Targeting Tumor-Derived Exosomes using a Lectin Affinity Hemofiltration Device

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Abstract

Background: Exosomes are 30-150 nm vesicles that are released by tumor cells and have systemic disease-promoting effects. Despite the abundant research showing that tumor-derived exosomes are purveyors of tumor growth-promoting and immunosuppressive cargo, a clinical strategy for addressing exosomes in oncology is lacking. **Aethlon Medical is advancing the Hemopurifier, a hollow fiber hemofiltration device that is operated using dialysis infrastructure, for removing exosomes from circulating blood. The Hemopurifier incorporates size exclusion and a lectin (*Galanthus nivalis* agglutinin; GNA) affinity matrix for capturing nanoparticles having high mannose glycoprotein surface structures.**

Experimental Procedures: A benchtop version of the clinical Hemopurifier device was operated by recirculating exosomes from cancer patients' plasma through the hollow fiber filters *ex vivo*. The vesicles populations captured by the Hemopurifier were assessed using exosomes isolated by a proven and reliable method, size exclusion chromatography, which utilizes small-size ion exchange columns (mini-SEC) to obtain total exosome fractions from plasma (Ref 1). The Hemopurifier-captured exosomes were quantified and phenotyped using nanoparticle tracking analysis (NTA) and the ExoView instrument

Results: The Hemopurifier can effectively clear 92-99% of plasma exosomes originating from diverse tumor types, including head and neck cancer, melanoma, ovarian cancer, esophageal cancer and breast cancer, from recirculating fluids. Analyses using plasma samples from Stages III and IV triple negative and human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer showed that the Hemopurifier exhibits specificity for exosomes as shown by size and phenotype analysis of the vesicles that are captured.

Conclusions: The Hemopurifier effectively clears exosomes present in plasma that originate from diverse types of cancer. Hemopurifier-captured exosomes exhibit signatures of malignancy and immunosuppression and are therefore relevant therapeutic targets. Aethlon is advancing the Hemopurifier as a therapeutic device to remove tumor-derived exosomes from cancer patients' circulation in combination with standard of care therapies.

Tumor-derived exosomes as therapeutic targets in oncology

- Exosomes are 30-150 nm, membrane-bound vesicles containing cellular cargo.
- Abundant in cancer patients' plasma; typically 10^8-10^{11} vesicles/mL and positive correlations of exosome concentrations with tumor stage (Ref 2).
- Exosomes may have a multitude of cargo including tumor antigens and microRNAs.
- Nonclinical studies show that cancer exosomes have disease-promoting functions:
  - Tumor growth
  - Metastasis
  - Immune suppression
  - Chemotherapy & immunotherapy resistance
- Exosomal PD-L1 suppresses anti-tumor immunity and has been implicated in resistance to immune checkpoint inhibitors (Ref 3).
- There are no approved therapies for addressing exosomes.

The Hemopurifier® : A first-in-class candidate for targeting tumor-derived exosomes in oncology

Mechanism of Action

- The Hemopurifier is hollow fiber plasma separator that filled with a proprietary lectin affinity resin in the extra-capillary space outside the hollow fibers and is compatible with dialysis infrastructure.
- The lectin is *Galanthus nivalis* agglutinin (GNA); has specificity for high-mannose glycoproteins.
- The Hemopurifier has a dual mechanism of action:
  - Plasma separation based on size (<200 nm);
  - GNA capture based on high-mannose surface glycoproteins.

Hemopurifier Oncology Program

- FDA granted "Breakthrough Device" designation for the removal of exosomes for advanced and metastatic cancer patients who are unresponsive or intolerant to standard of care therapy (Ref 4).
- FDA approved an Investigational Device Exemption (IDE) for an Early Feasibility study of the Hemopurifier for exosome removal in head and neck cancer (Ref 5).
- A scaled-down Hemoupurifier device is being advanced for exosome isolation for exosome-based diagnostic applications in oncology.

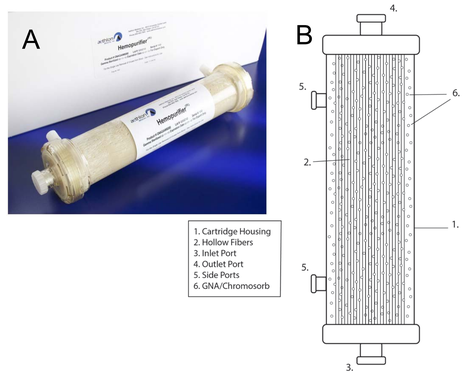

Figure 1: (A) The clinical Hemopurifier; (B) Schematic of the Hemopurifier cartridge

Results: The Hemopurifier® captures and removes exosomes from recirculating fluids *in vitro*

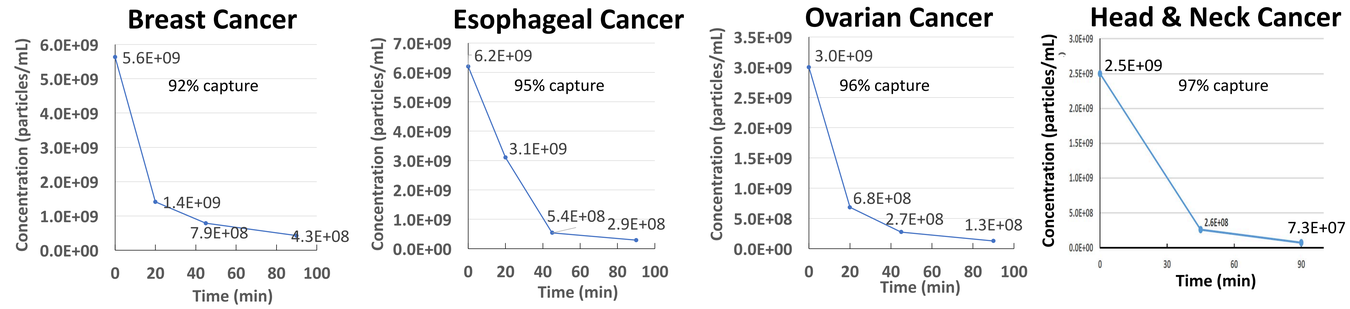

Figure 2: Capture of exosomes by the Hemopurifier. Plasma was from Stages III/IV cancer patients prior to treatment (University of California, Irvine IRB approval CPA#13990, HS# 2014-1523). Exosomes were enriched using mini-SEC or ultracentrifugation to determine the input exosome concentrations by nanoparticle tracking analysis (NTA). Exosomes were applied to medium that was recirculated continuously over small-scale Hemopurifiers using a peristaltic pump. Fluid aliquots were taken at the indicated time points to analyze exosomes remaining in solution (i.e. unbound) vs. time by NTA.

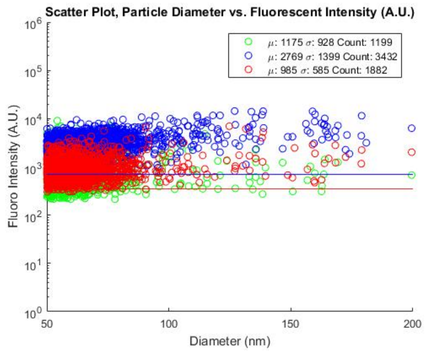

Figure 3 (left): Characteristics of exosomes that are captured by the Hemopurifier. Representative analysis of exosomes enriched from a breast cancer patient's plasma using the mini-SEC method. The vesicles are predominantly 50-100 nm in size, express tetraspanins, which are vital for exosome formation and function (Ref 6), and are captured by the Hemopurifier *in vitro* (as per Figure 2). ExoView (NanoView Biosciences) analysis was performed whereby exosomes are bound to spots on chips via tetraspanin-specific antibodies (anti-CD9 shown) and are then stained with Alexa Fluor 488, 555, and 647 antibodies to detect CD9 (blue), CD81 (green) and CD63 (red), respectively.

Conclusions

- **The Hemopurifier captures exosomes that are abundant in cancer patients' plasma.**
- **Ongoing studies are evaluating the immunophenotypes and functions of captured exosomes.**
- **The Hemopurifier is being advanced as a clinical hemofiltration device for removing circulating exosomes from cancer patients as an adjunct to immunotherapy.**
- **Therapeutic exosome removal may improve patients' immune status and responses to therapy.**

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