An innate immune modulator displays deep and durable antitumor activity in TNBC and other myeloid-rich immunosuppressive tumor models with a favorable preclinical safety profile

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HEPHAISTOS

SUMMARY

Background In triple-negative breast cancer (TNBC), B-cell lymphoma (BCL) and colorectal cancer (CRC), immunosuppressive myeloid cells, particularly M2 tumor-associated macrophages (TAMs), contribute to resistance to immunotherapies. HEPHA440 is a **chemically detoxified liposomal TLR4 agonist** optimized for **systemic administration** to address **disseminated tumors.** It demonstrated potent antitumor and immune memory properties against primary tumors and metastases through TAM activation/repolarization and induction of Th1 adaptive immune responses [1-3].

Method Antitumor and immunomodulatory activities of HEPHA440 were evaluated in murine **myeloid-rich immunosuppressive tumor models**, including EMT6 (TNBC), A20 (BCL), and CT26 (CRC). Cytokine profiles were assessed *in vivo* in mice and rabbits and compared with *in vitro* human blood data. Nonclinical toxicity was evaluated in a dose escalation toxicity study in rabbit and *ex vivo* on patient liver biopsies.

Results HEPHA440 demonstrated **robust antitumor activity** in EMT6, A20 and CT26 models and potentiated **anti-PD1** & **anti-CD20** efficacies (**Fig. 1**). In mice, HEPHA440 **reduces systemic** MyD88-associated **inflammatory** cytokine induction compared with natural TLR4 agonists, while **preserving** strong TRIF/IFN-associated and effector-recruiting chemokine signatures. A comparable cytokine profile was observed in human blood, supporting a potentially **favorable** clinical benefit-risk profile (**Fig. 2**). In rabbit toxicity study, HEPHA440 showed **favorable tolerability** and **PK** profile up to doses of **2 200µg/kg**, with controlled and reproducible immune cell stimulation and induction of pro-inflammatory cytokines (**Fig. 3A**). Importantly, HEPHA440 was not associated with gross toxicities typically limiting earlier-generation systemic TLR agonists, including macroscopic or acute hepatic toxicity. *Ex vivo* toxicity studies using patient liver biopsies showed that, unlike natural TLR4 agonists, HEPHA440 did not induce detectable liver function impairment or transaminase elevation, even at high concentrations, while triggering only moderate inflammatory activation (**Fig. 3B**).

HEPHA440 drives TRIF-biased TLR4 signaling while limiting inflammation

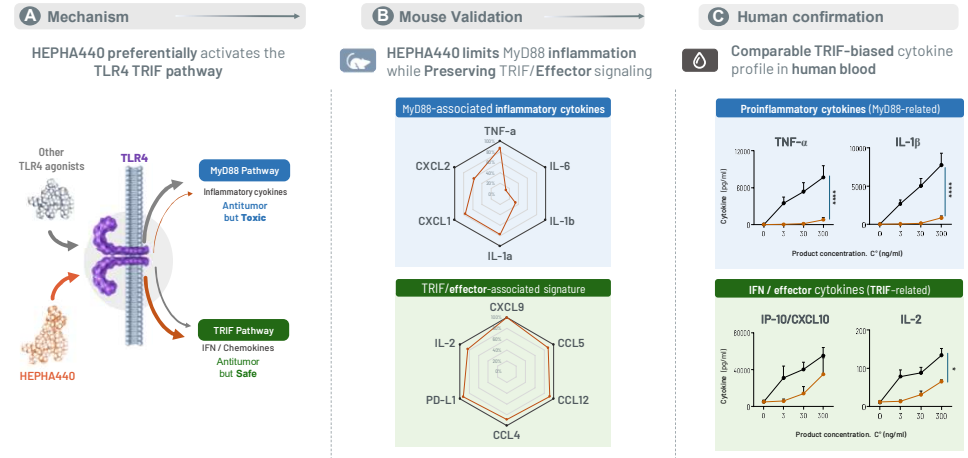


Figure 2. Cytokine/chemokine profile of HEPHA440 vs. natural TLR4 agonist

(**A**) Schematic representation of the differential TLR4 signaling induced by HEPHA440 versus natural TLR4 agonists, with preferential TRIF-associated responses.

(**B**) Circulating cytokine/chemokine profile in C57BL/6 mice (N = 6), evaluated using Olink technology on blood samples collected at 1, 3, 6, and 24 h post-injection. Data are presented as the mean percentage of the peak production of each cytokine/chemokine induced by HEPHA440 relative to natural TLR4 agonist following a 25µg IV dose.

(**C**) Confirmation of the TRIF-biased cytokine profile of HEPHA440 on human WB cells (5 donors) incubated 24h with increased concentrations (3 -> 300ng/ml) of HEPHA440 or a natural TLR4 agonist (LPS E.coli 026:B6). Cytokine production were analyzed with MSD U-PLEX Inflammation Panel 1 (human). Data are presented as mean concentration of each cytokine +/- SEM. Two-way ANOVA – * P<0,05, ****P<0,0001

HEPHA440 — Natural TLR4 Agonist

HEPHA440 – Potent Activity and Enhanced ICI and mAb Responses in Myeloid-Rich Tumors

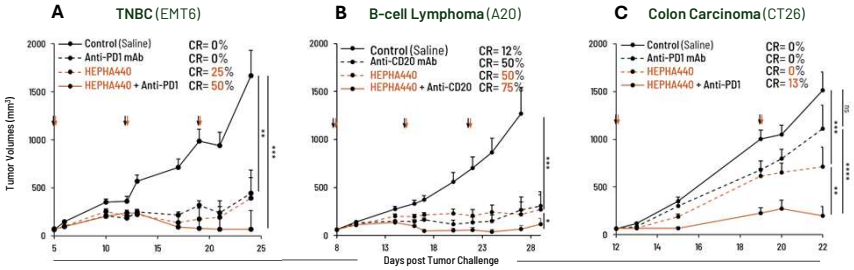


Figure 1. HEPHA440 antitumor activity as monotherapy and combination in mouse tumor models.

HEPHA440 antitumor activity as monotherapy (25µg in IV) or in combination with anti-mouse PD-1 (12.5 mg/kg in IP) or anti-mouse CD20 (12.5 mg/kg in IP) mAbs in group of mice (N=8) with established (**A**) Triple negative breast cancer (EMT6), (**B**) B-cell lymphoma (A20) and aggressive colorectal tumor model (**C**) (CT26). Mice treated with saline were used as controls. Data are presented as mean tumor growth +/- SEM over time. Two-way ANOVA * P<0.05, ** P<0.01, *** P<0.001; **** P<0.0001. CR= % complete regression. ns = not significant. Arrows indicate times of treatment.

HEPHA440 – Favorable Preclinical Tolerability and PK profiles in rabbits and ex-vivo patients' liver biopsies

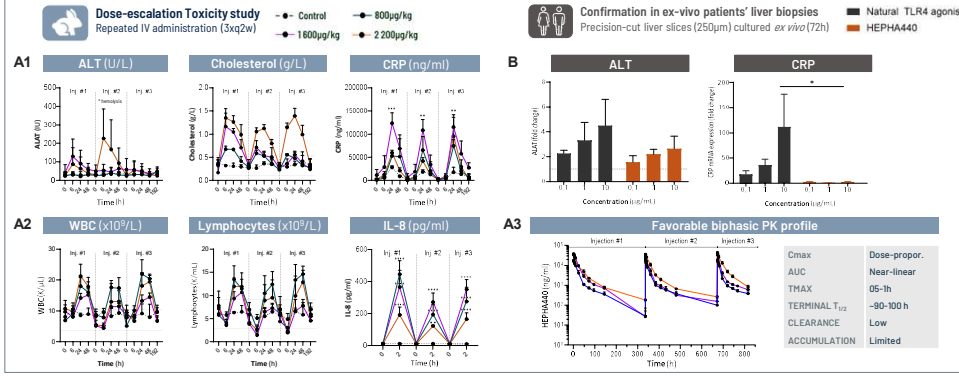


Figure 3. In vivo and ex vivo toxicity studies

A. Dose-escalation toxicity study in NZW rabbits the **only animal specie** presenting a **similar sensitivity than human** to TLR4 agonists. Toxicological (**A1**) and hematological (**A2**) parameters over time, quantified by ELISA (CRP, IL-8), enzymatic dosages (ALAT, cholesterol), or using ProCyte Dx Hematology analyser (WBC, lymphocytes). (**A3**) HEPHA440 pharmacokinetic profile in rabbit plasma quantified by HPLC-MS-MS. **B. Ex vivo studies on patient liver biopsies** stimulated 72h with HEPHA440 or a natural TLR4 agonist. Biological parameters were quantified by ELISA (ALAT) and RT-qPCR (CRP). Data are expressed as fold change relative to untreated cells.Two-way ANOVA – * P<0,05, **P<0.01, ***P<0.001, ****P<0,0001.

- **Favorable tolerability** profile in rabbits up to **2,200 µg/kg**, with **no cumulative** toxicity and **no severe** hepatotoxicity, **in contrast** to the **dose-limiting liver** toxicity reported with earlier-generation TLR4 agonists.
- **Ex vivo human liver** data support this **favorable hepatic safety profile**, showing no impairment of liver function and only moderate inflammatory responses compared with a natural TLR4 agonist.
- **Predictable PK profile** supporting intermittent dosing.

Conclusions

These data support clinical investigation of HEPHA440 in **TNBC** and other **myeloid-rich immunosuppressive tumors** and support the initiation of clinical-trial-enabling studies. Combining broad **systemic** antitumor activity, **potentiation** of anti-PD-1 therapy, preserved IFN-associated immune activation, and **low** systemic toxicity with **no evidence** of severe or dose-limiting hepatotoxicity reported with earlier-generation TLR4 agonists, HEPHA440 represents a **promising investigational drug** for patients with myeloid-rich, advanced recurrent or metastatic solid tumors who derive limited benefit from standard immunotherapies.

Partners

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