Abstract 2737

LIS22, a first in class polyclonal antibody immunotherapy against T Cell blood cancers

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XENOTHERA

INTRODUCTION

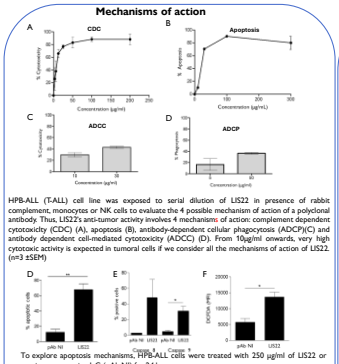
Several approach such as antibody drug conjugate (ADC), chimeric antigen receptor T cells (CAR T) and more recently bispecific antibodies are being successfully introduced as innovative weapon in B Cell lymphoma treatment arsenal. However, for rare T cell lymphoma and leukemia such as PTCL, there has been no improvement in five years survival rates in over 20 years, and there is an urgent need for new therapies.

LIS22 is a first in class glycol-humanized polyclonal antibody (GH-pAb), targeting multiple tumor associated antigens simultaneously. In this study, we extensively characterized the safety and efficacy of LIS22 in preclinical models of T cell blood cancers.

METHODS AND MATERIALS

- LIS22 is obtained by hyper immunizing pig double knock-out for the two main xenoantigens (Neu5GC and α1,3 galactosidase) with a human T-cell acute lymphoblastic leukemia (T-ALL) property of Xenothera
- ***In vitro Assays***
  - Anti-tumor activity was assessed by complement dependent cytotoxicity (CDC), apoptosis , antibody dependent cellular phagocytosis (ADCP), and antibody dependent cellular cytotoxicity (ADCC) using serial concentrations of LIS22 in a T-ALL cell line (HPB-ALL).
  - Activated caspase 8 and caspase 9 were measured in HPB -ALL, treated with 250 µg/ml of LIS22 after 20h of incubat ion and ROS was investigated after 4h with cell permeable H_2DCFDA
  - Repeated administrations of LIS22 were performed on tumor cells: HPB-ALL, T1301 and HUT78 seeded at 0.410^5 cells per ml. The cells were incubated for 24 h with LIS22, then the medium was changed, and cell density was monitored over time.
- ***In vivo studies***
  - 5.10^6 cells of each human tumor cell line (T1301, Jurkat cell lines) were injected subcutaneously in the right flank of NMRI nude mice to generate 2 human cancer models: T cell lymph oma and T-ALL, respectively. Treatment with LIS22 was initiated when tumor size reach 50 mm^3 and occurred twice a week for four weeks at a dose of 35 mg/kg by intraperitoneal route.
  - T1301 cell line was injected subcutaneously (15.10^6 cells) into SRG immunodeficient rats in the lower right flank. LIS22 was administrated at a dose 40 mg/kg intraperitoneally, twice a week for 4 weeks. CHOP was injected at the highest tolerable dose: 1 cycle[a] at D0 Cyclophosphamide 37.5 mg/kg, Doxorubicin 2.5 mg/kg, and vincristine 0.07 mg/kg, and at D0, D1, D2, D3, D4, D5 prednisolone 1.47 mg/kg. Tumor growth was measured twice a week using a caliper

Mechanisms of action



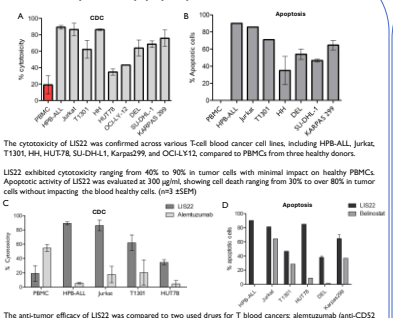
HPB-ALL (T-ALL) cell line was exposed to serial dilution of LIS22 in presence of rabbit complement, monocytes or NK cells to evaluate the 4 possible mechanism of action of a polyclonal antibody. Thus, LIS22's anti-tumor activity involves 4 mechanisms of action: complement dependent cytotoxicity (CDC) (A), apoptosis (B), antibody-dependent cellular phagocytosis (ADCP)(C) and antibody dependent cell-mediated cytotoxicity (ADCC) (D). From 10µg/ml onwards, very high cytotoxic activity is expected in tumoral cells if we consider all the mechanisms of action of LIS22. (n=3 ±SEM)

To explore apoptosis mechanisms, HPB-ALL cells were treated with 250 µg/ml of LIS22 or non-immune porcine IgG (pAb NI) for 24 hours.

Following LIS22 exposure, 80% of the cells underwent apoptosis. Activation of caspases 8 and 9 occurred after 20 hours, indicating involvement of both extrinsic and intrinsic pathways

Reactive oxygen species (ROS) accumulation was also examined using H2DCFDA, revealing a transient ROS increase after 4 hours of LIS22 treatment. (n=3 ±SEM - Paired t test *p<0.05,***p<0.01)

LIS22 induces cytotoxicity specific to malignant T-cells, with no toxicity to healthy lymphocytes



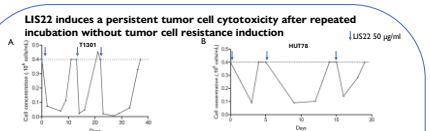
The cytotoxicity of LIS22 was confirmed across various T-cell blood cancer cell lines, including HPB-ALL, Jurkat, T1301, HH, HUT-78, SU-DH-L1, Karpas299, and OCI-LY12, compared to PBMCs from three healthy donors.

LIS22 exhibited cytotoxicity ranging from 40% to 90% in tumor cells with minimal impact on healthy PBMCs. Apoptotic activity of LIS22 was evaluated at 300 µg/ml, showing cell death ranging from 30% to over 80% in tumor cells without impacting the blood healthy cells. (n=3 ±SEM)

The anti-tumor efficacy of LIS22 was compared to two used drugs for T blood cancers: alemtuzumab (anti-CD52 mAb) and Belinostat (HDAC inhibitor), each tested at 100 µg/ml for antibodies and 100 µM for belinostat across T tumor cell lines. Alemtuzumab showed minimal cytotoxicity (< 20%) in tumor lines but induced significant toxicity in healthy PBMCs (nearly 60%), consistent with clinical observations. LIS22 displayed low cytotoxicity (20%) in PBMCs.

Belinostat elicited a variable response among T leukemia/lymphoma cell lines, with apoptotic rates ranging from 1.45% to 64.37%, consistent with clinical observations of diverse responses due to innate and acquired resistance mechanisms in tumor cells.

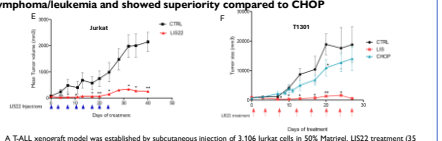
LIS22 induces a persistent tumor cell cytotoxicity after repeated incubation without tumor cell resistance induction



To assess resistance development in tumor cells, various leukemia/T-cell lymphoma lines were exposed to multiple doses of LIS22 at sub-lethal concentrations. The aim was to apply pressure on cancer cells without inducing complete cell death, potentially selecting for treatment-resistant cells.

Consistent cytotoxicity was observed across all cell lines after each LIS22 administration, indicating no development of resistance mechanisms. Interestingly, tumor cells showed increased susceptibility to LIS22 over time, with longer recovery periods between administrations. For example, recovery times increased from 10 to 16 days for the T1301 cell line (A) and from 3 to 5 days for HUT78 cells (B).

LIS22 can effectively reduce tumor growth in xenograft models of T-cell lymphoma/leukemia and showed superiority compared to CHOP



A T-ALL xenograft model was established by subcutaneous injection of 3.106 Jurkat cells in 50% Matrigel. LIS22 treatment (35 mg/kg) was initiated when tumors reached 50 mm3, administered twice weekly for 4 weeks. Significant tumor size reduction occurred after the second LIS22 treatment, with complete inhibition of tumor growth throughout the treatment. After treatment was stopped, tumor growth gradually resumed, but remained limited, reaching approximately 338 mm3 from 150 mm3. At D40, an 86% reduction in tumor size was sustained in the LIS22-treated group. No tumor escape was observed with LIS22 treatment. In comparison, CHOP chemotherapy slowed tumor growth by 40% at 21 days after cessation of treatment in a rat T-cell lymphoma xenograft model.

CONCLUSION

LIS22 is a new immunotherapy against T cell hematological malignancies:

- Acts against T-cell lymphoma mainly via plural MOA : CDC, Apoptosis, ADCC and ADCP.
- Selectively and effectively eliminates various T-cell hematological malignancies: including T-ALL, PTCL-NOS, ALCL, and cutaneous T-cell lymphoma, 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 85%) in T-ALL and T-cell lymphoma xenograft models.
- Demonstrates superior anti-tumor cytotoxicity and tolerance compared to the currently used T-cell blood cancer therapies (CHOP and belinostat,).

Context: 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.

Material and methods: XON7 ability to induce Complement Dependent Cytotoxicity (CDC) and apoptosis was tested in a panel of cancer cell lines and PBMC. XON7 was also evaluated in sphere formation assay to predict its effects on cancer resistant. Specific binding to human tumors and healthy tissues was assessed by immunochemistry on tissue micro-array. Cross-cancer activity was evaluated on biopsies from patients with solid tumor such as CRC, NSCLC, osteosarcoma, and breast cancers. In vivo XON7 efficacy was evaluated in NMRI nude mice using A549: NSCLC, HCT-116: colon cancer, LNCaP: prostate cancer and MDA-MB-231: breast cancer cells.A human NSCLC chorio-allantoic membrane (CAM) model was used to evaluate the efficacy of XON7 + anti PD-1 on tumor growth and metastasis. Pharmacokinetics and safety of this drug were assessed in marmoset after single and repeated IV dosing up to 60mg/kg.

Results: XON7 showed a potent in vitro antitumor activity in a panel of cancer cell lines, it induced specific tumor cell CDC (EC50=50ug/mL) and apoptosis (IC50= 100ug/mL).it was able to kill up to 100% of the cancer cells without affecting PBMC. In addition, it induced a striking decrease of tumor sphere formation in HCT116, A549 and MDA-MB-231cancer cell lines. XON7 showed preferential recognition of tumor cells as compared to normal cells, it demonstrated cross-reaction to tumor patient biopsy without staining of the healthy tissues.

In vitro XON7 potency was translated into in vivo efficacy in different mice xenograft models. XON7 induced a significant reduction of tumor growth ranging from 40 to 90% across several tested tumors. Furthermore, it showed, significant increase of anti-tumor response rate and decrease of metastasis when itis associated with anti PD-1 in vivo CAM model (>90%, p=0.001). XON7wasalso characterized by high tolerance and satisfactory exposure in marmoset.
Conclusion: Based on its safe toxicity profile and potent activity, XON7 appeared as a novel and promising cancer immunotherapy to fight against recurrent solid tumor, it is now aimed to reach clinical development, FIH in patients is planned to start before the end of 2023.
Keywords:
Solid cancers, polyclonal antibodies, XON7, Immunotherapy, metastasis, anti-PD-1