. XON9 represents a promising and selective immunotherapy against refractory hepatocarcinoma.

Abstract 4766

Characterization of XON9, an effective glyco-humanized antibody against hepatocarcinoma

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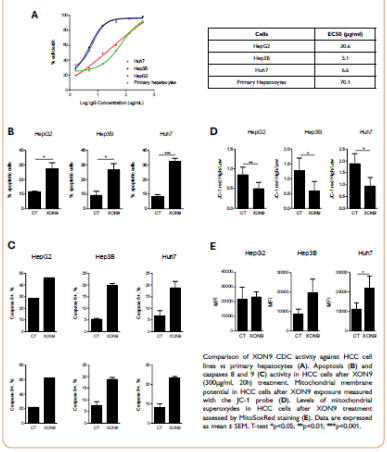
INTRODUCTION

Hepatocarcinoma (HCC) is the second leading cause of cancer-related death, and its incidence is increasing. Multikinase inhibitors (Sorafenib or Lenvatinib) are the conventional first line treatment for unresectable HCC, with limited response rates and serious side effects. Although immune base therapies such as checkpoint inhibitors have recently showed promising results, they remain applicable to a small fraction of patients. Thus, new treatments and strategies are desperately needed to improve the management of HCC. We evaluate here the *in vitro* and *in vivo* efficacy and safety of XON9, first-in-class glyco-humanized polyclonal antibody (GH-pAb) against HCC.

MATERIAL AND METHODS

- XON9 is obtained by hyperimmunization of double knock-out pigs for the two main xenoantigens, N-Glycolylneuraminic acid (Neu5GC) and α1,3 galactose (α-Gal), with a human tumor cell line.
- ***In vitro Assays***
  - Anti-tumor activity was assessed by complement dependent cytotoxicity (CDC), using serial concentrations of XON9 in three HCC cell lines (HepG2, Hep3B and Huh7). Primary hepatocytes were used as control.
  - Apoptosis, activity of caspases 8 and 9, level of reactive oxygen species and mitochondrial membrane potential was investigated after treatment with XON9.
- ***In vivo studies***
  - A xenograft mice model was obtained by subcutaneous injection of $4.5x10^6$ Huh7 cells in NMRI nude. Treatments (XON9 or Sorafenib) were initiated at the onset of tumor growth (approximately 50 mm^3). Tumor growth was assessed by measuring tumor volume twice weekly for a total of 25 days (n=10 mice/groups).
  - Pharmacokinetics and safety parameters were evaluated in a non-human primate after once weekly intravenous dosing at 50mg/kg over 2 weeks.

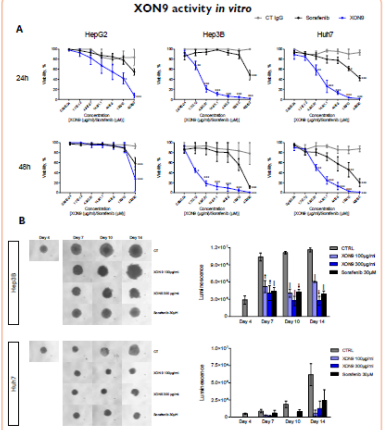
XON9 – Mechanism of action and tumor specificity



Cells	EC50 (µg/ml)
HepG2	30.6
Hep3B	5.1
Huh7	6.6
Primary Hepatocytes	70.1

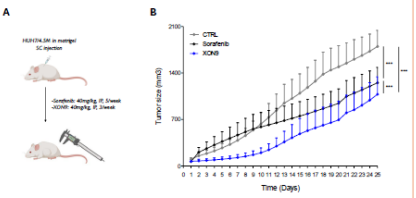
Comparison of XON9 CDC activity against HCC cell lines vs primary hepatocytes (**A**). Apoptosis (**B**) and caspases 8 and 9 (**C**) activity in HCC cells after XON9 (300µg/mL 20h) treatment. Mitochondrial membrane potential in HCC cells after XON9 exposure measured with the JC-1 probe (**D**). Levels of mitochondrial superoxydes in HCC cells after XON9 treatment assessed by MitoSoxRed staining (**E**). Data are expressed as mean ± SEM. T-test *p<0.05; **p<0.01; ***p<0.001.

XON9 activity *in vitro*



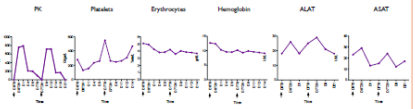
Overall oncolytic activity (CDC + apoptosis) at 24h and 48h of XON9 against HCC cell lines and comparison with the standard of care Sorafenib (**A**). Oncolytic activity of XON9 on Hep3B and Huh7 spheroid models, and comparison with sorafenib (**B**). XON9 or sorafenib treatments were performed at day 4, 7 and 10 in the presence of rabbit complement (1/6). Data are expressed as mean ± SEM. Two-way ANOVA with Bonferroni post-hoc test, *p<0.05; **p<0.01; ***p<0.001.

XON9 anti-tumor activity *in vivo* in a xenograft mice model



Xenograft mice model (**A**). NMRI nude mice were injected SC with $4.5x10^6$ Huh7 cells. Treatments (XON9 or Sorafenib, n=10 mice/groups) were initiated at the onset of tumor growth (approximately 50 mm^3). Tumor growth was assessed by measuring tumor volume twice weekly for a total of 25 days (**B**). Reduction of tumor growth by 40% in the XON9 group. Data are expressed as mean ± SEM. Two-way ANOVA, *p<0.05; **p<0.01; ***p<0.001.

Pharmacokinetic and safety parameters in a non-human primate



Pharmacokinetics and safety parameters were evaluated in a non-human primate after once weekly intravenous dosing at 50mg/kg over 2 weeks. No XON9 accumulation after 2 IV injections.

CONCLUSION

- XON9 shows a potent complement dependent cytotoxicity (CDC) against HepG2, Hep3B and Huh7 cells. By contrast, primary hepatocytes were relatively spared by XON9.
- XON9 treatment induced apoptosis of the three cell lines tested with activation of caspases 8 and 9, increase of reactive oxygen species levels and drop of mitochondrial membrane potential.
- Overall, *in vitro* lytic activity of XON9 towards HepG2, Hep3B and Huh7 was superior to that of Sorafenib, the current standards of care for HCC.
- *In vivo*, XON9 significantly reduced tumor progression in a mouse xenograft model (40% reduction at day 25). No toxicity was observed after repeated intravenous injections of 50mg/kg of XON9 in a non-human primate.